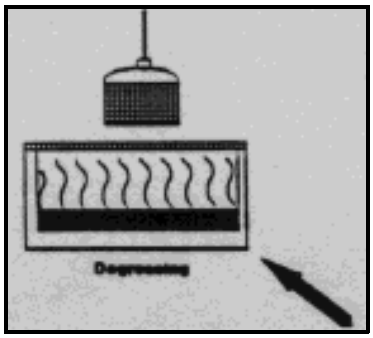
STAFF REPORT

PROPOSED IDENTIFICATION OF METHYLENE CHLORIDE AS A TOXIC AIR CONTAMINANT



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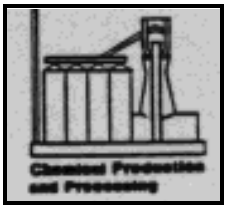


State California
Air Resources Board
Stationary Source Division

May 1989

Proposed Identification of Methylene Chloride as a Toxic Air Contaminant

Prepared by the Staffs of
the Air Resources Board and
the Department of Health Services

Stationary Source Division
Air Resources Board
May 1989

Proposed Identification of Methylene Chloride
as a Toxic Air Contaminant

Date and Address of Board Hearing:

July 13 and 14, 1989

Lincoln Plaza
Auditorium, First Floor
400 P Street
Sacramento, California 95814

Address of the Air Resources Board:

Air Resources Board
Stationary Source Division
1102 'Q' Street
Sacramento, California 95814

(This report has been reviewed by the staffs of the California Air Resources Board and the California Department of Health Services and approved for publication. Approval does not signify that the contents necessarily reflect the views and policies of the Air Resources Board or the Department of Health Services, nor does mention of trade names of commercial products constitute endorsement or recommendation for use.)

INTRODUCTION AND RECOMMENDATION

Definition of a Toxic Air Contaminant. Health and Safety Coda section 39655 defines a toxic air contaminant as an air pollutant which the Air Resources Board or the Department of Food and Agriculture finds "may cause or contribute to an increase in mortality or an increase in serious illness, or which may pose a present or potential hazard to human health."

Evaluation and Recommendation. The staffs of the Air Resources Board (ARB) and the Department of Health Services (DHS) have reviewed the available scientific evidence on the presence of methylene chloride in the atmosphere of California and its potential adverse effect on public health. Based on the finding of carcinogenicity and the results of the risk assessment, the DHS staff finds that methylene chloride meets the definition of a toxic air contaminant.

Findings of the Scientific Review Panel. The Scientific Review Panel (SRP), which is established pursuant to Health and Safety Code section 39670, reviewed the report in accordance with Health and Safety Code section 39661, and found the report to be without serious deficiency. The findings of the SRP are attached at the end of this Report.

Recommendation. The staff of the Air Resources Board recommends that the Board identify methylene chloride as a toxic air contaminant. In making this recommendation, the ARB and DHS staffs found that there is not sufficient available scientific evidence at this time to support the identification of an exposure level below which carcinogenic effects would not have some probability of occurring. Therefore, we recommend that methylene chloride be treated as having no identified threshold.

Reasons for Recommendation. Methylene chloride was chosen for evaluation because: the International Agency for Research on Cancer (IARC) and the U.S. Environmental Protection Agency (U.S. EPA) have concluded that there is ample evidence that methylene chloride is

carcinogenic in animals. (The IARC considers methylene chloride a possible human carcinogen and the U.S. EPA considers methylene chloride to be a probable human carcinogen.) Methylene Chloride is emitted from a variety of sources in the State, and it does not break down at a rate that would significantly reduce public exposure.

About these Documents. In addition to making a recommendation, this report summarizes the information in the accompanied technical support document. The technical support document includes four parts which are the “Overview and Recommendation,” the Part A report, the Part B report, and the Part C report. The “Overview and Recommendation" summarizes and integrates the information in Parts A and B; Part A was prepared by the ARB staff and reviews the public exposure to, environmental fate of, and sources of atmospheric methylene chloride in California; Part B was prepared by the DHS and reviews the health effects and risks associated with exposure to methylene chloride; and Part C contains the public comments that were received during the public comment periods and the ARB and the DHS staffs' responses to those comments.

I.

SOURCES OF METHYLENE CHLORIDE

Methylene chloride is a popular chemical, primarily because of its excellent solvent characteristics, low flammability, and low boiling point. Although methylene chloride is not produced in California, it is widely used in the state with estimated emissions of approximately 20,000 tons per year.

Methylene chloride is a constituent in products used by industry as well as by the general population. The major use categories, representing about 90 percent of the estimated annual emissions of methylene chloride, in decreasing order are paint removers, aerosols, degreasers, and polyurethane foam manufacturing. In addition to these, several other source categories use hundreds of tons of methylene chloride each year. These categories include pharmaceuticals, electronics, chemical production and processing, and pesticide manufacturing. For some source categories such as aerosols and decreasing, the consumption of methylene chloride has or is expected to decline. This decrease has resulted from concern over health effects as well as proposed restrictions on the use of methylene chloride.

Because emissions from many of the uses for methylene chloride are not controlled, a high percentage (about 80 percent) of the methylene chloride used in California is emitted to the atmosphere. For some categories, such as paint removers and aerosols, emissions from evaporation equal the amount used.

II.

EXPOSURE. HEALTH EFFECTS. AND RISK

A. EXPOSURE TO METHYLENE CHLORIDE

Persistence in the atmosphere. In the atmosphere, methylene chloride is a relatively stable pollutant. Methylene chloride's lifetime in the atmosphere is estimated to range from 80 to 250 days. Thus, methylene chloride is sufficiently persistent that it will be transported throughout an air basin and beyond before it is degraded.

Ambient concentrations. Since ARB's air toxics monitoring network was instituted in January of 1985, methylene chloride has been detected at eacthehe 20 monitoring stations in California. The mean concentrations have been determined for each of the monitoring stations during the 27 month period covering January 1985 through March 1987. Mean concentrations range from 0.4 ppb at the Merced station to 2.5 ppb at the El Monte station. Peak 24-hour average concentrations range from 2.0 ppb at the Concord, Richmond, and Stockton stations to 21 ppb at the Santa Barbara station.

Exposure based on monitoring data. General population exposure to atmospheric methylene chloride was estimated for several areas in California by using ambient monitoring data from January 1985 through March 1987. The staff estimates that 20.3 million people (approximately 80 percent of the state's population) are exposed to a population-weighted mean methylene chloride concentration of 1.1 to 2.4 ppb. For the South Coast Air Basin (SCAB), where the highest mean concentrations occurred, approximately 10.9 million people are exposed to a population-weighted mean concentration of 1.5 to 3.1 ppb.

Exposure based on modeling. In addition to estimating general population exposure, dispersion modeling was used to estimate population exposure for people living near methylene chloride emission sources in the SCAB. Dispersion modeling of emissions from three large sources (annual emissions greater than 45 tons) indicates that many people may be exposed to concentrations significantly above the mean concentration for the SCAB. The three sources are a mobile home manufacturer, an automobile assembler, and a polyurethane foam manufacturer. Based on the emissions from the polyurethane foam manufacturer, the exposure results show that approximately 1,300 people are exposed to a maximum annual average concentration of 10.7 ppb. For the motor home manufacturer the results show that approximately 1000 people are exposed to a maximum annual average concentration of 5.7 ppb, while the results for the automobile assembler show that approximately 2,000 people are exposed to a maximum annual average concentration of 1.0 ppb. Because these exposure estimates consider emissions from the modeled sources only, and because of the extensive use and emissions of methylene chloride by industry as well as consumers throughout the SCAB, the actual population exposure near these sources may be under-represented.

Indoor exposure. Because only limited data are available, an estimate of exposure to methylene chloride through indoor air is not presented in this report. However, because methylene chloride is a constituent in many consumer products, short-term indoor concentrations may be several orders of magnitude higher than ambient concentrations. Inhalation of methylene chloride from the indoor environment is expected to vary depending on the degree and manner of use of products containing methylene chloride.

A limited indoor monitoring study, conducted in Italy, monitored the indoor air of 15 structures for several organic compounds including methylene chloride. The mean indoor concentration of methylene chloride over a period of four to seven days was 193 ppb with the concentration for one of the structures reported as 1,450 ppb.

As part of a study conducted in Los Angeles County, the indoor and outdoor air of eight homes during the summer season was sampled and subsequently analyzed for several compounds including methylene chloride. For these homes, draft results show overnight indoor concentrations to range from 0.3 to 3.6 ppb with daytime indoor concentrations ranging from 0.3 to 3.9 ppb. Overnight outdoor concentrations range from 0.1 to 1.3 ppb while daytime outdoor concentrations range from 0.2 to 3.9 ppb. The results for the this study indicate that indoor concentrations of methylene chloride in some homes may not be substantially higher than outdoor concentrations. In addition, the contrasting results reported by the Italian and Los Angeles study illustrate the need for a comprehensive indoor monitoring study where methylene chloride is sampled and analyzed throughout California.

Other studies have evaluated personal breathing zone concentrations resulting from the use of consumer products containing methylene chloride. Results from a chamber study where a paint stripper was being used resulted in breathing zone exposures up to 2,000 ppm averaged over an hour with peak breathing zone concentrations of up to 3,300 ppm.

Breathing zone concentrations of the magnitude reported above are generally found during the time period the product is being applied and declines rapidly, depending on ventilation rate, once application is finished.

Other routes of exposure. In addition to inhalation, exposure to methylene chloride may occur when drinking water and food products which contain methylene chloride are ingested. The ARB staff reviewed the results of studies that analyzed food-products and drinking water for the presence of methylene chloride. The data indicate that concentrations of methylene chloride in drinking water are typically below detection levels of approximately 0.5 micrograms per liter. The study results also indicate that, for most people, the ingestion of food is not a substantial route of exposure to methylene chloride. Therefore, the ARB staff believes that inhalation is the most important route of exposure for the majority of California's population.

B. HEALTH EFFECTS OF METHYLENE CHLORIDE

The health effects of methylene chloride have been reviewed and evaluated to determine whether methylene chloride meets the definition of a toxic air contaminant. What follows is a summary of the DHS's findings regarding the health effects of methylene chloride.

Non-carcinogenic health effects. Methylene chloride has a relatively low acute toxicity. The median LC50 values for rats and mice exposed for six hours to methylene chloride vapors are approximately 17,000 ppm and 14,100 ppm, respectively. Environmental concentrations of methylene chloride estimated in California (24-hour average concentrations up to 21 ppb) are well below any levels that may cause adverse acute and noncarcinogenic chronic health effects.

The EPA concluded that methylene chloride is capable of inducing mutations in exposed human cells; the IARC reviewed short-term tests of DNA damage for methylene chloride and concluded that there was sufficient evidence to classify methylene chloride as genetically active. IARC also concluded that methylene chloride causes cell transformation in mammalian cells cultured *in vitro*.

The staff of the DHS concurs with the IARC's and the EPA's evaluations but stresses that the high concentrations of methylene chloride necessary to induce mutagenic and cellular transformations demonstrate that methylene chloride should be considered weakly genotoxic.

Carcinogenic health effects. Several long-term rodent studies have shown that methylene chloride increases tumor rates in some organs, primarily the mouse liver and lung and the rat mammary gland at concentrations ranging from 1,000 ppm to 4,000 ppm.

Both the EPA and the IARC have concluded that methylene chloride is carcinogenic in animals with inadequate evidence for carcinogenicity in humans. Overall, the EPA assigned methylene chloride to category B2, which means that methylene chloride should be considered a "probable" human carcinogen. IARC assigned methylene chloride a rating of 2B and considers methylene chloride a “possible” human carcinogen.

The DHS staff agrees with the EPA and the IARC that methylene chloride is carcinogenic in animals with inadequate evidence for carcinogenicity in humans. The DHS staff have concluded that methylene chloride is a potential human carcinogen. The DHS staff recommends that methylene chloride be considered as not having a threshold for carcinogenicity because:

1) The DHS staff found no evidence for a carcinogenic threshold level; and

2) because there are several short-term tests suggesting that methylene chloride is mutagenic.

C. RISK DUE TO METHYLENE CHLORIDE

The DHS staff recommends that the range of risks for ambient exposures to methylene chloride be based on the 95 percent upper confidence limit predicted from fitting two different mathematical models to the animal data. The DHS report (Part B) also includes the range of risks based on EPA's application of a physiologically based pharmacokinetic (PBPK) model to estimate exposure. The DHS staff estimate that the unit risk from a lifetime continuous exposure to 0.29 ppb (1 g/μm^3) of methyleng chloride ranges from 0.3 x 10^{-6} to 3 x 10^{-6} (1 x 10^{-6}/ppb to 10 x 10^{-6}/ppb). The SRP estimates that the range of lifetime excess cancer risk from exposure to 1 ppb of atmospheric methylene chloride based on the upper 95% confidence limit is from 9 x 10^{-8}/ppb (PBPK model without surface area correction) to

10 x 10^{-6}/ppb (applied dose). The DHS staff and the SRP conclude that the most plausible estimate of the unit risk based on application Of the PBPK model high-to-low dose adjustment, is 1 x 10^{-6} per 1 μg/m^3, which is equivalent to 4 x 10^{-6}/ppb.

Exposure to the range of mean ambient concentrations (weighted by population) of 1.1 to 2.4 ppb, as estimated by the Air Resources Board staff for a population of 20.3 million people, could result in up to 20 to 500 excess lifetime cancers, based on the upper-bound of the 95% confidence interval of the models used by the DHS. Applying the range of risk estimated by the SRP (9 x 10^{-8}/ppb to 10 x 10^{-6}/ppb) to the mean range of ambient exposure (weighted by population) for a population of 20.3 million people, could result in up to 2 to 500 excess lifetime cancers. Applying the most plausible estimate of upper limit risk (4 x 10^{-6}/ppb) to the mean range of ambient exposure (weighted by population) for a population of 20.3 million people, could result in up to 90 to 200 excess lifetime cancers. These calculations which are summarized in Table 1 are for the upper range of plausible excess cancer risks, the actual risk which cannot be calculated may approach zero.

Table 1

Estimate of Statewide Excess Lifetime Cancer Cases
from Exposure to Methylene Chloride

Group	**Estimate of Risk**	**Lifetime Cancer Cases[a]**
DHS/SRP	4 x 10^{-6}/ppb (most plausible)[b]	90 to 200
DHS	1 x 10-6/ppb to 10 x 10^{-6}/ppb	20 to 500
SRP	9 x 10^{-8}/ppb to 10 x 10^{-6}/ppb	2 to 500

[a] - Based on exposure to mean ambient concentrations (weighted by population) ranging from 1.1 to 2.4 ppb for a population of 20.3 million people.

[b] - The most plausible estimate of risk according to the DHS and the SRP.

Individuals exposed to the maximum annual mean concentration, estimated for people living near a ethylene chloride emission source, of 10.7 ppb for a lifetime would have a risk of

developing cancer equal to 40 cases per million exposed. (This estimate of cancer cases is obtained by applying the most plausible estimate of upper limit risk of 4×10^{-6}/ppb to the lifetime exposure estimate.) These calculations are for the upper range of plausible excess cancer risks, the actual risk, which cannot be calculated, may approach zero.

Based on the finding of methylene chloride-induced carcinogenicity and the results of the risk assessment, the DHS staff finds that at ambient concentrations, methylene chloride is an air pollutant which may cause or contribute to an increase in mortality or an increase in serious illness, or which may pose a present or potential hazard to human health.

III.

ALTERNATIVES AND ENVIRONMENTAL IMPACTS

A. ALTERNATIVES

Government Code Section 11346.14 requires agencies to describe alternatives to the regulation considered by the agency and the agency's reasons for rejecting those alternatives. The only alternative to identifying methylene chloride as a toxic air contaminant is to not identify it. The ARB staff is not recommending this alternative because the ARB staff believes that methylene chloride meets the statutory definition of a toxic air contaminant.

There are no alternatives considered by the ARB staff which would be more effective in carrying out the purpose for which the amendment is proposed or would be as effective and less burdensome to affected private persons than the proposed amendment.

B. ENVIRONMENTAL IMPACTS

The identification of methylene chloride as a toxic air contaminant is not in itself expected to result in any environmental effects. The identification of methylene chloride as a toxic air contaminant by the Board may result in the Board and air pollution control districts adopting control measures in accordance with the provisions of state law (Health and Safety Code sections 39665 and 39666). Any such toxic control measures would result in reduced emissions of methylene chloride to the atmosphere, resulting in reduced ambient concentrations, thereby reducing the health risk due to methylene chloride exposure. Therefore, the identification of methylene chloride as a toxic air contaminant may ultimately result in environmental benefits. Environmental impacts identified with respect to specific control measures will be included in the consideration of such control measures pursuant to Health and Safety Code sections 39665 and 39666.

Also considered was methylene chloride's importance in contributing to global warming as well as depletion of the stratospheric ozone layer. After consulting with the U.S. EPA and other scientists in the field it was determined that methylene chloride is not a significant contributor to either of these phenomenon. Thus, any reduction in emissions of methylene chloride would not be expected to significantly reduce global warming or stratospheric ozone depletion.

STATE OF CALIFORNIA ATTACHMENT George Deukmejian

AIR RESOURCES BOARD
1102 Q STREET P.O. BOX 2815
SACRAMENTO. CA 95812

May 15, 1989

Mr. William C. Lockett, Chief
Office of External Affairs
California Air Resources Board
1102 Q Street
Sacramento, California 95814

Dear Bill:

The Scientific Review Panel on Toxic Air Contaminants has reviewed the Report on Methylene Chloride, which includes Tables 8-5 and 8-7 from Part B of the report by the Department of Health Services, and has formulated its findings regarding the report. I am formally submitting the Scientific Review Panel's findings to the Air Resources Board.

Sincerely,

//s//
Dr. James N. Pitts
Acting Chairman, SRP

Enclosure

cc: Scientific Review Panel

Findings of the Scientific Review Panel on
THE REPORT ON METHYLENE CHLORIDE
As Adopted at the Panel's April 18, 1989 Meeting

In accordance with the provisions of Health and Safety Code section 39661, the Scientific Review Panel (SRP) has reviewed the reports of the staffs of the ARB and DHS on the public exposure and biologic and health effects of methylene chloride, and the public comments on these reports. Based on this review, the SRP finds that the reports are without serious deficiencies ant further finds that:

1. Methylene chloride has been identified as an animal carcinogen and should be regarded as a potential human carcinogen.

2. Methylene chloride is emitted into the air by a variety of stationary sources in California.

3. Based on its gas-phase reactivity with hydroxyl radicals, methylene chloride has an atmospheric lifetime estimated to range from 80 to 250 days.

4. Approximately 20.3 million people in California are estimated to be exposed to a population-weighted mean methylene chloride concentration of 1.1 to 2.4 parts per billion.

5. Adverse health effects other than cancer are not known to occur at predicted concentrations of methylene chloride in ambient outdoor air.

6. Based on available scientific information, a methylene chloride exposure level below which carcinogenic effects are not expected to occur cannot be identified.

7. Based on an interpretation of available scientific evidence, DHS staff estimated risks using both the applied dose and a physiologically based pharmacokinetic model (PBPK) (see attached table). The range of lifetime excess cancer risk from exposure to 1 ppb (3.5 $\mu g/m^3$) of atmospheric methylene chloride based on the upper 95% confidence limit is from 9×10^{-8}/ppb (PBPK model without surface area correction)[(a)] to 10×10^{-6}/ppb (applied dose). This includes EPA's application of the PBPK model which would estimate a risk of 1×10^{-6}/ppb with a surface area correction). DHS uses a PBPK model with a high to low dose adjustment which generates a risk of 4×10^{-6}/ ppb. Based on available data it is the most plausible estimate of the upper limit of risk. These upper bound excess lifetime risks are health protective estimates; the actual risk may be below these values.

8. Exposure to the range of mean ambient concentrations (weighted by population) of 1.1 to 2.4 ppb for a population of 20.3 million people, could result in up to 2 to 500 excess lifetime cancers, based on the upper-bound of the 95% confidence interval of the models.

For these reasons, we agree with the ARB staff recommendation to its Board that methylene chloride be listed by the ARB as a toxic air contaminant.

(a) **NOTE**: The DHS staff chose only to report a range using a surface area correction.

I certify that the above is a true and correct copy of the findings adopted by the Scientific Review Panel on April 18, 1989

//s//

Dr. James N. Pitts
Acting Chairman, SRP

Attachments

CONDENSATION OF DHS TABLES 8-5 AND 8-7

COMPARISON OF HUMAN CANCER RISK ESTIMATES FROM A CH_2Cl_2 EXPOSURE, BASED ON LUNG TUMORS IN FEMALE MICE, USING VARIOUS MODELS AND ASSUMPTIONS [a]

Approach	Mathematical Model	Lifetime Risk	
Applied Dose	Linearized Multistage	ppb^{-1}	$(\mu g/m^3)^{-1}$
Applied Dose	Time-Dependent Multistage	9×10^{-6}	3×10^{-6}
High-to-Low Dose[b]	Linearized Multistage	10×10^{-6}	3×10^{-6}
High to Low Dose	Time Dependent Multistage	4×10^{-6}	1×10^{-6}
Total PBPK[c]	Linearized Multistage	5×10^{-6}	1×10^{-6}
Total PBPK[d]	Linearized Multistage	1×10^{-6}	3×10^{-7}

[a] All values were adjusted for continuous exposure. That is, the mouse exposure in ppm was multiplied by (6 hours/day)/(24 hours/day) x (5 days/week)/(7 days/week). The concentrations are calculated from the female mouse lung tumor data (NTP 1986). The extrapolation was also corrected for surface area (a factor of 12.7) unless indicated otherwise. The risks are based on the linearized multistage model. Surface area conversion for rodent to human risks, and risk estimates are reported at 95% upper bound values.

[b] High-to-low adjustment for saturation of MFO pathway based on the HRAC report (EPA 1987a).

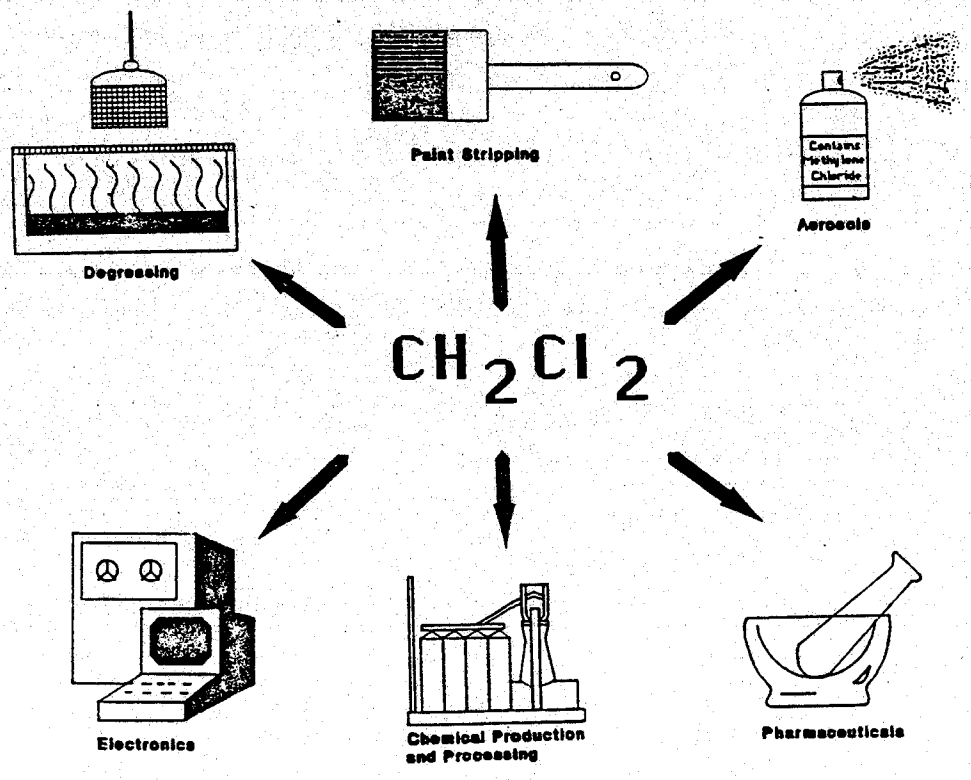
[c] High-to-low adjustment for saturation of MFO pathway and the species to-species adjustment based on the HRAC report which includes a 12.7 surface area correction (EPA 1987a).

[d] High-to-low adjustment for saturation of MFO pathway and the species adjustment based on the HRAC report (EPA 1987a), except without use of a surface area correction.

TECHNICAL SUPPORT DOCUMENT

PROPOSED IDENTIFICATION OF METHYLENE CHLORIDE AS A TOXIC AIR CONTAMINANT

Part A Report



State of California
Air Resources Board
Stationary Source Division

May 1989

FINAL DRAFT

TECHNICAL SUPPORT DOCUMENT

REPORT TO THE AIR RESOURCES BOARD ON METHYLENE CHLORIDE

Prepared by the Staff of the Air Resources Board

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May 1989

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May 1989

(This report has been reviewed by the staff of the California Air Resources Board and approved for publication. Approval does not signify that the contents necessarily reflects the views and policies of the Air Resources Board, nor does mention of trade names or commercial products constitute endorsement or recommendation for use.)

FINAL DRAFT

REPORT TO THE AIR RESOURCES BOARD ON METHYLENE CHLORIDE

Part A - Public Exposure to, Atmospheric fate of, and Sources of Atmospheric Methylene Chloride in California

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I.

INTRODUCTION

Methylene chloride is one of the family of chemicals known as chlorinated hydrocarbons and has the chemical formula CH_2Cl_2. It has several chemical synonyms and trade names including: dichloromethane, methylene dichloride, methane dichloride, Aerothene NM, Somethine^R, F30^R, Freon 30^R, and F-30^R. It is used in California in the following products and processes: paint removers, aerosols, degreasing operations, pesticide manufacturing and use, photographic film processing, pharmaceuticals, food processing, chemical processing and production, and photoresist stripping. Methylene chloride is also used in a variety of miscellaneous applications primarily for its solvent properties.

This report is an evaluation of methylene chloride uses, emission sources, ambient and indoor concentrations, and population exposure in California. Also included are discussions of the atmospheric fate and the physical properties of methylene chloride. The Air Resources Board will consider the findings of this report together with the health related effects findings of the Department of Health Services to determine if methylene chloride should be identified as a toxic air contaminant (TAC).

The ARB is the state agency responsible for the identification of TACs in their non-pesticidal uses. State law defines a TAC as an air pollutant which the Air Resources Board (ARB) or the Department of Food and Agriculture (DFA) finds "...may cause or contribute to an increase in mortality or an increase in serious illness, or which may pose a present or potential hazard to human health" (California Health and Safety Code Section 39655).

Because all of the methylene chloride consumed for many use categories is emitted to the atmosphere, annual emissions in California are significant. Once in the atmosphere methylene chloride is persistent with an atmospheric lifetime which may be on the order of several months. The ARB's monitoring network has detected methylene

chloride throughout the state. Monitoring results show that the highest concentrations occur in the South Coast Air Basin where the majority of the state's identified emission sources and roughly 40 percent of the state's population are located.

This report presents estimates of the general population's exposure to methylene chloride. The results show that people living within the studied areas (approximately 80 percent of the state's population) are exposed to airborne methylene chloride throughout the year. Because there is some uncertainty about the absolute concentrations that people are exposed to, a range of concentrations is presented. In addition, exposure was estimated for people living near methylene chloride emission sources in the South Coast Air Basin. Modeling results show that people living near emission sources are exposed to levels of methylene chloride that are higher than average concentrations in that air basin.

Although the data are limited, it appears that indoor exposure to methylene chloride as well as exposure from the use of consumer products may represent the most important source of exposure for some people. However, in order to adequately characterize these exposures will require further research.

II.

EXPOSURE TO METHYLENE CHLORIDE

A. AMBIENT MONITORING IN CALIFORNIA

In January 1985, the Air Resources Board (ARB) initiated ambient monitoring for methylene chloride at 20 monitoring stations located throughout California. ARB's monitoring and Laboratory Division (MLD) carried out the sampling and analysis for the nine monitoring stations located in southern California and for the eleven monitoring stations located in northern California.

For each monitoring station, approximately two samples are taken each month. Samples are collected in 30 liter Tedlar bags over a twenty-four hour period using a low volume sampler. Samples are subsequently preconcentrated and analyzed by gas chromatography employing an electron capture detector. Resultant peaks are identified by retention time and quantified by reference to calibration standards. The ARB's limit of detection (LOD) for the analytical method for methylene chloride has been determined to be 1.0 ppb. The precision for the analytical method has been determined and shows that one standard deviation for the analysis is on the order of ±10 percent. However, the overall error associated with the sampling and analysis method has not been determined. The standard operating procedures for the sampling and analysis conducted by ARB are provided in Appendix I.

Monitoring data for the time period of January 1985 through March 1987 have been evaluated for this report. At each monitoring station there are some months for which no data have been reported. For these months either no samples were collected or laboratory staff have judged that the sampling and analysis did not meet ARB's quality control criteria and therefore the data were deleted. Table II-1 presents the months for which data are available and the total number of samples reported for each station during the twenty-seven month period.

B. CONCENTRATIONS IN CALIFORNIA

Since the monitoring network was instituted in January of 1985, methylene chloride has been detected at each of the 20 monitoring

TABLE II-1

SUMMARY OF AVAILABILITY OF METHYLENE CHLORIDE DATA
DURING JANUARY 1985 THROUGH MARCH 1987*

Air Basin Site Location	1985 JFMAMJJASOND	1986 JFMAMJJASOND	1987 JFM	# of Samples
SOUTHERN CALIFORNIA SITES				
SOUTH COAST AIR BASIN:				
El Monte	●●●●●●○○●●●●	●○●●●●●●●●●●	●●●	68
Los Angeles	●●●●●●○○●●●●	●○●●●●●●●●●●	●●●	55
No. Long Beach	●●●●●○○○●●●●	●○●●●●●●●●●●	●●●	53
Riverside	●●●●●●○○●●●●	●●●●●●●●●●●●	●●●	52
Upland	●●●●●●○○●●●●	●●●●●●●●●●●●	●●●	54
SOUTH CENTRAL COAST AIR BASIN:				
Santa Barbara	●●●●●○○○●●●●	●○●●●●●●●●●●	●●●	46
Simi Valley	●●●●●○○○●●●●	●●●●●●●●●●●●	●●●	56
SAN DIEGO AIR BASIN:				
Chula Vista	●●●●●●○○●●●●	●○●●●●●●●●●●	●●●	56
El Cajon	●●●●●●○○●●●●	●○●●●●●●●●●●	○●●	50
NORTHERN CALIFORNIA SITES				
SAN FRANCISCO BAY AREA AIR BASIN:				
Concord	○○○○○○○●●●●●	●●○○○●●●●●○	●●●	24
Fremont	○○○○○○○●●●●●	●●○○○●○○●●●○	●●●	26
Richmond	○○○○○○○●●●●●	●●○○○●●●●●●	●●●	29
San Francisco	○○○○○○○●○●●●	○●○○○●●●●●●	○●●	19
San Jose	○○○○○○○●●●●●	●●○○○●●○●●●●	●●●	28
SAN JOAQUIN VALLEY AIR BASIN:				
Bakersfield	○○○○○○○●●●●●	●●○○○●●●○●○	●○●	30
Fresno	○○○○○○○●●●●●	●●○○○●○●●●●	●●●	32
Merced	○○○○○○○●●●●●	●●○○○○○●●●●	●●●	26
Modesto	○○○○○○○●●●●●	●●○○○●○●●●●	●●●	28
Stockton	○○○○○○○●●●●●	●●○○○●●●●●●	●●●	34
SACRAMENTO VALLEY AIR BASIN:				
Citrus Heights	○○○○○○○●●●●●	●●○○○●○●●●●	●●●	31

* A solid dot indicates at least one sample is available for that particular month.

stations in California. Summary statistics have been compiled using all the available monitoring data for the twenty-seven month period covering January 1985 through March 1987.

As stated earlier, for each of the monitoring stations there are months for which data are not available. Thus, the number of samples representing each month of the year for each station is variable. For instance, no data are available for the second calendar quarter at the Merced station for 1985, 1986 and 1987. If a seasonal variation of the ambient concentration occurs each year, the variation in the number of samples available for each month could bias the overall mean concentration. However, after evaluating the monthly means in each air basin, ARB staff did not detect any discernable seasonal variation in the ambient concentration of methylene chloride.

For each monitoring station Table II-2 reports the number of samples in the data base, the mean concentration for the samples, the peak 24-hour average concentrations measured, the percentage of samples below the LOD, and an estimate of the concentration of the samples below the LOD.

The number of samples available at each station ranges from 19 to 68 with a total of 797 samples reported for the 20 station network. Forty-eight percent of the 797 samples are below the LOD . However, for individual monitoring stations the percentage of samples below the LOD ranges from 16 percent at the El Monte station to 91 percent at the Stockton station. In general, northern California stations have a higher percentage of samples below the LOD than do southern California stations.

Calculation of mean concentrations for stations is complicated by the presence of concentrations below the LOD. The concentrations below the LOD must be somehow included in the calculation although their exact values are not known.

Staff has used a method proposed by Gleit (1985) to calculate the means. Gleit's method assumes that the sample of concentrations is a random sample from a normal distribution. Data that are judged not to be normally distributed may be transformed to approximate normality. Inspection of the methylene chloride data suggested that they were lognormally distributed, and Gleit's method was applied to the logarithms of these data. The calculated means were then transformed back to the original units.

Gleit's method accounts for the concentrations below the LOD by setting them equal to the "below-LOD mean", the mean of the portion of the normal distribution below the LOD. Setting the unknown concentrations to their average value seems intuitively reasonable, and

TABLE II-2

SUMMARY STATISTICS OF THE JANUARY 1985 THROUGH MARCH 1987
METHYLENE CHLORIDE MONITORING DATA

Air Basin Site Location	Number of Samples	Mean Concentration[a] (ppb)	Peak Concentration (ppb)	Percentage of Samples Below LOD[b]	Estimated Concentration for Samples below the LOD[b] (ppb)
SOUTHERN CALIFORNIA SITES					
SOUTH COAST AIR BASIN:					
El Monte	68	2.5	7.0	16	0.8
Los Angeles	55	2.1	9.2	20	0.7
North Long Beach	53	2.2	7.0	19	0.7
Riverside	52	1.6	6.0	42	0.6
Upland	54	1.8	9.0	30	0.7
BASIN AVERAGE:	56	2.0		25	0.7
SOUTH CENTRAL COAST AIR BASIN:					
Santa Barbara	46	1.9	21.0	50	0.5
Simi Valley	56	1.4	5.0	46	0.6
BASIN AVERAGE:	51	1.7		48	0.6
SAN DIEGO AIR BASIN:					
Chula Vista	56	1.8	12.0	39	0.6
El Cajon	50	1.5	5.0	46	0.6
BASIN AVERAGE:	53	1.7		42	0.6
NORTHERN CALIFORNIA SITES					
SAN FRANCISCO BAY AREA AIR BASIN:					
Concord	24	NA	2.0	75	NA
Fremont	26	1.6	7.0	50	0.5
Richmond	29	0.8	2.0	69	0.6
San Francisco	19	0.5	4.0	84	0.2
San Jose	28	1.1	4.0	57	0.6
BASIN AVERAGE:	25	1.0		66	0.5
SAN JOAQUIN VALLEY AIR BASIN:					
Bakersfield	30	1.6	6.0	43	0.6
Fresno	32	1.0	5.0	66	0.5
Merced	26	0.4	3.0	88	0.1
Modesto	28	0.5	3.0	86	0.2
Stockton	34	0.4	2.0	91	0.3
BASIN AVERAGE:	30	0.8		75	0.3
SACRAMENTO COUNTY[c]:					
Citrus Heights	31	0.5	3.0	87	0.2

a Gleit's method was used to estimate the concentration of samples below the quantitation limit. NA indicates the site data were not appropriate for the method and no value could be calculated.

b LOD is the limit of quantitation of the analysis method. The LOD for all samples is 1.0 part per billion.

c Since there is only one monitoring station located in the Sacramento Valley Air Basin, the concentration was estimated for Sacramento County.

the simulations reported in Gleit's paper show that his method is more accurate than other commonly used approximations. A detailed description of the method used to estimate the concentration of data below the LOD is provided in Appendix II.

The values for samples below the LOD estimated by this method range from 0.1 ppb to 0.8 ppb. The specific values for each station are shown in Table II-2. Note that as the percentage of samples above the LOD increases the estimated value for the samples below the LOD also increases. Gleit's method could not be applied to the data from the Concord station because the data above the LOD exhibit little or no variation, which is necessary for the application of the method.

The mean concentration for the monitoring stations ranges from 0.4 ppb at the Merced and Stockton sites to 2.5 ppb at the El Monte site. The mean concentrations of the data reported for each monitoring station are displayed in Table II-2. Mean concentrations calculated for the southern California stations are generally higher than those calculated for the northern California stations. The overall mean for the southern California stations is 1.9 ppb compared with 0.8 ppb for the northern California stations.

In addition to reporting the mean concentration for each monitoring station, Table II-2 also reports the peak 24-hour average concentrations. The peak concentrations range from 2.0 ppb at the Concord, Richmond, and Stockton stations to 21.0 ppb at the Santa Barbara station.

The observation that methylene chloride concentrations tend to be higher at southern California stations, particularly in the South Coast Air Basin (SCAB), is consistent with what is known about the uses and emissions of methylene chloride. First, the majority of the state's large methylene chloride emissions sources identified thus far are located in the SCAB. Second, about 40 percent of the state's population is located in the SCAB and since the amount of methylene chloride consumed for several use types is proportional to population, emissions from these uses would be expected to be concentrated in the SCAB. Therefore, the mean concentrations as well as the peak concentrations of methylene chloride are expected to be higher for stations in the SCAB. Chapter III of this report discusses the various categories for which methylene chloride is used as well as the annual emissions associated with each use.

Results from two short-term monitoring studies in the SCAB show concentrations of methylene chloride similar to ARB monitoring data. Based on two weeks of monitoring in 1979, the mean concentration of methylene chloride in Los Angeles was reported to be 3.8 ppb with the

maximum concentration reported to be 12 ppb (Singh, et al., 1981). Another study reports a mean concentration of 2.0 ppb with a maximum of 9.4 ppb for Riverside, based on 10 days of monitoring in 1980 (Singh, et al., 1982). The concentrations reported in these studies are consistent with the range of concentrations measured by the ARB in the SCAB. However, the results from these two studies are not sufficient to determine an annual average concentration of methylene chloride.

The methylene chloride monitoring data available for January 1985 through March 1987 at any one station represent only a small (2 to 8 percent) fraction of all days during the time period. Because of the limited number of samples, the high percentage of samples below the LOD, the variability in meteorology from year to year, and the unquantified error associated with the sampling and analysis procedure, there is some degree of uncertainty associated with assuming that the mean concentration of all data collected for each monitoring site is representative of an annual average. In an attempt to try to quantify this uncertainty we have calculated the upper and lower bound estimates of the mean concentrations which are intended to estimate the probable range of the annual average concentration at each monitoring station.

ARB staff developed a statistical treatment for calculating upper and lower bound estimates of the mean concentration at each monitoring station. This treatment provides an estimate of the uncertainty associated with the mean concentration. This method takes into account factors such as sample size, variance of the data, and an estimate of the uncertainty associated with the sampling and analysis procedure. The following text is a discussion of the statistical treatment used.

A scatter plot of the methylene chloride monitoring data indicates that they are distributed lognormally. Because available software only analyze data that are normally distributed, methylene chloride monitoring data were first converted from a lognormal distribution to a normal distribution. This was done by using the logarithms of the data for the analysis and then converting the results back to concentration units. The Statistical Analysis System (SAS, 1982) was used to calculate the standard error about the mean. The standard error calculated from the logarithms of the data are then converted back into concentration units. The upper and lower bound estimates reported for the mean represent two standard errors. Though the error in the sampling and analysis has not been determined, we assumed an overall uncertainty factor of $\pm$ 20 percent at one standard deviation to calculate the upper and lower bound estimates of the mean. The lower bound estimate represents two standard errors for the data with each sample concentration reduced by 20 percent. The upper bound estimate represents two standard errors for the data with each sample concentration increased by 20 percent. Upper and lower bound estimates for each station are shown in Table II-3.

TABLE II-3

RANGE OF ESTIMATED MEAN METHYLENE CHLORIDE CONCENTRATIONS FOR JANUARY 1985 THROUGH MARCH 1987[a] (units are parts per billion ppb)

Air Basin Site Location	Lower Bound Estimate	Estimated Mean	Upper Bound Estimate
SOUTH COAST AIR BASIN (SCAB):			
El Monte	1.8	2.5	3.5
Los Angeles	1.4	2.1	3.0
North Long Beach	1.5	2.2	3.2
Riverside	1.1	1.6	2.4
Upland	1.2	1.8	2.6
SOUTH CENTRAL COAST AIR BASIN (SCCAB):			
Santa Barbara	1.2	1.9	3.9
Simi Valley	0.9	1.4	1.9
SAN DIEGO AIR BASIN (SDAB):			
Chula Vista	1.2	1.8	2.7
El Cajon	1.0	1.5	2.2
SAN FRANCISCO BAY AREA AIR BASIN (SFBAAB):			
Concord	NA	NA	NA
Fremont	0.9	1.6	2.7
Richmond	0.6	0.8	1.2
San Francisco	0.3	0.5	1.0
San Jose	0.7	1.1	1.7
SAN JOAQUIN VALLEY AIR BASIN (SJVAB):			
Bakersfield	1.0	1.6	2.5
Fresno	0.6	1.0	1.4
Merced	0.2	0.4	0.6
Modesto	0.3	0.5	0.7
Stockton	0.3	0.4	0.5
SACRAMENTO COUNTY[b]:			
Citrus Heights	0.3	0.5	0.7

[a] Gleit's method was used to estimate the concentration of samples below the quantitation limit. NA indicates the site data were not appropriate for the method and no value could be calculated.

[b] Since there is only one monitoring station located in the Sacramento Valley Air Basin, the concentration was estimated for Sacramento County.

C. EXPOSURE IN CALIFORNIA

Population exposure in California for six areas (five air basins and one county) was estimated using the mean concentrations calculated for each monitoring station. The mean population exposure estimates for the SCAB and the San Francisco Bay Area Air Basin (SFBAAB) were estimated by interpolating station means to census tract centroids. For the other areas, a single estimated mean concentration was calculated, based on the means at monitoring stations in the area, and it was assumed that all the people in the area were exposed to this estimated mean concentration.

In order to present the possible range of exposure, upper and lower bound estimates of the mean were determined for each of the six areas. This required using the upper and lower bound estimates of the mean concentration for each monitoring station as presented in Table II-3. For the SCAB and SFBAAB the upper and lower bound estimates were calculated by the same method used to estimate the mean concentration. As for the other areas, the upper and lower bound estimates are simply the average of the station values. For each of the six areas, Table II-4 gives the mean, the upper and lower bound estimates of the mean, and the number of people exposed. The population residing in these six areas is approximately 20.3 million people which represents roughly eighty percent of California's population during 1985. Population estimates are based on 1980 census data projected to 1985.

The areas for which no monitoring data exist are predominantly rural areas. Generally, these areas are not densely populated nor are there many industrial sources. Thus, methylene chloride emissions, and consequently ambient concentrations are expected to be lower than those reported for the more urbanized areas listed in Table II-4. However, it is possible that people residing in the vicinity of methylene chloride emission sources could be exposed to higher concentrations.

Based on our analysis of the monitoring data, ARB staff estimate a population-weighted mean concentration of 2.2 ppb for over ten million people residing in the South Coast Air Basin. In the San Francisco Bay Area Air Basin the population-weighted mean concentration is 0.9 ppb for the Basin's 4.4 million residents. For all six areas studied, the mean annual methylene chloride concentration, weighted by population, is estimated to range from 1.1 to 2.4 ppb. Figure II-1 shows the statewide cumulative exposure to methylene chloride with upper and lower bound estimates of the mean.

TABLE II-4

RANGE OF ESTIMATED MEAN POPULATION-WEIGHTED
EXPOSURE ESTIMATES FOR JANUARY 1985 THROUGH MARCH 1987[a]
units are parts per billion (ppb)

Air Basin	Lower Bound Estimate	Mean	Upper Bound Estimate	Population (millions)
South Coast[b]	1.5	2.2	3.1	10.09
San Francisco Bay Area[b]	0.6	0.9	1.6	4.39
San Diego	1.1	1.7	2.5	2.14
San Joaquin Valley	0.5	0.8	1.1	1.90
South Central Coast	1.1	1.7	2.5	0.92
Sacramento County	0.3	0.5	0.7	0.89
Overall Population-Weighted Average	1.1	1.6	2.4	20.33[c]

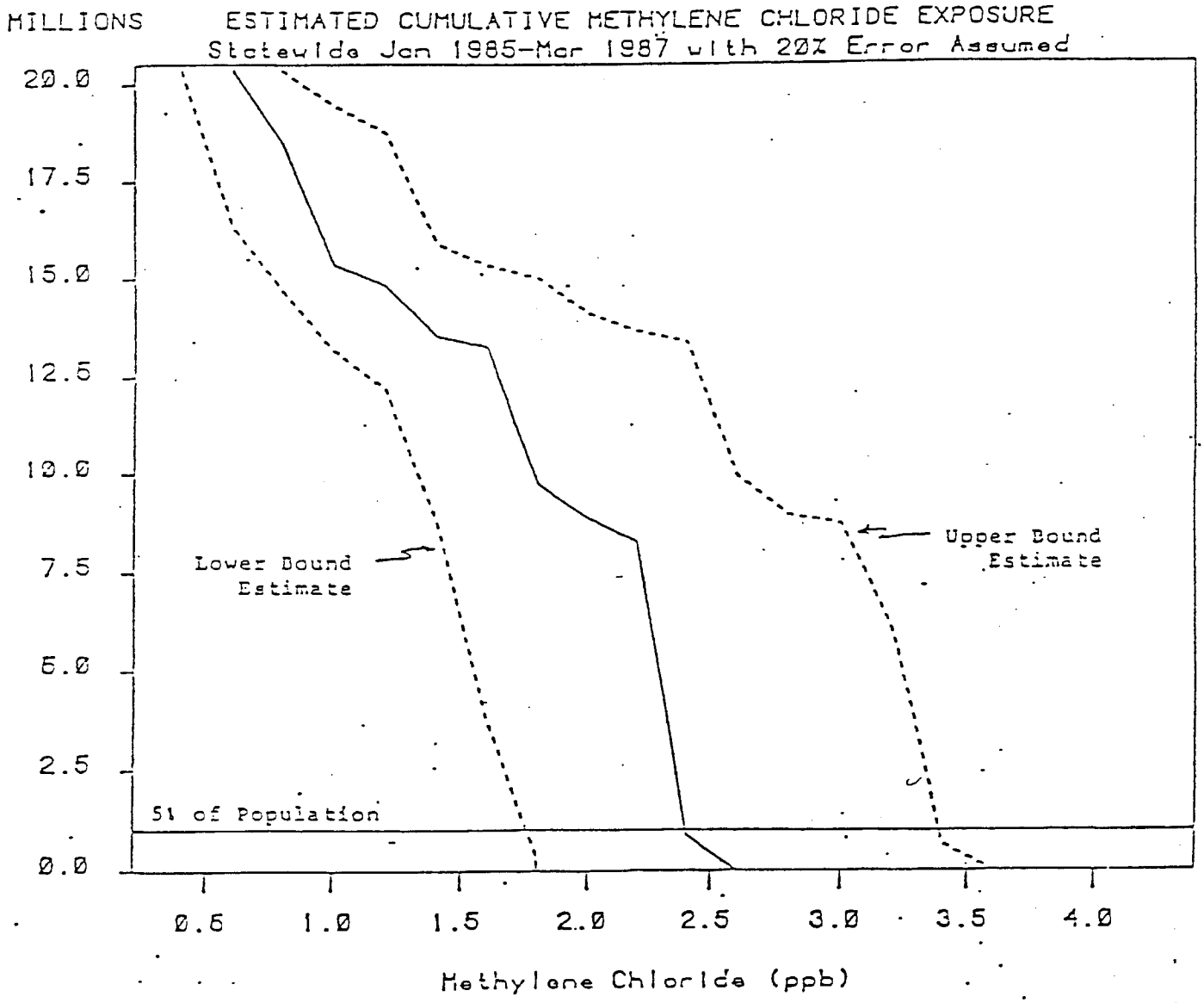
---

a Gleit's method was used to estimate the concentration of samples below the quantitation limit.

b The exposure estimates for the South Coast Air Basin and San Francisco Bay Area Air Basin are estimated by interpolating the mean concentrations at monitoring stations to census track centroids.

c Represents approximately 80 percent of California's population during 1985. The remaining 20 percent reside in areas in which no monitoring stations are located.

FIGURE II-1


MILLIONS
ESTIMATED CUMULATIVE METHYLENE CHLORIDE EXPOSURE
Statewide Jan 1985-Mar 1987 with 20% Error Assumed
20.0
17.5
15.0
12.5
10.0
7.5
5.0
2.5
0.0
Lower Bound Estimate
Upper Bound Estimate
51 of Population
0.5
1.0
1.5
2.0
2.5
3.0
3.5
4.0
Methylene Chloride (ppb)

D. EXPOSURE NEAR EMISSION SOURCES

To assess the impact of methylene chloride emission sources on nearby populations, the ARB used emissions information in conjunction with meteorological data to estimate population exposure in census tracts surrounding three facilities that emit methylene chloride. The following text is a discussion of the data and modeling methodology used to estimate population exposure along with the exposure results.

The concentration estimates presented in this section do not represent the total ambient air exposure of the people living in the vicinity of the modeled sources. The concentrations reported are those which would exist if the modeled facility was the only methylene chloride emission source affecting the nearby population. In reality there are other emission sources, large and small, located in the basin which contribute to the total methylene chloride concentration that the modeled population is exposed to. The modeled population is expected to be exposed to ambient concentrations similar to those monitored by the toxic monitoring stations in the SCAB (1.5 to 3.1 ppb) which are additive to the concentrations solely due to the nearby modeled emission source. Other nearby sources, similar in both emissions and proximity, could produce additive concentrations on the order of those presented below.

The three facilities ARB modeled are located in the South Coast Air Basin (SCAB) and have annual emissions ranging from 52 to 587 tons. They are: Source A, a manufacturer of urethane foam; Source B, a motor home manufacturer which uses a methylene chloride containing adhesive; and Source C, an automobile assembler, which uses methylene chloride as a paint stripper. These facilities were chosen to model population exposure because they are large emission sources, their processes are typical of other SCAB sources and because they are located in densely populated areas.

For each facility, the Industrial Source Complex Short Term (ISCST) Gaussian model was used to predict the annual average concentration of methylene chloride in 1 square kilometer (km) cells for a 30 x 30 km grid. In addition, a 3 x 3 km grid of 0.01 km^2 cells was used to obtain a higher resolution of concentrations for the 1 km^2 cells nearest to the source. In order to predict the annual average concentration in each of the cells, the ISCST model requires emissions information and meteorological data as input for each facility.

Information on emissions was provided by each facility for the year 1985 in response to an ARB survey (CARB, 1986a). The information obtained for each facility includes emission rates, stack parameters, the typical operating schedule, and an estimate of the size of any area source. Annual emissions of methylene chloride from point and/or area

sources along with the stack height are given in Table II-5 for each facility that was modeled.

Point source emissions are typically released from specific stacks while area source emissions are released over a larger area. Source A and Source B have both point and area methylene chloride emission sources. The stack heights given for these two facilities refer to point source emissions. Although Source C emits methylene chloride from

TABLE II-5

ANNUAL EMISSIONS AND STACK HEIGHTS OF MODELED FACILITIES

Source	Point Source Emissions (tons/yr)	Stack Height (ft)	Area Source Emissions (tons/yr)
Source A	351.8	27	18.5
Source B	15.5	30	30.0
Source C	-	175	386.4

64 stacks, it was modeled as an area source with emissions released at an effective stack height of approximately 289 feet. This was determined to be appropriate due to the similarity in stack parameters and the relative closeness of the stacks, and because the emissions are released at ambient temperatures.

Meteorological data, as required by the ISCST model, were obtained from the nearest station to each facility. When more than one year of meteorological data were available, the year which yields the poorest pollutant dispersion was used. The meteorological data used to model Source C, Source A and Source B are from Los Angeles International Airport for 1978, Long Beach Airport for 1968, and Ontario Airport for 1972, respectively.

An annual average concentration is determined for each 1 square km cell (a total of 900 cells) in the grid surrounding each source. The modeled concentrations in each of the cells surrounding the source ranged from 0 to 1 ppb at Source C; from 0.01 to 10.8 ppb at Source A; and from 0 to 5.8 ppb at Source B.

Population exposure near each facility is determined by using the results from the ISCST model in conjunction with 1985 census data. The number of people living within each 1 square km cell in the modeled grid are exposed to the annual average concentration that was determined for

that cell. Additional methylene chloride emission sources in or near the modeled grid of each facility would increase the average population exposure. However, additional emission sources are not represented in this analysis.

Figures II-2 through II-4 show the cumulative population exposure for each facility. As shown in Figure II-2, approximately 1 million people are exposed to an annual average concentration of at least 0.10 ppb of methylene chloride resulting from Source A's emissions. Only concentrations less than 1 ppb are shown in Figures II-2 through II-4. Thus, the complete range in concentration for each facility is not shown.

The exposure results show that over 82,000 people are exposed to an annual average concentration of at least 0.5 ppb as a result of emissions from Source A. Of these 82,000, over 30,000 people are exposed to an annual average concentration of at least 1.0 ppb. More than 12,000 people are exposed to an annual average concentration of at least 0.5 ppb as a result of emissions from Source C, while more than 8,000 people are exposed to at least 0.5 ppb as a result of emissions from Source B. The modeling was also used to determine the population exposure in the 1 square km cell with the highest annual average concentration around each facility. For these cells, the annual average concentrations and the populations exposed are: 10.7 ppb/1,318 persons; 5.7 ppb/986 persons and 1.0 ppb/1,982 persons for Source A, Source B and Source C, respectively.

The modeling results demonstrate that several thousand people living near these facilities are exposed to methylene chloride at concentrations higher than annual average concentrations monitored in the SCAB. In addition, these results imply that other methylene chloride emission sources in California may expose nearby populations to elevated concentrations. In the SCAB there are 33 facilities with annual methylene chloride emissions of over 25 tons (SCAQMD, 1985). Of these 33 facilities, nine have annual emissions in excess of 100 tons.

To assess the impact these other facilities have on nearby populations requires facility specific information. The source with the greatest emissions does not necessarily produce the highest annual average concentrations in the surrounding area. This is apparent from the modeling results. The concentrations in the nearby community resulting from Source C's emissions are significantly lower than those produced by Source B, a facility with one-tenth Source C's annual emissions. This may in part be attributed to meteorology. However, the primary reason is the height at which emissions are released from Source C.

Figure II-2

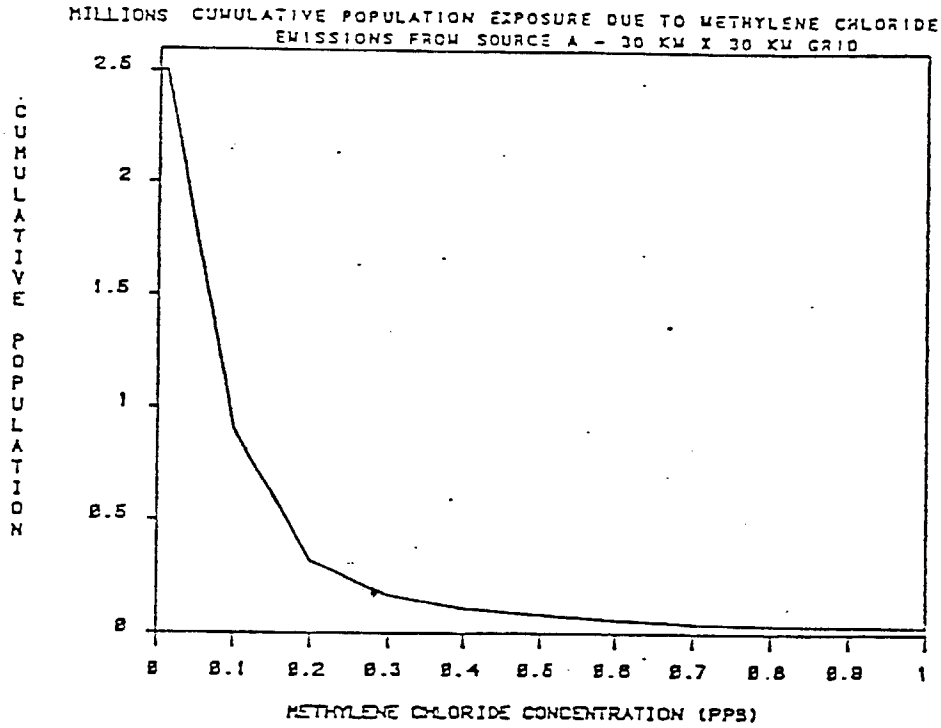


Figure II-3.

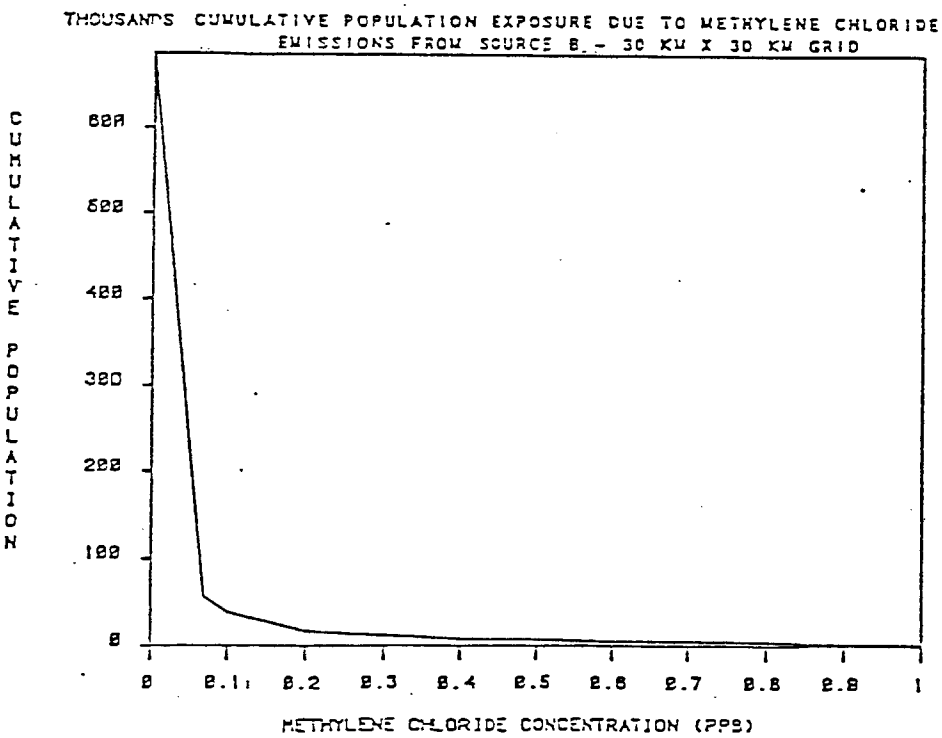
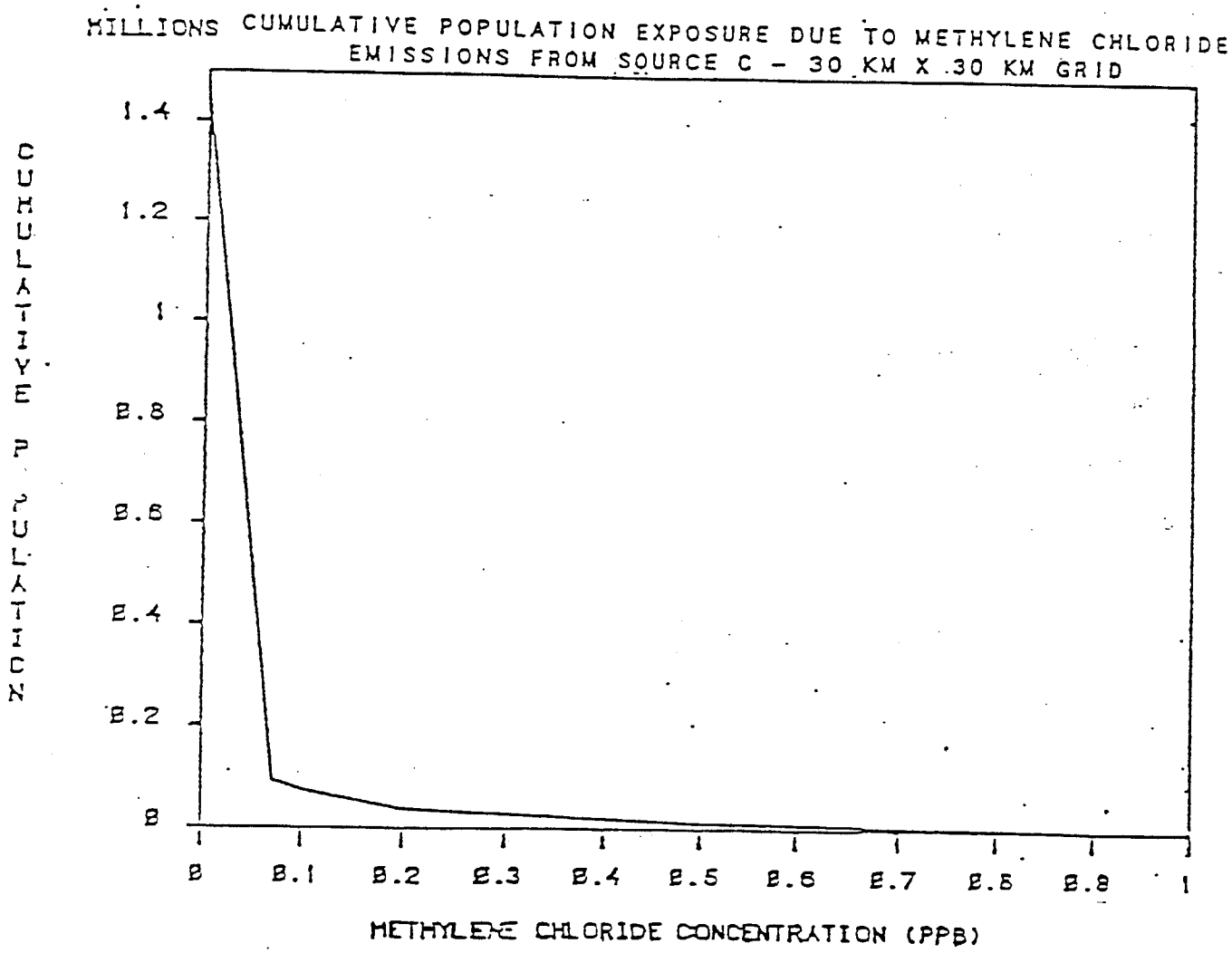


FIGURE II-4


MILLIONS CUMULATIVE POPULATION EXPOSURE DUE TO METHYLENE CHLORIDE
EMISSIONS FROM SOURCE C - 30 KM X 30 KM GRID
CUMULATIVE POPULATION
1.4
1.2
1
0.8
0.6
0.4
0.2
0
0
0.1
0.2
0.3
0.4
0.5
0.6
0.7
0.8
0.9
1
METHYLENE CHLORIDE CONCENTRATION (PPB)

E. INDOOR EXPOSURE

An estimate of exposure to methylene chloride from indoor air is not provided in this section because the data necessary to make such an estimate are not available. However, because methylene chloride is a constituent in many household consumer products, indoor concentrations may be substantially higher than ambient concentrations. Inhalation of methylene chloride from the indoor environment is expected to vary depending on many factors including the degree and manner of use of products containing methylene chloride. This section presents information concerning what is known about indoor concentrations of methylene chloride as well as the levels of exposure that may occur from using products that contain methylene chloride. For methylene chloride it is reasonable to assume that ambient concentrations establish baseline indoor concentrations. Thus, any reduction in ambient levels of methylene chloride will also act to reduce indoor concentrations by a similar margin.

There are several studies that have monitored for indoor air pollutants. However, ARB staff are only aware of two indoor studies that have monitored for methylene chloride. A limited study, conducted in Italy, monitored the indoor air of 15 structures (nine houses, five apartments and one office) for several compounds including methylene chloride (De Bortoli, et al., 1985). Based on charcoal tube collection and gas chromatography (electron-capture detector) analysis, the mean indoor concentration of methylene chloride for all data from four to seven days of monitoring at each structure was 193 ppb with the indoor concentration for one of the structures reported as 1450 ppb. The mean outdoor concentration was reported as less than 4 ppb. Although the study is limited, the results show that indoor concentrations of methylene chloride can be many times higher than outdoor concentrations. This conclusion is supported by the National Research Council (NRC, 1978). When considering the significance of De Bortoli's findings to indoor exposure in California, it is necessary to note that building materials as well as consumer products used in Italy may differ from those used in California. For example, the peak concentrations reported in De Bortoli's study correlated with the use of hairsprays which contain methylene chloride, these products are no longer produced or sold in the United States. However, because a number of consumer products containing methylene chloride are used in California, it is likely that indoor concentrations of methylene chloride in some homes may be similar or greater to those reported by De Bortoli.

As part of a study conducted in Los Angeles County, the indoor and outdoor air of 51 homes during the winter season and 43 homes during the summer season was sampled and subsequently analyzed for several organic compounds (Pellizzari, et al., 1988). However, because Tenax is not an

effective media for collecting methylene chloride and because Tenax was used to collect the majority of the samples that were taken, methylene chloride results are only available for eight homes during the summer season. For these homes, draft results show overnight indoor concentrations (based on canister collection and mass spectrometer analysis) to range from 0.3 to 3.6 ppb with a mean concentration of 1.7 ppb while daytime indoor concentrations range from 0.3 to 3.9 ppb with a mean concentration of 1.6 ppb. Overnight outdoor concentrations range from 0.1 to 1.3 ppb with a mean outdoor concentration of 0.5 ppb while daytime outdoor concentrations range from 0.2 to 3.9 ppb with a mean concentration of 1.0 ppb. The results reported by Pellizzari et al. for methylene chloride are for a limited number of homes. However, they do indicate that indoor concentrations of methylene chloride in some homes may not be substantially higher than outdoor concentrations. Most likely, this is the case when products which contain methylene chloride are not used or stored indoors.

Because of the sampling and analysis methods used, ARB staff believe that the draft results from the Los Angeles study are more reliable than the results for the Italian study. However, the differences between the results for the two studies as well as the fact that both studies are very limited illustrates the need for a comprehensive indoor monitoring study where methylene chloride is sampled and analyzed throughout California. Such a study would provide the information needed to better characterize indoor exposure to methylene chloride in California.

Several studies have shown that personal breathing zone concentrations resulting from the use of consumer products containing methylene chloride may be several orders of magnitude higher than ambient concentrations. These products, which include spray paints and paint strippers, are used widely by the general population. Results from a chamber study where a paint stripper containing methylene chloride was being used show breathing zone exposures up to about 2,000 ppm averaged over one hour with peak concentrations as high as 3,300 ppm (Girman and Hodgson, 1986). For this study participants were placed in a chamber (20 $meters^3$) and supplied with a pressure demand breathing apparatus and therefore not exposed to elevated levels of methylene chloride. In another study, participants were placed in a chamber (5.4 $meters^3$) and requested to use personal-care-products containing methylene chloride. Peak use concentrations ranged from 95 to over 500 ppm and the fifteen minute time-weighted-average concentration was 102 ppm (Skory et al., 1975). The purpose of the study was to determine the participants levels of carboxy hemoglobin following normal use exposures to aerosol products containing methylene chloride. Otson et al. (1981) reported one hour time-weighted-average concentrations ranging from 37 to 1460 ppm as a result of paint stripper use in a chamber with a volume of approximately 28 $meters^3$. For this study, laboratory personnel

participants were supplied with respirators and thus were not exposed to elevated levels of methylene chloride. The purpose of the study was to evaluate air levels of methylene chloride generated by the use of small quantities of paint removers under poor ventilation conditions. Stevenson et al. (1978) reported a 26 minute average exposure of 450 ppm when test subjects placed in a chamber (approximately 75 $meters^3$) were exposed to methylene chloride through the use of aerosol spray paint. The purpose of this study was to determine airborne levels of methylene chloride and its effects on carboxy hemoglobin levels under simulated home-use conditions using typical commercial spray paint formulations.

Breathing zone concentrations of the magnitude reported above are generally found during the time period the product is being applied and can decline rapidly, depending on ventilation rate, once application is finished. Several factors are responsible for the range of results reported by these studies. These factors include: the concentration of methylene chloride in the consumer product used, the manner and duration of use, the volume of the study chamber, the ventilation rate of the study area, and the averaging time for the concentration reported.

The magnitude of exposures from the use of consumer products containing methylene chloride is put into perspective when the intake of methylene chloride from these exposures is compared to the intake from the ambient air. However, the following comparison is not intended to imply that the health effects associated with these two exposure scenarios are equivalent. The intake of methylene chloride from inhalation for approximately 20 minutes each year at a concentration of 100 ppm (Cal/OSHA's 8 hour time-weighted permissible exposure limit for methylene chloride) is equal to the annual intake from continuously breathing methylene chloride at a concentration of 2.0 ppb (the annual average concentration in the SCAB for January 1985 through March 1987). Exposure to methylene chloride from indoor air is an additional route of exposure and adds to the total risk.

Exposure to methylene chloride from the use of consumer products is expected to decline over the next several years. The reasons for the decrease are: 1) the FDA has proposed a rule to ban the use of methylene chloride as an ingredient of personal-care-products and, 2) the Consumer Product Safety Commission has announced that it will require certain consumer products which contain methylene chloride to be labelled in a manner which adequately describes the potential for chronic health effects. The labelling requirement is expected to cause manufacturers of these consumer products to switch to safer substitutes when available.

ARB staff did not make an estimate of the annual exposure to methylene chloride through use of consumer products. To do so would require detailed information on the manner and frequency with which methylene chloride containing products are used. Currently, there are

studies, both planned and underway, which may be helpful in evaluating indoor air exposure to methylene chloride.

The EPA in conjunction with the Consumer Product Safety Commission has conducted a survey on consumer use habits. The survey included responses from roughly 5,000 people about the incidence and frequency of their use of products, quantities used and typical protective measures taken. A solvent shelf survey was also conducted to analyze household products for the presence of chlorinated solvents including methylene chloride. The EPA plans to develop exposure estimates based on these surveys. ARB staff plan to review the results when they become available.

F. EXPOSURE THROUGH OTHER ROUTES

The primary focus of this report is to assess the adverse effects on health due to methylene chloride exposure in ambient air. In the six areas for which ambient monitoring data are available the population-weighted-average ambient concentration for 20.3 million people is estimated as 1.6 ppb. Based on this concentration and a breathing rate of 20 m^3/day, the population-weighted-average intake of methylene chloride through inhalation is 40,500 micrograms/year. For many California residents, nearby emission sources and indoor air contribute to additional exposure; resulting in even higher yearly intakes.

Exposure may also occur through the ingestion of methylene chloride, if present, in drinking water and food products. The following comparisons simply illustrate the extent of exposures to methylene chloride by routes other than inhalation. The comparisons do not imply that equivalent doses via different exposure routes necessarily result in health effects that are equivalent. ARB staff believe that the greatest contribution to total intake is from inhalation of methylene chloride.

The ARB staff estimate that for the majority of California residents, the intake of methylene chloride through drinking water is less than 365 ug/year. Between January, 1984 and December, 1985, the Department of Health Services conducted a study in which groundwater from 2,947 wells, representing 819 public water systems, was analyzed for methylene chloride. Less than one percent of the wells sampled (eleven wells) contained methylene chloride at concentrations above the 0.5 ug/liter detection limit. For these eleven wells the median concentration was 3.0 ug/liter, the maximum was 10.0 ug/liter and the minimum was 0.65 ug/liter (DHS, 1986).

Groundwater supplies roughly 40 percent of California's domestic use with surface water making up the other 60 percent. The DHS study did not monitor surface waters for methylene chloride. Methylene chloride released into surface waters is not expected to remain due to

its high volatility. The EPA used results from two major surveys (the National Organics Monitoring Survey and the National Screening Program for Organics in Drinking Water) to predict methylene chloride concentrations in the potable water of public water systems nationwide. Based on data from both groundwater and surface water, the EPA has estimated that 93.5 percent of the U.S. population who are served by public drinking water systems receive water with no methylene chloride or levels less than 0.5 ug/liter. Furthermore, 99.6 percent of the population receive water with concentrations at or below 10 ug/liter (U.S. EPA, 1984).

ARB staff estimated a range of annual intake through drinking water based on the concentrations found in the DHS monitoring study (less than 0.5 to 10.0 ug/liter). Intake is based on an average drinking water consumption of two liters per day, resulting in an intake ranging from less than 365 ug/year to 7300 ug/yr. Because methylene chloride is not expected to remain in surface waters and because methylene chloride was not detected in over 99 percent of the groundwater wells that DHS tested, ARB staff believe that the overwhelming majority of California population would have annual intakes less than those reported above.

Decaffeinated coffee was considered as a source of methylene chloride in food products. Because the United States population consumes a large volume of decaffeinated coffee that is manufactured using methylene chloride, the Food and Drug Administration (FDA) has estimated a maximum likely intake through this route. The FDA's food and color additive regulations limit the methylene chloride residual allowed in roasted and instant coffee to 10 ppm. To estimate upper-bound exposure, the FDA assumed that all decaffeinated coffee products contain methylene chloride at the maximum level allowed (10 ppm) and that all of it is extracted during brewing and becomes part of the beverage. Using information available on the average consumption of decaffeinated coffee, FDA estimated that the daily intake is not likely to exceed 140 micrograms (51,000 micrograms/year) due to ingestion of decaffeinated coffee (FR, 50(243): 51551).

The assumptions made by FDA were choosen so that the estimated intake represents a maximum which is not likely to be exceeded by consumers of decaffeinated coffee. Actual intakes are probably much lower for two reasons. First, most coffee products are not expected to contain methylene chloride at the maximum level allowed. One manufacturer, General Foods Corporation, found residuals of 0.10 ppm or less in 100 percent of 69 ground coffee samples and 54 instant coffee samples (FR, 50, (243): 51551). Second, all methylene chloride present in coffee may not be ingested with the beverage. Some residual may remain in coffee grounds and it is likely that methylene chloride which does enter the beverage may volatilize at the high temperatures experienced during brewing. The boiling point of methylene chloride is

40°C whereas coffee is brewed at temperatures near the boiling point of water, 100°C. However, all of the methylene chloride that volatilizes during brewing would be expected to enter the indoor environment and thus be available for inhalation by the residents.

The FDA is not aware of any possible public health hazards posed by the presence of methylene chloride in foods other than decaffeinated coffee.

REFERENCES TO CHAPTER II

CARB, 1986a. Responses to memorandum from Bill Loscutoff to facilities that emit methylene chloride, "Methylene Chloride Emissions Survey." July 22, 1986.

CARB, 1986b. "A Report on the Parallel Ambient Sampling Project for Selected Organic Toxics", ARB/HS86-02, February.

De Bortoli, M., et al., 1985. "Measurements of Indoor Air Quality and Comparison with Ambient Air; A Study on 15 Homes in Northern Italy," Commission of the European Communities.

DHS, 1986. The State of California Department of Health Services, Organic Chemical Contamination of Large Public Water Systems in California. April 1986.

FR, 50, (243): 51551. Federal Register Notice. Cosmetics; Proposed Ban on the Use of Methylene Chloride as an Ingredient in Aerosol Cosmetic Products. December 18, 1985.

Girman, J.R. and Hodgson, A.T., 1986. Source Characterization and Personal Exposure to Methylene Chloride from Consumer Products. Prepared by Lawrence Berkeley Laboratory, Applied Science Division for U.S. Department of Energy under contract DE-AC03-76SF00098.

Gleit, A., 1985. "Estimation for Small Normal Data Sets with Detection Limits," Environmental Science and Technology, 19:1201-1206.

HSIA, no date. Methylene Chloride A Significant Solvent in Consumer Products submitted to the ARB by Michael Neale, Chairman, HSIA, Western Committee, 1985.

National Research Council, 1978. "Panel on Low Molecular Weight Halogenated Hydrocarbons. Chloroform, Carbon Tetrachloride...", National Academy of Sciences, Washington, D.C.

Otson, R.; Williams, D. T.; and Bothwell, P.D. 1981. "Dichloromethane Levels in Air After Application of Paint Removers" American Industrial Hygiene Association Journal Vol 42, pp. 56-60, 1981.

Pellizzari, E.D.; Michael, L.C.; Perritt, K.; Smith, D.J.; Hartwell, T.D.; Sebestik, J., 1988. "Comparison of Indoor and Outdoor Toxic Air Pollutant Levels in Several Southern California Communities", Draft Final Report, Contract Number: A5-174-33, Research Triangle Institute, Research Triangle Park, N.C.

Figure III-1

U.S. Production of Methylene Chloride

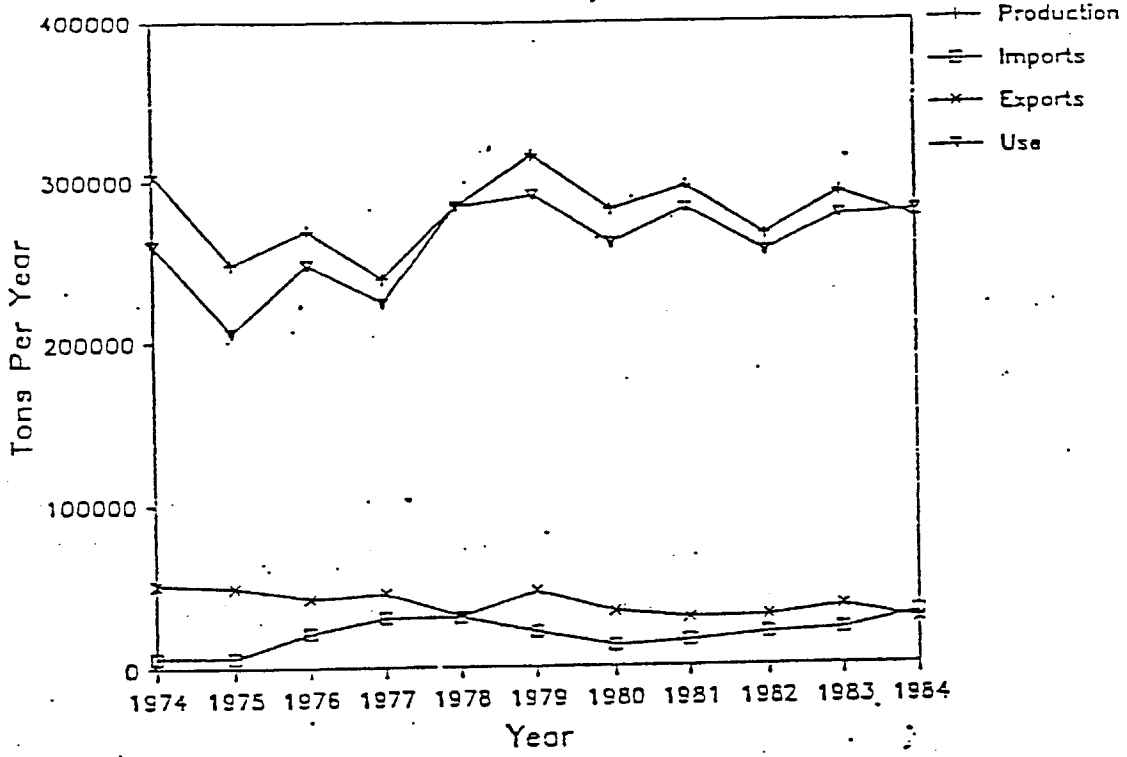
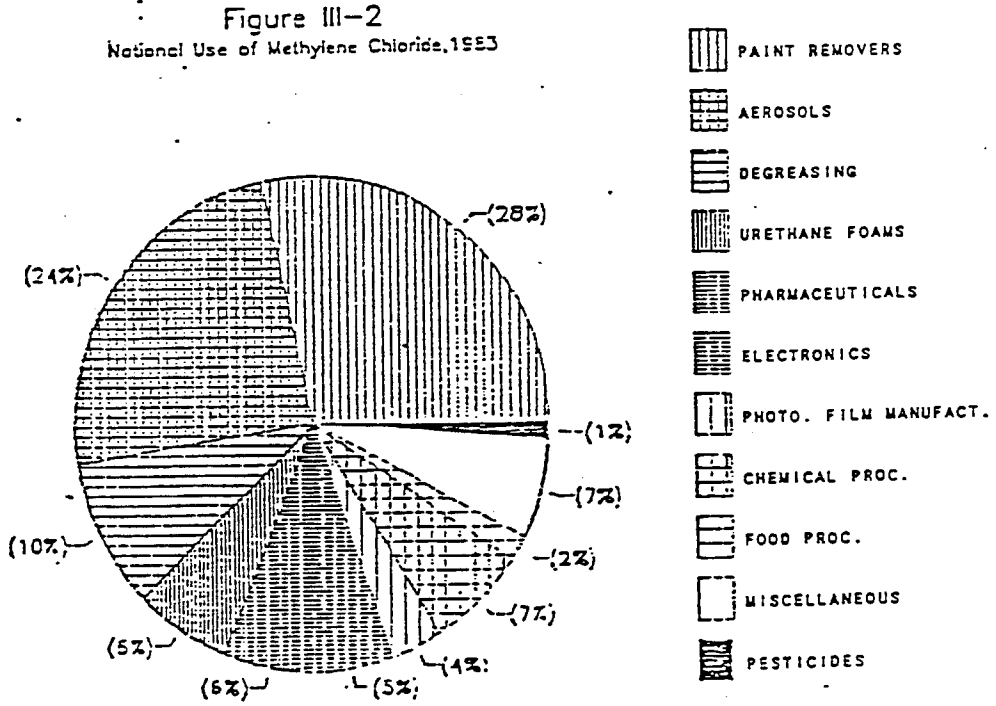


Figure III-2

National Use of Methylene Chloride,1983



B. USES AND EMISSIONS

Methylene chloride is used in California in the following products and processes: paint removers, aerosols, foam manufacturing, degreasing operations, pesticide manufacturing, pesticide application, pharmaceutical manufacturing, food processing, and chemical production. Methylene chloride is also used in miscellaneous applications such as a thinning agent in adhesives, a solvent in surface coatings, a mold release agent in plastics manufacturing, and as a shrink fitting agent.

The Halogenated Solvents Industry Alliance (HSIA) supplied the ARB with data on the amounts of methylene chloride shipped to California by domestic manufacturers of the chemical (HSIA, 1986a). Data are presented below for the time period 1980 through 1985 (data for 1981 are not available).

Year	Domestic Manufacturers CH_2Cl_2 Shipments to California (tons)
1980	30,408
1981	NA
1982	25,054
1983	26,501
1984	23,123
1985	23,462

The shipping data submitted by HSIA do not account for several possible distribution alternatives:

(1) Accounts receiving shipments in California may reship portions of those shipments outside the state.
(2) Organizations other than domestic manufacturers may ship methylene chloride to California after purchasing it in another state. Such organizations may include chemical distributors, private companies who use the chemical, and military agencies.
(3) There may be a small fraction of California use which is imported from foreign methylene chloride producers.

These distribution alternatives are expected to represent a small fraction of total California use. Thus the figures supplied by HSIA are thought to be a good estimate of total California consumption.

Figure III-2 presents the percent of methylene chloride consumed in the U.S. by use type. Table III-1 presents the California and U.S. consumption of methylene chloride by use type in tons per year (TPY).

TABLE III-1

SUMMARY OF METHYLENE CHLORIDE USE (TPY)

Use Type	Estimated California Use[a]	Estimated United States Use[b]
Paint Removers	7,500	68,300
Aerosols	6,400	58,300
Degreasing	3,100	23,700
Urethane Foam Manufacturing	1,600[c]	15,600
Pharmaceuticals	500[d]	14,600
Electronics (Photoresist Stripping)	640[d]	13,100
Photographic Film Manufacturing	0	8,900
Specialty Chemical Production	800[d]	8,700
Chemical Processing Industry	1,200[d]	7,500
Food Processing	25[d]	3,700
Triacetate Fibers	0	2,600
Pesticide Manufacturing	800[e]	3,000
Miscellaneous	1,500	13,400
TOTAL USE[f]	24,000	241,000[g]

a. Appendix II describes the methods used to estimate California use. California use is based on information from 1983 unless otherwise noted.
b. All U.S. use is based on information from the Halogenated Solvents Industry Alliance for Calendar Year 1983. (HSIA, 1985)
c. Foam manufacturing use is based on 1984 emission estimates for eight slabstock foam plants identified by an EPA contractor (Pandullo and Nash, 1986a). This is does not include use for the manufacture of molded foam.
d. Use estimates for these categories are assumed to equal the amounts the domestic manufacturers reported shipping to California for these end-uses during 1985 (HSIA, 1986b). These are lower-end use estimates (See Appendix II).
e. Methylene chloride use in pesticide manufacturing includes only process use. It does not include methylene chloride consumed as an ingredient in pesticide formulations. Use information is for 1983.
f. Total use is rounded to the nearest 1000 TPY.
g. U.S. use for 1987 is estimated at 248,500 tons (HSIA, 1988).

The California use breakdown for methylene chloride as presented in Table III-1 is based on 1983 figures for all uses except urethane foam manufacture (1984 data), specialty chemical production (1985 data), the chemical processing industry (1985 data), food processing (1985 data), photoresist stripping (1985 data) and pharmaceutical manufacturing (1985 data). Discussion of the methodology used to estimate California consumption is included in Appendix II.

Emissions of methylene chloride in California result entirely from stationary sources and are estimated to be approximately 20,000 TPY. There is not sufficient information available to estimate emissions for several of the end uses listed in Table III-1. Additional emissions may result from sources that have not been identified in this report. Table III-2 presents the emissions by source type as well as the percentage of use that is expected to be emitted. Because methylene chloride is frequently used in a dispersive manner where few controls are in place, emissions of methylene chloride for several source types are assumed to equal 100 percent of its use.

Source types for which there are facilities in California with methylene chloride emissions greater than 100 TPY include: Foam manufacturing, paint stripping operations, degreasing operations, publicly owned treatment works, and hazardous waste landfills. Other source types that include facilities with emissions greater than 10 TPY include: paint remover formulators, pesticide manufacturing, chemical production, and possibly solvent reclaimers. Some facilities included as miscellaneous users may also emit substantial amounts of methylene chloride. Potential emission sources not evaluated include on-site solvent recovery and industrial waste water treatment. The following text discusses the various uses of methylene chloride as well as the emissions associated with each use when known.

Paint Removers

Paint removers account for the largest use of methylene chloride in California. An estimated 7,500 tons of methylene chloride were used in paint removers in California in 1983. Methylene chloride is the primary ingredient in paint stripping formulations used for industrial, commercial, military, and domestic applications. The majority of the methylene chloride in paint removers is used by the industrial sector. Factory and shop production for original equipment manufacture and after-market goods accounted for 70 percent of paint remover use in 1983 (Mullen and Mould, 1985).

In paint stripping applications, all of the methylene chloride used is eventually emitted to the atmosphere (U.S. EPA, 1985a). Thus, an estimated 7,500 tons of methylene chloride were emitted in 1983 from the formulation and use of paint removers. Emissions result from the use of

TABLE III-2

ESTIMATED EMISSIONS OF METHYLENE CHLORIDE IN CALIFORNIA[a]

Source	Emissions (TPY)	Percent of Use Emitted Percent	Reference
Paint Removers	7,500	100	U.S. EPA, 1985a
Aerosol Products	6,400	100	U.S. EPA, 1985a
Urethane Foam Manufacturing	1,600	100	U.S. EPA, 1985a
Degreasing Operations	2,600	83	U.S. EPA, 1985a
Pesticide Manufacturing	90	ND[b]	-
Chemical Production and Processing	57	Small Percent	U.S. EPA, 1985a
Distribution Facilities	11	0.2	U.S. EPA, 1985a
Solvent Reclamation	35	1-2	(O'Morrow, 1986; Schneider, 1986)
POTWs	225	ND	ARB, 1987
Photoresist Stripping	360	57	U.S. EPA, 1985a
Miscellaneous Uses	1,500	100	U.S. EPA, 1985a
Total Emissions[c]	20,000		

a. Emission estimates are for 1983 for all source categories except urethane foam manufacturing and pesticide manufacturing (1984 data), solvent reclamation (1985 data), photoresist stripping (1985 data), and POTWs (1987 data). Data on total emissions were not available for pharmaceutical manufacturing, food processing, specialty chemical production, the chemical process industry, pesticide application, municipal landfills, surface impoundments, and hazardous waste landfills.

b. ND - not determined.

c. Total emission estimate is rounded to the nearest 1000 TPY.

paint removers as well as from paint remover formulation facilities. Some paint removal operations emit greater than 100 TPY of methylene chloride to the atmosphere (Taback, et al., 1983). Methylene chloride is also emitted at paint remover formulation facilities during mixing, packaging, and storage (U.S. EPA, 1985a). It is estimated that there are seven paint remover formulation facilities in California with emissions estimated to equal 0.1 percent of use (PEI Assoc. Inc., 1985).

Aerosols

Aerosol products have historically been one of the largest uses of methylene chloride in California. California use of methylene chloride in 1983 was an estimated 6,400 tons. Use of methylene chloride in aerosol products throughout the U.S. appears to be decreasing in 1986; however, summary data are not available (Wind, 1986; Chemical Marketing Reporter, 1986). In response to a proposed FDA ban on the use of methylene chloride in aerosol cosmetics, most manufacturers of aerosol cosmetics (primarily hairsprays) stopped using methylene chloride in their products during 1986 (Wind, 1986).

Methylene chloride is used in aerosols as a solvent, a flammability suppresser, vapor pressure depressant, and viscosity thinner. It is used in hair sprays, paints, insecticides, cleaners, room deodorants, and various household and personal care products. The percentage of methylene chloride in the total aerosol formulation depends on what the product is. Aerosol paint strippers may contain as much as 85% methylene chloride. Aerosol products are packaged at central facilities before being distributed. Aerosols are largely consumer products. Thus, use and emissions are assumed to be distributed with population.

Aerosol packing and product use resulted in estimated emissions of 6,400 tons in California in 1983. Methylene chloride is emitted from aerosol products during the volatilization of suspended droplets or by evaporation from sprayed surfaces. All methylene chloride used in aerosol products is assumed to be emitted to the atmosphere (U.S. EPA, 1985a). Emissions also occur at aerosol packaging plants during spills, mixing, and aerosol can charging (PEI Assoc. Inc., 1985). There are at least 20 aerosol packaging plants in California (Johnson, 1982; Verhagen, 1984).

Foam Manufacturing

An estimated 1,600 tons of methylene chloride were used for manufacturing slabstock polyurethane foam in California in 1984 (Pandullo and Nash, 1986a). Methylene chloride is used in the manufacture of two types of foam products, slabstock and molded. Methylene chloride is used in different ways in these two processes. In

the slabstock foam manufacturing process, methylene chloride is used as a foam blowing agent. Methylene chloride, mixed with other ingredients, is discharged into a foam tunnel in which an exothermic reaction vaporizes the methylene chloride, forming air cells in the foam. In molded foam manufacture methylene chloride is used as a cleanup solvent (Pandullo and Nash, 1986a).

Methylene chloride is emitted from both slabstock and molded foam manufacturing processes. Mass balance data from manufacturers of slabstock foam indicate that about 60 percent of the initial charge of methylene chloride is emitted from the tunnel and about 40 percent from ventilation of the foam curing area (Pandullo and Nash, 1986a). Methylene chloride emissions from foam manufacturing plants are typically uncontrolled. Thus, all of the methylene chloride used in the process is emitted to the atmosphere (U.S. EPA, 1985a). An EPA contractor estimated emissions from eight slabstock foam manufacturing facilities in California in 1984 as 1,600 tons (Pandullo and Nash, 1986a). The majority of the identified emissions of methylene chloride from its use as a foam blowing agent are in the South Coast Air Basin. The number of molded foam manufacturing facilities in California and the methylene chloride emissions from such facilities have not been determined.

Degreasing Operations

Methylene chloride is also used in degreasing operations in California. California use of methylene chloride for degreasing was an estimated 3,100 tons in 1983. Degreasing is an integral part of many metal fabrication processes such as the manufacture of automobiles, electronics, furniture, appliances, jewelry, and plumbing fixtures. Methylene chloride is also used as a degreaser to a minor extent in the textile, paper, plastic, and glass manufacturing industries. Cold cleaning units account for 96 percent of all degreasers in the U.S. (PEI Assoc. Inc., 1985). An estimated 77 percent of the methylene chloride used as a degreasing solvent in the U.S. is used in cold cleaning units (PEI Assoc. Inc., 1985). Substantial quantities of the methylene chloride used for degreasing are sent to solvent reclaimers (U.S. EPA, 1985a).

Emissions of methylene chloride from degreasing operations in California are estimated as 2,600 tons for 1983. Approximately 83 percent of the fresh methylene chloride used for degreasing operations is emitted. This percentage estimate includes a weighted average emission factor for cold cleaning, conveyor and vapor degreasers and takes into account the emissions resulting from solvent returned to the degreasing operation from solvent reclaimers (U.S. EPA, 1985a; PEI Assoc. Inc., 1985; U.S. EPA, 1977). In this approach,

it is assumed that all solvent not consumed at the degreasing facility is sent to solvent reclaimers (U.S. EPA, 1985a). There are estimated to be several thousand cold cleaning facilities in California (Federal Register, 1985; PEI Assoc. Inc.,1985). The methylene chloride emissions for the average facility are 0.3 TPY (PEI Assoc. Inc., 1985). In addition, there are estimated to be over 100 facilities with vapor degreasers, with average methylene chloride emissions of 2.4 TPY per plant (PEI Assoc. Inc., 1985).

Pesticide Manufacturing

Methylene chloride is used as a solvent in some pesticide manufacturing processes. An EPA report lists the methylene chloride consumed by one pesticide manufacturing facility in California as 800 tons in 1983. This estimate includes only process use, and excludes that which may be added to pesticide formulations as an active or inert ingredient. No other pesticide manufacturing facilities in California that use methylene chloride have been identified.

Methylene chloride is used for extraction, phase separation, purification, crystallization, and as a general transport solvent (U.S. EPA, 1985a). The pesticide industry employs a variety of unit operations similar to those used in the chemical processing industry. Emissions of methylene chloride from the pesticide manufacturing facility identified above are estimated at 90 tons for 1984. The facility has reported that methylene chloride emissions from one process vent are controlled through incineration. (Pandullo and Nash, 1986b). Additional methylene chloride emissions from other pesticide manufacturing facilities in California may exist. However, staff of the EPA contractor researching this source category believes that most of the emissions have been identified (Pandullo, 1986c).

Chemical Processing and Specialty Chemical Production

Two facilities in California that use methylene chloride in chemical production have been identified. Based on information from an EPA contractor, one facility uses methylene chloride in the production of a specialty chemical (Howie, 1986) while the other uses methylene chloride in the production of rubber cement (U.S. EPA, 1985a). However, the total amount of methylene chloride used at these facilities is not known.

Emissions of methylene chloride from the production of rubber cement are estimated as 3 TPY (U.S. EPA 1985a). Emissions from the specialty chemical production facility are estimated as 54 TPY (Howie, 1986). These emissions are primarily from equipment leaks, equipment openings, and process vents. The staff is unaware of additional methylene chloride used by other chemical processing and production facilities in California.

Photoresist Stripping

In 1983 five percent of the methylene chloride consumed in the United States was used as a photoresist stripping solvent. Photoresist stripping occurs during the manufacture of copper clad laminated circuit boards. Recent information from electronics industry personnel and suppliers of photoresist films indicates a trend away from organic-solvent stripping has been occurring over the past few years (Waterhouse, 1986; ARB Survey 1986). The trend appears to be more pronounced in California than in the rest of the country.

Aqueous photoresist films which use alkaline stripping solution have gained popularity due to concerns about exposure to organic solvents. However, some methylene chloride based stripping is expected to continue for application to circuit boards requiring high resolution. Industry sources have stated that organic solvents must be used to meet the specifications of some military contracts (Rameriz, 1986; Waterhouse, 1986).

Emissions from photoresist stripping in California were estimated as 360 tons for calendar year 1985. This estimate is based on the assumption that total use for this category is equivalent to 640 tons, the HSIA's estimate of methylene chloride shipments to the electronics industry. An emission factor reported by EPA (1985a) was applied to this use estimate. There is some uncertainty as to whether this estimate is representative of current emissions due to shifting technology in the circuit board manufacturing industry.

Chemical Distributors

Virtually all methylene chloride produced is sold by chemical distributors. Distribution operations involve transport, storage, and repackaging of methylene chloride. There are estimated to be 500 regional distribution facilities in the U.S. (U.S. EPA, 1985a). An estimated 25,000 TPY of methylene chloride are sold through distribution facilities in California. California distribution was estimated as U.S. distribution times the ratio of the quantity of methylene chloride used in California to that used in the U.S.

Emissions of methylene chloride from distribution facilities in California were estimated to be 50 tons in 1983. Emissions resulting from storage tanks were calculated using assumptions about the throughput of methylene chloride and the number and size of storage tanks (U.S. EPA, 1985a; U.S. EPA, 1985b). Estimated emissions are 0.2 percent of the methylene chloride that is distributed.

Solvent Reclaimers

There are about 20 commercial solvent reclaimers in California. The largest three reclaimers handle 85 percent of the business (DHS, 1986). The largest of these accounts for almost half of the off-site solvent recovery in California. It is estimated that 2,400 tons of methylene chloride were sent for recycling in California in 1985. The quantity of methylene chloride sent for recycling in California was estimated by using: 1) the quantity of halogenated solvent reported on hazardous waste manifests (PEI Assoc. Inc., 1985), and 2) the estimated percent of methylene chloride contained in the halogenated solvents (O'Morrow, 1986; Schneider, 1986).

It is estimated that 35 (± 15) tons of methylene chloride were emitted from solvent reclamation facilities in California during 1983. Most emissions result from storage and handling of waste and reclaimed solvents, although some emissions come from distillation condensers at reclamation facilities. Industry representatives estimated emission losses for a solvent as volatile as methylene chloride to be one to two percent of the methylene chloride received (O'Morrow, 1986; Schneider, 1986). Assuming that facility emissions are proportional to the throughput of solvents, the largest solvent reclaimer in the State may emit as much as 25 TPY of methylene chloride (McCormack, 1985).

Miscellaneous Uses

Miscellaneous use of methylene chloride in California was estimated to be 1,500 tons. Examples of miscellaneous uses of methylene chloride include: 1) a solvent for cleaning ink from printing equipment and as a thinning agent in some ink formations, 2) a solvent to bond plastic pieces together, 3) a thinning agent for adhesives, 4) a constituent in specialty cleaners, 5) a solvent for coatings where a fast-drying time is required, as for traffic paints, and 6) a constituent in photographic film cement. The magnitude of methylene chloride used and the resulting emissions for each of these individual applications has not been determined. Miscellaneous use is primarily dispersive and thus 100 percent of use is assumed to be emitted to the atmosphere.

C. SOURCES WITH UNQUANTIFIED EMISSIONS

Pesticide Application

Approximately 14 pesticidal products registered with the U.S. government contain methylene chloride as an active ingredient, and more than 1,750 registered pesticidal products contain methylene chloride as an inert ingredient (Federal Register, 1985). The quantity of methylene chloride applied in California as an active pesticidal ingredient was

minor in 1984 (less than 0.2 tons) (CDFA, 1984). The quantity of methylene chloride used in California as an inert ingredient in pesticides has not been determined.

Pharmaceutical Manufacturing

Methylene chloride is used in California in pharmaceutical manufacturing. Use is estimated as 500 tons in 1985 (HSIA, 1986b). Methylene chloride is used in pharmaceutical manufacturing in the U.S. in the following ways: as an extraction solvent and reaction media in production of bulk pharmaceutical chemicals, and as a carrier for tablet coatings such as cellulose ether films. The one pharmaceutical production facility in California identified by the EPA as using methylene chloride closed production operations in May 1985 (Blanchard, 1985; Pandullo, 1986a). Methylene chloride may be used in tablet coating operations at pharmaceutical manufacturing facilities in the State, although an EPA contractor assumed such usage to be small (Pandullo, 1986a; White, 1986). Emissions of methylene chloride from pharmaceutical manufacturing are probably small, based on information from an EPA contractor studying methylene chloride. The number of pharmaceutical manufacturing facilities in California that use methylene chloride was not determined.

Food Processing

Methylene chloride is used to extract heat sensitive substances such as caffeine, cocoa, hops, and edible fats (U.S. EPA, 1985a; HSIA, no date). For 1985, use of methylene chloride in food processing is estimated at 25 tons (HSIA, 1986b). Based on the available information at least two food processing facilities in California use methylene chloride. These facilities use methylene chloride as an extraction solvent for decaffeination of coffee (Hall, 1986).

Methylene chloride emissions from food processing operations in California were not determined. However, the majority of emissions are expected from the extraction process, and from storage and handling facilities (U.S. EPA, 1985a). Two coffee decaffeination facilities in California were identified (Hall, 1986). There may be other food processors with methylene chloride emissions. However, none have been identified.

POTWS and Landfills

In addition to the uses discussed above, methylene chloride may be released from municipal waste water treatment facilities, known as publicly owned treatment works (POTWs), municipal landfills, surface impoundments, and hazardous waste landfills. Methylene chloride emitted

from landfills and POTWs originates as waste streams from source types listed in Table III-1. For several source types emissions have been estimated as 100 percent of use. Thus, a portion of the emissions attributed to these source types may actually be released to the air at POTWs and landfills.

The quantities of methylene chloride that are disposed at municipal landfills throughout California have not been determined. None of the information reviewed for this report indicated the quantities of methylene chloride that are disposed of at municipal landfills. Although industrial wastes contaminated with methylene chloride can not be legally disposed in municipal landfills, the chemical inadvertently enters landfills as residues in consumer products such as aerosol cans and paint remover containers. In California, there are approximately 1,000 active and 1,200 inactive municipal landfills (Barnickol, 1986).

Hazardous waste landfills and surface impoundments may handle large quantities of methylene chloride. At least 1,500 tons of halogenated solvent containing wastes were disposed in landfills and at least 3,200 tons of halogenated solvent containing wastes were disposed at surface impoundments in California in 1985 (DHS, 1986).

In response to the Resource Conservation and Recovery Act, the Environmental Protection Agency is promulgating restrictions on land disposal of hazardous wastes. Effective November 8, 1986, spent solvent wastes are prohibited from land disposal. As detailed in the federal register (FR 50 (216): 40572) land disposal of solvent wastes which meet listed requirements will be allowed until November 8, 1988. Between November 1988 and November 1990 exemptions can be requested on a case by case basis. Beyond November 1990 no land disposal of solvent wastes is allowed.

In the past, fewer restrictions were placed on the land disposal of halogenated solvents, and the amounts disposed could have been greater. Methylene chloride previously disposed at closed and existing landfills escapes into the atmosphere over a period of several decades (U.S. EPA, 1986). The HSIA estimated that roughly 15 percent of the methylene chloride shipped to California ends up being disposed of by incineration or landfilling (HSIA, 1986a).

In response to AB 3374 all active disposal sites (municipal and hazardous waste) are required to conduct tests to determine: (1) the composition of landfill gases; (2) the presence of specified air contaminants in the ambient air at the disposal site perimeter; (3) whether or not off-site subsurface migration of landfill gas is occurring. Disposal site operators are required to report test results to the air pollution control officer by July 1, 1987 with the

possibility of an extension up to January 1, 1989. Methylene chloride is specified as one of the compounds for which testing will be performed. The test results should be useful in determining which disposal sites in the state may pose a potential public health risk.

POTWs are another source of methylene chloride emissions. Available information indicates that POTWs may be significant emission sources of methylene chloride. The Association of Bay Area Governments has estimated that the three major POTWs in Santa Clara County emit 44 TPY of methylene chloride (Hinman, et al., 1985). The largest POTW in the County was estimated to emit 26 TPY of methylene chloride. A more recent study estimates emissions of methylene chloride from POTWs in California to be 225 tons. For one facility in Los Angeles, emissions of methylene chloride were estimated to be 121 TPY (ARB, 1987). Methylene chloride is also emitted in route to POTWs at pump stations and at high points in the sewer line venting through manhole covers (Caulkins, 1986). Discharges of methylene chloride to POTWs are assumed to result primarily from industrial sources.

D. EMISSION TRENDS

Emissions of methylene chloride are expected to decline over the next few years as a result of a decline in use. Estimated production of methylene chloride in 1985 was down 10 to 15 percent from 1984. The demand for methylene chloride was forecasted to remain nearly stagnant in 1986 (Chemical and Engineering News, 1985). National consumption data for methylene chloride show a thirteen percent reduction from 286,100 tons in 1985 to 247,400 tons in 1987 (HSIA, 1988). Currently, we do not have any information which suggests that a similar reduction in consumption has not occurred in California. The decline in use for most of the source types are based on concerns about the health impacts of methylene chloride and the potential for governmental regulation (Chemical and Engineering News, 1985b; Wind, 1986; Chemical Marketing Reporter, 1986; Smart and Landels, 1985).

The emission trend for methylene chloride used as a paint remover is uncertain. The Consumer Products Safety Commission (CPSC) has determined that there are no good substitutes for methylene chloride in paint removers. The CPSC voted to institute rule making in February, 1986, which requires consumer products containing methylene chloride that are packaged after September 14, 1988 to comply with additional labeling requirements.

Declines in the use of methylene chloride in personal care products as well as spray paints have occured. The Federal Food and Drug Administration (FDA) has issued a notice of proposed rule making to ban methylene chloride in personal care products (Vogel, 1985). According

to the FDA as well as industry representatives, methylene chloride is not currently used in personal care products in the United States. The paint industry is also working to reformulate aerosol spray paints without methylene chloride (Chemical Marketing Reporter, 1986). National consumption data show that there has been a fourty six percent decline in the consumption of methylene chloride for use in aerosols from 1985 to 1987 (HSIA, 1988). This decline in consumption is expected to continue as suitable substitutes are identified.

The shift to less metal processing in producing automobiles and other durable goods tends to reduce the demand for the use of methylene chloride in industrial cleaners and degreasers. A slight decline is expected in the emissions of methylene chloride from pesticide manufacturing. This may result as 1,1,1-trichloroethane and other solvents are substituted for methylene chloride because of concern about health effects (Smart and Landels, 1985). The trend in emissions of methylene chloride in food processing is uncertain. EPA has determined that there is not sufficient justification to ban the chemical from use in decaffeinating coffee (Chemical Marketing Reporter, 1986).

Despite the expected decline in overall methylene chloride emissions, governmental regulation could cause an increase in methylene chloride emissions from some source types. Efforts to control methylene chloride residuals in waste water effluent might result in increased emissions from pharmaceutical manufacturing, POTWs, and solvent reclamation. EPA is currently seeking comment on the use of steam stripping technology for the removal of methylene chloride from pharmaceutical manufacturing waste waters (Federal Register, 1985). Steam stripping is an effective method of removing volatile organics such as methylene chloride by releasing them to the atmosphere. EPA is also considering approval of the use of steam stripping at POTWs to remove volatile organics (Federal Register, 1985). One reference estimated that about ten percent of the total methylene chloride used in the U.S. is released to water (Cothern, et al., 1984).

Methylene chloride emissions from solvent reclamation may also increase in the future. The quantities of methylene chloride received by solvent reclaimers have increased over the past few years and the trend may continue. Concerns about land disposal of halogenated solvents have prompted much of this trend.

REFERENCES TO CHAPTER III

ARB, 1987. "Emissions of Volatile and Potentially Toxic Organic Compounds from Sewage Treatment Plants and Collection Systems," Contract No. A5-127-32, July 1987.

ARB Survey, 1986. Responses to memorandum from Bill Loscutoff to facilities that emits methylene chloride, "Methylene Chloride Emissions Survey". July 22, 1986.

Barnickol, J., 1986. Telephone conversation between Ronald A. Rothacker of CARB and Jeff Barnickol of the California Water Resources Control Board. July 16, 1986.

Blanchard, K., 1985. EPA memorandum to file entitled "Methylene Chloride Exposure and Risk Analysis for Cancer as the Endpoint of Concern." September 3, 1985.

Caulkins, P., 1986. Telephone conversation between Ronald A. Rothacker of CARB and Peter Caulkins, U.S. EPA, Washington D.C. June 27, 1986.

Chemical and Engineering News, 1985a, "Production by the U.S. Chemical Industry," June 10, 1985, p. 27.

Chemical and Engineering News, 1985b, Vol. 63 No. 50, December 16, 1985.

Chemical Marketing Reporter, "Vote Takes Chemical A Step...". March 3, 1986.

CPSC, no date. Consumer Products Safety Commission. Use of Methylene Chloride in Consumer Products: A Preliminary Report

Cothern, C.R. et al., 1984. Techniques for the Assessment of the Carcinogenic Risk to the U.S. Population Due to Exposure from Selected Volatile Organic Compounds from Drinking Water Via the ....., U.S. EPA. May 1, 1984.

DFA, 1984. California Department of Food and Agriculture, Pesticide Use Report, Annual 1984. Sacramento, CA.

DHS, 1984. California Department of Health Services' Directory of Industrial Recyclers, California Waste Exchange. March 1984.

DHS, 1986. California Department of Health Services. Computer printout from hazardous waste information system giving disposal method information for 1985. April 11, 1986.

(FR), 50(201): 42037, Federal Register Notice. "Methylene Chloride: Initiation of Regulatory Investigation." October 17, 1985

(FR), 51(216): 40572. Federal Register Notice Hazardous Waste Management System; Land Disposal Restrictions. November 7, 1986.

Gas Research Institute, 1982. Landfill Methane Recovery Part II: Gas Characterization. December.

Hall, L., 1986. Telephone conversation between Loren Hall of EPA/OTS, Washington, D.C., and John Marconi of ARB, Sacramento, CA. March 27, 1986.

Hinman, K., et al., Santa Clara Valley Integrated Environmental Management Project, Draft Stage One Report. U.S. EPA. October 11, 1985.

Howle, R., 1986. Telephone conversation between Ronald A. Rothacker of CARB and Reese Howle of Radian Corporation, Research Triangle Park, NC. July 22, 1986.

HSIA, No Date. Halogenated Solvents Industry Alliance. A Summary of the Primary Uses of Methylene Chloride in Industry and Consumer Products.

HSIA, 1985. Letters from D.L. Morgan of Cleary, Gottlieb, Steen and Hamilton to R. E. Rosensteel, U.S. EPA, OAQPS, Emission Standards and Engineering Division regarding methylene chloride end use data from HSIA member companies. Dated March 8, April 11 and June 28, 1985.

HSIA, 1986a. Letter from D.L. Morgan of Cleary Gottlieb, Steen and Hamilton to J.A. Marconi of ARB regarding amounts of methylene chloride shipped to California. Dated Oct. 28, 1986.

HSIA, 1986b Letter from Michael Neale of Dow Chemical To Ron Rothacker of ARB regarding the consumption of methylene chloride in California. Dated June 18, 1986.

HSIA, 1988. Comments on Parts A and B of the Preliminary Draft Report to the Air Resources Board on Methylene Chloride, January 14, 1988.

Hinman, K., et al., Santa Clara Valley Integrated Environmental Management Project, Draft Stage One Report. U.S. EPA. October 11, 1985.

Howie, R., 1986. Telephone conversation between Ronald A. Rothacker of California Air Resources Board and Reese Howie of Radian Corporation, Research Triangle Park, NC. July 22, 1986.

Johnson, A., 1982. The Aerosol Handbook, Second Edition.

McCormack, B., 1985. California Department of Health Services. Lecture presented to a class on air toxics at University of California, Davis. July 22.

Mullen, W.F. and Mould, C.B., 1985. Integrated Solvents Analysis: Paints, Draft Report, SRI International, prepared for U.S. EPA. December.

O'Morrow, K., 1986. Telephone conversation between Ronald Rothacker of CARB and Ken O'Morrow of Oil and Solvent Process Co., Azusa, CA. June 9, 1986.

Pandullo, R., 1986a. Telephone conversation between Ronald A. Rothacker of California Air Resources Board and Rich Pandullo of Radian Corporation, Research Triangle Park, NC. July 14, 1986.

Pandullo, R., 1986b., Telephone conversation between Ronald A. Rothacker of California Air Resources Board and Rich Pandullo of Radian Corporation, Research Triangle Park$NC. June 4, 1986.

Pandullo, R., 1986c. Telephone conversation between Ronald A. Rothacker of California Air Resources Board and Rich Pandullo of Radian Corporation, Research Triangle Park, NC. August 5, 1986.

Pandullo, R. and Nash, R., 1986a. Radian Corporation memorandum to file entitled: "Estimates of Methylene Chloride Emissions from Polyurethane Foam Slabstock Facilities and Emission Reductions Achievable with Additional Controls," April 28.

Pandullo, R. and Nash, R., 1986b. Radian Corporation memorandum to file entitled: "Estimates of Hazardous Compound Emissions From Pesticide Facilities and Emission Reductions Achievable with Additional Controls." July 24.

PEI Associates, Inc., 1985. Occupational Exposure and Environmental Release Assessment of Methylene Chloride. April 12.

Ramirez, J.A., 1986. Telephone conversation between J.A. Marconi of ARB and J.A. Ramirez, Manager, Process Control Lab, Xerox Corporation, Pomona, California. September 1986.

San Francisco Chronicle, 1985. "State Warning on Cleaning Solvent," August 16.

Schneider, P., 1986. Telephone conversation between Ronald Rothacker of CARB and Peter Schneider of Romic Chemical Corporation, East Palo Alto, CA. June 9, 1986.

Smart, Marilynne M., and Sara P. Landels, 1985. Integrated Solvents Analysis: Pesticide Uses, Draft Report. Prepared for U.S. EPA by SRI International, July.

Taback, H.J., et al., 1983. Airborne Toxic Materials Study for Sacramento County, Prepared for Sacramento Air Pollution Control District, Sacramento, CA., July.

U.S. Department of Commerce, Bureau of Census. U.S. Imports for Consumption and General Imports, TSUSA Commodity by County of Origin. FT 246/Annual 1974-1984.

U.S. Department of Commerce, Bureau of the Census. U.S. Exports, Schedule B Commodity By County. FT 446/Annual, 1975-1984; FT 410, 1974.

U.S. Department of Commerce, Bureau of the Census, 1982. Statistical Abstract of the United States 1982-83. 103rd Edition. December 1982.

U.S. EPA, 1977. Control of Volatile Organic Emissions from Solvent Metal Cleaning OAQPS Guidelines. EPA-450/2-77-022. November 1977.

U.S. EPA, 1984. Emission Test Report No. 85-HWS-2. November 21, 1984.

U.S. EPA, 1985a. "Survey of Methylene Chloride Emission Sources," EPA-450/3-85-015, Research Triangle Park, NC.

U.S. EPA, 1985b. Compilation of Air Pollutant Emission Factors, AP-42, Fourth Edition. September 1985.

U.S. EPA, 1985c. Emission Test Report No. 85-HWS-1. May 1985.

U.S. EPA., 1986. Regulatory Analysis of Proposed Restrictions on Land Disposal of Certain Solvent Wastes, Draft Report, Office of Solid Waste. January 13, 1986.

Verhagen, D., 1984. Correspondence from Dirk Verhagen of Western Aerosol Information Bureau, Inc. to David Craft of CARB. May 1, 1984.

Vogel, G.A., 1985 "Air Emission Control at Hazardous Waste Management Facilities," Journal of the Air Pollution Control Association. 1985.

Waterhouse, B., 1986. Telephone conversation between Ronald A. Rothacker of CARB and Bert Waterhouse of SRI International, Palo Alto, CA. June 12, 1986.

White, T., 1986. Telephone conversation between Ronald A. Rothacker of CARB and Tom White of the Pharmaceutical Manufacturing Association, Washington, D.C. June 25, 1986.

Wind, M., 1986. Telephone conversation between Ronald A. Rothacker of CARB and Marilyn Wind of Consumer Products Safety Commission, Washington, D.C. May 29, 1986.

IV.

PROPERTIES AND ATMOSPHERIC FATE

A. PROPERTIES OF METHYLENE CHLORIDE

Methylene chloride (dichloromethane, DCM) is a nonflammable volatile liquid that is completely miscible with a variety of solvents (Anthony, 1979). In the absence of moisture, at ordinary temperatures, methylene chloride is relatively stable. In dry air, methylene chloride decomposes at temperatures exceeding 120°C. Methylene chloride evaporates relatively quickly from water. Selected properties of methylene chloride are given in Table IV-1.

TABLE IV-1

PHYSICAL PROPERTIES OF METHYLENE CHLORIDE

Properties	Value	References
Molecular Weight	84.94	U.S. EPA, 1982
Boiling Point (760 mmHg)	40°C	U.S. EPA, 1982
Melting Point	-95°C to -97°C	U.S. EPA, 1982
Vapor Pressure, 20°C	349 mm Hg	U.S. EPA, 1982
Solubility in H_2O, 20°C	20 g/liter	U.S. EPA, 1982
Density, 20°C	1.33 g/ml	WHO, 1984
Log Octanol/H_2O Partition Coeff.	1.25	WHO, 1984
Relative Evaporative Rate	14 (water = 1)	U.S. EPA, 1982
Color	Colorless	WHO, 1984
Odor	Ethereal	WHO, 1984
Flash Point	none	U.S. EPA, 1982

B. ATMOSPHERIC FATE

Reaction with hydroxyl radicals is the dominant mechanism removing methylene chloride from the troposphere. Estimates provided in the literature for methylene chloride's tropospheric lifetime range from 27 to 559 days. However, for reasons provided later in this Section, we believe that a range of 80 to 250 days is representative of typical atmospheric conditions. The rate at which this reaction proceeds depends on temperature and the tropospheric concentrations of both methylene chloride and hydroxyl radical. The temperature dependence of the reaction rate is incorporated in the rate constant for the reaction of CH_2Cl_2 with hydroxyl radical. The product of the rate constant and both species concentrations gives the rate at which methylene chloride is being degraded (Finlayson-Pitts and Pitts, 1986).

The tropospheric lifetime of a compound is an estimate of the time required for a given amount of the compound to decrease to 1/e (0.368) of its original value (at time zero). The tropospheric lifetime (τ) of methylene chloride is related to the rate constant (k) and the hydroxyl radical concentration ([.OH]) by the equation:

$$\tau = (k[.OH])^{-1} \qquad (1)$$

In deriving the above equation, it is assumed that hydroxyl radical is at a constant or steady state concentration in the troposphere.

ARB staff reviewed the literature published in the last ten years to obtain pertinent data on the rate of reaction of methylene chloride with the hydroxyl radical. We found five research groups that determined reaction rate constants from empirical data in the laboratory (Jeong & Kaufman, 1982; Howard & Evenson, 1976; Perry, et al., 1976; Davis, et al., 1976; Cox, et al., 1976). Other researcher's have used the data from one or more of these studies to estimate the tropospheric lifetime of methylene chloride.

Table IV-2 is a summary of the rate constant and tropospheric lifetime estimates resulting from our literature search. The table includes a temperature dependent expression for the rate constant and the room temperature rate constant. Also included in the table are the authors' estimates of tropospheric lifetime and the average tropospheric values of temperature and hydroxyl radical concentration.

The estimates of hydroxyl radical concentration and tropospheric temperature are responsible for much of the variation in the estimated tropospheric lifetimes. Because there is no clear agreement on the value of these parameters, we used a range for each to develop a range of atmospheric lifetime estimates.

TABLE IV-2

TROPOSPHERIC LIFETIME ESTIMATES AND RATE CONSTANT ESTIMATES FOR METHYLENE CHLORIDE'S REACTION WITH HYDROXYL RADICALS

Temperature Dependent Rate Expression for the Hydroxyl Radical Reaction (cm^3/molecule.sec)	Room Temperature Rate Constant for the Hydroxyl Radical Reaction (10^{-14} cm^3/molecule.sec)	Atmospheric Lifetime (Days)	Average Tropospheric Values Used to Calculate Lifetime: Hydroxyl Concentration (molecules/cm^3)	Average Tropospheric Values Used to Calculate Lifetime: Temperature (Kelvin)	References
$8.54\ ^{+8.18}_{-4.18}) \times 10^{-18} T^2 \exp(-500 + 212)/T$	14.2	N.R.	a		Atkinson, 1986
$8.58 \times 10^{-18} T^2 \exp(-502/T)$	14.1	N.R.	N.R.	N.R.	Demoore, 1985
$4.27 \times 10^{-12} \exp(-1094/T)$	10.9	329 $\pm$ 110	$(5.1 \pm 1.7) \times 10^5$	265	Singh, 1983
$6.86 \times 10^{-18} T^2 \exp(-348/T)$d	14.6	N.R.	N.R.	N.R.	Jeong & Kaufman, 1982
N.R.	14	83	1×10^6	300	Cupitt, 1980
$4.27 \times 10^{-12} \exp(-1095/T)$	11	559	3.3×10^5	265	Altshuller, 1980
$4.27 \times 10^{-12} \exp(-1098/T)$	11	365	b	b	Derwent & Eggleton, 1978
$2.1 \times 10^{-12} \exp(-770/T)^d$	15.9	N.R.	N.R.	N.R.	Howard and Evenson, 1976
N.R.	14.5^d	27^c	3×10^6	298	Perry et al., 1976
$(4.27 \pm 0.63) \times 10^{-12} \exp(-1094 \pm 81)/T^d$	10.9	142	9×10^5	265	Davis et al., 1976
N.R.	10.4^d	111	1×10^6	298	Cox et al., 1976

a Lifetime was not reported but, the author suggested using $[.OH] = 5 \times 10^5$ molecules/cm^3 for the Northern Hemisphere.
b Author used model with seasonal and meridional distributions for [.OH] and Temperature.
c Author reported atmospheric half-life , $T_{\frac{1}{2}}$, atmospheric lifetime was calculated as $T_{\frac{1}{2}}/0.693$.
d Authors conducted their own laboratory experiments to determine rate constants.
N.R. Not Reported

Two researchers, Atkinson 1986 and DeMoore 1985, fitted the experimental data of Jeong & Kaufman, Howard & Evenson, Perry et al. and Davis et al. to a temperature dependent expression for the rate constant. Their results are shown in Table IV-2 and are in good agreement. Evaluating Atkinson's equation at the low and high end of the temperature range (265-300K) seen in Table IV-2 gives a rate constant in the range of:

$$9.1 \times 10^{-14} < k < 14.5 \times 10^{-14} \text{ cm}^3/\text{molecules.second}$$

for the reaction of methylene chloride with hydroxyl radical in the troposphere.

The 24-hour average hydroxyl radical concentration in the troposphere has been estimated to range from 3×10^5 to 3×10^6 molecules/cm^3 (Hewitt & Harrison, 1985). Because hydroxyl radicals are only present during daylight, the actual range for daytime concentrations are twice the 24-hour averages given above while nighttime concentrations are essentially zero. Daytime hydroxyl radical concentrations vary depending on many factors including photolytic activity and the concentration of ozone and other pollutants in the troposphere.

Using the rate constants obtained from Atkinson's equation for 265K and 300K and a range of hydroxyl radical concentrations, the estimated tropospheric lifetime for methylene chloride ranges from:

27 days for [.OH] = 3×10^6 molecules/cm^3, T = 300K
426 days for [.OH] = 3×10^5 molecules/cm^3, T = 265K

As previously stated, the concentration of hydroxyl radicals in the troposphere can vary considerably. However, several researchers recommend 24-hour average hydroxyl radical concentrations which are between 0.5×10^6 and approximately 1×10^6 molecules/cm^3 (Prinn, et al., 1987; Winer, 1987; Singh, 1983; Cupitt, 1980; Cox, et al., 1976; Davis, et al., 1976). By using this range of hydroxyl radical concentrations (0.5×10^6 to 1×10^6 molecules/ cm^3) in conjunction with the rate constants determined from Atkinson's equation for 265K and 300K, the resulting atmospheric lifetimes range from 80 to 250 days.

Even for the shortest atmospheric lifetime estimate of 27 days, methylene chloride will become widely dispersed from its emission source. Thus, it is a persistent pollutant, likely to be transported throughout an air basin before it is degraded by chemical reaction.

The initial reaction of methylene chloride with hydroxyl radical is believed to proceed by hydrogen atom abstraction resulting in the

formation of water and dichloromethyl radical (Howard and Evenson, 1976; Davis, et al., 1976; Cox, et al., 1976). Equation 2 summarizes the initial step in the reaction of methylene chloride with hydroxyl radical.

$$CH_2Cl_2 + .OH \longrightarrow .CHCl_2 + H_2O \qquad (2)$$

The subsequent reactions of this radical are as follows (Equation 3) (Atkinson and Winer, 1987). The $CHCl_2$ radical will rapidly react with O_2 to form the peroxy radical.

$$CHCl_2 + O_2 \longrightarrow OOCHCl_2 \qquad (3)$$

This peroxy radical will then react with NO (if present), HO_2 radicals (to form dichloromethylhydroperoxide) or other peroxy (RO_2) radicals (Equations 4 through 6):

$$OOCHCl_2 + NO \longrightarrow OCHCl_2 + NO_2 \qquad (4)$$

$$OOCHCl_2 + HO_2 \longrightarrow HOOCHCl_2 + O_2 \qquad (5)$$

$$OOCHCl_2 + RO_2 \longrightarrow OCHCl_2 + \text{other products} \qquad (6)$$

These reactions have not been experimentally studied; rather they are expected to occur based on our knowledge of the atmospheric reactions of other simple alkyl peroxy and haloalkyl peroxy radicals. The $CHCl_2O$ radical is known to eliminate a chlorine atom to form formyl chloride (reaction 7) (Niki, et al., 1980):

$$OCHCl_2 \longrightarrow HC(O)Cl + Cl \qquad (7)$$

The Cl atom will react with whichever organics are present (in the cleaner troposphere this will be mainly with methane) to yield HCl and lead to the formation of O_3 if NO_x is present.

As previously stated, the most important atmospheric removal mechanism for methylene chloride involves its reaction with hydroxyl radicals. Although other atmospheric removal mechanisms such as photolysis and physical removal play an important role for some compounds that enter the atmosphere, they are expected to be insignificant in the removal of methylene chloride (Winer, 1986).

A 1980 EPA document reports photolysis as a possible process by which methylene chloride can be removed from the atmosphere (U.S. EPA, 1980). Photolysis was listed as possible because chlorinated compounds often absorb in the solar radiation region of the troposphere. However, if photolysis does occur, it is expected to be negligible compared to

removal by the reaction of methylene chloride with hydroxyl radicals (Cupitt, 1986). This is supported by Dilling who attempted to determine the rate of photolysis for methylene chloride in the presence of NO and NO_2. The results demonstrate that methylene chloride is not reactive in the presence of NO or NO_2 when exposed to ultraviolet light at an intensity near that of natural sunlight (Dilling, 1976).

Hydrolysis is not expected to be an important mechanism by which methylene chloride is degraded in the environment. Dilling experimentally determined the hydrolytic half-life to be 18 months in water at 25°C (Dilling et al., 1975). Most methylene chloride entering natural waters is expected to evaporate long before hydrolysis would occur. Under experimental conditions Dilling determined an evaporative half-life of 18 to 25 minutes. Water initially containing approximately 1 ppm methylene chloride was held at 25°C and stirred at 200 rpm. The average solution depth was 6.5 cm (Dilling et al., 1977).

Information concerning physical removal processes for atmospheric methylene chloride is sparse; however, both wet and dry deposition have been reported to be unlikely to occur at a significant rate (U.S. EPA, 1980).

REFERENCES TO CHAPTER IV

Altshuller, A.P., 1980. "Lifetime of Organic Molecules in the Troposphere and Lower Stratosphere", Environmental Science and Technology, 10:180-219.

Anthony, T., 1979. Chlorocarbons and Chlorohydrocarbons. In: Kirk Othmer's Encyclopedia of Chemical Technology. Third Edition, Volume 5, Wiley Interscience.

Atkinson,R., and Winer, A., 1987. Comments on the preliminary draft report entitled "Report to the Air Resources Board on Methylene Chloride" December 22, 1987.

Atkinson, R., 1986. "Kinetics and Mechanisms of the Gas-Phase Reactions of the Hydroxyl Radical with Organic Compounds Under Atmospheric Conditions", Chemical Reviews, 6: 69-201.

Butler, R.; Solomon, I.J.; Snelson, A., 1978. "Rate Constants for the Reaction of OH with Halocarbons in the Presence of O_2 + N_2.", Journal of the Air Pollution Control Association, 28(11): 1131-1133.

Cox, R.A; Derwent, R.G.; Eggleton, A.E.J.; Lovelock, J.E., 1976. "Photochemical Oxidation of Halocarbons in the Troposphere", Atmospheric Environment, 10:305-308.

Cupitt, L., 1986. Personal communication between Richard Corey of ARB staff and Larry Cupitt of EPA. August 20, 1986.

Davis, D.D.; Machado, G.; Conaway, B.; Oh, Y.; Watson, R., 1976. "A Temperature Dependent Kinetics Study of the Reaction of OH with CH_3Cl, CH_2Cl_2, $CHCl_3$, and CH_3Br", Journal of Chemical Physics, 65(4): 1268-1274.

DeMoore, W.B., 1985. "Chemical Kinetics and Photochemical Data for Use in Stratospheric Modeling", Jet Propulsion Lab. NASA-CR-176198.

Derwent, R.G., and Eggleton, A.E.J., 1978. "Halocarbon Lifetime and Concentration Distributions Calculated Using a Two-Dimensional Tropospheric Model", Atmospheric Environment, 12:1261-1269.

Dilling, W.L.; Tefertiller, N. B.; Kallos, G. J., 1975. "Evaporation Rates and Reactivities of Methylene Chloride, Chloroform, 1,1,1-Trichloroethane, Trichloroethylene, Tetrachloroethylene, and Other Chlorinated Compounds in Dilute Aqueous Solutions," Environmental Science and Technology, 9(9): 833-837.

Dilling, W.L., Bredeweg, C.J., Tefertiller, N.B., 1976. "Organic Photochemistry: Simulated Atmospheric Photodecomposition Rates of Methylene Chloride, 1,1,1-Trichloroethane, Trichloroethylene, Tetrachloroethylene, and Other Compounds," Environmental Science and Technology, 10(4): 351-356.

Dilling, W.L., 1977. "Interphase Transfer Processes. II. Evaporation Rates of Chloromethanes, Ethanes, Ethylenes, Propanes, and Propylenes from Dilute Aqueous Solutions. Comparisons with Theoretical Predictions.", Environmental Science and Technology, 11(4): 405-409.

Fells, I. and Moelwyn-Hughes, E.A., 1958. "The Kinetics of the Hydrolysis of Methylene Dichloride," Journal of the Chemical Society, 1326-1333.

Finlayson-Pitts, B.J. and Pitts, Jr., J.N., 1986. "Atmospheric Chemistry: Fundamentals and Experimental Techniques", John Wiley & Sons Inc., 1986.

Hewitt, C.N. and Harrison, R.M., 1985. "Tropospheric Concentrations of the Hydroxyl Radical - A Review", Atmospheric Environment, 19(4): 545-554.

Howard, C.J. and Evenson, K.M., 1976. "Rate Constants for the Reactions of OH with CH_4 and Fluorine, Chlorine, and Bromine Substituted Methanes at 296K", Journal of Chemical Physics 64(1): 197-202.

Jeong, K. and Kaufman, F., 1982. "Kinetics of the Reaction of Hydroxyl Radical With Methane and with Nine Cl- and F-Substituted Methanes. 1. Experimental Results, Comparisons, and Applications", Journal of Physical Chemistry, 86: 1808-1815.

Niki, H., Maker, P.D., Savage, C.M., Breitenbach, L.P., 1980. "An FTIR Study of the Cl Atom-initiated Oxidation of CH_2Cl_2 and CH_3Cl", International Journal of Chemical Kinetics, 12:1001-1012.

Perry, R.A.; Atkinson, R.; Pitts, J. N., Jr., 1976. "Rate Constants for the Reaction of OH Radicals with $CHFCl_2$ and CH_3Cl Over the Temperature Range 298-423k, and with CH_2Cl_2 at 298K", Journal of Chemical Physics, 64(4): 1618-1620.

Prinn, R.; Cunnold, D.; Rasmussen, R.; Simmonds, P.; Alyea, F.; Crawford, A.; Fraser, P.; Rosen, R., 1987. "Atmospheric Trends in Methylchloroform and the Global Average for the Hydroxyl Radical", Science, 238(13): 945-950.

Singh, H.B.; Salas, L. J.; Stiles, R. E., 1983. "Selected Man-made Halogenated Chemicals in the Air and Oceanic Environment", Journal of Geophysical Research, 88(C6): 3675-3683.

Spence, J.W.; Hanst, P. L.; Gay, B. W. Jr., 1976. "Atmospheric Oxidation of Methyl Chloride, Methylene Chloride, and Chloroform", Journal of the Air Pollution Control Association, 26(10): 994-996.

U.S. EPA, 1977. "Review of the Environmental Fate of Selected Chemicals", Stanford Research Inst., Menlo Park, Ca., EPA Report No. EPA/560/5-77/003.

U.S. EPA, 1980. "Fate of Toxic and Hazardous Materials in the Air Environment", Cupitt, L., Environmental Science Research Laboratory, Research Triangle Park, N.C., Report No. EPA 600/3-80-084.

U.S. EPA, 1982. "Health Assessment Document for Dichloromethane (Methylene Chloride), Draft Report", Office of Health and Environmental Assessment, Washington, D.C., EPA-600/8-82-004.

Winer, A., 1986. Personal communication between John Marconi of ARB staff and Arthur Winer of UCR. August, 1986.

Winer, A., 1987. Personal communication between Richard Corey of ARB staff and Arthur Winer of UCR. October, 1987.

World Health Organization, 1984. Environmental Health Criteria Report,

APPENDIX I

ARB ANALYTICAL METHODS FOR SAMPLING AND ANALYSIS OF ATMOSPHERIC METHYLENE CHLORIDE

October 1986

AIR RESOURCES BOARD
PROCEDURE FOR THE SAMPLING AND ANALYSIS OF ATMOSPHERIC C_1 TO C_2 HALOGENATED HYDROCARBONS

Method 103
Revision 2

Haagen-Smit Laboratory Division
State of California
Air Resources Board
9528 Telstar Avenue
El Monte, CA 91731

Procedure for the Sampling and Analysis of Atmospheric C_1 to C_2 Halogenated Hydrocarbons Method 103

1 Introduction

1.1 This procedure describes a method of sampling and analyzing atmospheric concentrations of C_1 to C_2 halogenated hydrocarbons in the range of 0.004 to 1.0 parts per billion (ppb).

1.2 Lower concentrations may be analyzed by increasing the sample volume and using a cryogenic trap to concentrate the sample.

1.3 Higher concentrations may be analyzed by direct injection of a diluted sample into a sample loop of a gas chromatograph.

1.4 Compounds which can be analyzed by this method are:

1.4.1 Dichloromethane, CH_2Cl_2, (methylene chloride)

1.4.2 Trichloromethane, $CHCl_3$, (chloroform)

1.4.3 1,2-Dichloroethane, $ClCH_2CH_2Cl$, (ethylene dichloride, EDC)

1.4.4 1,1,1-Trichloroethane, Cl_3CCH_3, (methyl chloroform)

1.4.5 Tetrachloromethane, CCl_4, (carbon tetrachloride)

1.4.6 Trichloroethene, $Cl_2C{=}CHCl$, (trichloroethylene, TCE)

1.4.7 1,2-Dibromoethane, $BrCH_2CH_2Br$, (ethylene dibromide, EDB)

1.4.8 Tetrachloroethene, $Cl_2C{=}CCl_2$, (perchloroethylene, PERC)

2 Method

2.1 Air is sampled into a Tedlar bag at a calibrated and controlled flow during selected time intervals as described in Appendix A, "Procedure for Atmospheric Tedlar Bag Sampling".

2.2 A measured volume of the air sample is transferred by a syringe into the chromatograph.

2.3 The components are separated by a specified column and analyzed by an electron capture detector.

2.4 An electronic integrator quantitates the halogenated hydrocarbons by integrating the peak areas and calculating concentrations from a factor determined during calibration with a halogenated hydrocarbons

standard mixture.

3 Apparatus

3.1 A sampler with bags is required for each site. The sampler and bags are prepared and operated as described in the "Procedure for Atmospheric Tedlar Bag Sampling".

3.2 A gas chromatograph (GC) equipped with a gas injection valve and freeze-out trap inlet system is required. An electron capture detector is used.

3.3 One GC column is required: A glass column (6 ft x 1/4 in O.D.) packed with 0.2 percent Carbowax 1500 on Supelco 80/100 mesh Carbopac C.

3.4 Other GC supportive apparatus used are a strip chart recorder, a remote controller, and an electronic integrator.

3.5 Ground glass syringes (50, 100, and 250 mL capacity) or other suitable devices to accurately transfer air samples from Tedlar bags to the sample inlet of the GC are used.

3.6 A large air-tight chamber is used to prepare standard gas mixtures.

3.7 The cryogenic traps holding the liquid nitrogen are Dewar containers.

4 Reagents

4.1 All gases used in the GC analysis shall be of the highest commercial quality available.

4.2 Helium shall have a purity of 99.995%.

4.3 Halogenated hydrocarbons reference liquid standards, 99% purity as listed in 1.4 are used to prepare a 10 ppb working standard mixture which is used as a span gas.

4.4 A mixture of 10 percent methane in argon is used as make-up gas in the GC.

4.5 Commercial liquid nitrogen (b.p. = -196°C) is used to cool the freeze-out trap.

5 Procedure

5.1 Bags and samplers are fabricated, tested, and operated as described in Appendix B, "Procedure for the Fabrication and Testing of Sample Bags".

5.2 The air sample is analyzed for C_1 to C_2 halogenated hydrocarbons by using either the loop method or the freeze-out trap method. The freeze-out trap method is used for ppb to ppt (parts per trillion) concentrations.

5.2.1 The procedure for the loop method follows:

5.2.2 The air sample is transferred from the gas sample bag and injected into the sample loop of the GC using a clean 100 mL syringe fitted with a Luer-lok to quick-connect adapter.

5.2.3 The gas sampling valve (rotary type) is equipped with a 1 mL loop.

5.2.4 The gas sampling valve is rotated and the sample enters the GC analyzer and is separated into component compounds.

5.2.5 A Carbowax 1500/Carbopak C column is used to separate the halogenated hydrocarbons. Typical operating conditions for the gas chromatograph are:

25 mL/min helium carrier gas flow
40 mL/min 10% methane in argon make-up flow gas
80°C 10-port valve compartment temperature
150°C injection port temperature
350°C detector temperature
6° to 160°C at 8°C/min programming column temperature
Backflush: 23 min.

5.2.6 Each separated component passes through the electron capture detector and yields a response proportional to its response factor and concentration.

5.2.7 Concentrations of halogenated hydrocarbons may be calculated using an electronic integrator.

5.3.1 The procedure for the freeze-out method follows:

5.3.2 Immerse the sample trap in liquid nitrogen (LN_2) and allow the temperature to stabilize while maintaining a flow of helium through the system.

5.3.3 After discarding about 50 mL of the sample, withdraw exactly 100 mL from the sample bag with a 100 mL syringe and transfer the sample into the trap.

5.3.4 Backfill the syringe with another 40 mL of helium and flush the 40 mL through the trap; then flush the carrier helium through the trap for three minutes.

5.3.5 Isolate the cryogenic trap by using the isolation valve which allows the carrier gas to by-pass the trap.

5.3.6 Replace the LN_2 Dewar flask with a Dewar containing hot water at about 90 deg C.

5.3.7 Allow the trap to warm up.

5.3.8 Inject the sample into the carrier gas stream by turning the GC sampling valve. The gas sample enters the GC analyzer and is separated into component compounds.

5.3.9 The instrument operating conditions are the same as those described in Section 5.2.5 above.

5.3.10 Each separated component passes through the electron capture detector and yields a response proportional to its response factor and concentration.

5.3.11 Figure 5.3.11 is a typical halogenated hydrocarbon chromatogram.

6 Calculations

6.1 The concentrations of halogenated hydrocarbons, in ppb, are calculated by an electronic integrator using the external standard method.

6.1.1 Concentration = Area x Response Factor x Dilution Factor

6.2 The Response Factor (RF) is calculated during calibration by the equation:

$$RF = \frac{\text{Concentration}}{\text{Area}}$$

6.2.1 Dilution Factor = $\frac{\text{Total volume of diluted sample}}{\text{Initial sample volume before dilution}}$

6.2.2 Replicate calibrations are averaged and the arithmetic mean is stored as the RF for subsequent analyses.

6.3 Concentrations may be converted from ppb to mg/m^3 by means of the following formula:

$$mg/m^3 = \frac{P \times (M.W.) \times (ppb) \times (10^6)}{(82.05) \times (T)}$$

Where:

P	=	Pressure in atmospheres
M.W.	=	Molecular weight of corresponding halogenated hydrocarbon
82.05	=	Gas constant in $cm^3 \times atm. / {}^oK$-mole
T	=	Absolute temperature (^oK).

6.4 The concentration unit mg/m^3 is equivalent to ng/cm^3

6.5 The limit of detection (LOD) for each compound is calculated by means of the following formula:

$$LOD = 3(RSD)\frac{(avg.\ conc.)}{m}$$

Where:

RSD = Relative standard deviation.
avg. conc. = Mean count of the smallest discernable analytical signal.
m = Instrument calibration factor.

7 Quality Control

7.1 Quality control procedures are followed in two areas: sampling and analysis.

7.2 The quality control procedures used in sampling are:

7.2.1 The Tedlar bag samplers are checked every 6 months for leakage and contamination. The interval is shortened if any malfunction is suspected. A written record is maintained of the history of each sampler. (See Appendix A).

7.2.2 The Tedlar bags are checked for leakage and contamination before being used for sampling. A log book is maintained with a complete history of bag usage. (See Appendix B).

7.3 The quality control procedures used in analyzing the sample are:

7.3.1 The accuracy of the method has not been determined.

7.3.1.1 Every six to nine months a calibration standard is prepared in a glass-lined Pfaudler Chamber maintained by the Environmental Laboratory Section of the Haagen-Smit Laboratory.

7.3.1.2 The chamber is repeatedly evacuated and flushed with zero air until it is shown by gas chromatographic analysis to be free of any significant contamination.

7.3.1.3 To prepare the standard, the chamber is re-evacuated and filled with zero air to a pressure of 5 psia.

7.3.1.4 A measured volume of a volumetrically prepared solution of halogenated hydrocarbons in methanol is injected via a heated injector into a stream of zero air as it is flowing into the chamber. The volume of the solution injected into the chamber is chosen so as to give the

desired gas phase concentration of halogenated hydrocarbons when the chamber is pressurized to 16 psia with zero air.

7.3.2 Calibration standards are prepared periodically. The accuracy of the standard is verified and the procedure validated by comparing the concentration of tetrachloroethene in the chamber to that of an NBS standard.

7.3.2.1 A newly prepared chamber working standard is rejected unless the tetrachloroethene concentration based on calculation agrees within +/- 5% of the value determined by analysis, using the NBS standard for calibration.

7.3.2.2 A newly prepared chamber working standard is rejected unless the relative response factors for all eight halogenated hydrocarbons of interest fall within +/- 10% of the historically established mean values.

7.3.3 A working chamber standard is checked at least every three months for conformity to criteria 7.3.2.1 and 7.3.2.2.

7.3.3.1 A new standard is prepared as frequently as required as determined by the above mentioned criteria.

7.3.3.2 Any reports generated after the standard ceases to be demonstratively within the established tolerances shall contain a cautionary explanation.

7.4 The gas chromatograph is calibrated periodically.

7.4.1 Calibration factors are determined on the basis of the mean values of the previous calibration runs which meet the criteria of 7.4.3.

7.4.2 Each day a calibration check is performed using the Pfaudler chamber standard to span the instrument.

7.4.3 If the response for each compound of interest is within 10% of the established calibration value, the established calibration factors are retained.

7.4.4 The calibration check is repeated if the response of the instrument has changed by more than 10% from the established values.

7.4.5 If the response is still out of tolerance, a quality assurance report is submitted, remedial action is initiated, and new calibration factors calculated.

7.4.6 Blank samples shall be analyzed daily after the calibration

is completed and, whenever necessary, between samples.

7.5 The linearity of the instrument is checked periodically.

7.5.1 A gas chromatographic multipoint linearity check is performed annually with standards of at least four different concentrations and four replicate runs for each concentration. The concentrations should include the anticipated range of sample concentrations above the limit of detection.

7.5.2 The mean-square error due to lack of fit about the regression line is compared to the total mean-square error of the independent replicates about their individual means. The calibration is accepted if the F-ratio is less than the 95% rejection limit.

7.5.3 A repeated multipoint calibration should not differ from the previous calibration by more than 10%.

7.5.4 Any region of concentration that deviates more than 5% from the least-square line is considered nonlinear.

7.5.5 Data is reported only for compounds whose concentrations lie in the linear range.

7.6 Limits of detection are established.

7.6.1 The limit of detection (LOD) is based on three standard deviations (SD) of runs near the LOD (within 10 SD of the LOD, Winefordner and Long, 1983).

7.6.2 The LOD should be determined at least on an annual basis.

7.6.3 If the instrument response changes by more than 15%, the instrument must be checked and the LOD redetermined.

7.6.4 The presence in a sample of a very large adjacent peak will often raise the LOD in the sample.

7.7 Analytical instruments have quality control procedures.

7.7.1 Column conditions are checked periodically and as needed.

7.7.1.1 All GC accessible parameters is logged when a column is first installed. These parameters are checked daily and recorded on integrator reports.

7.7.1.2 The efficiency and resolution of the column are checked every month. If the tests show more than a 10% change, the column is replaced.

7.7.1.3 If the headpressure required to maintain a specified

flow through the column increases by more than 100%, the column is replaced.

7.7.1.4 If the drift of retention times of peaks results in peak misidentification, all instrument parameters are checked.

7.7.2 Replicate analyses are a quality control procedure.

7.7.2.1 A duplicate analysis is performed on at least one sample per day.

7.7.2.2 If the duplicate analysis (replicate) differs by more than 20%, and if the concentration of the sample is higher than 3X LOD, then an additional analysis is performed.

7.7.2.3 If the range of the replicate analyses is greater than the mean and if the concentration of the sample is greater than 3X LOD, the analyses are not acceptable.

7.7.2.4 If the range is within 20%, the mean and the standard deviation are reported.

7.7.2.5 If there is any reason to suspect the presence of an interferent (peak broadening, shift of retention time, shoulder formation, etc.), peak identification is verified using another analyzer (GC/MS), detector, or column.

7.7.2.6 When spiked samples are analyzed, the peak height and peak area ratios of the spiked and unspiked samples must be consistent.

7.7.3 Compound confirmation is a quality control procedure.

7.7.3.1 Ten percent of the analyses are confirmed by a different analytical system (different column or different detector, e.g. GC/MS).

7.7.3.2 If the confirmatory and the routine analyses differ by more than 20%, none of the analyses are acceptable.

7.8 Analytical reports undergo quality control procedures.

7.8.1 Data storage: raw data transmitted from the integrator are stored unmodified in electronic storage. Data are archived according to date, site, analyses, and project for easy retrieval. These data are kept for 3 years in the laboratory electronic storage.

7.8.2 All data above the minimum detection limits are reported to the requesting agency in hard copy or electronic format.

7.8.3 All reports are reviewed by at least two qualified staff before they are released.

8 Critique and Comments

8.1 Lower limits of detection have been established using the prescribed instrument conditions and using a 100 mL sample with the freeze-out trap technique.

8.1.1 Table 8.1.1 lists the lower limits of detection for the the compounds analyzed by this method.

8.2 Interferences are not usually a serious problem for light halogenated hydrocarbon analysis when the electron capture detector is used.

8.2.1 The electron capture detector is selective for the measurement of halogenated hydrocarbons. It is virtually insensitive to other hydrocarbons thus eliminating interferences from non-halogenated hydrocarbons.

8.2.2 Any halogenated hydrocarbons present in the sample having retention times very similar to the compounds of interest under the operating conditions described in this method will interfere. Therefore, proof of chemical identity requires confirmation.

8.2.3 Water vapor at normal ambient humidity in the sample does not interfere with the separation and quantification of halogenated hydrocarbons.

8.2.4 High concentrations of nitrogen oxides (500 ppm) and sulfur oxides (50 ppm) interfere in the determination of methylene chloride in the samples of stack emission sources.

8.3 The procedure described herein has both advantages and disadvantages:

8.3.1 This method provides a simple way of air sampling. The concentrations of halogenated hydrocarbons in the range of interest are stable for more than 24 hours in the bag, providing sufficient time for the analysis.

8.3.2 The sample is easily and repeatedly introduced into the instrument by means of a gas sampling valve.

8.3.3 A representative composite sample is readily obtained for any selected time interval because the air sampling flow rate is constant.

8.3.4 Both the upper and the lower limits of detection can

be extended by concentrating a larger volume of the sample with a freeze-out trap or by diluting the sample in a Tedlar bag with nitrogen or by loop injection.

8.3.5 Interferences can be eliminated by selecting chromatographic conditions.

9 References

9.1 U.S. Environmental Protection Agency (1976O, "Quality Assurance Handbook for Air Pollution Measurement Systems, Volume I-Principles", EPA-600/9-76-005 Environmental Monitoring and Support Laboratory, Research Triangle Park, North Carolina 27711.

9.2 Grimsrud, E. P., and Knighton, W. B., Anal. Chem. 54, 565 (1982)

9.3 Bennett, C. A., and Franklin, M. L., "Statistical Analysis in Chemistry and the Chemical Industry", John Wiley and Sons, Inc., New York, (1954), pp. 222-232.

9.4 Ullman, N. R., (1973), "Elementary Statistics", John Wiley and Sons, Inc., New York, pp. 282-298.

9.5 Winefordner, J. D. and Long, G. L., Anal. Chem. 55, 712 A (1983).

CAUTION Laboratory Operations Involving Carcinogens

Most halogenated hydrocarbons are identified as human carcinogens; therefore, appropriate precautions should be observed when handling these compounds. Do not release halogenated hydrocarbon vapors to the laboratory atmosphere at any time. When venting or purging, the vapor must be routed to outside air. The OSHA regulations pertaining to the use and handling of halogenated hydrocarbons are published in Title 29 of the Code of Federal Regulations available in the Federal Register, Volume 40, May 28, 1975, pp. 23073.

TABLE 8.1.1
LIMITS OF DETECTION

Compound	Limit of Detection ppb	Concentration ppb	Mean Area	Area St.Dev.	n	% Rel St.Dev.
Methylene Chloride	1	1.37	8,230	800	6	9.7
Chloroform	0.004	0.006	8,290	197	5	2.4
Methyl Chloroform	0.004	0.004	34,000	3600	5	10.6
Carbon Tetrachloride	0.02	0.028	13,900	676	5	4.9
		0.01	2,400	320	6	13.3
Trichloroethylene	0.005	0.0064	15,600	515	5	3.3
Ethylene Dibromide	0.01	0.009	3,150	430	5	13.7
Perchloroethylene	0.004	0.0047	102,700	6080	5	5.9
Ethylene Dichloride	0.2	0.3	61,778	4811	6	7.8
		0.09	26,677	2143	5	8

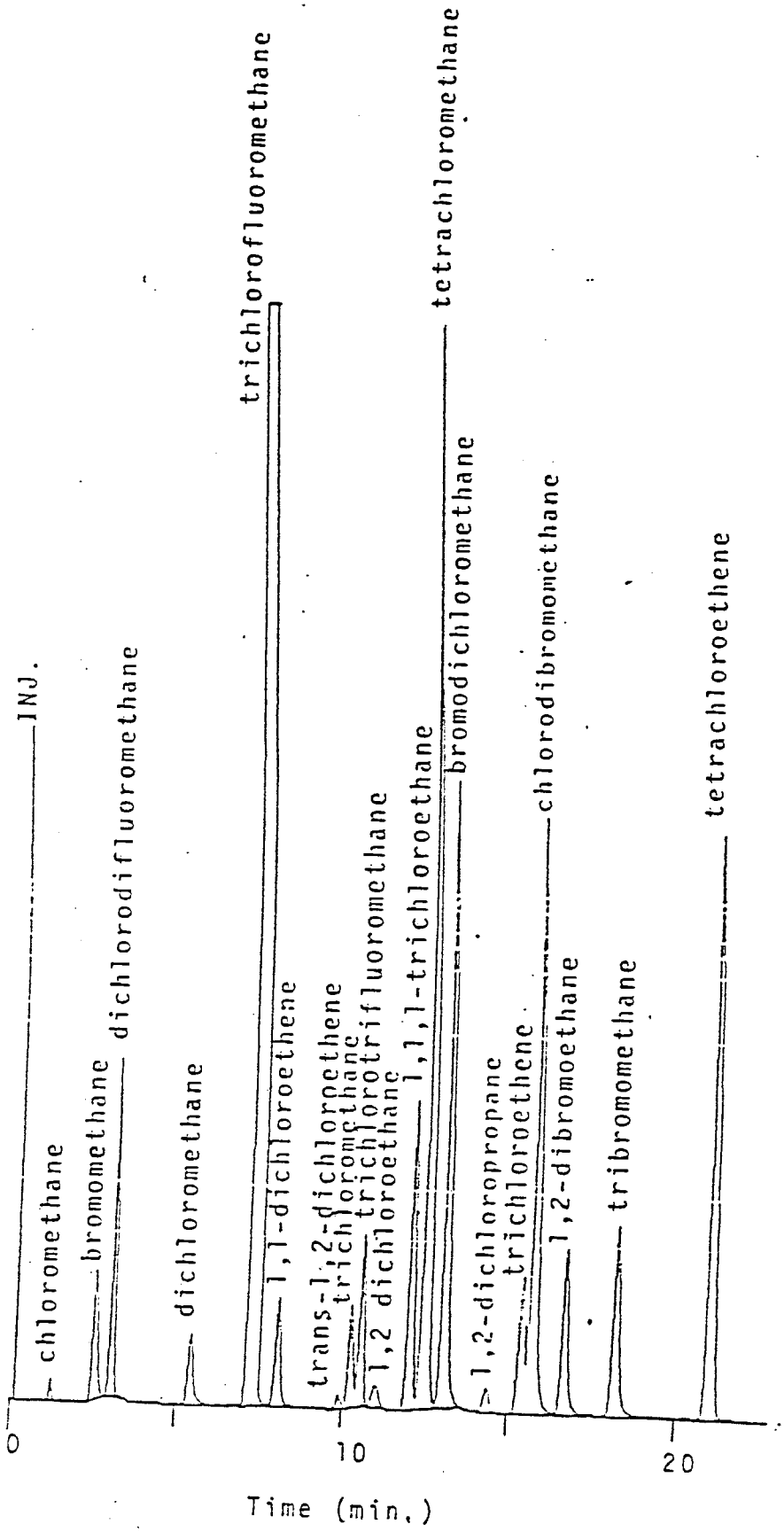


Figure 5.3.11 - Typical Halogenated Hydrocarbon Chromatogram

METHOD NO. ADDL002

STANDARD OPERATING PROCEDURE FOR THE DETERMINATION OF VOLATILE ORGANICS IN AMBIENT AIR USING TENAX TRAP PRECONCENTRATION GAS CHROMATOGRAPHY AND TANDEM PHOTOIONIZATION/ELECTRON CAPTURE DETECTORS

1.0 SCOPE

This document describes a procedure for the determination of volatile halogenated hydrocarbons and aromatics having a boiling point of less than 120°C. This procedure is based on documents received from the ARB Haagen-Smit Laboratory, El Monte, as well as EPA Method TO1.

2.0 SUMMARY OF PROCEDURE

Ambient air is continuously sampled and collected in a Tedlar bag over a 24 hour period and immediately sent to the laboratory for analysis. A sample from the bag is drawn through a sampling valve attached to a Tekmar LSC-2 Tenax Sample Concentrator (see Figure I) with a vacuum pump at 50 cc/min for four minutes (total sample volume: 200 cc). The organic constituents are trapped on Tenax and when the collection is complete, the Tenax is purged with 40 cc of helium to remove any trapped moisture. The sample is then thermally desorbed onto the head of the GC column. The GC column is temperature programmed and component peaks

eluting from the column are sequentially detected and quantified, first by a photoionization detector (PID) and then by an electron capture detector (ECD). The components are identified based on retention times. Positive identification or confirmation requires the use of an appropriately configured GC/MS.

3.0 INTERFERENCES/LIMITATIONS

a. Components having similar GC retention times will interfere, causing misidentification and/or faulty quantitation.

b. Because of the very low sample concentrations, extreme care must be taken to insure that the sample is not degraded or contaminated by the Tedlar sampling bag, sampling apparatus, or delayed delivery to the laboratory. Exposure of the Tedlar sampling bag to temperatures greater than 25°C should be minimized.

c. Only components of the sample which can be detected by PID/ECD detectors will be quantified.

4.0 APPARATUS

a. Varian Model 6000 Gas Chromatograph/PID/ECD system equipped with a Varian Vista 402 dual channel data system.

b. Tekmar LSC-2 Sample Concentrator equipped with Tenax trap and sampling valves as shown in Figure 1.

c. Matheson Model 8240 Mass Flow Controller accurately calibrated in the 5-100 cc/min range.

d. Laboratory timer, accurate to within 0.1 minutes.

e. Gas tight microliter syringe, 50 ul.

f. GC column - 10' x 2 mm i.d. glass column packed with 1 percent SP-1000 on Carbopack B, 60/80 mesh.

5.0 REAGENTS

a. Primary Gas Standard (Scott Specialty Gases - Research Triangle Institute Certified Series 1)

Compound	Concentration (ppb)
Chloroform	107
Carbon tetrachloride	105
Perchloroethene	106
Vinyl chloride	104
Benzene	107

b. Primary Gas Standard (Scott Specialty Gases - Research Triangle Institute Certified Series 2)

Compound	Concentration (ppb)
1,2-Dichloroethane	101
1,1,1-Trichloroethane	98
Trichloroethene	100
1,2-Dibromoethane	102

c. Stock Gas Standard - Scott-Marrin Blend (assayed against primary cylinders)

Compound	Concentration (ppb)
Dichloromethane	4272
Chloroform	528
1,2-Dichloroethane	3104
1,1,1-Trichloroethane	424
Carbon tetrachloride	46
Trichloroethene	336
1,2-Dibromoethane	5
Perchloroethene	43
Vinyl chloride	4736
Benzene	1888

d. Control Gas Standard - Scott-Marrin blend (assayed against primary cylinder)

Compound	Concentration (ppb)
Dichloromethane	6
Chloroform	0.2
1,2-Dichloroethane	0.2
1,1,1-Trichloroethane	3.6
Carbon tetrachloride	0.3
Trichloroethene	1.8
1,2-Dibromoethane	2.5
Perchloroethene	1.2
Vinyl chloride	3.3
Benzene	4.8

e. Surrogate Gas Standard (Scott-Marrin Blend)

Compound	Concentration (ppm)
Bromochloromethane	10
1,3-Bromochloropropane	33

6.0 PROCEDURES

a. Sample Trapping

1. The preconcentration system is shown in Figure 1.

2. The high concentration inlet is used for high concentration calibration standards and for other samples with concentrations higher than ambient levels. The sample is introduced through the high concentration inlet and 6 port valve into an appropriate size loop of known volume. The sample then passes through a 10 port valve, mass flow meter, and vacuum pump. Before an analysis, the system is leak checked by blocking the sample inlet port and observing that the mass flow meter reading drops to zero. The high concentration inlet then is connected to a Tedlar sample bag valve and the gas bag valve is opened. The loop is then flushed with sample gas for three minutes. After three minutes of flushing, the 6 port valve is reset so that the sample contained in the loop is carried into the trap by the helium purge gas. This continues for three minutes to ensure that all of the contents of the loop are trapped.

3. Ambient samples are introduced from Tedlar bags as described above, except that the sample loop is bypassed and the sample goes directly to the 10 port valve. After flushing the system with sample for three minutes, the 10 port valve is reset so that 200 cc's of sample is trapped (50 cc/min. for four minutes). After sample trapping is complete, the Tenax trap is flushed with 40 cc of helium to remove water vapor and any nonadsorbed reactive gases.

4. In both ambient and high concentration cases, after the sample has been trapped, the Tekmar LSC-2 heats the Tenax trap to 180°C while the trap is swept with the G.C.'s internal carrier gas for four minutes. The contents of the trap are thus desorbed and collected on the head of the G.C. column. The trap is baked out after the end of the desorption cycle. In the bakeout cycle, the trap is flushed with helium purge gas for eight minutes while being held at 225°C in order to prepare the trap for the next cycle. After bakeout the trap is isolated from the system and ready for the next sample.

b. Analysis

1. The concentrated sample is separated under the chromatographic condition detailed below. The resulting chromatogram (see Figure II) is then integrated and quantified by reference to calibration standard gases.

2. Instrument Conditions:

GC: Column: 10' x 2 mm i.d. glass column, packed with 1 percent SP-1000 on Carbopack B 60/80 mesh

Temperatures: Injection: 200°C

Detector: 350°C

Oven: 45°C, hold for four minutes, 5°C/min ramp, to 210°C, hold for eight minutes

Flow Rates: Carrier: He, 20 cc/min

ECD make up: N_2, 40 cc/min

Detectors: ECD: Range X 10, Attenuation X 32

PID: Range X 1, Attenuation X 32, 10.2 ev lamp

Conc: Tekmar LSC-2: Purge: 4 minutes

Desorb: 4 minutes at 180°C

Bake: 8 minutes at 225°C

3. All blanks, standards, control samples, and ambient samples are spiked with surrogate compounds by injecting 50 microliters of the surrogate gas standard (5.e.) during sample trapping. The surrogate compounds, chosen such that they simulate the characteristics of the analytes of interest and are unlikely to occur in the environment, are added to insure that systematic errors or equipment failures will be noted and corrected promptly.

4. The first step in a calibration is to analyze a system blank. This is done by trapping and analyzing a 200 cc sample of auxiliary carrier gas. The system blank must be free of interfering peaks. A system blank must also be run after a high concentration sample is analyzed in order to detect any carry-over within the system.

5. A calibration is performed using a 1.25 cc loop of stock standard gas (5.c.). Two hundred cubic centimeters of helium gas is passed through the loop to carry the standard onto the trap. The calibration analysis is made as a normal analysis. The calculated concentration value for each component should be inspected to insure consistency with previous analyses. The stored chromatographic information may then be used to recalculate the response factors for the subsequent analyses. The G.C. data system will not accept updated response factors which are in excess of plus or minus 15 percent of historic data.

6. Following calibration, 200 cc of the control sample (5.d.) is concentrated on the trap and analyzed. The control sample data are plotted on control charts of the normal Shewhart type. Upper and lower warning limits are plus or minus two times the standard deviation. Any analysis which falls outside the upper and lower warning limits is repeated and the laboratory quality control officer is advised. Upper and lower control limits are plus or minus three times the standard deviation. If any analysis falls outside the upper or lower control limit, the method is discontinued until the out of control situation is remedied. The laboratory quality control officer is advised and provided with written documentation of the out of control condition and how it was remedied. All data generated prior to the out of control situation must be reviewed for possible decertification by laboratory management.

7. Multipoint calibrations are conducted monthly. Each multipoint calibration includes a trap blank and three standard concentration levels to bracket the concentration ranges expected in ambient air. If subsequent data indicate that the resulting least squares analyses are consistently acceptable, less frequent multipoint calibrations may be made.

7.0 PERFORMANCE

a. All ambient field samples are analyzed in duplicate. The relative error between analyses must be less than 20 percent. Duplicate analyses having greater than 20 percent relative error must be decertified.

b. The percent recovery of the surrogate is recorded in the instrument laboratory workbook for each analysis. If this value is outside the 80% to 120% range, the sample analysis must be repeated.

8.0 METHOD SENSITIVITY, PRECISION AND ACCURACY

The method sensitivity, precision and accuracy are outlined in Table I. These data were produced with gaseous calibration standards, and using carrier gas as the sample matrix. The relative accuracy of the method, with the exception of dichloromethane, is based on reference to the Research Triangle Institute Certified Gas Standards (NBS traceable). Authoritative reference calibration standards for dichloromethane are under development at NBS but are not yet available. The concentration value of the present standard was assigned by the commercial manufacturer and found to be in good agreement with diluted pure dichloromethane prepared in our laboratory. The absolute accuracy of the method has not been determined by interlaboratory testing.

Figure I

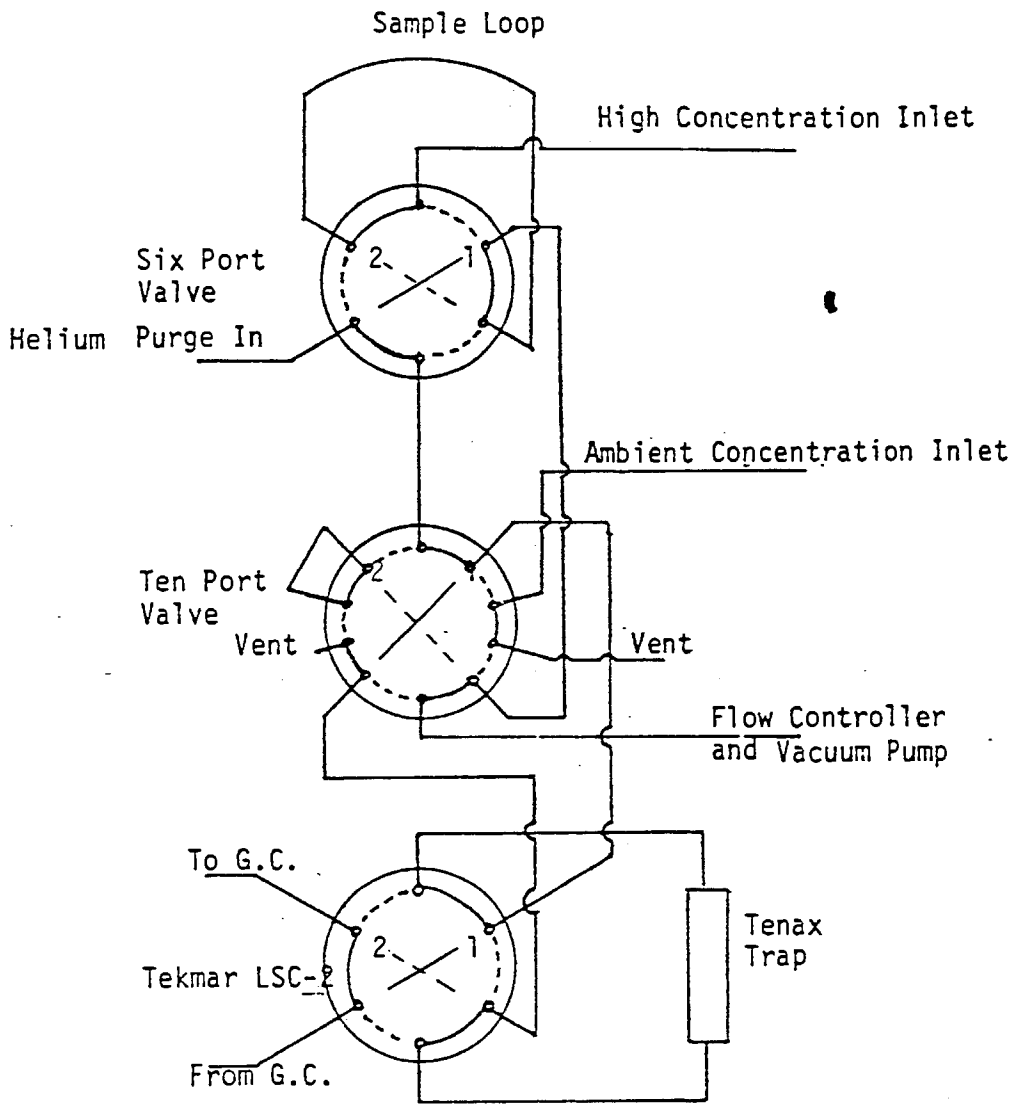


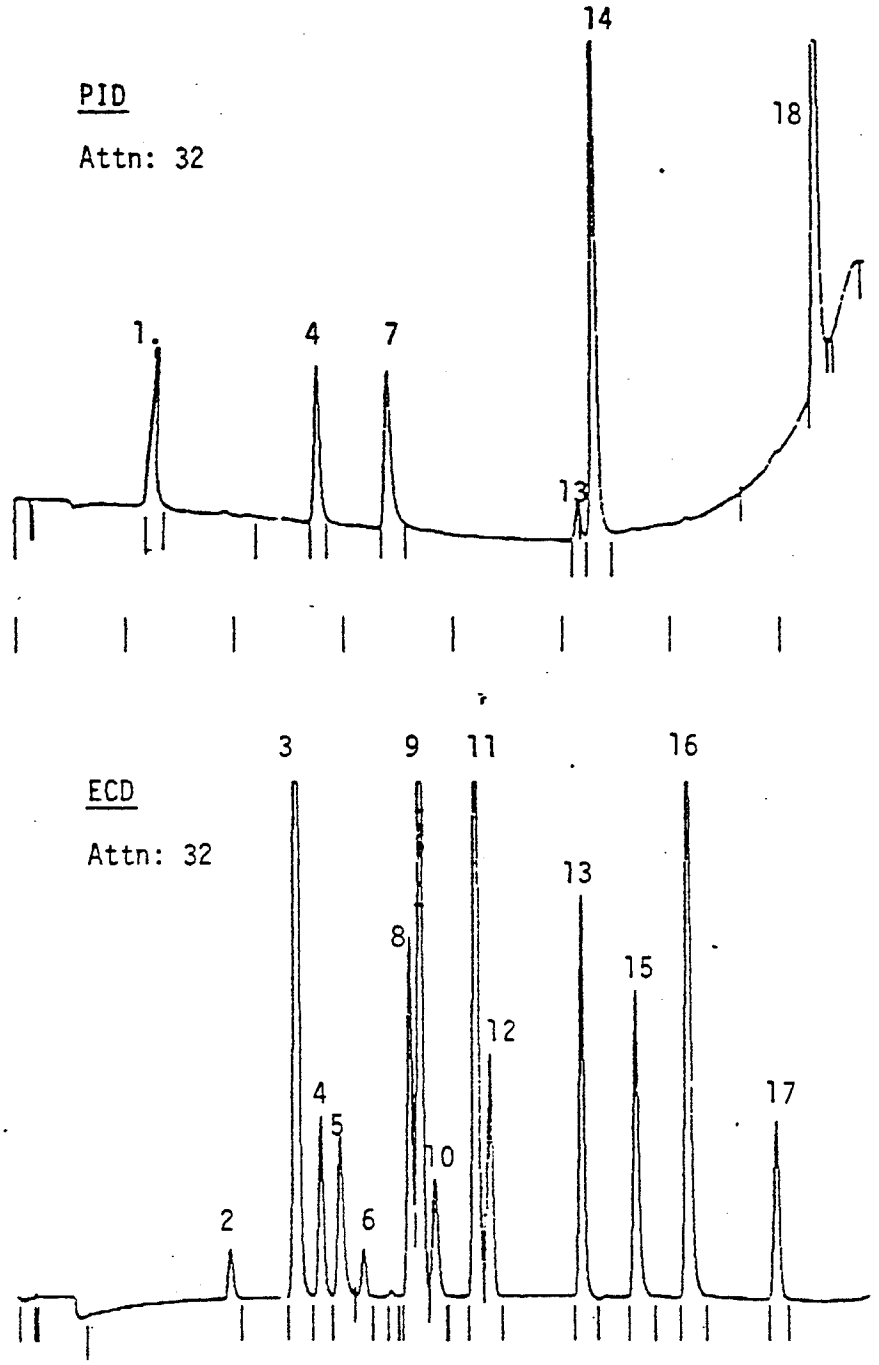
Figure 1. Schematic of concentrator system. Sampling Conditions are: 200 cc volume, purge at 40cc/min, 1 min., desorb at 180 C for 4 min., bake for 8 min. at 225 C.

SYSTEM GUIDE

Operational Step	Valve Position			
	6-Port	10-Port	LSC-2	Purge Gas
Loop Fill	1	1	1	Off
Loop Trap	2	1	1	On
Ambient Trap	1	2	1	Off
Trap Desorb	1	1	2	Off
Trap Bake Out	1	1	1	On

Figure II

Standard PID/ECD Organic Analysis



1. Vinyl Chloride
2. Dichloromethane
3. Trichlorofluoromethane
4. 1,1-Dichloroethylene
5. Bromochloromethane
6. 1,1-Dichloroethane
7. t-1,2-Dichloroethylene
8. Chloroform
9. Freon 113
10. 1,2-Dichloroethane
11. 1,1,1-Trichloroethane
12. Carbon Tetrachloride
13. Trichloroethylene
14. Benzene
15. 1,2-Dibromoethane
16. Bromochloropropane
17. Tetrachloroethylene
18. Toluene

Table I

Method Sensitivity and Precision

Compound	Correlation Coefficient	Slope	R.S.D* (Percent)	Detector	LOD ppbv
Vinyl Chloride	0.997	0.946	16	PID	0.8
Dichloromethane	0.999	0.975	5	ECD	0.6
1,1-Dichloroethylene	0.991	0.966	6	ECD	0.05
Chloroform	0.999	0.901	3	ECD	0.02
1,2-Dichloroethane	0.999	1.054	7	ECD	0.1
1,1,1-Trichloroethane	0.999	0.989	9	ECD	0.01
Carbon Tetrachloride	0.999	0.980	6	ECD	0.005
Trichloroethylene	0.999	0.992	6	ECD	0.02
Benzene	0.998	0.950	10	PID	0.5
1,2-Dibromoethane	0.974	1.067	9	ECD	0.005
Tetrachloroethylene	0.994	1.080	10	ECD	0.01

* R.S.D. - Relative Standard Deviation at 5 x LOD, n = 5

APPENDIX II

DESCRIPTION OF GLEIT'S METHOD

APPENDIX II

DESCRIPTION OF GLEIT'S METHOD

Gleit's method accounts for the concentrations below the LOD by setting them equal to the "below -LOD mean" μ_{BLOD}, the mean of the portion of the normal distribution below the LOD. Setting the unknown concentrations to their average value seems intuitively reasonable, and the simulations reported in Gleit's paper show that his method is more accurate than other commonly used approximations.

The below-LOD mean of a normal distribution of a variable with a limit of detection L is given, in terms of L and the mean μ and the standard deviation σ of the distribution, by equation 1:

$$\mu_{BLOD} = \mu - \sigma * [\, f((L-\mu)/\sigma) \,/\, F((L-\mu)/\sigma) \,] \qquad (1)$$

In equation (1), f and F are, respectively, the probability density function and cumulative distribution function of the standard normal distribution. The "Estimated Concentrations for Samples Below the LOD" reported in Table II-2 are the below-LOD means of the assumed lognormal distributions of the concentrations. These below-LOD means are computed from equation (2) in terms of parameters of the associated normal distribution: the LOD L, the mean concentration from Table II-2, and the estimated standard deviation (which is not tabulated).

$$\exp\,(\mu+0.5^{*}\,\sigma^{2})^{*}\,F((L-\mu-\sigma^{2})/\sigma) \,/\, F(L-\mu/\sigma) \qquad (2)$$

We now describe how Gleit's method estimates the mean and variance of the assumed normal distribution. The mean and variance cannot be estimated by merely substituting into standard formulas, if below-LOD concentrations are to be set to the below-LOD mean. On the one hand, the mean and variance must be known in order to calculate the below-LOD mean from (1); on the other hand,

the below-LOD mean must be known if it is to be used in the calculation of the mean and variance. Statistical theory, by asserting that a "best -fitting " mean and variance for the distribution exist, provides a way out of this dilemma. Gleit uses a simple iterative procedure to compute these best-fitting parameters. Since his procedure can be simply described in words, a written description is given, supplemented where necessary by equations written in a notation more convenient than Gleit's.

Starting with initial guesses $\mu(0)$ and $\sigma^2(0)$ for the mean and variance, the procedure repeatedly generates new estimates of the mean and variance by the two-step computation described below until successive estimates of the mean and variance converge sufficiently (The K-th pair of estimates are denoted by $\mu(K)$ and $\sigma^2(K)$.). The two steps are:

(a) The K+1-st below-LOD mean $\mu_{BLOD}(K+1)$ is computed by substituting $\mu(K)$ and $\sigma(K)$ (the square root of $\sigma^2(K)$) into equation (1).

(b) The K+1-st estimate of the mean, $\mu(K+1)$, is computed in the usual way with $\mu_{BLOD}(K+1)$ substituted for the sample values below the LOD. The K+1-st estimate of the variance, $\sigma^2(K+1)$, is also computed in the usual way, with an analogous substitution for sample values below the LOD: the squared deviations from the mean of concentrations below the LOD are set equal to the average squared deviation from the mean of the below-LOD portion of the distribution.

Let the N sample items be X(1),....,X(N), and let p be the number of sample items below the LOD. $\mu(K+1)$ is computed by:

$$\mu(K+1) = (1/N)\,\Sigma\, Y(J), \text{ where } Y(J) = X(J) \text{ if } X(J) \geq L$$
$$\text{and } Y(J) = \mu_{BLOD}(K+1) \text{ otherwise.}$$

$\sigma^2(K+1)$ is computed by:

$$\sigma^2(K+1) = (1/N)\,\Sigma\, D^2(J), \quad \text{where } D^2(J) = (X(J) - \mu(K+1))^2$$
$$\text{if } X(J) \geq L, \text{ and } D^2(J) = \sigma^2_{BLOD}(K+1) \text{ otherwise.}$$

The quantity $\sigma^2_{BLOD}(K+1)$, the average squared deviation of the below-LOD portion of the distribution, is computed from the following equation:

$$\sigma^2_{BLOD}(K+1) = \sigma^2(K)*[1 - Z(K)* (f(Z(K)) / F(Z(K)))],$$
$$\text{where } Z(K) = ((L-\mu (K)) / \sigma(K)).$$

Gleit's method nearly always converges in a few steps unless there are only a few distinct values above the detection limit, in which case it may converge very slowly. Gleit's method and closely related methods appear to be the best available estimators of the mean when the sample includes values below the LOD, as is demonstrated by the simulations reported in Gleit's paper.

APPENDIX III

METHODS FOR ESTIMATING CALIFORNIA METHYLENE CHLORIDE USE

APPENDIX TO CHAPTER III

Methods For Estimating California Methylene Chloride Usage

Methylene chloride data, collected by source category, was used as the basis for estimating emissions and for comparing the relative consumption of different categories. Thirteen major source categories of methylene chloride use in the U.S. were identified. Estimates of California consumption of methylene chloride were developed for these categories.

Different methods were used to estimate California use for the 13 categories. For several categories, California use was estimated from estimated U.S. use compiled by the Halogenated Solvents Industry Alliance (HSIA, 1985). For other categories, information providing a more direct estimate of California use was available.

Paint removers, aerosols, and miscellaneous:

Population fractions were used to estimate California use from U.S. use for these four categories. California's population was 11 percent of the U.S population in 1980 (U.S. Dept. of Commerce). Thus, California use was estimated as 11 percent of U.S use for these categories.

Pharmaceuticals, Photoresist Stripping, Specialty Chemicals, Chemical Processing & Food Processing:

Domestic manufacturers reported the amounts of methylene chloride that they shipped to California for eleven different end use categories during 1985 (HSIA, 1986b). These figures were reported in Table III-1 as an estimate of California use for the five categories above. They are considered as lower-end estimates because fractions of the 5,400 tons that manufacturers report shipping to California for unidentified miscellaneous use could have been consumed by these five end uses.

Degreasing Operations

Methylene chloride use for degreasing operations in California was determined as the ratio of California employment in applicable SIC categories to U.S. employment in those categories, multiplied by the U.S. use. Five major industry groups used methylene chloride in degreasing operations, including: furniture and fixtures (SIC 25), fabricated metal products (SIC 34), electric and electronic equipment (SIC 36), transportation equipment (SIC 37), and miscellaneous manufacturing industries (SIC 39). Employment data by two-digit SIC

code is available from the Bureau of Census. An EPA report discussed this approach and listed the California use of methylene chloride by degreasing operations as 3,100 TPY in 1983 (U.S. EPA, 1985).

Distribution Facilities:

The quantity of methylene chloride handled by distribution facilities in California was estimated as a ratio of California use to U.S. use times the quantity of methylene chloride handled by U.S. distribution facilities. Use in this case was considered to be the total usage determined for all direct users, as presented in Table III-1 of the text. An EPA report estimated the quantity of methylene chloride handled by U.S. distribution facilities to be 240,000 TPY (U.S. EPA, 1985).

Urethane Foam Manufacturing:

California consumption for foam manufacture is based on estimates made by an EPA contractor. (Pandullo and Nash, 1986). The contractor identified eight California facilities which use methylene chloride. At this time ARB has not verified whether or not these are the only facilities which use methylene chloride for manufacturing urethane foam products in California. Total use by all eight facilities was reported as 1,600 tons in 1984. The manufacturers supplied methylene chloride use for four of the eight facilities. The contractor assumed that for the remaining facilities use was equivalent to the average use (194 tons) of all 43 foam manufacturing facilities in the United States for which methylene chloride consumption was obtained.

Pesticide Manufacturing:

Pesticide manufacturing use is based on information for the one facility in the state that the EPA identified as a user of methylene chloride. Use at this facility was 800 tons in 1983 (U.S. EPA, 1985). It is possible that other pesticide manufacturing facilities located in California are using methylene chloride.

Solvent Reclaimers:

The quantity of methylene chloride sent to solvent reclaimers in California was based on two sources of information: 1) the California Department of Health Services and 2) private industry. The California Department of Health Services collects data from transportation manifests on the quantities of hazardous waste shipped in California. According to these data, 9,685 tons of halogenated solvents were sent to solvent reclaimers in 1985 (DHS, 1986). Representatives of two of the

largest solvent reclaimers in California estimated the percent of methylene chloride in the halogenated solvents received (O'Morrow, 1986; Schneider, 1986). From this information, it was estimated that one fourth or 2,400 tons of the halogenated solvents were methylene chloride in 1985.

Chemical Processing & Specialty Chemical Production:

The HSIA reported that in 1985 1,200 tons of methylene chloride were shipped to California for the chemical processing industry and that 800 tons were shipped into the state for specialty chemical production. Only two facilities in California which fall into these end use categories have been identified. Methylene chloride consumption for both facilities is unknown. It is possible that other chemical processing and production facilities in California use and emit methylene chloride.

The two facilities that have been identified are both in the San Francisco Bay Area Air Basin. One uses methylene chloride in the production of an unidentified specialty chemical and the other uses it to manufacture rubber cement. Both facilities reported emissions data to EPA. Methylene chloride emission estimates are 3 tons per year at the rubber cement production facility (Howie, 1986), and 54 tons per year at the specialty chemical production facility (U.S. EPA, 1985).

Triacetate Fiber Production

Methylene chloride is used as a solvent for triacetate fiber production at one facility in Rockhill, South Carolina (U.S. EPA, 1985). Methylene chloride is not used in this manner in California.

REFERENCES

DHS, 1986. California Department of Health Services. Computer Printout from HazardousWaste Information System Giving Disposal Information for 1985. April 11, 1986.

Howle, R., 1986. Telephone conversation between Ronald A. Rothacker of CARB and Reese Howle of Radian Corporation, Research Triangle Park, NC. July 22, 1986.

HSIA, 1985. Letters from D.L. Morgan of Cleary, Gottlieb, Steen and Hamilton to R.E. Rosensteel, U.S. IPA, OAQPS, Emission Standards and Engineering Division. Regarding methylene chloride end use data from HSIA member companies. Dated March 8, April 11 and June 28, 1985.

HSIA, 1986a. Letter from D.L. Morgan of Cleary, Gottlieb, Steen and Hamilton to J.A. Marconi of CARB regarding amount of methylene chloride shipped to California. October 28, 1986.

HSIA, 1986b. Letter from D.L. Morgan of Cleary, Gottlieb, Steen and Hamilton to Donald J. Ames of CARB regarding end use of methylene chloride shipped to California by domestic manufacturers. December 23, 1986.

O'Morrow, K., 1986. Telephone conversation between Ronald A. Rothacker of CARB and Ken O'Morrow of Oil and Solvent Process Co., Azusa, Ca., June 9, 1986.

Pandullo, R. and Nash, R., 1986. Radian Corporation memorandum to file entitled: "Estimates of Methylene Chloride Emissions from Polyurethane Foam Slabstock Facilities and Emission Reductions Achievable with Additional Controls," April 28, 1986.

Schneider, P., 1986. Telephone conversation between Ronald A. Rothacker of CARB and Peter Schneider of Romic Chemical Corporation, East Palo Alto, Ca., June 9, 1986.

U.S. Department of Commerce, Bureau of the Census, 1982. Statistical Abstract of the United States 1982-83. 103rd. Edition., December, 1982.

U.S. EPA, 1985. "Survey of Methylene Chloride Emission Sources," EPA-450/3-85-015, Research Triangle Park, NC.

APPENDIX IV

INFORMATION REQUEST LETTER WITH ATTACHMENTS AND RESPONSES

STATE OF CALIFORNIA

GEORGE DEUKMEJIAN, Governor

AIR RESOURCES BOARD
1102 Q STREET
P.O. BOX 2815
SACRAMENTO, CA 95812

Dear Sir or Madam:

Request for Information Regarding
Methylene Chloride (dichloromethane)

I am writing to request information on the health effects of methylene chloride (dichloromethane) as part of our toxic air contaminant program. This program is based on Health and Safety Code Sections 39650, et seq. which require the Air Resources Board (ARB) to prepare a report which would serve as the basis for regulatory action and to determine by regulation, whether a substance is a toxic air contaminant. Once identified as a toxic air contaminant, the law further requires that the ARB prepare a report on the need and appropriate degree of regulation for the substance. After consultation with the staff of the Department of Health Services (DHS), we have selected methylene chloride as a candidate toxic air contaminant to be evaluated in accordance with the provisions of Health and Safety Code Sections 39650, et seq. During our evaluation of methylene chloride, we will consider all available health information regarding this substance. Additionally, we are soliciting information regarding possible biological transformations of methylene chloride.

Before the ARB can formally identify a substance as a toxic air contaminant, several steps must be taken. First, the ARB must request the Department of Health Services to evaluate the health effects of the candidate substance. Second, the ARB staff must prepare a report which includes the health effects evaluation and then submit the report to a Scientific Review Panel for its review. The report submitted to the Panel will be made available to the public. Information submitted in response to this request will be considered in the report to the Panel. Although any person may also submit information directly to the Panel for its consideration, I urge you to submit all information at this time for our consideration in the development of the report for the Panel. The Panel reviews the sufficiency of the

information, methods, and data used by the DHS in its evaluation. Last, after review by the Scientific Review Panel, the report with the written findings of the Panel will be considered by the Air Resources Board and will be the basis for any regulatory action by the Board officially to identify a substance as a toxic air contaminant.

Prior to formally requesting the DHS to prepare a health effects evaluation of methylene chloride, we are providing, pursuant to the provisions of Section 39660(e) of the Health and Safety Code, an opportunity for interested parties to submit information on the health effects of methylene chloride which he or she believes would be important in DHS' evaluation of methylene chloride as a candidate toxic air contaminant.

In January 1986, we conducted a reference search on methylene chloride health effects using the MEDLINE and TOXLINE data bases available from the National Library of Medicine. These information services include material published from 1965 to late-1985. The attached bibliography lists the references from this information search. We are requesting pertinent information on methylene chloride health effects, including any material that may not be available to the public and/or that is not included in the attached bibliography.

Pursuant to the provisions of the Public Records Act (Government Code Sections 6280 et seq.), the information you provide will be public record and subject to public disclosure, except for trade secrets which are not emission data or other information which is exempt from disclosure or the disclosure of which is prohibited by law. The information may also be released to the Environmental Protection Agency, which protects trade secrets and confidential information in accordance with federal law, and to other public agencies, which are also required to protect such information.

To expedite the review process, we ask that any information which you believe should be regarded as "trade secret" be clearly marked and separated from other information. You may identify portions of the information you submit as "trade secret" in accordance with Health and Safety Code Section 39660(e). The claim of trade secrecy must be supported upon the request of the Air Resources Board. Other information claimed to be trade secret and information otherwise claimed to be exempt from disclosure may be identified as confidential in accordance with Section 91011, Title 17, California Administrative Code. Section 91011 requires that the claim of confidentiality be

accompanied by specified supporting information.

I would appreciate receiving any relevant information you wish to submit by March 17, 1986. Your help in expediting our review will be greatly appreciated. Please send the information in duplicate to the attention of:

William V. Loscutoff, Chief
Toxic Pollutants Branch
Re: Methylene Chloride
California Air Resources Board
P. O. Box 2815
Sacramento, CA 95812

If you have any further questions regarding health effects information, please contact Mr. John Batchelder at (916) 323-1505. For any other questions, please contact Mr. Don Ames at (916) 322-8285.

If you are not the person to whom this request should be addressed, please forward it to the appropriate person in your organization. Also, please let us know whether you would like to continue to receive information inquiries for other candidate substances, and if not, if there is anyone in your organization to whom such requests should be sent.

Sincerely,

Peter D. Venturini, Chief
Stationary Source Division

Attachment

cc: Alex Kelter, DHS
Lori Johnston, DFA
Wayne Morgan, President, CAPCOA
Jan Bush, Executive Secretary, CAPCOA
David Howekamp, EPA Region IX
Assemblywoman Sally Tanner, Chairwoman, Committee on Toxic Materials
Senator Ralph Dills, Chairman, Committee on Governmental Organization
Senator Art Torres, Chairman, Committee on Toxics and Public Safety Management
Emil Mrak, Chairman, and Scientific Review Panel Members
APCOs

HSIA HALOGENATED SOLVENTS INDUSTRY ALLIANCE
2315 M Street, N.W., Third Floor, Washington, D.C. 20037 • (202) 223-5890

March 27, 1986

Mr. William V. Loscutoff, Chief
Toxic Pollutants Branch
California Air Resources Branch
P.O. Box 2815
Sacramento, CA 95812

Re: Methylene Chloride

Dear Mr. Loscutoff:

We thank you for providing us the opportunity to submit health information on methylene chloride. We are enclosing a variety of recent documents and articles which were not included in your list of references. Please note that the last two references listed are not enclosed. They are currently being cleared for outside release and should be sent within two weeks.

HSIA understands that this information will be used by the State Department of Health Services in its process of considering all available scientific data in its evaluation of health effects and recommendation as to whether or not methylene chloride should be determined to be a toxic air contaminant.

A toxic air contaminant is defined in AB 1807 as:

> An air pollutant which may cause or contribute to an increase in mortality or an increase in serious illness, or which may pose a present or potential hazard to human health.

HSIA believes that methylene chloride does not cause adverse health effects when used according to current industrial hygiene guidelines or labeled instructions. Furthermore, there is no scientific evidence to indicate that ambient concentrations of methylene chloride cause or contribute to an increase in mortality or an increase in serious illness, or which may pose a present or potential hazard to human health. HSIA believes that adequate scientific evidence exists to determine that methylene chloride does not meet the above definition of a toxic air contaminant and should not be identified as one.

We submit the following scientific information as the basis for our conclusion that methylene chloride should not be identified as a toxic air contaminant.

1. Two well-conducted epidemiologic studies have been completed on worker populations exposed to methylene chloride. Neither study indicated a human cancer risk associated with methylene chloride exposure. The most definitive study compare the mortality rate of over 1,000 employees with identified methylene chloride exposure to an unexposed worker population. The findings of this study indicate that workers to methylene chloride for a minimum of 20 years and observed over 20 additional years actually had a decreased incidence of cancer when compared to the New York State control population.

   The other epidemiologic study of methylene chloride examined the health and mortality of over 1200 employees exposed to a mixture of methylene chloride, ethanol, and acetone in a fiber production plant. The study showed no excess mortality cancer in the workers exposed to methylene chloride, even though exposures had ranged up to 475 parts per million and over 300 of them had been followed at least 17-1/2 years after exposure. This study is highly relevant to an understanding of the health risk posed by methylene chloride.

2. The only sites in which methylene chloride has produced tumors are those sites in experimental rodents where the tumors occur spontaneously in unexposed animals (mouse lung and liver tumors and benign mammary gland tumors in rats with no progression to malignancy). The only exception is a low but statistically significant incidence of ventral neck region tumors in male rats only in one study. This response was reviewed and its relevance severely questioned by the U.S. EPA Science Advisory Board and at the Nutrition Foundation Food Solvents Workshop on Methylene Chloride in 1984. Most importantly and of extreme toxicological significance is the fact that the ventral neck region tumor response was not repeatable in the NTP bioassay in rats in any other species evaluated (mouse and hamster).

3. Methylene chloride has been evaluated in numerous short-term *in vitro* tests, from microorganisms to mammalian cells in culture, as well as in whole animal assays to determine its genotoxic potential. Aside from positive activity in certain microorganisms (primarily bacteria), results in mammalian cells and whole animals, have, for the most part, been negative. A genotoxic component, if present at all in exposed animals is likely to be very weak to nil and not a significant factor in the toxicity of methylene chloride. Thus the principle of practical no-effect-levels should apply in assessing risk for humans.

4. Mechanistic studies have been conducted which provide plausible hypotheses for the observed enhancement of spontaneous tumors in animals exposed to high concentrations of methylene chloride (mouse lung/liver; cytotoxicity/physiological stimulation: rat benign mammary gland; elevated prolactin levels). HSIA believes that humans are not exposed to levels of methylene chloride in the ambient environment which could cause is chain of events to occur. Furthermore, development of a physiologically-based pharmacokinetic model for methylene chloride has confirmed that markedly lower tissue levels of bioactive metabolites of methylene chloride (lower internal dose) would be found in humans than in experimental animals exposed to comparable external concentrations of methylene chloride.

To summarize, HSIA believes that methylene chloride should not be identified as a toxic air contaminant. We have enclosed additional pertinent references to support this belief. In addition we offer our cooperation in answering any questions which might arise in this review of methylene chloride.

Please feel free to call me at (202) 223-5890.

Sincerely yours,

Paul A. Cammer

Paul Cammer, Ph.D.
Executive Director

Enclosure

Gail, D. B. and Lenfant, D. J. M., State of the Art, Cells of the Lung: Biology and Clinical Implications, Am. Rev. Respir. Dis. 127(3):366-387 (1983).

Schairer, L. A., Sautkulis, R. D. and Tempel, N. R., Detection and Identification of Genotoxic Agents in Ambient Air Using the Tradescantia Bioassay, Environ. Mutagenesis 4:379 (1982).

Te-Hsui, M. A., Harris, M. M., Anderson, V. A., Ahmed, I., Mohammad, K., Bare, J. L. and Lin, G., Tradescantia -- Micronucleus (TRAD-MCN), Tests on 140 Health Related Agents, Mutat. Research 138:157-167 (1984).

Abrahamson, S., and Valencia, R., Evaluation of Substance of Interest for Genetic Damage Using Drosophila melanogaster (1980), prepared for FDA Contract No. 233-77-2119.

Sivak, A., Capitals Balb/C-3T3 Neoplastic Transformation Assay with Methylene Chloride (Food Grade Test Specification), Report to National Coffee Association, Inc., 1978.

Thilagar, A. K., Back, A. M., Kirby, P. E., Kumaroo, P. V., Pant, K. J., Clarke, J. E., Kight, R. and Haworth, S. R., Evaluation of Dichloromethane in Short-term In Vitro Genetic Toxicity Assays, Environ. Mutagenesis 6:418-419 (1984).

Thilagar, A. K., Kumaroo, P. V., Clarke, J. J., Kott, S., Back, A. M. and Kirby, P. E., Induction of Chromosome Damage by Dichloromethane in Cultured Human Peripherial Lymphocytes, CHO Cells and Mouse Lymphoma L5178Y Cells, Environ. Mutagenesis 6:422 (1984).

Dutton, D. R. and Bowden, G. T., Indirect Induction of a Plastogenic Effect on Epidermal Cells by a Tumor Promoter, Carcinogenesis 6:1279-1284 (1985).

Henry, James, M.E., et. al., Force Breeding, Social Disorder and Mammory Tumor Formation in CBA/WSC Mouse Colonies: A Pilot Study, Psychosomatic Medicine 37(3):277-283 (1975).

Schumann, A. M., Nitschke, K. D., Fox, T. R. and Watanabe, P. G. (1984). [^{14}C] Methylene Chloride: pharmacokinetics and interaction with hamster and rat tissue macromolecules. Report of The Dow Chemical Co., Midland, MI, 48640.

Welsch, C. W. and Nagaswa, H. (1977). Prolactin and murine mammary tumorigenesis: a review. Cancer Research 37, 951-963.

Letter of July 18, 1984 to William D. Ruckelshaus from Herschel E. Griffin and Norton Nelson.

** Breslein, W. J. and Landry, T. D. (1986). Methylene chloride: effects on estrous cycling and serum prolactin in Sprague-Dawley rats. Report of the Dow Chemical Co., Midland, MI 48640.

** Schuman, A. M. (1986). Perspective on the genetic toxicology data base for methylene chloride. The Dow Chemical Co., Midland, MI 48640.

** Not enclosed. Currently being cleared for outside release.

HALOGENATED SOLVENTS INDUSTRY ALLIANCE
2315 M Street, N.W., Third Floor, Washington, D.C. 20037 • (202) 223-5890

March 31, 1986

Mr. William V. Loscutoff, Chief
Toxic Pollutants Branch
California Air Resources Branch
P.O. Box 2815
Sacramento, CA 95812

Dear Mr. Loscutoff:

Our submission of March 27, 1986, contained a typographical error. On page 3, line 8 the word is should be changed to this.

Sincerely,

Thomas Cortina
Assistant Director

HSIA HALOGENATED SOLVENTS INDUSTRY ALLIANCE
2315 M Street, N.W., Third Floor, Washington, D.C. 20037 • (202) 223-5890

April 10, 1986

Mr. William V. Loscutoff, Chief
Toxic Pollutants Branch
California Air Resources Board
P.O. Box 2815
Sacramento, CA 95812

Re: Methylene Chloride

Dear Mr. Loscutoff:

In my letter of March 27, 1986, I noted that the last two documents listed in our submission would be sent at a later date. Those documents have now been cleared for outside release and are enclosed.

We appreciate your allowing us to submit these documents on a delayed basis and offer our cooperation in answering any questions which might arise in your review of methylene chloride.

Please feel free to call me at (202) 223-5890.

Sincerely,

Paul A. Cammer

Paul A. Cammer, Ph.D.
Executive Director

Enclosures

Received
APR 11 1986

HSIA HALOGENATED SOLVENTS INDUSTRY ALLIANCE
2315 M Street, N.W., Third Floor, Washington, D.C. 20037 • (202) 223-5890

July 8, 1986

Mr. William V. Loscutoff, Chief
Toxic Pollutants Branch
California Air Resources Board
P.O. Box 2815
Sacramento, CA 95812

Re: Methylene Chloride

Dear Mr. Loscutoff:

Dr. Alan Schumann, chairman of HSIA's Health and Science Committee, presented a seminar on methylene chloride at DOHS' Berkeley offices on June 4. Enclosed are two copies of the June 24 summary letter which Dr. Schumann sent to Dr. Neutra.

Also enclosed are two copies of the final report for a two-generation inhalation reproduction study in Fisher 344 rats, and the most recent update from CEFIC of their ongoing research program on the species differences in the metabolism of methylene chloride. The reports from the first phase of the CEFIC research program were submitted in March by CEFIC, and they have asked that HSIA submit this latest update.

We would like you to consider the enclosed materials as part of the health information used by the Department of Health Services in its recommendation as to whether or not methylene chloride should be determined to be a toxic air contaminant.

Please feel free to call me at (202) 223-5890 if you have any questions or need further information.

Sincerely yours,

Paul A. Cammer

Paul A. Cammer, Ph.D.
Executive Director

Enclosures

cc: Dr. Raymond Neutra

Received
JUL 17 1986
DOHS

METHYLENE CHLORIDE PRODUCERS ASSOCIATION

A CEFIC Sector Group

Conseil Européen des
Fédérations de l'Industrie Chimique

European Council of Chemical
Manufacturer's Federations

Your Ref.

Our Ref. SG METHYLENE CHLORIDE

REPLIES TO: Dr M R Harris
Regulatory Affairs
Imperial Chemical Industries PLC
Mond Division, PO Box 13
The Heath, Runcorn, WA7 4QF
CHESHIRE, England

William V Loscutoff
Chief of Toxic Pollutants Branch
Re: Methylene Chloride
California Air Resources Board
PO Box 2815
Sacramento
California 95812
USA

Your ref	Our ref	Date
	MRH/CL	04 Mar 86

Dear Sirs

METHYLENE CHLORIDE (=DICHLOROMETHANE)

I am writing to you on behalf of the Methylene Chloride Workgroup of the European Council of Chemical Manufacturers' Federations (CEFIC) with regard to your 5 February 1986 'Request for Information' regarding methylene chloride.

This group has sponsored a 2-year program of research on the species differences in the mechanism of action and biological transformation of methylene chloride in an effort better to understand the marked species differences in tumorigenicity as evidenced by a number of long term bioassays in the rat, the hamster and the mouse in which only the mouse shows clear evidnece of a carcinogenic response - and this only at the high doses of the recent NTP inhalation bioassay, and yet not at the similar internal doses of the recent NCA ingestion (drinking water) bioassay. Further evidence suggestive of a species difference - or a dose-response difference, or both - is provided by the human epidemiology (particularly the 1985 and 1986 extensions of Friedlander's studies at Kodak, NY). This provides data which (while inevitably unable to "prove" a negative conclusion) makes any significant human risk a highly improbable hypothesis, and whose most probable explanation (by far) is the hypothesis of zero human risk.

Avenue Louise 250, Bte 72 B-1050 BRUXELLES Tél.: 640.20.95 Tlx : 62 498 Telefax : 640 1981

Our own studies are being conducted at the Central Toxicology Laboratory of Imperial Chemical Industries PLC in England, with external scientific monitoring and peer review b the European Chemical Industry Ecology & Toxicology Centre (ECETOC). The work will in due course be published in the scientific literature, and the laboratory studies are fully open to independent audit. The first stage of these studies has recently been completed, and copies of the three full final reports are enclosed; this work has also recently been presented at Toxicology Forum in Washington DC (February 1986) and at the Annual Meeting of the US Society of Toxicologists in New Orleans (March 1986); it has also been submitted to EPA, FDA, CPSC and OSHA.

This stage of our work has included a 10-day inhalation cytotoxicity study, DNA binding studies, and unscheduled DNA synthesis (UDS) studies (in vivo/ in vitro and in vitro) - in the B6C3F1 mouse and Fisher F344/N rat. We have found evidence of cytotoxicity to a specific cell type (the Clara cell) of the mouse lung, but not in the rat lung nor in mouse or rat liver. Under our experimental conditions we found no evidence for DNA-binding or UDS, strongly suggesting that under these conditions there is no interaction between methylene chloride or its metabolites and mammalian DNA. This would be consistent with a non-genotoxic action of methylene chloride, not necessarily because of "instantaneous detoxification of a carcinogen" (apparently the only rationale, among the many possible, considered in the November 1985 California DHS carcinogen risk assessment guidelines) but more probably because (bearing in mind the existence of at least two independent metabolic pathways, at least one of which is saturable) no stable genotoxic intermediates were generated.

The second phase of our work, now in progress and expected to complete during the third quarter of 1986, is studying quantitatively the metabolic pathways involved in mouse, rat, hamster and man. Collection of the relevant pharmacokinetic data, and its incorporation into appropriate pharmacokinetic models, should allow us to establish and quantify interspecies differences in metabolism, the existence and level of any thresholds and, more generally, the shape of possible dose-response curves for comparison with animal bioassay findings, and with the findings of epidemiological studies.

It is our belief that this approach should assist the risk assessment process by allowing the use of experimentally-determined and scientifically valid dose-response relationships, rather than simplistic linear extrapolations between the high doses of animal bioassays and the much lower levels of human exposure, and the even greater extrapolation from mouse to man under circumstances where the mouse fails even to predict the bioassay response of such other rodents as the rat and the hamster.

Yours sincerely

DR M R HARRIS

Encs

DOCUMENTS ENCLOSED:

1. CEFIC submission to EPA. 20 September 1985. Interim report.
2. CEFIC submission to EPA. 13 December 1985. Interim report.
3. CEFIC Research Report No: CTL/P/1432. 10 Day Inhalation Toxicity Study.
4. CEFIC Research Report No: CTL/R/851. Interaction with Rat and Mouse Liver and Lung DNA *In Vivo.*
5. CEFIC Research Report No: CTL/P/1444. *In Vivo* and *In Vitro* UDS Studies.

METHYLENE CHLORIDE PRODUCERS ASSOCIATION

A CEFIC Sector Group

Conseil Europeen des
Federations de l'Industrie Chimique

European Council of Chemical
Manufacturer's Federations

Your Ref

Our Ref SG METHYLENE CHLORIDE

REPLIES TO:

M R Harris General Chemicals Business Group
Imperial Chemical Industries PLC
Mond Division
P O Box 13
RUNCORN Cheshire WA7 4QF

Telephone Runcorn (0928) 511344 (Direct)

William V Loscutoff
Chief of Toxic Pollutants Branch
Re: Methylene Chloride
California Air Resources Board
PO Box 2815
Sacramento
California 95812
USA

Your ref	Our ref	Date
	MRH/CL	08 Oct 86

Dear Sirs

METHYLENE CHLORIDE

We refer to our letter of 4 March 1986 with which we enclosed reports on the earlier phases of the research programme being conducted by us in Europe into species differences in response to methylene chloride, and their relevance to human health.

With our earlier letter we provided two interim and three final reports on the first phase of this work, in which no evidence could be found for any genotoxicity of methylene chloride in rats or mice - strongly suggesting that any quantitative carcinogenicity risk assessment could not simply extrapolate from high-dose bioassays to low occupational exposures, and would need to reflect cross-species differences.

We now have pleasure in submitting four final reports on the second phase of the CEFIC studies (Annexes 1 - 4) which describe the results of a study of S-phase hepatocyte induction in the mouse, of an evaluation of methylene chloride in the mouse micronucleus test, of **in vivo** studies on the pharmacokinetics and metabolism of methylene chloride in mouse and rat, and of **in vitro** studies on metabolism in rat, mouse, hamster and man.

Avenue Louise 250, Bte 72 B-1050 BRUXELLES Tél 640 20 95 Tlx 62 498 Telefax 640 1981

We believe that these latest studies from our research programme considerably add to the weight of evidence suggesting that methylene chloride does not express genotoxic effects in vivo in mammalian systems, and that the mouse is a quite inappropriate model for man in view of major differences in its metabolism of methylene chloride. Instead, the hamster would appear to be the most appropriate species for evaluating the human carcinogenic potential of methylene chloride. These results fit well with the observed absence of carcinogenicity in the hamster lifetime bioassay (Burek et al), and in the human epidemiology (Friedlander et al, as updated June 1986 with enhanced statistical power, and showing absence of dose-response).

We trust that this further new scientific information will assist the Air Resources Board and the Department of Health Services in assessing the level of any toxic hazard arising from methylene chloride. We believe that these results provide even greater confidence in the view that the recent NTP inhalation bioassay findings are without direct relevance for human health, and that methylene chloride does not pose a carcinogenic threat to humans.

We hope that Dr Trevor Green, who has been responsible for much of the work described in the enclosed reports, will be in California in November. If you feel that discussions at that time with scientists from DOHS or CARB would be helpful, perhaps you would let me know as soon as possible so that we may discuss dates. In any case, we will do our best to respond to any further queries you may have.

DR M R HARRIS
European Council of Chemical Manufacturers' Federations (CEFIC)
International Office for Chlorinated Solvents (BITSC)
Methylene Chloride Workgroup

Enclosed:

Annex 1. Methylene Chloride: An Evaluation in the Mouse Micronucleus Test
Report No: CTL/P/1603

Annex 2. Methylene Chloride: Induction of S-phase Hepatocytes in the Mouse after in vivo Exposure
Report No: CTL/R/885

Annex 3. Methylene Chloride: In vitro Metabolism in Rat, Mouse and Hamster Liver and Lung Fractions and in Human Liver Fractions
Report No: CTL/R/879

Annex 4. Methylene Chloride: In vivo Inhalation Pharmacokinetics and Metabolism in F344 Rats and B6C3F1 Mice
Report No: CTL/R/880

METHYLENE CHLORIDE PRODUCERS ASSOCIATION

A CEFIC Sector Group

Conseil Européen des
Fédérations de l'Industrie Chimique

European Council of Chemical
Manufacturer's Federations

Your Ref.

Our Ref. SG METHYLENE CHLORIDE

Replies to:

Dr M R Harris
Imperial Chemical Industries PLC
Mond Division
PO Box 13
The Heath
RUNCORN Cheshire WA7 4QF
ENGLAND

Tel: (0928) 511344 (Direct)

17th November 1986

William V Loscutoff
Chief of Toxic Pollutants Branch
Re: Methylene Chloride
California Air Resources Board
PO Box 2815
Sacramento
California 95812
USA

Dear Sirs

METHYLENE CHLORIDE - TOXICOLOGY REPORTS FROM CEFIC

We recently sent to you four reports on our toxicological investigations of methylene chloride, numbered and titled as follows:

Report No: CTL/P/1603 ... Evaluation in the Mouse Micronucleus Test

CTL/R/879 ... In Vitro Metabolism in Rat, Mouse and Hamster Liver and Lung Fractions and in Human Liver Fractions

CTL/R/880 ... In Vivo Inhalation Pharmacokinetics and Metabolism in F344 Rats and B6C3F1 Mice

CTL/R/885 ... Induction of S-phase Hepatocytes in the Mouse after In Vivo Exposure

Avenue Louise 250, Bte 72 B-1050 BRUXELLES Tél.: 640.20.95 Tlx : 62 498 Telefax : 640 1981

Due to an administrative oversight, these reports were marked 'Confidential - not to be copied' on their title page. We would like to assure you that these reports are NOT confidential, contain no confidential business information, and are intended for placement on the public record for public comment and review.

We have also noted a calculation error in the data in Table 4 of Report No CTL/R/879 (In Vitro Metabolism) and enclose an audited correction sheet with the corresponding Quality Assurance Statement from the contracting Laboratory. The nature of the error is such that it does not in any way alter the conclusions in the text or the interpretation of the report.

We apologise for any inconvenience these errors may have caused.

Yours sincerely

DR M R HARRIS
Regulatory Affairs

Enc

METHYLENE CHLORIDE PRODUCERS ASSOCIATION

A CEFIC Sector Group

Conseil Européen des
Fédérations de l'Industrie Chimique

European Council of Chemical
Manufacturer's Federations

Your Ref.

Our Ref. SG METHYLENE CHLORIDE

Replies to:

Dr M R Harris
Imperial Chemical Industries PLC
Chemicals & Polymers Group
PO Box 13
The Heath
RUNCORN Cheshire WA7 4QF
ENGLAND

Tel: (0928) 511344 (Direct)

8th May, 1987

METHYLENE CHLORIDE (DICHLOROMETHANE). SPECIES DIFFERENCES IN METABOLISM.

Further to our earlier submissions, we are pleased to enclose an interim statement giving the latest results of metabolic studies conducted on our behalf at the Central Toxicology Laboratory of ICI in England, and confirmed by parallel independent studies by the Mammalian and Environmental Toxicology Laboratory of Dow Chemical Company in the USA.

We expect to report in more detail on our own further studies by means of a further interim submission in early June in order that the Science Advisory Board may have as much information as possible available for the June meeting of the Halogenated Solvents Subcommittee. A full report should be available in July/August 1987 and should contain more detailed metabolic and pharmacokinetic findings.

M R Harris

Dr M R Harris
Chairman, Methylene Chloride Workgroup
Bureau International des Solvants Chlores (BITSC), CEFIC

Avenue Louise 250, Bte 72 B-1050 BRUXELLES Tél.: 640.20.95 Tlx : 62 498 Telefax : 640 1981

METHYLENE CHLORIDE PRODUCERS ASSOCIATION

A CEFIC Sector Group

Conseil Européen des
Fédérations de l'Industrie Chimique

European Council of Chemical
Manufacturer's Federations

Your Ref.

Our Ref. SG METHYLENE CHLORIDE

Replies to:

Dr M R Harris
Imperial Chemical Industries PLC
Chemicals & Polymers Group
PO Box 13
The Heath
RUNCORN Cheshire WA7 4QF
ENGLAND

Tel: (0928) 511344 (Direct)

15th July, 1987

Dr W V Loscutoff
Chief of Toxic Pollutants Branch
California Air Resources Board
P O Box 2815
Sacramento
California 95812
USA

Dear Mr Loscutoff

METHYLENE CHLORIDE (DICHLOROMETHANE):

NEW RESULTS FROM RESEARCH INTO SPECIES DIFFERENCES IN TOXICOLOGY:

Please find attached ECETOC Statement No. 4 (June 1987) being a further submission of results from ongoing research sponsored by CEFIC and conducted at the Central Toxicology Laboratory of ICI in England.

The results further extend and further quantify our understanding of the marked cross-dose and cross-species differences in the toxicology of methylene chloride in three rodent species and in man.

Full reports of these studies will be available in October 1987.

Yours sincerely

pp

Dr M R Harris
Methylene Chloride Workgroup
Bureau International Technique des Solvants Chlores
Conseil Europeen des Federations de l'Industrie Chimique

Encl

Avenue Louise 250, Bte 72 B-1050 BRUXELLES Tél.: 640.20.95 Tlx : 62 498 Telefax : 640 1981

E. I. DU PONT DE NEMOURS & CO. (INC.)
WILMINGTON, DELAWARE 19898

MARKETING COMMUNICATIONS DEPARTMENT

February 18, 1986

Mr. Peter D. Venturini
State of California
Air Resources Board
P.O. Box 2815
Sacramento, CA 95812

Dear Mr. Venturini:

Thank you for your recent correspondence requesting information concerning methylene chloride.

The Du Pont Company is not currently engaged in the manufacture or supply of this item.

Listed below is the name and address of the firm indicated as manufacturer or supplier of the product involved.

Dow Chemical USA
2020 Dow Center
Midland, MI 48640
517-636-1000

We acknowledge and appreciate your interest in our Company.

Product Information Center
800-441-7515

Chevron

Chevron Environmental Health Center, Inc.
A Chevron Research Company Subsidiary
15299 San Pablo Avenue, Richmond, California
Mail Address P.O. Box 4054, Richmond, CA 94804

R. D. Cavalli
Manager
Product Evaluation

3/3

February 24, 1986

Methylene Chloride

William V. Loscutoff, Chief
Toxic Pollutants Branch
California Air Resources Board
P.O. Box 2875
Sacramento, California 95812

Dear Mr. Loscutoff:

As per your request, in accordance with Health and Safety Code Sections 39650, et seq., we have reviewed our files on the health effects of methylene chloride. We have no relevant "in-house" data on methylene chloride, but feel the following references may be useful additions to the bibliography for the Department of Health Services:

1. Heppel, L. A., Neal, P. A., Perrin, T. L., Orr, M. L., Porterfield: Toxicology of Dichloromethane (Methylene Chloride). I. Studies on Effects of Daily Inhalation. J. Ind. Hyg. Toxicol. 26: 8-16, 1944.

2. Heppel, L. A., Neal, P. A.: Toxicology of Dichloromehtane (Methylene Chloride). II. Its Effect Upon Running Activity in the Male Rat. J. Ind. Hyg. Toxicol. 26: 17-21, 1944.

Thank you for the opportunity to contribute to the review of candidate toxic air contaminants.

Sincerely,

RD Cavalli / GFSM

MAR 03 1986

New York State Department of Environmental Conservation
50 Wolf Road, Albany, New York 12233-0001

Henry G. Williams
Commissioner

Chang M
John Marconi

March 7, 1986

RECEIVED

MAR 11 1986

Stationary Source
Division
Air Resources Board

Mr. Peter Venturini
Chief
Stationary Source Division
State of California
Air Resources Board
1102 Q Street
P. O. Box 2815
Sacramento, California 95812

Dear Mr. Venturini:

This is in response to your letter of February 5, 1986 in which you requested information on the health effects of methylene chloride. At this time, we are conducting a quantitative risk assessment by applying statistical models to the bioassay data obtained from the inhalation study completed by the National Toxicology Program (NTP) in 1985. In addition, we will be completing a search of the same references which were included with your letter.

When we have completed this risk assessment, we will be glad to send you a copy.

Sincerely,

Virginia M Rest

Virginia M. Rest
Environmental Chemist
Section of Toxics Assessment
Bureau of Air Toxics

VMR:cb

BASIC PRODUCTS GROUP
CHLOR-ALKALI, PHOSPHORUS
& SULFURIC PRODUCTS
DIVISIONS

Stauffer CHEMICALS

Stauffer Chemical Company
636 California St./San Francisco, CA 94108/Tel. (415) 544-9311/Cable "Stauffer"

REPLY TO:
P. O. Box 7766
San Francisco, CA 94120

March 3, 1986

William V. Loscutoff, Chief
Toxic Pollutants Branch
RE: Methylene Chloride
California Air Resources Board
P. O. Box 2815
Sacramento, CA 95812

Dear Mr. Loscutoff:

Per your request, attached please find a copy of our Product Safety Information Sheet for Methylene Chloride.

Also, please be advised that as of March 1, 1986, Stauffer Chemical Company is no longer in the Methylene Chloride business.

Sincerely,

W. J. Meier
Senior Technical Service Representative
Basic Chemicals Division

WJM:el
enclosure

Product Safety Information

METHYLENE CHLORIDE

This Product Safety Information Sheet is principally directed to managerial, safety, hygiene and medical personnel. The description of physical, chemical and toxicological properties and handling advice is based on experimental results and past experience. It is intended as a starting point for the development of health and safety procedures.

Synonyms: Dichloromethane; Methylene dichloride
CAS Registry Number: 75-09-2

I. PHYSICAL AND CHEMICAL PROPERTIES

Formula
CH_2Cl_2
Formula Weight
84.94
Physical State/Description
Colorless volatile liquid at 68°F (20°C), 14.7 psia
Autoignition Temperature
1224°F (662°C)
Boiling Point
104°F (40°C)
Density
11 lbs/gallon at 68°F (20°C)
Evaporation Rate
1.47 (Carbon tetrachloride = 1)
Explosive Limits In Pure Oxygen
LEL: 15.5%; UEL: 66.4%
Flammability Limits In Air
13.9 to 19.1 [% volume at 212°F (100°C)]
Freezing Point
-142°F (-96.7°C)
Miscibility
Miscible with alcohol and ether
Odor
Penetrating, ether-like, pleasantly aromatic, sweet odor
pH
Slightly alkaline (water extract)
Solubility
Sparingly soluble in water
[1.95 g/100g in water at 68°F (20°C)]
Specific Gravity
1.326 at 68°F/68°F (20°C/20°C)
Vapor Pressure
140 mmHg at 32°F (0°C)
350 mmHg at 68°F (20°C)
700 mmHg at 100°F (37.7°C)
Viscosity
0.43 cp at 68°F (20°C)

IN CASE OF SUSPECTED POISONING, REFER TO THE PROCEDURE AND EMERGENCY CONTACTS IN SECTION VIII: FIRST AID.

IN CASE OF SPILLAGE, REFER TO THE PROCEDURE AND EMERGENCY CONTACTS IN SECTION X: SPILL HANDLING OR CALL (CHEMTREC) (800) 424-9300.

II. CHEMICAL REACTIVITY

Does not undergo hazardous polymerization. However, a serious chemical reaction, with the possibility of explosion, could result if halogenated hydrocarbon solvents are used in pressurizable fluid systems having aluminum or galvanized wetted parts. Prolonged exposure to water at elevated temperatures (140°F/60°C) may cause noticeable hydrolysis. May react violently or explode upon contact with alkali or chemically active metals such as finely powdered aluminum, magnesium, potassium and/or sodium.

III. STABILITY

Stable at ambient temperatures and atmospheric pressure. However, exposure to high temperature sources (such as open flame and welding arcs) causes thermal-oxidative decomposition yielding toxic phosgene gas and corrosive hydrogen chloride.

IV. FIRE HAZARD

Methylene chloride does not have a measurable flash point by conventional test methods. It is not considered flammable but forms flammable vapor/air mixtures at approximately 212°F (100°C) or higher. Under fire conditions, decomposes to form toxic phosgene gas and corrosive hydrogen chloride.

V. FIREFIGHTING TECHNIQUE

Vapors are irritating to the respiratory tract and may cause breathing difficulty and pulmonary edema. Symptoms may be delayed several hours or longer depending upon the extent of exposure.

As in any fire, prevent human exposure to fire, smoke, fumes or products of combustion. Evacuate nonessential personnel from the fire area. Firefighters should wear full-face, self-contained breathing apparatus and protective clothing.

Use standard firefighting techniques to extinguish fires involving this material—use water spray, dry chemicals or carbon dioxide.

If not leaking, use a water spray to keep fire-exposed containers cool to prevent rupture due to excessive heat. High pressure water hose may spread product from broken containers increasing contamination or fire hazard.

VI. HUMAN HEALTH

WARNING: Vapor and liquid may be harmful if inhaled, swallowed, or absorbed by skin. Avoid prolonged or repeated breathing of vapor. May cause skin and eye irritation. Avoid contact with skin, eyes, and clothing.

The primary route of entry for methylene chloride is inhalation.

Inhalation of large amounts of the vapor may cause nausea, chills, headache, lightheadedness, drowsiness, and irritation of the respiratory tract. Continued overex-

posure may produce unconsciousness, kidney and lung damage, and death.

Eye irritation and blurred vision may result from eye contact by the liquid or large quantities of the vapor.

Prolonged skin contact with the liquid may cause burns. Repeated skin exposures may produce dermatitis.

LONG TERM EXPOSURE: An epidemiological study performed on a large group of male workers at an Eastman Kodak facility in New York, who were continuously exposed to estimated TWA exposures of 30 – 125 ppm of methylene chloride for up to 30 years, has been reported. There was no increase in mortality among exposed workers as compared to industrial controls and the general New York State male population. (1)

VII. TOXICOLOGY

Ingestion

The acute oral LD50 is 2,710 mg/kg in male rats and 2,330 mg/kg in female rats. A single oral dose of 2,150 mg/kg produced mild depression and 20 percent mortality in male rats, and produced moderate to severe depression, tremors and 40 percent mortality in female rats. A single oral dose of 4,640 mg/kg resulted in dyspnea, ataxia, and narcosis in female rats and 100% mortality in male and female rats.

Skin Contact

The acute dermal LD50 is greater than 4,640 mg/kg in rabbits. A single dermal application of 4,640 mg/kg did not produce signs of toxicity in male or female rabbits. Local effects included a mild erythema and moderate edema.

Mild to moderate irritant to rabbit skin following a 4-hour exposure.

Eye Contact

A single ocular dose of 0.1 mL produced lacrimation, inflammation, conjunctival edema, and an increased corneal thickness (reversible). (2)

Inhalation

The acute inhalation LC50 is greater than 19.5 mg/L (nominal concentration) in both male and female rats. A single 1-hour inhalation exposure of 19.5 mg/L (nominal concentration) produced slight depression, moderate ataxia, and ocular protrusion. The acute inhalation LC50 in mice is approximately 14,400 – 17,400 ppm. Seven hour exposures to doses ranging from 13,000 – 17,000 ppm produced ataxia, labored respiration, muscular twitching, and narcosis. (3)

Monkeys, dogs, rabbits, guinea pigs and rats exposed to 500 ppm for 8 hour/day, 5 days/week, for 15 weeks showed 20% mortality (in rats), and a slight increase in eosinophils (rabbits). (3)

Monkeys, dogs, rats, and mice were continuously exposed to 25 or 100 ppm for 100 days. No overt signs of stress were observed. The exposure produced: histopathological effects in mouse livers (100 ppm) and rat kidneys (25 and 100 ppm); no significant findings in hematology, clinical chemistry or histopathology in dogs and monkeys; and significantly increased carboxyhemoglobin in dogs and monkeys (monkeys 100 and 25 ppm, dogs 100 ppm). (3)

Rats and hamsters were exposed by inhalation to 0, 500, 1,500 or 3,500 ppm of methylene chloride for 6 hours per day, 5 days a week for 2 years. No organ-specific toxicity was observed in hamsters. Carboxyhemoglobin levels were elevated in hamsters and rats at all dose levels, but this effect was not cumulative. (4)

Histopathological effects in the liver were observed in male and female rats at all dose levels and considered to be exposure-related. Despite these effects, there was no increase in neoplastic (hyperplastic) liver nodules and no increase in hepatocellular carcinomas. (4)

Increased total numbers of benign mammary tumors were seen in male and female rats, but no increase in malignant mammary tumors was observed. There were no significant lung tumor findings in any exposed group compared to the controls. (4)

Male rats in the 3,500 ppm dose group exhibited a significant increase in sarcomas in or around the salivary glands. A significant increase in this finding was not observed in males exposed at 500 or 1,500 ppm or any of the exposed females. (4)

In a second study, male and female rats were exposed to 0, 50, 200, or 500 ppm for 6 hrs/day, 5 days/week for 2 years. Additionally, one group of females was exposed at 500 ppm for the first 12 months only, and another group for the second 12 months only. (5)

A significant increase in histopathological effects in the liver was observed in females exposed to 500 ppm for 2 years and to 500 ppm for the first 12 months. (5)

Mutagenicity

Methylene chloride was examined for mutagenic activity in *in vitro* microbial assays, with and without metabolic activation, using the TA-100 and TA-98 strain of *Salmonella typhimurium* as the indicator organism. The compound demonstrated mutagenic activity in both assays. (6)

Methylene chloride did not induce chromosomal aberrations in a rat bone marrow assay. (4)

Methylene chloride induced cellular transformation in Fischer rat embryo cells. (7)

T-4054, T-4171, T-5528

VIII. FIRST AID

CALL A POISON CENTER OR
A PHYSICIAN IMMEDIATELY.

If a known exposure occurs or is suspected, immediately start the recommended procedures below. Simultaneously contact a Poison Center, a physician or the nearest hospital. Inform the person contacted of the type and extent of exposure, describe the victim's symptoms, and follow the advice given.

NOTE TO PHYSICIAN

Methylene chloride is metabolized to carbon monoxide and carbon dioxide. The resultant elevation of carboxyhemoglobin levels is of short duration (e.g. usually less

(1) J. Occ. Med. 20 (10):657 (1978).
(2) Toxicol. 6:173-187 (1976).
(3) HEW (NIOSH) Publication No. 76-138.
(4) Burek, J. D. et. al (1980) Methylene Chloride: "A Two-Year Inhalation Toxicity and Oncogenicity Study in Rats and Hamsters." Final Report Issued December 31, 1980 by Dow Chemical, USA.
(5) Nitschke, K. D. et. al (1982) Methylene Chloride: "A Two-Year Toxicity and Oncogenicity Study." Final Report Issued October 11, 1982 by Dow Chemical, USA.
(6) Mut.Res. 56:245-248 (1978)
(7) In Vitro 14:290-293 (1978)

than 48 hours) unless overexposure is prolonged. Inhalation of large amounts of methylene chloride may cause pulmonary edema and could be fatal. Exposure to high concentrations of methylene chloride may cause depression of the central nervous system.

FOR ADDITIONAL MEDICAL OR TOXICOLOGICAL INFORMATION, CALL COLLECT, DAY OR NIGHT, STAUFFER CHEMICAL COMPANY (203) 225-6602 OR CHEMTREC (800) 424-9300.

Ingestion

If swallowed, immediately give several glasses of water but *do not* induce vomiting. If vomiting does occur, give fluids again. Have a physician determine if condition of patient will permit induction of vomiting or evacuation of stomach. Do not give anything by mouth to an unconscious or convulsing person.

Skin Contact

Immediately flush all affected areas with large amounts of water for at least 15 minutes while removing any contaminated clothing and shoes. Do not attempt to neutralize with chemical agents. Get medical attention immediately. Wash clothing before reuse.

Eye Contact

Immediately flush the eyes with large quantities of running water for a minimum of 15 minutes. Hold the eyelids apart during the flushing to ensure rinsing of the entire surface of the eye and lids with water. Do not attempt to neutralize with chemical agents. Obtain medical attention as soon as possible. Oils or ointments should not be used. Continue the flushing for an additional 15 minutes if the physician is not immediately available.

Inhalation

Remove from contaminated atmosphere. Seek medical attention if respiratory irritation occurs. If the breathing becomes difficult, oxygen may be delivered from a demand-type or continuous flow inhaler, preferably with a physician's advice. If not breathing, give artificial respiration, preferably mouth-to-mouth. Get medical attention.

IX. INDUSTRIAL HYGIENE

Ingestion

All food should be kept in a separate area away from the storage/use location. Eating, drinking and smoking should be prohibited in areas where there is a potential for significant exposure to this material. Before eating, hands and face should be thoroughly washed.

Skin Contact

Skin contact with liquid or its aerosol should be prevented through the use of suitable protective clothing, gloves and footwear selected with regard for use condition exposure potential.

Eye Contact

Eye contact with liquid or its aerosol should be prevented through the use of chemical safety glasses, goggles or a face shield selected with regard for use condition exposure potential.

Inhalation

If use conditions generate airborne liquid, aerosol or vapor handle this material only in an open (e.g., outdoor) or well ventilated area. Where adequate ventilation is not available, use NIOSH-approved organic vapor respirators to reduce exposure. Where exposure potential under the use conditions necessitates a higher level of protection, use a positive-pressure, air-supplied respirator.

Exposure Limits

The Federal OSHA Permissible Exposure Limit (PEL) is an 8-hour, time-weighted average of 500 ppm (1,700 mg/m^3) and a ceiling concentration of 1,000 ppm for methylene chloride. ([8])

The American Conference of Governmental Industrial Hygienists (ACGIH) has recommended a Threshold Limit Value (TLV) of 100 ppm (360 mg/m^3) as an 8-hour time-weighted average, and a Short Term Exposure Limit (STEL) of 500 ppm (1,700 mg/m^3) for methylene chloride. ([9])

X. SPILL HANDLING

Make sure all personnel involved in the spill cleanup follow good industrial hygiene practices (refer to SECTION IX: INDUSTRIAL HYGIENE). Any person entering a significant spill area or an unknown concentration of a gas or vapor, should wear a positive-pressure, self-contained breathing apparatus or a positive-pressure, supplied-air respirator with escape pack.

Small spills can be handled routinely. Use adequate ventilation and wear a respirator to prevent inhalation exposure. Wear protective clothing to prevent skin and eye contact. Use the following procedures:

Evacuate spill area and remove sources of combustion or extreme heat. Ventilate the area and let small spills evaporate. Somewhat larger spills should be contained and recovered where possible. Mop up spilled methylene chloride or adsorb with a suitable adsorbent such as clay, sawdust or kitty litter. Reclaim waste solvent by distillation procedures or offer for disposal to a licensed hydrocarbon solvent reclaimer.

Large spills should be handled according to a predetermined plan. For assistance in developing a plan, contact Stauffer Chemical Company, Westport, CT 06881.

IN CASE OF SPILL EMERGENCY, DAY OR NIGHT, CALL (800) 424-9300 CHEMTREC.

XI. CORROSIVITY TO MATERIALS OF CONSTRUCTION

In the absence of contamination by water or moisture, methylene chloride is not corrosive to common materials such as copper, iron or stainless steel. In contact with water or moisture, particularly at elevated temperatures, it can be corrosive. Methylene chloride may soften or dissolve certain plastics and rubbers. Avoid contact with alkali or chemically active metals because an explosion may occur (See SECTION II: CHEMICAL REACTIVITY).

([8]) 29 CFR 1910.1000

([9]) The American Conference of Governmental Industrial Hygienists (ACGIH). 1982. *Threshold Limit Values for chemical substances and physical agents in the workroom environment with intended changes for 1982.* ACGIH: Cincinnati, Ohio.

XII. STORAGE REQUIREMENTS

Containers should be stored in a cool, dry, well ventilated area away from flammable materials and sources of heat or flame. Store away from foodstuffs or animal feed. Exercise due caution to prevent damage to or leakage from the container.

Avoid airborne concentration buildup. No smoking where vapors of this material are present. Open containers with caution.

When methylene chloride vapors are drawn into the combustion chamber of a space heater, even at low levels, severe corrosion damage to the heater can occur.

XIII. DISPOSAL OF UNUSED MATERIAL

Material that cannot be used or chemically reprocessed should be disposed of at an approved facility in accordance with any applicable regulations under the Resource Conservation and Recovery Act.

NOTE: State and local regulations may be more stringent than Federal.

Contact the nearest Stauffer Sales Office for assistance in disposing of unused material. Waste material can be sent to a licensed hydrocarbon solvent reclaimer.

XIV. DISPOSAL OF CONTAINER

Dispose of empty containers according to any applicable regulations under the Resource Conservation and Recovery Act.

NOTE: State and local regulations may be more stringent than Federal.

XV. ENVIRONMENTAL PRECAUTIONS

Avoid runoff to sewers.

PACIFIC GAS AND ELECTRIC COMPANY

PG&E 77 BEALE STREET • SAN FRANCISCO, CALIFORNIA 94106 • (415) 781-4211 • TWX 910-372-6587

June 4, 1986

Mr. William V. Loscutoff, Chief
Toxic Pollutants Branch
Re: Methylene Chloride
California Air Resources Board
P.O. Box 2815
Sacramento, California 95812

Dear Mr. Loscutoff:

Request for Public Health
Information Regarding Methylene Chloride

Pacific Gas and Electric Company received your February 5, 1986 request for additional public health information regarding Methylene Chloride. We have reviewed the bibliography attached to your request and concluded that we are unaware of any additional information which would be of use to you.

When the risk assessment report becomes available, please send me a copy.

Sincerely,

J. F. McKenzie

APPENDIX V

HEALTH EFFECTS REQUEST TO DHS AND LETTER OF RESPONSE

ATTACHMENT I

HSIA HALOGENATED SOLVENTS INDUSTRY ALLIANCE
2315 M Street, N.W., Third Floor, Washington, D.C. 20037 • (202) 223-5890

March 27, 1986

Mr. William V. Loscutoff, Chief
Toxic Pollutants Branch
California Air Resources Branch
P.O. Box 2815
Sacramento, CA 95812

Re: Methylene Chloride

Dear Mr. Loscutoff:

We thank you for providing us the opportunity to submit health information on methylene chloride. We are enclosing a variety of recent documents and articles which were not included in your list of references. Please note that the last two references listed are not enclosed. They are currently being cleared for outside release and should be sent within two weeks.

HSIA understands that this information will be used by the State Department of Health Services in its process of considering all available scientific data in its evaluation of health effects and recommendation as to whether or not methylene chloride should be determined to be a toxic air contaminant.

A toxic air contaminant is defined in AB 1807 as:

> An air pollutant which may cause or contribute to an increase in mortality or an increase in serious illness, or which may pose a present or potential hazard to human health.

HSIA believes that methylene chloride does not cause adverse health effects when used according to current industrial hygiene guidelines or labeled instructions. Furthermore, there is no scientific evidence to indicate that ambient concentrations of methylene chloride cause or contribute to an increase in mortality or an increase in serious illness, or which may pose a present or potential hazard to human health. HSIA believes that adequate scientific evidence exists to determine that methylene chloride does not meet the above definition of a toxic air contaminant and should not be identified as one.

We submit the following scientific information as the basis for our conclusion that methylene chloride should not be identified as a toxic air contaminant.

1. Two well-conducted epidemiologic studies have been completed on worker populations exposed to methylene chloride. Neither study indicated a human cancer risk associated with methylene chloride exposure. The most definitive study compare the mortality rate of over 1,000 employees with identified methylene chloride exposure to an unexposed worker population. The findings of this study indicate that workers to methylene chloride for a minimum of 20 years and observed over 20 additional years actually had a decreased incidence of cancer when compared to the New York State control population.

   The other epidemiologic study of methylene chloride examined the health and mortality of over 1200 employees exposed to a mixture of methylene chloride, ethanol, and acetone in a fiber production plant. The study showed no excess mortality cancer in the workers exposed to methylene chloride, even though exposures had ranged up to 475 parts per million and over 300 of them had been followed at least 17-1/2 years after exposure. This study is highly relevant to an understanding of the health risk posed by methylene chloride.

2. The only sites in which methylene chloride has produced tumors are those sites in experimental rodents where the tumors occur spontaneously in unexposed animals (mouse lung and liver tumors and <u>benign</u> mammary gland tumors in rats with no progression to malignancy). The only exception is a low but statistically significant incidence of ventral neck region tumors in male rats only in one study. This response was reviewed and its relevance severely questioned by the U.S. EPA Science Advisory Board and at the Nutrition Foundation Food Solvents Workshop on Methylene Chloride in 1984. Most importantly and of extreme toxicological significance is the fact that the ventral neck region tumor response was not repeatable in the NTP bioassay in rats in any other species evaluated (mouse and hamster).

3. Methylene chloride has been evaluated in numerous short-term <u>in vitro</u> tests, from microorganisms to mammalian cells in culture, as well as in whole animal assays to determine its genotoxic potential. Aside from positive activity in certain microorganisms (primarily bacteria), results in mammalian cells and whole animals, have, for the most part, been negative. A genotoxic component, if present at all in exposed animals is likely to be very weak to nil and not a significant factor in the toxicity of methylene chloride. Thus the principle of practical no-effect-levels should apply in assessing risk for humans.

4. Mechanistic studies have been conducted which provide plausible hypotheses for the observed enhancement of spontaneous tumors in animals exposed to high concentrations of methylene chloride (mouse lung/liver; cytotoxicity/physiological stimulation: rat benign mammary gland; elevated prolactin levels). HSIA believes that humans are not exposed to levels of methylene chloride in the ambient environment which could cause is chain of events to occur. Furthermore, development of a physiologically-based pharmacokinetic model for methylene chloride has confirmed that markedly lower tissue levels of bioactive metabolites of methylene chloride (lower internal dose) would be found in humans than in experimental animals exposed to comparable external concentrations of methylene chloride.

To summarize, HSIA believes that methylene chloride should not be identified as a toxic air contaminant. We have enclosed additional pertinent references to support this belief. In addition we offer our cooperation in answering any questions which might arise in this review of methylene chloride.

Please feel free to call me at (202) 223-5890.

Sincerely yours,

Paul A. Cammer

Paul Cammer, Ph.D.
Executive Director

Enclosure

ADDITIONAL REFERENCES SUBMITTED TO
THE CALIFORNIA AIR RESOURCES BOARD
ON METHYLENE CHLORIDE

Comments of HSIA before the U.S. Environmental Protection Agency, Docket No. OPTS-62045: Methylene Chloride; Initiation of Regulatory Investigation, December 16, 1985.

Comments of HSIA before the U.S. Environmental Protection Agency, Regarding Docket No 1 OPTS-48503: Methylene Chloride; Initiation of Accelerated Review Under Section 4(f) of the Toxic Substance Control Act, July 15, 1985.

Letter of July 30, 1985 to Lee M. Thomas from Drs. Griesemer and Nelson, concerning the HAD Addendum.

Anderson, M. E., Clewell, H. J. II, Gargas, M. J., Smith, F. A., and Reitz, R. H.,Physiologically-Based Pharmacokinetics and the Risk Assessment Process for Methylene Chloride, Toxicol.Appl.Pharmacol. (submitted for publication, Jan 1986).

Friedlander, B. R., Hearne, F. T., and Pifer, J. W., 1964 Methylene Chloride Cohort Mortality Study, Updated Through 1984, July 2, 1985.

Friedlander, B. R., Hearne, F. T., Pifer, J. W., and Grose, F. H., Epidemiological Evidence Regarding Methylene Chloride, Presented at the Winter Toxicology Forum, Washington, D.C., February 18, 1986.

Food Solvents Workshop I: Methylene Chloride, Proceedings of the Workshop, March 8-9, 1984, Bethesda, MD.

Smith, M. N., Greenberg, S. D., and Spjut, H. J., The Clara Cell: A Comparative Ultrastructural Study in Mammals, Am. J. Anat. 155:15-30 (1979).

Plopper, C. G., Hill, L. H., and Mariassy, A. T., Ultrastructure of the Nonciliated Bronchiolar Epithelial (Clara) Cell of Mammalian Lung. III. A Study of Man with Comparison of 15 Mammalian Species, Exp. Lung Research 1:171-180 (1980).

Widdicombe, J. G. and Pack, R. J., The Clara Cell, Eur. J. Respir. Dis. 63:202-220 (1982).

Gail, D. B. and Lenfant, D. J. M., State of the Art, Cells of the Lung: Biology and Clinical Implications, Am. Rev. Respir. Dis. 127(3):366-387 (1983).

Schairer, L. A., Sautkulis, R. D. and Tempel, N. R., Detection and Identification of Genotoxic Agents in Ambient Air Using the Tradescantia Bioassay, Environ. Mutagenesis 4:379 (1982).

Te-Hsui, M. A., Harris, M. M., Anderson, V. A., Ahmed, I., Mohammad, K., Bare, J. L. and Lin, G., Tradescantia -- Micronucleus (TRAD-MCN), Tests on 140 Health Related Agents, Mutat. Research 138:157-167 (1984).

Abrahamson, S., and Valencia, R., Evaluation of Substance of Interest for Genetic Damage Using Drosophila melanogaster (1980), prepared for FDA Contract No. 233-77-2119.

Sivak, A., Capitals Balb/C-3T3 Neoplastic Transformation Assay with Methylene Chloride (Food Grade Test Specification), Report to National Coffee Association, Inc., 1978.

Thilagar, A. K., Back, A. M., Kirby, P. E., Kumaroo, P. V., Pant, K. J., Clarke, J. E., Kight, R. and Haworth, S. R., Evaluation of Dichloromethane in Short-term In Vitro Genetic Toxicity Assays, Environ. Mutagenesis 6:418-419 (1984).

Thilagar, A. K., Kumaroo, P. V., Clarke, J. J., Kott, S., Back, A. M. and Kirby, P. E., Induction of Chromosome Damage by Dichloromethane in Cultured Human Peripherial Lymphocytes, CHO Cells and Mouse Lymphoma L5178Y Cells, Environ. Mutagenesis 6:422 (1984).

Dutton, D. R. and Bowden, G. T., Indirect Induction of a Plastogenic Effect on Epidermal Cells by a Tumor Promoter, Carcinogenesis 6:1279-1284 (1985).

Henry, James, M.E., et. al., Force Breeding, Social Disorder and Mammory Tumor Formation in CBA/WSC Mouse Colonies: A Pilot Study, Psychosomatic Medicine 37(3):277-283 (1975).

Schumann, A. M., Nitschke, K. D., Fox, T. R. and Watanabe, P. G. (1984). [^{14}C] Methylene Chloride: pharmacokinetics and interaction with hamster and rat tissue macromolecules. Report of The Dow Chemical Co., Midland, MI, 48640.

Welsch, C. W. and Nagaswa, H. (1977). Prolactin and murine mammary tumorigenesis: a review. Cancer Research 37, 951-963.

Letter of July 18, 1984 to William D. Ruckelshaus from Herschel E. Griffin and Norton Nelson.

** Breslein, W. J. and Landry, T. D. (1986). Methylene chloride: effects on estrous cycling and serum prolactin in Sprague-Dawley rats. Report of the Dow Chemical Co., Midland, MI 48640.

** Schuman, A. M. (1986). Perspective on the genetic toxicology data base for methylene chloride. The Dow Chemical Co., Midland, MI 48640.

** Not enclosed. Currently being cleared for outside release.

METHYLENE CHLORIDE PRODUCERS ASSOCIATION

A CEFIC Sector Group

Conseil Européen des
Fédérations de l'Industrie Chimique

. opean Council of Chemical
Manufacturer's Federations

Your Ref.

Our Ref. SG METHYLENE CHLORIDE

REPLIES TO: Dr M R Harris
Regulatory Affairs
Imperial Chemical Industries PLC
Mond Division, PO Box 13
The Heath, Runcorn, WA7 4QF
CHESHIRE, England

William V Loscutoff
Chief of Toxic Pollutants Branch
Re: Methylene Chloride
California Air Resources Board
PO Box 2815
Sacramento
California 95812
USA

Your ref	Our ref	Date
	MRE/CL	04 Mar 86

Dear Sirs

METEYLENE CHLORIDE (=DICHLOROMETHANE)

I am writing to you on behalf of the Methylene Chloride Workgroup of the European Council of Chemical Manufacturers' Federations (CEFIC) with regard to your 5 February 1986 'Request for Information' regarding methylene chloride.

This group has sponsored a 2-year program of research on the species differences in the mechanism of action and biological transformation of methylene chloride in an effort better to understand the marked species differences in tumorigenicity as evidenced by a number of long term bioassays in the rat, the hamster and the mouse in which only the mouse shows clear evidnece of a carcinogenic response - and this only at the high doses of the recent NTP inhalation bioassay, and yet not at the similar internal doses of the recent NCA ingestion (drinking water) bioassay. Further evidence suggestive of a species difference - or a dose-response difference, or both - is provided by the human epidemiology (particularly the 1985 and 1986 extensions of Friedlander's studies at Kodak, NY). This provides data which (while inevitably unable to "prove" a negative conclusion) makes any significant human risk a highly improbable hypothesis, and whose most probable explanation (by far) is the hypothesis of zero human risk.

Our own studies are being conducted at the Central Toxicology Laboratory of Imperial Chemical Industries PLC in England, with external scientific monitoring and peer review b the European Chemical Industry Ecology & Toxicology Centre (ECETOC). The work will in due course be published in the scientific literature, and the laboratory studies are fully open to independent audit. The first stage of these studies has recently been completed, and copies of the three full final reports are enclosed; this work has also recently been presented at Toxicology Forum in Washington DC (February 1986) and at the Annual Meeting of the US Society of Toxicologists in New Orleans (March 1986); it has also been submitted to EPA, FDA, CPSC and OSHA.

This stage of our work has included a 10-day inhalation cytotoxicity study, DNA binding studies, and unscheduled DNA synthesis (UDS) studies (in vivo/in vitro and in vitro) - in the B6C3F1 mouse and Fisher F344/N rat. We have found evidence of cytotoxicity to a specific cell type (the Clara cell) of the mouse lung, but not in the rat lung nor in mouse or rat liver. Under our experimental conditions we found no evidence for DNA-binding or UDS, strongly suggesting that under these conditions there is no interaction between methylene chloride or its metabolites and mammalian DNA. This would be consistent with a non-genotoxic action of methylene chloride, not necessarily because of "instantaneous detoxification of a carcinogen" (apparently the only rationale, among the many possible, considered in the November 1985 California DHS carcinogen risk assessment guidelines) but more probably because (bearing in mind the existence of at least two independent metabolic pathways, at least one of which is saturable) no stable genotoxic intermediates were generated.

The second phase of our work, now in progress and expected to complete during the third quarter of 1986, is studying quantitatively the metabolic pathways involved in mouse, rat, hamster and man. Collection of the relevant pharmacokinetic data, and its incorporation into appropriate pharmacokinetic models, should allow us to establish and quantify interspecies differences in metabolism, the existence and level of any thresholds and, more generally, the shape of possible dose-response curves for comparison with animal bioassay findings, and with the findings of epidemiological studies.

It is our belief that this approach should assist the risk assessment process by allowing the use of experimentally-determined and scientifically valid dose-response relationships, rather than simplistic linear extrapolations between the high doses of animal bioassays and the much lower levels of human exposure, and the even greater extrapolation from mouse to man under circumstances where the mouse fails even to predict the bioassay response of such other rodents as the rat and the hamster.

Yours sincerely

DR M R HARRIS

Encs

DOCUMENTS ENCLOSED:

1. CEFIC submission to EPA. 20 September 1985. Interim report.

2. CEFIC submission to EPA. 13 December 1985. Interim report.

3. CEFIC Research Report No: CTL/P/1432. 10 Day Inhalation Toxicity Study.

4. CEFIC Research Report No: CTL/R/851. Interaction with Rat and Mouse Liver and Lung DNA In Vivo.

5. CEFIC Research Report No: CTL/P/1444. In Vivo and In Vitro UDS Studies.

BASIC PRODUCTS GROUP
CHLOR-ALKALI PHOSPHORUS
& SULFURIC PRODUCTS
DIVISIONS

Stauffer CHEMICALS

Stauffer Chemical Company
636 California St./San Francisco, CA 94108/Tel. (415) 544-9311/Cable "Stauffer"

REPLY TO:
P. O. Box 7766
San Francisco, CA 94120

March 3, 1986

William V. Loscutoff, Chief
Toxic Pollutants Branch
RE: Methylene Chloride
California Air Resources Board
P. O. Box 2815
Sacramento, CA 95812

Dear Mr. Loscutoff:

Per your request, attached please find a copy of our Product Safety Information Sheet for Methylene Chloride.

Also, please be advised that as of March 1, 1986, Stauffer Chemical Company is no longer in the Methylene Chloride business.

Sincerely,

W. J. Meier
Senior Technical Service Representative
Basic Chemicals Division

WJM:el
enclosure

Product Safety Information

METHYLENE CHLORIDE

This Product Safety Information Sheet is principally directed to managerial, safety, hygiene and medical personnel. The description of physical, chemical and toxicological properties and handling advice is based on experimental results and past experience. It is intended as a starting point for the development of health and safety procedures.

Synonyms: Dichloromethane; Methylene dichloride
CAS Registry Number: 75-09-2

I. PHYSICAL AND CHEMICAL PROPERTIES

Formula
CH_2Cl_2
Formula Weight
84.94
Physical State/Description
Colorless volatile liquid at 68°F (20°C), 14.7 psia
Autoignition Temperature
1224°F (662°C)
Boiling Point
104°F (40°C)
Density
11 lbs/gallon at 68°F (20°C)
Evaporation Rate
1.47 (Carbon tetrachloride = 1)
Explosive Limits In Pure Oxygen
LEL: 15.5%; UEL: 66.4%
Flammability Limits In Air
15.9 to 19.1 [% volume at 212°F (100°C)]
Freezing Point
-142°F (-96.7°C)
Miscibility
Miscible with alcohol and ether
Odor
Penetrating, ether-like, pleasantly aromatic, sweet odor
pH
Slightly alkaline (water extract)
Solubility
Sparingly soluble in water
[1.96 g/100g in water at 68°F (20°C)]
Specific Gravity
1.326 at 68°F/68°F (20°C/20°C)
Vapor Pressure
140 mmHg at 32°F (0°C)
350 mmHg at 68°F (20°C)
700 mmHg at 100°F (37.7°C)
Viscosity
0.43 cp at 68°F (20°C)

IN CASE OF SUSPECTED POISONING, REFER TO THE PROCEDURE AND EMERGENCY CONTACTS IN SECTION VIII: FIRST AID.

IN CASE OF SPILLAGE, REFER TO THE PROCEDURE AND EMERGENCY CONTACTS IN SECTION X: SPILL HANDLING OR CALL (CHEMTREC) (800) 424-9300.

II. CHEMICAL REACTIVITY

Does not undergo hazardous polymerization. However, a serious chemical reaction, with the possibility of explosion, could result if halogenated hydrocarbon solvents are used in pressurizable fluid systems having aluminum or galvanized wetted parts. Prolonged exposure to water at elevated temperatures (140°F/60°C) may cause noticeable hydrolysis. May react violently or explode upon contact with alkali or chemically active metals such as finely powdered aluminum, magnesium, potassium and/or sodium.

III. STABILITY

Stable at ambient temperatures and atmospheric pressure. However, exposure to high temperature sources (such as open flame and welding arcs) causes thermal-oxidative decomposition yielding toxic phosgene gas and corrosive hydrogen chloride.

IV. FIRE HAZARD

Methylene chloride does not have a measurable flash point by conventional test methods. It is not considered flammable but forms flammable vapor/air mixtures at approximately 212°F (100°C) or higher. Under fire conditions, decomposes to form toxic phosgene gas and corrosive hydrogen chloride.

V. FIREFIGHTING TECHNIQUE

Vapors are irritating to the respiratory tract and may cause breathing difficulty and pulmonary edema. Symptoms may be delayed several hours or longer depending upon the extent of exposure.

As in any fire, prevent human exposure to fire, smoke, fumes or products of combustion. Evacuate nonessential personnel from the fire area. Firefighters should wear full-face, self-contained breathing apparatus and protective clothing.

Use standard firefighting techniques to extinguish fires involving this material—use water spray, dry chemicals or carbon dioxide.

If not leaking, use a water spray to keep fire-exposed containers cool to prevent rupture due to excessive heat. High pressure water hose may spread product from broken containers increasing contamination or fire hazard.

VI. HUMAN HEALTH

WARNING: Vapor and liquid may be harmful if inhaled, swallowed, or absorbed by skin. Avoid prolonged or repeated breathing of vapor. May cause skin and eye irritation. Avoid contact with skin, eyes, and clothing.

The primary route of entry for methylene chloride is inhalation.

Inhalation of large amounts of the vapor may cause nausea, chills, headache, lightheadedness, drowsiness, and irritation of the respiratory tract. Continued overex-

[illegible] without guarantee or obligation for the accuracy or sufficiency [illegible] and is offered at user's risk. The uses referred to are for the purpose [illegible] and establish the suitability of such uses in every case [illegible] as a recommendation for uses which infringe valid patents or as [illegible]

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STAUFFER CHEMICAL COMPANY
BASIC PRODUCTS GROUP
Westport, Connecticut 06881

posure may produce unconsciousness, kidney and lung damage, and death.

Eye irritation and blurred vision may result from eye contact by the liquid or large quantities of the vapor.

Prolonged skin contact with the liquid may cause burns. Repeated skin exposures may produce dermatitis.

LONG TERM EXPOSURE: An epidemiological study performed on a large group of male workers at an Eastman Kodak facility in New York, who were continuously exposed to estimated TWA exposures of 30 - 125 ppm of methylene chloride for up to 30 years, has been reported. There was no increase in mortality among exposed workers as compared to industrial controls and the general New York State male population. (1)

VII. TOXICOLOGY

Ingestion

The acute oral LD50 is 2,710 mg/kg in male rats and 2,330 mg/kg in female rats. A single oral dose of 2,150 mg/kg produced mild depression and 20 percent mortality in male rats, and produced moderate to severe depression, tremors and 40 percent mortality in female rats. A single oral dose of 4,640 mg/kg resulted in dyspnea, ataxia, and narcosis in female rats and 100% mortality in male and female rats.

Skin Contact

The acute dermal LD50 is greater than 4,640 mg/kg in rabbits. A single dermal application of 4,640 mg/kg did not produce signs of toxicity in male or female rabbits. Local effects included a mild erythema and moderate edema.

Mild to moderate irritant to rabbit skin following a 4-hour exposure.

Eye Contact

A single ocular dose of 0.1 mL produced lacrimation, inflammation, conjunctival edema, and an increased corneal thickness (reversible). (2)

Inhalation

The acute inhalation LC50 is greater than 19.5 mg/L (nominal concentration) in both male and female rats. A single 1-hour inhalation exposure of 19.5 mg/L (nominal concentration) produced slight depression, moderate ataxia, and ocular protrusion. The acute inhalation LC50 in mice is approximately 14,400 - 17,400 ppm. Seven hour exposures to doses ranging from 13,000 - 17,000 ppm produced ataxia, labored respiration, muscular twitching, and narcosis. (3)

Monkeys, dogs, rabbits, guinea pigs and rats exposed to 500 ppm for 8 hour/day, 5 days/week, for 15 weeks showed 20% mortality (in rats), and a slight increase in eosinophils (rabbits). (3)

Monkeys, dogs, rats, and mice were continuously exposed to 25 or 100 ppm for 100 days. No overt signs of stress were observed. The exposure produced: histopathological effects in mouse livers (100 ppm) and rat kidneys (25 and 100 ppm); no significant findings in hematology, clinical chemistry or histopathology in dogs and monkeys; and significantly increased carboxyhemoglobin in dogs and monkeys (monkeys 100 and 25 ppm, dogs 100 ppm). (3)

Rats and hamsters were exposed by inhalation to 0, 500, 1,500 or 3,500 ppm of methylene chloride for 6 hours per day, 5 days a week for 2 years. No organ-specific toxicity was observed in hamsters. Carboxyhemoglobin levels were elevated in hamsters and rats at all dose levels, but this effect was not cumulative. (4)

Histopathological effects in the liver were observed in male and female rats at all dose levels and considered to be exposure-related. Despite these effects, there was no increase in neoplastic (hyperplastic) liver nodules and no increase in hepatocellular carcinomas. (4)

Increased total numbers of benign mammary tumors were seen in male and female rats, but no increase in malignant mammary tumors was observed. There were no significant lung tumor findings in any exposed group compared to the controls. (4)

Male rats in the 3,500 ppm dose group exhibited a significant increase in sarcomas in or around the salivary glands. A significant increase in this finding was not observed in males exposed at 500 or 1,500 ppm or any of the exposed females. (4)

In a second study, male and female rats were exposed to 0, 50, 200, or 500 ppm for 6 hrs/day, 5 days/week for 2 years. Additionally, one group of females was exposed at 500 ppm for the first 12 months only, and another group for the second 12 months only. (5)

A significant increase in histopathological effects in the liver was observed in females exposed to 500 ppm for 2 years and to 500 ppm for the first 12 months. (5)

Mutagenicity

Methylene chloride was examined for mutagenic activity in *in vitro* microbial assays, with and without metabolic activation, using the TA-100 and TA-98 strain of *Salmonella typhimurium* as the indicator organism. The compound demonstrated mutagenic activity in both assays. (6)

Methylene chloride did not induce chromosomal aberrations in a rat bone marrow assay. (4)

Methylene chloride induced cellular transformation in Fischer rat embryo cells. (7)

T-4054, T-4171, T-5628

VIII. FIRST AID

CALL A POISON CENTER OR
A PHYSICIAN IMMEDIATELY.

If a known exposure occurs or is suspected, immediately start the recommended procedures below. Simultaneously contact a Poison Center, a physician or the nearest hospital. Inform the person contacted of the type and extent of exposure, describe the victim's symptoms, and follow the advice given.

NOTE TO PHYSICIAN

Methylene chloride is metabolized to carbon monoxide and carbon dioxide. The resultant elevation of carboxyhemoglobin levels is of short duration (e.g. usually less

(1) J. Occ. Med. 20 (10):657 (1978).
(2) Toxicol. 6:173-187 (1976).
(3) HEW (NIOSH) Publication No. 76-138.
(4) Burek, J. D. et. al (1980) Methylene Chloride: "A Two-Year Inhalation Toxicity and Oncogenicity Study in Rats and Hamsters." Final Report Issued December 31, 1980 by Dow Chemical, USA.
(5) Nitschke, K. D. et. al (1982) Methylene Chloride: "A Two-Year Toxicity and Oncogenicity Study." Final Report Issued October 11, 1982 by Dow Chemical, USA.
(6) Mut.Res. 56:245-248 (1978)
(7) In Vitro 14:290-293 (1978)

than 48 hours) unless overexposure is prolonged. Inhalation of large amounts of methylene chloride may cause pulmonary edema and could be fatal. Exposure to high concentrations of methylene chloride may cause depression of the central nervous system.

FOR ADDITIONAL MEDICAL OR TOXICOLOGICAL INFORMATION, CALL COLLECT, DAY OR NIGHT, STAUFFER CHEMICAL COMPANY (203) 226-6602 OR CHEMTREC (800) 424-9300.

Ingestion

If swallowed, immediately give several glasses of water but *do not* induce vomiting. If vomiting does occur, give fluids again. Have a physician determine if condition of patient will permit induction of vomiting or evacuation of stomach. Do not give anything by mouth to an unconscious or convulsing person.

Skin Contact

Immediately flush all affected areas with large amounts of water for at least 15 minutes while removing any contaminated clothing and shoes. Do not attempt to neutralize with chemical agents. Get medical attention immediately. Wash clothing before reuse.

Eye Contact

Immediately flush the eyes with large quantities of running water for a minimum of 15 minutes. Hold the eyelids apart during the flushing to ensure rinsing of the entire surface of the eye and lids with water. Do not attempt to neutralize with chemical agents. Obtain medical attention as soon as possible. Oils or ointments should not be used. Continue the flushing for an additional 15 minutes if the physician is not immediately available.

Inhalation

Remove from contaminated atmosphere. Seek medical attention if respiratory irritation occurs. If the breathing becomes difficult, oxygen may be delivered from a demand-type or continuous flow inhaler, preferably with a physician's advice. If not breathing, give artificial respiration, preferably mouth-to-mouth. Get medical attention.

IX. INDUSTRIAL HYGIENE

Ingestion

All food should be kept in a separate area away from the storage/use location. Eating, drinking and smoking should be prohibited in areas where there is a potential for significant exposure to this material: Before eating, hands and face should be thoroughly washed.

Skin Contact

Skin contact with liquid or its aerosol should be prevented through the use of suitable protective clothing, gloves and footwear selected with regard for use condition exposure potential.

Eye Contact

Eye contact with liquid or its aerosol should be prevented through the use of chemical safety glasses, goggles or a face shield selected with regard for use condition exposure potential.

Inhalation

If use conditions generate airborne liquid, aerosol or vapor handle this material only in an open (e.g., outdoor) or well ventilated area. Where adequate ventilation is not available, use NIOSH-approved organic vapor respirators to reduce exposure. Where exposure potential under the use conditions necessitates a higher level of protection, use a positive-pressure, air-supplied respirator.

Exposure Limits

The Federal OSHA Permissible Exposure Limit (PEL) is an 8-hour, time-weighted average of 500 ppm (1,700 mg/m^3) and a ceiling concentration of 1,000 ppm for methylene chloride. ([8])

The American Conference of Governmental Industrial Hygienists (ACGIH) has recommended a Threshold Limit Value (TLV) of 100 ppm (360 mg/m^3) as an 8-hour time-weighted average, and a Short Term Exposure Limit (STEL) of 500 ppm (1,700 mg/m^3) for methylene chloride. ([9])

X. SPILL HANDLING

Make sure all personnel involved in the spill cleanup follow good industrial hygiene practices (refer to SECTION IX: INDUSTRIAL HYGIENE). Any person entering a significant spill area or an unknown concentration of a gas or vapor, should wear a positive-pressure, self-contained breathing apparatus or a positive-pressure, supplied-air respirator with escape pack.

Small spills can be handled routinely. Use adequate ventilation and wear a respirator to prevent inhalation exposure. Wear protective clothing to prevent skin and eye contact. Use the following procedures:

Evacuate spill area and remove sources of combustion or extreme heat. Ventilate the area and let small spills evaporate. Somewhat larger spills should be contained and recovered where possible. Mop up spilled methylene chloride or adsorb with a suitable adsorbent such as clay, sawdust or kitty litter. Reclaim waste solvent by distillation procedures or offer for disposal to a licensed hydrocarbon solvent reclaimer.

Large spills should be handled according to a predetermined plan. For assistance in developing a plan, contact Stauffer Chemical Company, Westport, CT 06881.

IN CASE OF SPILL EMERGENCY, DAY OR NIGHT, CALL (800) 424-9300 CHEMTREC.

XI. CORROSIVITY TO MATERIALS OF CONSTRUCTION

In the absence of contamination by water or moisture, methylene chloride is not corrosive to common materials such as copper, iron or stainless steel. In contact with water or moisture, particularly at elevated temperatures, it can be corrosive. Methylene chloride may soften or dissolve certain plastics and rubbers. Avoid contact with alkali or chemically active metals because an explosion may occur (See SECTION II: CHEMICAL REACTIVITY).

([8]) 29 CFR 1910.1000

([9]) The American Conference of Governmental Industrial Hygienists (ACGIH). 1982. *Threshold Limit Values for chemical substances and physical agents in the workroom environment with intended changes for 1982.* ACGIH: Cincinnati, Ohio.

XII. STORAGE REQUIREMENTS

Containers should be stored in a cool, dry, well ventilated area away from flammable materials and sources of heat or flame. Store away from foodstuffs or animal feed. Exercise due caution to prevent damage to or leakage from the container.

Avoid airborne concentration buildup. No smoking where vapors of this material are present. Open containers with caution.

When methylene chloride vapors are drawn into the combustion chamber of a space heater, even at low levels, severe corrosion damage to the heater can occur.

XIII. DISPOSAL OF UNUSED MATERIAL

Material that cannot be used or chemically reprocessed should be disposed of at an approved facility in accordance with any applicable regulations under the Resource Conservation and Recovery Act.

NOTE: State and local regulations may be more stringent than Federal.

Contact the nearest Stauffer Sales Office for assistance in disposing of unused material. Waste material can be sent to a licensed hydrocarbon solvent reclaimer.

XIV. DISPOSAL OF CONTAINER

Dispose of empty containers according to any applicable regulations under the Resource Conservation and Recovery Act.

NOTE: State and local regulations may be more stringent than Federal.

XV. ENVIRONMENTAL PRECAUTIONS

Avoid runoff to sewers.

Chevron

Chevron Environmental Health Center, Inc.
A Chevron Research Company Subsidiary
15299 San Pablo Avenue, Richmond, California
Mail Address: P.O. Box 4054, Richmond, CA 94804

R. D. Cavalli
Manager
Product Evaluation

3/3
Lynnette
pls route to
staff person
working on MC

February 24, 1986

Methylene Chloride

William V. Loscutoff, Chief
Toxic Pollutants Branch
California Air Resources Board
P.O. Box 2875
Sacramento, California 95812

Dear Mr. Loscutoff:

As per your request, in accordance with Health and Safety Code Sections 39650, et seq., we have reviewed our files on the health effects of methylene chloride. We have no relevant "in-house" data on methylene chloride, but feel the following references may be useful additions to the bibliography for the Department of Health Services:

1. Heppel, L. A., Neal, P. A., Perrin, T. L., Orr, M. L., Porterfield: Toxicology of Dichloromethane (Methylene Chloride). I. Studies on Effects of Daily Inhalation. J. Ind. Hyg. Toxicol. 26: 8-16, 1944.

2. Heppel, L. A., Neal, P. A.: Toxicology of Dichloromehtane (Methylene Chloride). II. Its Effect Upon Running Activity in the Male Rat. J. Ind. Hyg. Toxicol. 26: 17-21, 1944.

Thank you for the opportunity to contribute to the review of candidate toxic air contaminants.

Sincerely,

RD Cavalli / GFSH

MAR 03 1986

New York State Department of Environmental Conservation
50 Wolf Road, Albany, New York 12233-0001

Henry G. Williams
Commissioner

March 7, 1986

RECEIVED

MAR 11 1986

Stationary Source
Division
Air Resources Board

Mr. Peter Venturini
Chief
Stationary Source Division
State of California
Air Resources Board
1102 Q Street
P. O. Box 2815
Sacramento, California 95812

Dear Mr. Venturini:

This is in response to your letter of February 5, 1986 in which you requested information on the health effects of methylene chloride. At this time, we are conducting a quantitative risk assessment by applying statistical models to the bioassay data obtained from the inhalation study completed by the National Toxicology Program (NTP) in 1985. In addition, we will be completing a search of the same references which were included with your letter.

When we have completed this risk assessment, we will be glad to send you a copy.

Sincerely,

Virginia M Rest

Virginia M. Rest
Environmental Chemist
Section of Toxics Assessment
Bureau of Air Toxics

VMR:cb

ATTACHMENT II

Health Effects References for Methylene Chloride (1/28/1986)

1) Ahmed AE, Anders MW **Metabolism of dihalomethanes to formaldehyde and inorganic halide. II. Studies on the mechanism of the reaction **Biochem Pharmacol 27:2021-2025, 1978

2) Ahmed AE, Anders MW **Metabolism of dihalomethanes to formaldehyde and inorganic chloride **Drug Metab Dispos 4:356-361, 1976

3) Ahmed AE, Kubic VL, Stevens JL, Anders MW **Halogenated methanes: Metabolism and toxicity **Fed Proc 39(13):3150-3155, 1980

4) Alexander HC , McCarty WM , Bartlett EA **Toxicity of Perchloroethylene, Trichloroethylene, 1,1,1-trichloroethane, and Methylene Chloride to Fathead Minnows. **Bull Environ Contam Toxicol 1978 Sep,20(3):344-52

5) Alexeeff GV , Kilgore WW **Learning impairment in mice following acute exposure to dichloromethane and carbon tetrachloride. **J Toxicol Environ Health 1983 Apr-Jun,11(4-6):569-81

6) Anders MW , Sunram JM **Transplacental passage of dichloromethane and carbon monoxide. **Toxicol Lett 1982 Aug,12(4):231-4

7) Andrae U , Wolff T **Dichloromethane is not genotoxic in isolated rat hepatocytes. **Arch Toxicol 1983 Apr,52(4):287-90

8) Angelo MJ , Pritchard AB **Simulations of methylene chloride pharmacokinetics using a physiologically based model. **Regul Toxicol Pharmacol 1984 Dec,4(4):329-39

9) Anon **Update: methylene chloride **Mod. Paint Coat., VOL 68, ISS 10, 1978,113-17

10) Astrand I , Ovrum P , Carlsson A **Exposure to methylene chloride. I Its concentration in alveolar air and blood during rest and exercise and its metabolism. **Scand J Work Environ Health 1975 Jun,1(2):78-94

11) Baird R , Gute J , Jacks C , Jenkins R , Neisess L , Scheybeler E , Van Sluis R , Yanko W **Health effects of water reuse: a combination of toxicological and chemical methods for assessment **Water Chlorination: Environ. Impact Health Eff., VOL 3,, 1980,925-35

12) Ballantyne B , Gazzard MF , Swanston DW **The ophthalmic toxicology of dichloromethane. **Toxicology 1976 Aug-Sep,6(2):173-87

13) Balmer MF , Smith FA , Leach LJ , Yuile CL **Effects in the liver of methylene chloride inhaled alone and with ethyl alcohol. **Am Ind Hyg Assoc J 1976 Jun,37(6):345-52

14) Barber ED , Donish WH , Mueller KR **QUANTITATIVE MEASUREMENT OF THE MUTAGENICITY OF VOLATILE LIQUIDS IN THE AMES SALMONELLA/MICROSOME ASSAY **ENVIRON MUTAGENESIS, 2:307,1980

15) Barlow SM , Sullivan FM **Reproductive hazards of industrial chemicals **Academic Press (London) Ltd., 24-28 Oval Road, London NW1 7DX, United Kingdom, 1982. 610p. Bibl.

16) Beall JR , Ulsamer AG **TOXICITY OF VOLATILE ORGANIC COMPOUNDS PRESENT INDOORS **COMMITTEE ON PUBLIC HEALTH OF THE NEW YORK ACADEMY OF MEDICINE SYMPOSIUM ON HEALTH ASPECTS OF INDOOR AIR POLLUTION, NEW YORK, N.Y., USA, MAY 28-29, 1981. BULL N Y ACAD MED, 57 (10). 1981 (RECD. 1982). 978-996.

17) Bergman K **Whole-body autoradiography and allied tracer techniques in distribution and elimination studies of some organic solvents. **SCAND J WORK ENVIRON HEALTH, 5 (SUPPL. 1). 1979. 1-263.

18) Birge WJ , Black JA , Kuehne RA **Effects of organic compounds on amphibian reproduction **Report, ISS RR-121, W80-03438, OWRT-A-074-KY(2), Order No. PB80-147523,, 1980,50 pp.

19) Blair CA , Shibamoto T **Ames mutagenicity tests of overheated brewed coffee. **Food Chem Toxicol, VOL 22, ISS 12, 1984, P971-5

20) Bornschein RL , Hastings L , Manson JM **Behavioral toxicity in the offspring of rats following maternal exposure to dichloromethane. **Toxicol Appl Pharmacol 1980 Jan,52(1):29-37

21) Brooks AL , Royer RE , Sanchez A , Clark CR **The biological availability of mutagenic chemicals associated with diesel exhaust particles **Annu. Rep. Inhalation Toxicol. Res. Inst. (Lovelace Biomed. Environ. Res. Inst.), 1979,239-43

22) Buijs W , Van der Gen A , Mohn GR , Breimer DD **The direct mutagenic activity of alpha,omega-dihalogenoalkanes in Salmonella typhimurium. Strong correlation between chemical properties and mutagenic activity **Mutat. Res., VOL 141, ISS 1, 1984,11-14

23) Burek JD , Nitschke KD , Bell TJ , Wackerle DL , Childs RC , Beyer JE , Dittenber DA , Rampy LW , McKenna MJ **Methylene chloride: a two-year inhalation toxicity and oncogenicity study in rats and hamsters. **Fundam Appl Toxicol 1984 Feb,4(1):30-47

24) Caderni G , Dolara P **Detection of mutagens in human urine by means of XAD-2 adsorption-desorption techniques and bacterial mutagenesis **Pharmacol. Res Commun., VOL 15, ISS 8, 1983,775-82

25) Callen DF , Wolf CR , Philpot RM **Cytochrome P450-mediated genetic activity and cytotoxicity of 7 halogenated aliphatic hydrocarbons in Saccharomyces cerevisiae. **MUTAT RES, 77 (1). 1980. 55-64.

26) Carlsson A , Hultengren M **Exposure to methylene chloride. III. Metabolism of 14C-labelled methylene chloride in rat. **Scand J Work Environ Health 1975 Jun,1(2):104-8

27) Corsi GC , Valentini F , Bertazzon A **Effect of subtoxic amounts of furan, acetylfuran and methylene chloride on some serum enzymes of rat. **Boll Soc Ital Biol Sper 1983 Aug 30,59(8): 1049-52

28) Cothem CR , Coniglio WA , Marcus WL **Techniques for the assessment of the carcinogenic risk to the U. S. population due to exposure from selected volatile organic compounds from drinking water via the ingestion, inhalation and dermal routes **Report, ISS Order No. PB84-213941,, 1984,254 pp.

29) Crossland J **AEROSOLS **ENVIRONMENT (ST LOUIS), 16 (6). 1974 16-20, 25-26

30) Cunningham ML , Gandolfi AJ , Brendel K , Sipes IG **Covalent binding of halogenated volatile solvents to subcellular macromolecules in hepatocytes. **Life Sci 1981 Sep 21,29(12):1207-12

31) De Greef E , Morris JC , van Kreijl CF , Morra C FH **HEALTH EFFECTS IN THE CHEMICAL OXIDATION OF POLLUTED WATERS **JOLLEY, R. L., W. A. BRUNGS AND R. B. CUMMING (ED.). WATER CHLORINATION: ENVIRONMENTAL IMPACT AND HEALTH EFFECTS, VOL. 3., 3RD CONFERENCE, COLORADO SPRINGS, COLO., USA, OCT. 28-NOV. 2, 1979. XXVIII+1171P. ANN ARBOR SCIENCE PUBLISHERS, INC.: ANN ARBOR, MI

32) DiVincenzo GD , Halmilton ML **Fate and disposition of [14Ia1 methylene chloride in the rat. **Toxicol Appl Pharmacol 1975 May,32(2):385-93

33) DiVincenzo GD , Kaplan CJ **Effect of exercise or smoking on the uptake, metabolism, and excretion of methylene chloride vapor. **Toxicol Appl Pharmacol 1981 Jun 15,59(1):141-8

34) DiVincenzo GD , Kaplan CJ **Uptake, metabolism, and elimination of methylene chloride vapor by humans. **Toxicol Appl Pharmacol 1981 Jun 15,59(1):130-40

35) Dill DC , Watanabe PG , Norris JM **Effect of methylene chloride on the oxyhemoglobin dissociation curve of rat and human blood. **Toxicol Appl Pharmacol 1978 Oct,46(1):125-9

36) Douglas GR , Nestmann ER , Betts JL , Mueller JC , Lee EG H , Stich HF , San RH C , Brouzes RJ F , Chmelauskas AL **Mutagenic activity in pulp mill effluents **Water Chlorination: Environ. Impact Health Eff., VOL 3,, 1980,865-80

37) Dow Chemical Company **Methylene chloride: a two-year inhalation toxicity and oncogenicity study in rats . **Toxicology Research Laboratory, Health and Environmental Science, Dow Chemical Company, Midland, Mi, 1980

38) Elovaara E , Hemminki K , Vainio H **Effects of methylene chloride, trichloroethane, trichloroethylene, tetrachloroethylene and toluene on the development of chick embryos. **Toxicology 1979 Feb,12(2):111-9

39) Engstrom J , Bjurstrom R **Exposure to methylene chloride. Content in subcutaneous adipose tissue. **Scand J Work Environ Health 1977 Dec,3(4):215-24

40) Fishbein L **Potential halogenated industrial carcinogenic and mutagenic chemicals: II. Halogenated saturated hydrocarbons. **SCI TOTAL ENVIRON, 11 (2). 1979. 163-196.

41) Frinedlander BR, Hearne FT, Hall S **Epidemiologic investigation of employees chronically exposed to methylene chloride **J. Occup. Med. 20:657-666 1978

42) Fuxe K, Andersson K , Hansson T , Agnati LF , Eneroth P , Gustafsson JA **Central catecholamine neurons and exposure to dichloromethane. Selective changes in amine levels and turnover in tel- and diencephalic DA and NA nerve terminal systems and in the secretion of anterior pituitary hormones in the male rat. **Toxicology 1984 Feb,29(4):293-305

43) Gamberale F , Annwall G , Hultengren M **Exposure to methylene chloride. II. Psychological functions. **Scand J Work Environ Health 1975 Jun,1(2):95-103

44) Giniiatullina AKh , Khabirova FIu **[Disorders of the hydro- and hemodynamic processes in the workers' eyes under the effect of different groups of hydrocarbons (chlorinated, saturated and unsaturated)] **Oftalmol Zh 1977,32(6):424-7

45) Gocke E , King MT , Eckhardt K , Wild D **Mutagenicity of cosmetics ingredients licensed by the European Communities **Mutat. Res., VOL 90, ISS 2, 1981,91-109

46) Gopalan H NB , Njagi G DE , Hongo-Allego P **CYTOLOGICAL STUDIES ON METHYLENE CHLORIDE **11TH ANNUAL MEETING OF THE ENVIRONMENTAL MUTAGEN SOCIETY, NASHVILLE, TENN., USA, MARCH 16-19, 1980. ENVIRON MUT, 2 (2). 1980. 289.

47) Green T **The metabolic activation of dichloromethane and chlorofluoromethane in a bacterial mutation assay using Salmonella typhimurium. **Mutat Res 1983 Sep,118(4):277-88

48) Green T **MUTAGENICITY OF DICHLORO- AND CHLOROFLUORO-METHANE **THE TOXICOL, 1:26,1981

49) Hardin BD , Manson JM **Absence of dichloromethane teratogenicity with inhalation exposure in rats. **Toxicol Appl Pharmacol 1980 Jan,52(1):22-8

50) Haseman JK **Issues in carcinogenicity testing: dose selection **Fundam. Appl. Toxicol., VOL 5, ISS 1, 1985,66-78

51) Hatch G , Anderson T , Elmore E , Nesnow S **STATUS OF ENHANCEMENT OF DNA VIRAL TRANSFORMATION FOR DETERMINATION OF MUTAGENIC AND CARCINOGENIC POTENTIAL OF GASEOUS AND VOLATILE COMPOUNDS **ENVIRON MUTAGENESIS, 5:422,1983

52) Hatch GG , Mamay PD , Ayer ML , Casto BC , Nesnow S **Chemical enhancement of viral transformation in Syrian hamster embryo cells by gaseous and volatile chlorinated methanes and ethanes **Cancer Res., VOL 43, ISS 5, 1983,1945-50

53) Hatch GG , Mamay PD , Ayer ML , Casto BC , Nesnow S **Methods for detecting gaseous and volatile carcinogens using cell transformation assays **Environ. Sci. Res., VOL 25, ISS Genotoxic Eff. Airborne Agents, 1982,75-90

54) Hatch GG , Mamay PD , Christenson CC , Casto BC , Langenbach R , Nesnow S **IN-VITRO TRANSFORMATION OF HAMSTER EMBRYO CELLS EXPOSED TO GASEOUS OR VOLATILE CHLORINATED HYDRO CARBONS **72ND ANNUAL MEETING OF THE AMERICAN ASSOCIATION FOR CANCER RESEARCH, INC., WASHINGTON, D.C., USA, APRIL 27-30, 1981. PROC AM ASSOC CANCER RES AM SOC CLIN ONCOL, 22 (0). 1981. 119.

55) Hathway DE **MECHANISMS OF CHEMICAL CARCINOGENESIS **HATHWAY, D. E. SPECIALIST PERIODICAL REPORTS: FOREIGN COMPOUND METABOLISM IN MAMMALS, VOL. 6. A REVIEW OF LITERATURE PUBLISHED DURING 1978 AND 1979. XVI+390P. ROYAL SOCIETY OF CHEMISTRY: LONDON, ENGLAND. ILLUS. ISBN 0-85186-058-3., 0 (0). 1981. P160-197.

56) Hearne FT, Friedlander BR **Follow-up of methylene chloride study **J. Occup. Med 23:660, 1981

57) Helmes CT , Atkinson DL , Sigman CC , Thompsom KL , Kelsey MI , Kraybill HF , Munn JI **Evaluation and classification of the potential carcinogenicity of organic air pollutants. **J ENVIRON SCI HEALTH PART A ENVIRON SCI ENG, 17 (3). 1982. 321-390.

58) IARC **Dichloromethane. **IARC Monogr Eval Carcinog Risk Chem Hum 1979 Oct,20:449-65

59) Infante PF , Tsongas TA **MUTAGENIC AND ONCOGENIC EFFECTS OF CHLOROMETHANES, CHLOROETHANES AND HALOGENATED ANALOGS OF VINYL CHLORIDE, IN: GENOTOXIC EFFECTS OF AIRBORNE AGENTS **ENVIRON SCI RES, 25:301-327,1982

60) Jongen WM **Relationship between exposure time and metabolic activation of dichloromethane in Salmonella typhimurium. **Mutat Res 1984 May,136(2):107-8

61) Jongen WM , Alink GM , Koeman JH **Mutagenic effect of dichloromethane on Salmonella typhimurium. **Mutat Res 1978 Jan,56(3):245-8

62) Jongen WM , Harmsen EG , Alink GM , Koeman JH **The effect of glutathione conjugation and microsomal oxidation on the mutagenicity of dichloromethane in S. typhimurium. **Mutat Res 1982 Aug,95(2-3):183-9

63) Jongen WM , Lohman PH , Kottenhagen MJ , Alink GM , Berends F , Koeman JH **Mutagenicity testing of dichloromethane in short-term mammalian tests systems. **Mutat Res 1981 Apr,81(2):203-13

64) Kanada T , Uyeta M **MUTAGENICITY SCREENING OF ORGANIC SOLVENTS IN MICROBIAL SYSTEMS **MUTAT RES, 54:215,1978

65) Kennish JM , French SB **TRACE WATER-SOLUBLE COMPONENTS FROM PRUDHOE BAY USA CRUDE OIL CHEMICAL CHARACTERIZATION AND MUTAGENICITY **2ND INTERNATIONAL SYMPOSIUM ON RESPONSES OF MARINE ORGANISMS TO POLLUTANTS, WOODS HOLE, MASS., USA, APR. 27-29, 1983. MAR ENVIRON RES, 14 (1-4). 1984. 506-510.

66) King LC , Kohan MJ , Austin AC , Claxton LD , Huisibgh JL **Evaluation of the release of mutagens from diesel particles in the presence of physiological fluids. **ENVIRON MUTAGEN, 3 (2). 1981. 109-122.

67) Kirwin CJ , Thomas WC , Simmon VF **IN VITRO MICROBIOLOGICAL MUTAGENICITY STUDIES OF HYDROCARBON PROPELLANTS **J SOC COSMET CHEM, 31:367-370,1980

68) Kohler-Staub D , Leisinger T **Dichloromethane dehalogenase of Hyphomicrobium sp. strain DM2. **J Bacteriol 1985 May,162(2):676-81

69) Kool HJ , Van Kreijl CF , Van Kranen HJ , De Greef E **Toxicity assessment of organic compounds in drinking water in the Netherlands **Sci. Total Environ., VOL 18,, 1981,135-53

70) Kool HJ , van Kreijl CF , Zoeteman B CJ **TOXICOLOGY ASSESSMENT OF ORGANIC COMPOUNDS IN DRINKING WATER **CRIT REV ENVIRON CONTROL, 12 (4). 1982 (RECD. 1983). 307-357.

71) Kopfler FC , Melton RG **HUMAN EXPOSURE TO WATER POLLUTANTS **SUFFET, IRWIN H. (ED.). ADVANCES IN ENVIRONMENTAL SCIENCE AND TECHNOLOGY VOL. 8. FATE OF POLLUTANTS IN THE AIR AND WATER ENVIRONMENTS. PART 2. CHEMICAL AND BIOLOGICAL FATE OF POLLUTANTS IN THE ENVIRONMENT. John Wiley & Sons, Inc., 1977

72) Kraybill HF **ASSESSMENT OF HUMAN EXPOSURE AND HEALTH RISK TO ENVIRONMENTAL CONTAMINANTS IN THE ATMOSPHERE AND WATER WITH SPECIAL REFERENCE TO CANCER **J ENVIRON SCI HEALTH PART C ENVIRON CARCINOG REV, 1 (2). 1983. 175-232.

73) Kraybill HF **EVALUATION OF PUBLIC HEALTH ASPECTS OF CARCINOGENIC MUTAGENIC BIO REFRACTORIES IN DRINKING WATER **CONFERENCE ON THE PRIMARY PREVENTION OF CANCER: ASSESSMENT OF RISK FACTORS AND FUTURE DIRECTIONS, NEW YORK, N.Y., USA, JUNE 7-8, 1979. PREV MED, 9 (2). 1980. 212-218.

74) Kurppa K , Kivisto H , Vainio H **Dichloromethane and carbon monoxide inhalation: carboxyhemoglobin addition, and drug metabolizing enzymes in rat. **Int Arch Occup Environ Health 1981,49(1):83-7

75) Kurppa K , Vainio H **Effects of intermittent dichloromethane inhalation on blood carboxyhemoglobin concentration and drug metabolizing enzymes in rat. **Res Commun Chem Pathol Pharmacol 1981 Jun,32(3):535-44

76) LaPat-Polasko LT , McCarty PL , Zehnder AJ **Secondary substrate utilization of methylene chloride by an isolated strain of Pseudomonas sp. **Appl Environ Microbiol 1984 Apr,47(4):825-30

77) Laham S , Potvin M , Schrader K **[Toxicological studies on dichloromethane, a solvent simulating carbon monoxide poisoning (author's transl)] **Toxicol Eur Res 1978 Mar,1(2):63-73

78) Landry TD , Ramsey JC , McKenna MJ **Pulmonary physiology and inhalation dosimetry in rats: development of a method and two examples **Toxicol. Appl. Pharmacol., VOL 71, ISS 1, 1983,72-83

79) Leuschner F , Neumann BW , Hubscher F **Report on subacute toxicological studies with dichloromethane in rats and dogs by inhalation. **Arzneimittelforschung 1984,34(12):1772-4

80) Lioy PJ , Daisey JM **The New Jersey Project on Airborne Toxic Elements and Organic Substances (ATEOS): A summary of the 1981 summer and 1982 winter studies **J. Air Pollut. Control Assoc., VOL 33, ISS 7, 1983,649-57

81) Ma TH , Harris MM , Anderson VA , Ahmed I , Mohammad K , Bare JL , Lin G **Tradescantia-Micronucleus (Trad-MCN) tests on 140 health-related agents **Mutat. Res., VOL 138, ISS 2-3, 1984,157-67

82) Matsumoto T , Okura Y , Okawa Y , Mikami T , Nagase T , Hashimoto K , Suzuki M , Sugai N , Suzuki S **ACUTE TOXICITY TESTING OF SOME CHLORINATED LOWER HYDRO CARBONS DI CHLORO METHANE 1 2 DI CHLORO PROPANE AND 1 3 DI CHLORO PROPANE **8TH SYMPOSIUM ON ENVIRONMENTAL POLLUTANTS AND TOXICOLGY, SENDAI, MIYAGI, JAPAN, OCT. 8-9, 1981. EISEI KAGAKU, 28 (1). 1982. P-31.

83) Matthews RM , James TL **Effect of dichloromethane on sickle cells. An in vitro water proton magnetic resonance relaxation study. **Biochem Biophys Res Commun 1978 Feb 28,80(4):879-85

84) Mazzoli S **DETECTION OF URINARY MUTAGENS IN CHEMICAL WORKERS **9TH ANNUAL MEETING OF THE EUROPEAN ENVIRONMENTAL MUTAGEN SOCIETY, MARKASKA, YUGOSLOVIA, SEPT. 30-OCT. 5, 1979. MUTAT RES, 74 (3). 1980. 197.

85) McGregor DB **PRACTICAL EXPERIENCE IN TESTING UNKNOWNS IN-VITRO **PAGET, G. E. (ED.). TOPICS IN TOXICOLOGY, MUTAGENESIS IN SUB-MAMMALIAN SYSTEMS: STATUS AND SIGNIFICANCE. XIV+231P. UNIVERSITY PARK PRESS: BALTIMORE, MD., USA. ILLUS. ISBN 0-8391-1367-6., 0 (0). 1979. P53-72.

86) McKenna MJ , Zempel JA , Braun WH **The pharmacokinetics of inhaled methylene chloride in rats. **Toxicol Appl Pharmacol 1982 Aug,65(1):1-10

87) McKenna MJ , Zempel JA **The dose-dependent metabolism of [14C]methylene chloride following oral administration to rats. **Food Cosmet Toxicol 1981 Feb,19(1):73-8

88) McKenna MJ , Zempel JA , Braun WH **The pharmacokinetics of inhaled methylene chloride in rats **Aerosp. Med. Res. Lab., [Tech. Rep.] AMRL-TR (U. S.), ISS AMRL-TR-79-68 Proc. Conf. Environ. Toxicol.,, 1979,184-209

89) Mercier M , Lans M , de Gerlache J **MUTAGENICITY, CARCINOGENICITY, AND TERATOGENICITY OF HALOGENATED HYDROCARBON SOLVENTS **MUTAGEN CARCINOG TERATOG IND POLLUT, 281-324,1984

90) Meyers VK **CHEMICALS WHICH CAUSE BIRTH DEFECTS TERATOGENS A SPECIAL CONCERN OF RESEARCH CHEMISTS **SCI TOTAL ENVIRON, 32 (1). 1983 (RECD. 1984). 1-12.

91) Mortelmans KE , Griffin AF **MICROBIAL MUTAGENESIS TESTING OF SUBSTANCES, COMPOUND REPORT: F76-076, METHYLENE CHLORIDE (FOOD GRADE) GENERAL FOODS, CAS NO.75092 **REPORT, (LSU-6909):15 PP,1981

92) Mumford JL , Lewtas J **Mutagenicity and cytotoxicity of coal fly ash from fluidized-bed and conventional combustion **J. Toxicol. Environ. Health, VOL 10, ISS 4-5, 1982,565-86

93) National Coffee Association **Twenty-four month chronic toxicity and oncogenicity study of methylene chloride in mice. **Unpublished report prepared by Hazelton Laboratories America, Inc., Vienna, Va, 1983

94) National Coffee Association **Twenty-four month chronic toxicity and oncogenicity study of methylene chloride in rats. Addition to the final report. **Unpublished report prepared by Hazelton Laboratories America, Inc., Vienna, Va, 1982

95) National Coffee Association **Twenty-four month chronic toxicity and oncogenicity study of methylene chloride in rats. final report **Unpublished report prepared by Hazelton Laboratories America, Inc., Vienna, Va, 1982

96) National Toxicology Program **The toxicology and carcinogenesis studies of dichloromethane in F344/N rats and B6C3F1 mice, inhalation study. **NTP Board draft technical report NTP TR 306, 1985

97) National Toxicology Program **The carcinogenesis bioassay of dichloromethane, garage study. **NTP draft technical report, 1982

98) Nellor MH , Baird RB , Carrison WE **Health effects of water reuse by groundwater recharge **Proc. Water Reuse Symp., ISS 3, 1982,2138-66

99) Nestmann E , Kowbel DJ **MUTAGENICITY OF PAINT-REMOVING PRODUCTS DETECTED IN A MODIFIED SALMONELLA/MAMMALIAN-MICROSOME ASSAY **CAN J GENET CYTOL, 22:673,1980

100) Nestmann ER , Lee EG , Matula TI , Douglas GR , Mueller JC **Mutagenicity of constituents identified in pulp and paper mill effluents using the Salmonella/mammalian-microsome assay. **Mutat Res, VOL 79, ISS 3, 1980, P203-12

101) Nestmann ER , Otson R , Williams DT , Kowbel DJ **Mutagenicity of paint removers containing dichloromethane. **Cancer Lett 1981 Feb,11(4):295-302

102) Nestmann ER , Otson R , LeBel GL , Williams DT , Lee GH , Biggs DC **Correlation of water quality parameters with mutagenicity of drinking water extracts **Water Chlorination: Environ. Impact Health Eff., VOL 4, ISS Book 2, 1983,1151-64

103) Nestmann ER , Stephen ER , Kowbel DJ , Nasim A **DIFFERENTIAL SURVIVAL AS AN INDICATOR OF POTENTIAL MUTAGENICITY USING REPAIR DEFICIENT STRAINS OF SACCHAROMYCES CEREVISIAE AND SCHIZOSACCHAROMYCES POMBE **CAN J GENET CYTOL, 24:771-775,1982

104) Nitschke K et al **Methylene chloride: a two-year inhalation toxicity abd oncogenicity study. Final report of studies conducted by Dow Chemical Company **

105) Osterman-Golkar S , Hussain S , Walles S , Anderstam B , Sigvardsson K **Chemical reactivity and mutagenicity of some dihalomethanes. **Chem Biol Interact 1983 Aug 15,46(1):121-30

106) Ott MG, et al **Health evaluation of employees occupationally exposed to methylene chloride. Mortality **Scan. J. Work Environ. Health 9:8-16, 1983

107) Ott MG, et al **Health evaluation of employees occupationally exposed to methylene chloride. Clinical laboratory evaluation. **Scan. J. Work Environ. Health 9:17-25, 1983

108) Ott MG, et al **Health evaluation of employees occupationally exposed to methylene chloride. Twenty-four hour electrocardiographic monitoring. **Scan. J. Work Environ. Health 9:26-30, 1983

109) Ott MG, et al **Health evaluation of employees occupationally exposed to methylene chloride. Metabolism data and oxygen half-saturation pressures. **Scan. J. Work Environ. Health 9:31-38. 1983

110) Pankow D , Gutewort R , Ponsold W , Glatzel W , Tietze K **Effect of dichloromethane on the sciatic motor conduction velocity of rats. **Experientia 1979 Mar 15,35(3):373-4

111) Perbellini L , Brugnone F , Grigolini L , Cunegatti P , Tacconi A **Alveolar air and blood dichloromethane concentration in shoe sole factory workers. **Int Arch Occup Environ Health 1977 Dec 22,40(4):241-7

112) Pereverzeva EA , Tsulaia VR , Volokhova LI **[Action of methylene chloride on the body via the inhalatory route of entry] **Gig Sanit 1975 Sep,(9):105-6

113) Perocco P , Prodi G **DNA damage by haloalkanes in human lymphocytes cultured in vitro. **Cancer Lett, VOL 13, ISS 3, 1981, P213-8

114) Peterson JE **Modeling the uptake, metabolism and excretion of dichloromethane by man. **Am Ind Hyg Assoc J 1978 Jan,39(1):41-7

115) Price PJ , Hassett CM , MANSFIELD JI **Transforming activities of trichloroethylene and proposed industrial alternatives. **IN VITRO (ROCKVILLE), 14 (3). 1978 290-293

116) Putz VR , Johnson BL , Setzer JV **A comparative study of the effects of carbon monoxide and methylene chloride on human performance. **J Environ Pathol Toxicol 1979 May-Jun,2(5):97-112

117) Rapson W , Nazar MA , Bulsky VV **MUTAGENICITY PRODUCED BY AQUEOUS CHLORINATION OF ORGANIC COMPOUNDS **BULL ENVIRON CONTAM TOXICOL, 24:590-596,1980

118) Reznik GK **Methylene chloride: a two-year inhalation toxicity and oncogenicity study in rats and hamsters [letter] **Fundam Appl Toxicol 1984 Oct,4(5):883-4

119) Rodkey FL **Biological oxidation of [14C]methylene chloride to carbon monoxide and carbon dioxide by the rat. **Toxicol Appl Pharmacol 1977 Apr,40(1):33-8

120) Roth RP , Drew RT , Lo RJ , Fouts JR **Dichloromethane inhalation, carboxyhemoglobin concentrations, and drug metabolizing enzymes in rabbits. **Toxicol Appl Pharmacol 1975 Sep,33(3):427-37

121) Russell JF , Sippel ME , Krahn DF **METHODS FOR THE DETECTION OF MUTAGENIC GASES AND VOLATILE LIQUIDS IN THE SALMONELLA/MICROSOME ASSAY **ENVIRON MUTAGENESIS, 2:307,1980

122) Sahu SC , Lowther DK **Pulmonary reactions to inhalation of methylene chloride: effects on lipid peroxidation in rats. **Toxicol Lett 1981 Jun-Jul,8(4-5):253-6

123) Savolainen H , Kurppa K , Pfaffli P , Kivisto H **Dose-related effects of dichloromethane on rat brain in short-term inhalation exposure. **Chem Biol Interact 1981 Mar 15,34(3):315-22

124) Savolainen H , Pfaffli P , Tengen M , Vainio H **Biochemical and behavioural effects of inhalation exposure to tetrachlorethylene and dichlormethane. **J Neuropathol Exp Neurol 1977 Nov,36(6):941-9

125) Saxena AM , North BE , Schoenborn BP **The effect of dichloromethane on the oxygenation of hemoglobin. **Biochim Biophys Acta 1982 May 21,704(1):1-6

126) Schwetz BA , Leong KJ , Gehring PJ **The effect of maternally inhaled trichloroethylene, perchloroethylene, methyl chloroform, and methylene chloride on embryonal and fetal development in mice and rats. **Toxicol Appl Pharmacol 1975 Apr,32(1):84-96

127) Simmom VF , Tardiff RG **MUTAGENIC ACTIVITY OF HALOGENATED COMPOUNDS FOUND IN CHLORINATED DRINKING WATER **WATER CHLORINATION:ENVIRON IMPACT HEALTH EFF PROC CONF(1977), 2:417-431,1978

128) Simmon VF **Structural correlations of carcinogenic and mutagenic alkyl halides **DHEW Publ. (FDA) (U. S.), ISS FDA 78-1046, Struct. Correl. Carcinog. Mutagen.,, 1978,163-71

129) Simmon VF , Kauhanen K , Tardiff RG **Mutagenic activity of chemicals identified in drinking water **Dev. Toxicol. Environ. Sci., VOL 2, ISS Prog. Genet Toxicol., 1977,249-58

130) Sipes IG **ACTIVATION AND BINDING OF ORGANOHALOGEN CARCINOGENS **U S N T I S PB REPORT, (PB 81-929605):11,1981

131) Sipes IG , GANDOLFI AJ **IN-VITRO COMPARATIVE BIO ACTIVATION OF ALIPHATIC HALOGENATED HYDRO CARBONS **2ND INTERNATIONAL CONGRESS ON TOXICOLOGY, BRUSSELS, BELGIUM, JULY 6-11, 1980. TOXICOL LETTERS (AMST), 0 (SPEC. ISSUE 1). 1980. 33.

132) Sipes IG , Gandolfi AJ **In vitro comparative bioactivation of aliphatic halogenated hydrocarbons **Dev. Toxicol. Environ. Sci., VOL 8, ISS Mech. Toxic. Hazard Eval., 1980,501-6

133) Skrabalak DS , Babish JG **Safety standards for occupational exposure to dichloromethane. **Regul Toxicol Pharmacol 1983 Jun,3(2):139-43

134) Stevenson MF , Chenoweth MB , Cooper GL **Effect on carboxyhemoglobin of exposure to aerosol spray paints with methylene chloride. **Clin Toxicol 1978,12(5):551-6

135) Stewart RD , Hake CL **Paint-remover hazard. **JAMA 1976 Jan 26,235(4):398-401.

136) Thilagar AK , Kumaroo V **Induction of chromosome damage by methylene chloride in CHO cells. **Mutat Res 1983 Mar,116(3-4):361-7

137) Toftgaed R , Gustafsson J-A **BIO TRANSFORMATION OF ORGANIC SOLVENTS A REVIEW **SCAND J WORK ENVIRON HEALTH, 6 (1). 1980. 1-18.

138) Toftgard R , Nilsen OG , Gustafsson JA **Dose dependent induction of rat liver microsomal cytochrome P-450 and microsomal enzymatic activities after inhalation of toluene and dichloromethane. **Acta Pharmacol Toxicol (Copenh) 1982 Aug,51(2):108-14

139) U.S. EPA **Health assessment document for dichloromethane (methylene chloride) Final Report. **EPA-700/8-82-004F, 1985

140) U S. EPA **Addendum to the health assessment document for dichloromethane (methylene chloride): updated carcinogen assessment of dichloromethane **Revew Draft, EPA-600/8-82-004FA, 1985

141) Verrett MJ , SCOTT WF , REYNALDO EF , ALTERMAN EK , THOMAS CA **Toxicity and teratogenicity of food additive chemicals in the developing chicken embryo. **TOXICOL APPL PHARMACOL, 56 (2). 1980. 265-273.

142) Winneke G **The neurotoxicity of dichloromethane. **Neurobehav Toxicol Teratol 1981 Winter,3(4):391-5

143) Winneke G , Fodor GG **Dichloromethane produces narcotic effect. **Occup Health Saf 1976 Mar-Apr,45(2):34-5, 49

144) Withey JR , Collins BT **Chlorinated aliphatic hydrocarbons used in the foods industry: the comparative pharmacokinetics of methylene chloride, 1,2 dichloroethane, chloroform and trichloroethylene after i.v. administration in the rat **J. Environ. Pathol. Toxicol., VOL 3, ISS 5-6, 1980,313-32

145) Zelikoff JT , Daisey JM , Traul KA , Kneip TJ **Balbc/3T3 cell transformation response to extracts of organic air samples as seen by their survival in aggregate form. **Mutat Res, VOL 144, ISS 2, 1985, P107-16

ATTACHMENT III

ATTACHMENT III

During 1985, the ARB performed sampling for methylene chloride at twenty-two sites in seven air basins. The maximum concentration of methylene chloride measured was 47.0 ppb; the minimum concentration was below the detection limit of 0.6 ppb. Average concentrations at the monitoring stations range from 0.3 ppb at Merced to 5.6 ppb at Santa Barbara. The average concentration for all samples taken during 1985 at five sites in the South Coast Air Basin is 3.7 ppb.

The major uses of methylene chloride are as a paint remover, degreaser, aerosol propellent, and foam blowing agent. Potential point sources are vapor degreasing and cold cleaning solvent operations, polyurethane foam production facilities, and photo resist stripping operations associated with the electronics industry. Widespread emissions of methylene chloride occur through its use in consumer products such as aerosol sprays and paint and varnish removers.

State of California

Department of Health Services

Memorandum

To : James Boyd
Executive Officer
Air Resources Board
1102 Q Street
Sacramento, CA 95814

Date : September 29, 1986

Subject: Request for Extension of Methylene Chloride Evaluation

Air Resources Board
RECEIVED
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rug: SSO
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From : Office of the Director
714 P Street, Room 1253
Sacramento, CA
5-1248

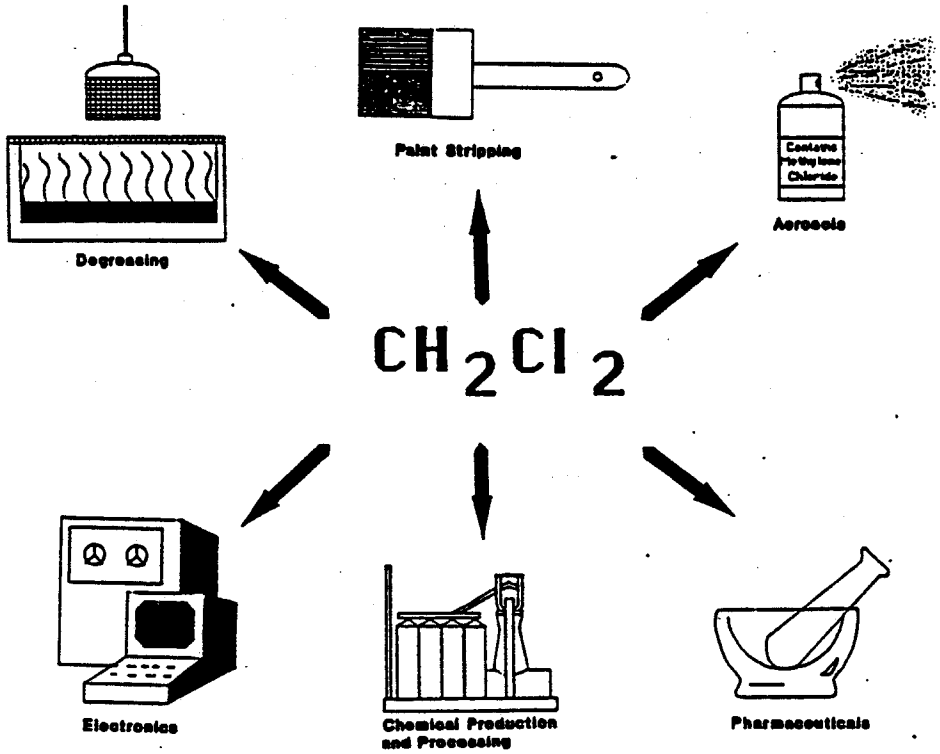
We are unable to complete the AB 1807 document on methylene chloride due on July 15, 1986. Therefore, we are requesting the statutory 30-day extension plus an additional 60 days to October 13, 1986. Much of this document has been performed under contract. Department staff have requested the contractor to make extensive changes to the draft, which necessitates the need for this extension.

Kenneth W. Kizer, M.D., M.P.H.
Director

TECHNICAL SUPPORT DOCUMENT

PROPOSED IDENTIFICATION OF METHYLENE CHLORIDE AS A TOXIC AIR CONTAMINANT

Part B Report



State of California

Air Resources Board

Stationary Source Division

May 1989

FINAL DRAFT

TECHNICAL SUPPORT DOCUMENT

PART B - HEALTH EFFECTS OF METHYLENE CHLORIDE

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May 1989

Based in part on document prepared by ICF-Clement, 1850 K Street NW, Suite 450, Washington, DC 20006, for the California Department of Health Services under contract 85-86676 (045A).

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Executive Summary

Methylene chloride (dichloromethane, CH_2Cl_2) is a solvent with a variety of uses in industry and food processing. It is rapidly absorbed through the lungs and is distributed throughout the body. It can cross the blood-brain barrier and the placental barrier to the fetus. Although it is not highly bioconcentrated, it reaches equilibrium in fat at concentrations six to eight times greater than in other tissues.

Two pathways have been identified for CH_2Cl_2 metabolism in rats and mice. A cytochrome P450 oxidative dehalogenation pathway yields primarily carbon monoxide (CO), which has also been identified as a metabolite in humans. A second pathway involves glutathione and glutathione-S-transferase. Each pathway theoretically involves the formation of metabolically active intermediates that may be capable of binding to cellular macromolecules, including DNA. The oxidative pathway appears to be saturated at CH_2Cl_2 inhalation exposure concentrations above 500 ppm, while even at 4,000 ppm there is no evidence of saturation of the glutathione pathway.

Methylene chloride has a relatively low acute toxicity. Animal and human studies indicate that the central nervous system (CNS) is the principal target organ, resulting in CNS depression at inhalation concentrations greater than 1000 ppm. Chronic and subchronic effects on lung and liver (other than cancer) are observed in laboratory animals at concentrations of 100 ppm or greater. The most sensitive animal toxicity study suggests an oral no-observed-adverse-effect level (NOAEL) of 5 mg/kg-day. Assuming

equivalency of toxicity by the oral and inhalation exposure routes, a calculated NOAEL for humans is 5 ppm. Since these levels are at least three to four orders of magnitude greater than ambient air concentrations (approximately 2 ppb), adverse health effects other than cancer are not expected from acute or chronic exposures to methylene chloride in ambient air.

Inhalation of maternally toxic concentrations of methylene chloride (1250-4500 ppm) by female rodents throughout pregnancy can result in fetotoxicity. CH_2Cl_2 has low teratogenic potential in rodents, but experimental data are inadequate to make inferences about effects on human reproduction.

The genotoxicity of CH_2Cl_2 has been shown in several assay systems, including bacteria (*S. typhimurium* and *E. coli*), yeast (*Saccharomyces cerevisiae*), and fruit flies (*Drosophila*). Methylene chloride induced sister chromatid exchange and chromosomal aberrations in cultured mammalian cells. These responses, albeit positive, were weak.

The U.S. Environmental Protection Agency (EPA) concluded that CH_2Cl_2 is capable of inducing mutations in exposed human cells. The International Agency for Research on Cancer (IARC) reviewed short-term tests of DNA damage for CH_2Cl_2 and concluded that there was sufficient evidence to classify CH_2Cl_2 as genetically active. IARC also concluded that CH_2Cl_2 causes cell transformation in mammalian cells cultured *in vitro*. Staff of the California Department of Health Services (DHS) concur with IARC's and EPA's evaluations but stress that the high concentrations of CH_2Cl_2 necessary to

induce mutagenic and cellular transformations demonstrate that CH_2Cl_2 should be considered weakly genotoxic.

A series of epidemiological studies by Ott and co-workers compared the mortality experience of workers exposed to CH_2Cl_2 in one fiber production plant to nonexposed workers in another. No effects on mortality were attributable to CH_2Cl_2 exposure, but the studies had low statistical power and confounding factors; thus increased health risks cannot be excluded.

The mortality experience of a male cohort of Eastman-Kodak employees exposed to low levels of CH_2Cl_2 was compared both to Kodak employees not exposed to CH_2Cl_2 and to the general New York State male population. This study demonstrated an increase in pancreatic cancer compared to the nonexposed Kodak cohort and compared to the state male population. The authors attributed the increase in pancreatic cancer to an artifact of multiple statistical testing. DHS staff conclude that the epidemiological data are inadequate either to establish or to rule out carcinogenicity of methylene chloride in humans.

Several chronic rodent bioassays have shown that CH_2Cl_2 increases tumor rates in some organs, primarily the mouse liver and lung and the rat mammary gland. A lifetime inhalation bioassay was conducted in 1986 by the National Toxicology Program (NTP) in which both male and female rats and mice were exposed to 1,000 to 4,000 ppm of CH_2Cl_2 in air. The NTP concluded, "Under the conditions of these inhalation studies, there was some evidence of carcinogenicity of dichloromethane for male F344/N rats...and clear evidence

of carcinogenicity ... for female F344/N rats (and) ... for male and female B6C3F$_1$ mice."

The EPA concluded that for CH_2Cl_2 the evidence for carcinogenicity in experimental animals is sufficient, and the human evidence is inadequate. Overall, the EPA assigned CH_2Cl_2 to category B2, meaning that methylene chloride should be considered a probable human carcinogen. IARC recently reviewed the carcinogenicity data and concluded that methylene chloride is carcinogenic in animals although there is inadequate evidence that it is a human carcinogen. IARC assigned CH_2Cl_2 a rating of 2B, a category comprised of possible human carcinogens. DHS staff concur with EPA's and IARC's conclusions. Since the staff found no evidence of a carcinogenic threshold level and because there are several short-term tests suggesting that CH_2Cl_2 is mutagenic, the staff recommends that CH_2Cl_2 be considered as not having a threshold for carcinogenicity.

DHS staff used female mouse lung tumors (the most sensitive sex, species and tumor site of the 1986 NTP inhalation bioassay) to calculate the low-dose risk from exposure to CH_2Cl_2. The mean environmental concentration measured by Air Resources Board staff is approximately 2 ppb (0.002 ppm), while the animal experimental concentrations were 2,000 to 4,000 ppm. The mice were exposed to these experimental concentrations for six hours per day, five days per week. Adjusting for lifetime daily exposure, the experimental concentrations correspond to environmental exposures of approximately 357 ppm to 714 ppm. The low-dose extrapolation for human exposure from the animal bioassays spans five to six orders of magnitude.

As shown below, DHS staff fit several low-dose risk assessment models to the mouse lung tumor data, including the multistage (Global 82 and Global 86), time-dependent multistage (Weibull 82), probit, logit, Weibull, gamma multihit, and two-stage models. DHS staff also applied a physiologically based pharmacokinetic model to estimate the internal dose. This model adjusts the expected exposure concentration and suggests lower human risks than predicted by an unadjusted or applied dose approach. The NTP bioassay results are consistent with an applied dose as well as a pharmacokinetic approach. The application of the pharmacokinetic approach to risk assessment is based on information developed by the EPA and the U. S. Consumer Product Safety Commission. DHS staff recommend that the range of risks for ambient exposures to CH_2Cl_2 be based on the upper 95% confidence limit predicted from fitting either the multistage (Global 82) model or the time-dependent multistage (Weibull 82) model to the animal data. These approaches suggest that the upper bound of excess carcinogenic risk from a lifetime exposure to a range of CH_2Cl_2 concentrations from 1.1 to 2.4 ppb is 1 to 24 cases per million persons exposed. The unit risk for a lifetime of continuous exposure to 1 ppb of CH_2Cl_2 is 1 to 10 x 10^{-6} (lifetime exposure to 1 $\mu g/m^3$ of CH_2Cl_2 is 0.3 to 3 x 10^{-6}). The most likely estimate of the upper limit of risk, based on application of the PBPK high-to-low dose adjustment, is 4 x 10^{-6} per ppb. These calculations are for the upper range of plausible excess cancer risks: the actual risk, which cannot be calculated, may be insignificant.

The California Air Resources Board estimates a range of CH_2Cl_2 concentrations from 1.5 to 3.1 in the South Coast air basin. Assuming that there are about 10.09 million residents of this area, this suggests that the

upper bound for the excess number of additional lifetime cases of cancer in the South Coast air basin ranges from 20 to 300.

Based on the finding of methylene chloride-induced carcinogenicity and the results of the risk assessment, DHS staff finds that at ambient concentrations, methylene chloride is an air pollutant which may cause or contribute to an increase in mortality or an increase in serious illness, or which may pose a present or potential hazard to human health.

METHYLENE CHLORIDE EVALUATION HIGHLIGHTS

I. National and International Evaluation (Other Agencies' Evaluations).

A. U.S. Environmental Protection Agency (EPA)

1. Short-Term Tests: Sufficient evidence of genetic activity and cell transformation.
2. Animal carcinogenicity assays: Sufficient evidence of animal carcinogenicity by inhalation.
3. Human Evidence: Inadequate evidence of human carcinogenicity.
4. Conclusion: Methylene chloride is a probable human carcinogen, ranked B2.

B. International Agency For Research on Cancer (IARC)

1. Short-Term Tests: Sufficient evidence of genetic activity and cell transformation.
2. Animal carcinogenicity assays: Sufficient evidence of animal carcinogenicity by inhalation.
3. Human Evidence: Inadequate evidence of human carcinogenicity.
4. Conclusion: Methylene chloride is a possible human carcinogen grouped under IARC category 2B.

C. Conclusions: Both EPA and IARC concluded that there is ample evidence that methylene chloride is carcinogenic in animals (mice and rats) by inhalation and that it is genotoxic. Other than a slight increase in pancreatic cancer, there was no excess risk of death from malignancies in three epidemiological studies; however, both EPA and

IARC noted that the studies had a very limited power to detect excess risk.

II. Carcinogenic Threshold.

A. Shape of the dose-response curve

1. Animal: The carcinogenic dose-response curve for female mice lung tumors (adenomas and carcinomas combined) is consistent with a linear extrapolation. However, it should be noted that the dose-response curve consisted of only three data points, a control and two exposure concentrations.

2. Human: NA

B. Animal pharmacokinetic information: Moderate to good. No evidence for the presence of a carcinogenic threshold at low doses; both metabolic pathways appear to act at low doses.

C. Human pharmacokinetic information: Very limited. At low doses humans are likely to produce metabolic products by both pathways.

D. Conclusions: The positive short-term genetic activity tests, the observed animal carcinogenic dose-response curve, and the activity of metabolic activation at low doses lead the staff of DHS to conclude that methylene chloride is genotoxic and should not be considered to have a carcinogenic threshold.

III. Exposure Sources

A. Air levels

1. Ambient levels measured in the Los Angeles basin area: 1 to 5 ppb

2. Ambient levels measured in "hot spots": 17 to 28 ppb

3. Indoor air: Concentrations are variable depending on the size of the room, ventilation, quantity released, and time since release. Measured methylene chloride concentrations for a 90-minute average use in controlled studies using commercially available paint removers ranged from 50 to 2200 ppm. An Italian study reported that the mean methylene chloride concentration in 15 homes was 193 ppb with a high concentration of 1.5 ppm.

B. Reported Levels in Water

1. Methylene chloride may result from water chlorination; thus, finished water may have higher methylene chloride concentrations than raw water.

2. National Data

a. ambient waters: range 0 to 120 ppb, detected in 60 of 118 samples.

b. drinking water: range 1 to 3 ppb.

3. California drinking water: In 11 wells sampled in Southern California, methylene chloride concentrations ranged from 0.65 ppb to 10 ppb.

C. Reported levels in food: No information

IV. Quantitative Risk Assessment

A. Range of Extrapolation: Animal to human exposures in air for calculated lifetime daily exposures

1. Experimental to ambient: approximately 10^5

2. Experimental to hot spots: approximately 10^4

B. Range of Risks from MLE to 95% UCL:

Model	MLE to 95% UCL
Multistage	1.3 fold
Time-dependent multistage	2.6 fold

C. Physiologically-Based Pharmacokinetic Adjustments

1. CPSC high-to-low dose adjustment reduced applied dose risk estimate by approximately 2.2-fold.
2. EPA species-to-species dose adjustment reduced applied dose risk estimate by approximately 8-fold.

D. Range of 95% Upper Bound Risk Estimate for Lifetime Excess Cancer Cases

1. Per $\mu g/m^3$: 3×10^{-7} to 3×10^{-6}
2. Per ppm: 1×10^{-3} to 10×10^{-3}
3. Per ppb: 1×10^{-6} to 10×10^{-6}

E. Comparison with other carcinogenic potencies: Methylene chloride is in the lower fourth quartile of 53 carcinogens for which EPA has calculated potencies.

1.0 INTRODUCTION

Methylene chloride (dichloromethane, CH_2Cl_2) is a volatile, synthetic, halogenated hydrocarbon of low flammability. It has gained wide use in industry as a paint stripper, cleaning agent, process solvent, decaffeinating agent for coffee, and as a component of aerosol propellants. In acute exposures CH_2Cl_2 is toxic to humans and animals, with central nervous system depression as the major grossly observable effect. Subchronic and chronic exposures have been reported to cause behavioral changes, hepatic dysfunction, and, less frequently, lung lesions. Based on available data, the reproductive and teratogenic effects of CH_2Cl_2 cannot be evaluated. CH_2Cl_2 is mutagenic in bacteria test systems, but other mutagenicity tests are negative or equivocal; several tests for clastogenic activity have been positive. An International Agency for Research on Cancer (IARC) working group recently concluded that, although the evidence for carcinogenicity in animals is sufficient, there is inadequate evidence of its carcinogenicity in humans. IARC considers substances such as this to be potential human carcinogens. Applying the criteria of the EPA (1984) for evaluating the overall weight of evidence of carcinogenicity to humans, CH_2Cl_2 is most appropriately classified in Group B2, meaning that it is a probable human carcinogen.

This report reviews and evaluates the literature on the toxicity of CH_2Cl_2 and develops a risk estimate for its carcinogenic potency based on mathematical models. A risk estimate is also presented based on exposure adjusted to account for metabolism of CH_2Cl_2 to a proposed biologically active metabolite.

2.0 METABOLISM AND PHARMACOKINETICS

2.1 Absorption, Distribution, and Elimination

The route of exposure has been found to have a significant effect on the disposition of CH_2Cl_2 in rats and mice (Angelo et al. 1986a,b). Unlike the oral administration pattern, there was a dramatic retention of unmetabolized CH_2Cl_2 in tissues of mice following i.v. administration and the blood profile did not resemble the tissue profile (Angelo et al. 1986a).

2.1.1 Oral Absorption Methylene chloride appears to be rapidly absorbed from the gastrointestinal tract following ingestion (EPA 1985a). Rats given a single oral dose of 1 or 50 mg/kg $^{14}CH_2Cl_2$ excreted 92% and 96%, respectively, as the combined metabolized and nonmetabolized dose (McKenna and Zempel 1981). Similarly, Angelo et al. (1986) found that 97% of an oral dose of 50 or 200 mg/kg was excreted within 24 hours. Peak blood levels of CH_2Cl_2 were attained in mice less than 10 minutes after oral administration of 50 mg/kg CH_2Cl_2 (Pritchard and Angelo 1982).

2.1.2 Dermal Absorption The available information indicates that CH_2Cl_2 is absorbed very slowly across the skin (Stewart and Dodd 1964).

2.1.3 Pulmonary Uptake Inhaled CH_2Cl_2 is rapidly absorbed in the lung. Pulmonary uptake is facilitated by the water and lipid solubilities of CH_2Cl_2 and by the large lung alveolar surface area (EPA 1985a). The actual rate of CH_2Cl_2 absorption depends upon several factors: concentration in inspired air, pulmonary ventilation, duration of exposure, diffusion rates into blood and

tissues, and solubilities in blood and tissues. The concentration of CH_2Cl_2 in alveolar air, which is in equilibrium with the concentration of CH_2Cl_2 in the pulmonary venous blood, asymptotically approaches the concentration in inspired air, at which point a steady-state condition is attained.

Data from Riley et al. (1966) can be used to illustrate the complex absorption of CH_2Cl_2 in humans. During a 2-hour inhalation exposure to air containing 100 ppm CH_2Cl_2, the alveolar air concentration could be described by an exponentially rising curve with three components. At the beginning of exposure, an initial rapid rate of uptake occurred (0 to 50 ppm alveolar air), followed by a second slower uptake (50 to 65 ppm alveolar air), and finally, a very slow rate of uptake as equilibrium was approached, but not reached, at 70 ppm alveolar air concentration (EPA 1985a). These observations can be interpreted as a function of both the tissue partition coefficients and the degree of tissue blood perfusion and suggest that there are three types of tissues serving as body stores for absorbed CH_2Cl_2: a rapidly equilibrating group of tissues (termed the "vessel-rich group") with high blood flows, an intermediate group (primarily muscles), and the slowly diffusing adipose tissues. Obese human subjects absorbed 30% more CH_2Cl_2 on a mg/kg basis than lean subjects from one hour exposure of 750 ppm (EPA 1985a).

Human exposure studies (Divincenzo and Kaplan, 1981b) indicate that exercise increased the CH_2Cl_2 blood concentrations, the carboxyhemoglobin levels, exhaled level of CO. Based on ventilation capacity in exercising individuals the percent CH_2Cl_2 absorbed decreased from 72 to 47%, but the net pulmonary uptake of CH_2Cl_2 actually increased up to 4-fold. Also reflective of the increased uptake, the extent of CO production increased from 26% during

sedentary conditions up to 40% during exercise. In an attempted follow-up study, Carlson and Kim (1986) examined whether a similar phenomenon could be demonstrated in rats. For rats exposed to 500 or 1000 ppm for 8 hours, exercise did not elevate carboxyhemoglobin levels and the CH_2Cl_2 blood levels were lower in the exercised animals than in the sendentary animals. The authors concluded that additional safety considerations are needed to include the influence of exercise which exacerbates CH_2Cl_2's effects in humans but not in rats.

2.1.4 Tissue Distribution Methylene chloride is probably distributed throughout the body fluid by virtue of its water solubility, and its lipid solubility allows it to diffuse through lipid-containing membranes into various tissues. Methylene chloride readily crosses the blood-brain barrier (Winneke and Fodor 1976, Winneke 1981) and the placental barrier (Schwetz et al. 1975, Anders and Sunram 1982).

Concentrations of CH_2Cl_2 in various tissues depend upon exposure concentrations and durations and on tissue partition coefficients (EPA 1985a). Savolainen et al. (1977) found similar levels of CH_2Cl_2 in the brain, blood, and liver of rats exposed to 200 ppm CH_2Cl_2 for 5 days for 6 hours per day, but found a cumulative increase in CH_2Cl_2 concentrations in perirenal fat after the fifth day of exposure. At termination of the last 6-hour exposure, the ratios of CH_2Cl_2 concentrations in tissues versus blood were approximately 0.83 to 0.90 for brain and liver, and 6.6 for perirenal fat.

Studies of CH_2Cl_2 distribution in rats 48 hours after a 6-hour inhalation exposure to 50, 500, or 1,500 ppm or after a single oral dose of 1 or 50 mg/kg

have been conducted by McKenna et al. (1982) and McKenna and Zempel (1981) using ^{14}C-labelled CH_2Cl_2. Both routes of exposure resulted in the liver, kidney, and lung having the highest levels of ^{14}C-activity. Methylene chloride was not detected in any of the tissues assayed; it was assumed that the radioactivity found was due to the presence of nonvolatile metabolites.

2.1.5 Elimination Virtually all unchanged CH_2Cl_2 is exhaled from the body (EPA 1985a). Less than 2% of absorbed CH_2Cl_2 has been found unchanged in the urine of human subjects exposed to 100 or 200 ppm for two hours (DiVincenzo et al. 1972), or in the urine of dogs exposed to 5,000 ppm (MacEwen et al. 1972).

After termination of exposure, CH_2Cl_2 is initially eliminated from the body via the lungs. Alveolar air rapidly equilibrates with pulmonary venous blood in which the CH_2Cl_2 concentration is a function of the first-order diffusion of CH_2Cl_2 from tissues, the arterial blood flow/tissue mass, and the relative solubility of CH_2Cl_2 in tissues (EPA 1985a).

Estimates of the half-times of pulmonary elimination of CH_2Cl_2 are given in Table 2-1. The alveolar air concentration of CH_2Cl_2 showed evidence of a three-phase decline with an initial rapid fall in alveolar concentration, a less rapid drop, and a longer-term slow decrease. The apparent three-component elimination for humans is relevant to the earlier discussion of tissues with high vascularity and blood flow (alpha), those with less flow (beta), and those with high amounts of adipose tissue (gamma). The values reported for the gamma components may not be indicative of actual diffusion

TABLE 2-1

COMPARISON OF HALF-TIMES OF PULMONARY ELIMINATION OF METHYLENE CHLORIDE FOR HUMANS AND RATS

Subject	Exposure Method	Methylene Chloride Concentration	$t_{1/2}$ First-Order Components[a] (minutes) Alpha[b]	Beta[c]	Gamma[d]
Human	Inhalation	50-500 ppm (1-7.5 hr)	8-23	40-80	360-390
Rat	Inhalation	500 ppm (6 hr)	2.4	14.8	--
		1,500 ppm(6 hr)	1.06	15.5	--
Rat	Gavage	1 mg/kg	--	12.6	46.5
		50 mg/kg	--	12.6	46.6

SOURCE: After EPA, 1985a

[a] Exponential elimination curve consistent with three distinct first-order rate constants.

[b] Attributed to elimination blood vessel-rich tissues with high blood flow, e.g., brain, heart, liver.

[c] Attributed to elimination from lean body mass, e.g., muscle and skin.

[d] Attributed to elimination from adipose tissues.

rates from fat tissues following repeated exposures since the gamma value reported in Table 2-1 was from a single exposure, and it has been previously shown that CH_2Cl_2 levels in fat tissues do not equilibrate after a single exposure. Fat biopsies of subcutaneous adipose tissue of obese subjects contained about 10 mg and 8 mg of CH_2Cl_2 at 1 and 4 hours repectively and between 1 and 2 mg/kg at 22 hours after exposure (EPA 1985a).

2.2 Metabolism

Methylene chloride is a single-carbon halogenated hydrocarbon for which few enzymatic pathways of metabolism are available. The primary known pathways for metabolizing CH_2Cl_2 are the mixed function oxidase system (i.e., microsomal oxidation) and a cytosolic pathway consisting of a glutathione transferase system which dehalogenates CH_2Cl_2. However, the specific mechanisms of metabolism and the formation of potentially reactive intermediates have not been directly demonstrated.

2.2.1 Enzymatic Metabolism

2.2.1.1 Microsomal oxidation Kubic and Anders (1975) first described the oxidative dehalogenation that appears to be a primary route of enzymatic metabolism of CH_2Cl_2 and other dihalomethanes. Working with an *in vitro* rat liver microsomal system, it was determined that the reaction required nicotinamide adenine dinucleotide phosphate (NADP), reduced NADP (NADPH), and molecular oxygen for maximal activity. Under optimal conditions, carbon monoxide (CO) was produced; in the absence of NADPH, CH_2Cl_2 and other dihalomethanes were dehalogenated without formation of carbon monoxide. When

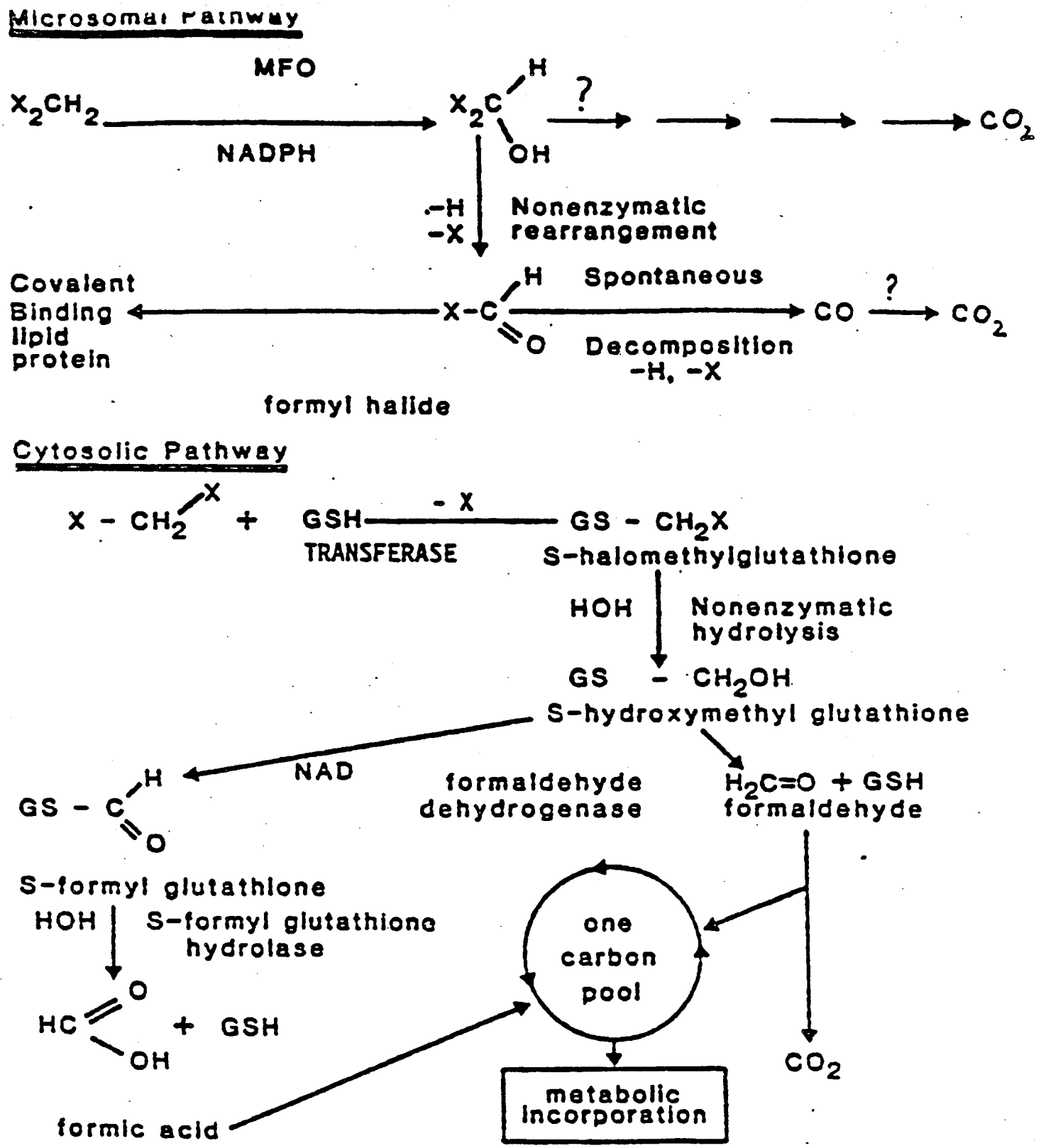


Figure 1. Proposed reaction mechanisms for the metabolism of dihalomethanes to carbon monoxide, carbon dioxide, formaldehyde, formic acid, and inorganic halide. (EPA 1985b; Gargas et al. 1986)

the reaction was carried out under anaerobic conditions, the yield of the reaction was reduced by approximately 80% (20% of the maximal carbon monoxide was produced). Tissue activity studies showed that liver microsomes were about 5 times more active than lung microsomes and 30 times more active than kidney microsomes. Hogan et al. (1976) confirmed that CH_2Cl_2 was converted to carbon monoxide by the rat liver microsomal system, and noted a high correlation between carbon monoxide production in vitro and microsomal cytochrome P450 activity. Further studies by Kubic and Anders (1978) with $^{18}O_2$ and CD_2Cl_2 led to the current suggested scheme for P450 metabolism of CH_2Cl_2 to CO as indicated in Figure 1. In a recent study, Takano and Miyazaki (1988) used a perfused rat liver system and reported that the CO produced by P450 during metabolism could bind to the P450 cytochrome. However, in another study (Halpert et al. 1986) CH_2Cl_2 did not inactivate the phenobarbital-inducible isozyme of rat liver cytochrome P-450 in an in vitro assay system.

Studies by Anders and coworkers (Kubic and Anders 1978; Ahmed and Anders 1978; Stevens and Anders 1978, 1979) have shown that molecular oxygen, not water, is the source of the oxygen in carbon monoxide. They postulated that the cytochrome P450 mixed-function oxidase system hydroxylates dihalomethanes to yield an intermediate compound, a hydroxydihalomethane, which spontaneously breaks down to a formylhalide with the loss of one halogen atom. The formylhalide then decomposes to carbon monoxide by elimination of a halide anion.

A recent study by Reitz et al. (1988) provided some in vitro data on the kinetics of MFO enzymes in several species. The CH_2Cl_2 concentrations used varied from 1 to 10 mM. Reaction rates from liver microsomes (nmoles/min/mg

protein) were measured for hamster, mouse, rat and human tissue. The reaction rates were greatest in the hamster, followed by the mouse; and the rates of the human and rat tissue were equivalent and lower (per mg protein). The smallest difference between the mouse and human reaction rates occurred at the 5 mM level where the mean reaction rate for the mouse tissue (11.4) was 2.6 times the mean reaction rate for the human tissue (4.5). Although some mean values were presented, standard deviations were not, so it is difficult to ascertain the closeness of the responses. Reaction rates from lung microsomes were also measured in the mouse, rat and hamster, but no activity was measured in the single pooled human sample. At the 5 mM level, the reaction rate from mouse liver microsomes (11.4) was 2.5 times the reaction rate for the mouse lung microsomes (4.6). However, the lung data appears to represent information from a single experiment; means and standard deviations were not presented. Kinetic constants were calculated for the male liver tissue in the study and the KM for the mouse data (1.8 mM) was within the human range of data (0.9 - 2.6 mM). The VMAX for the mouse data (15.9 mmoles product/min/mg protein) was close to the upper range reported for humans (1.53 - 13.0 mmoles product/min/mg protein). However, the four human samples exhibited a 8.5-fold range indicating considerable human variability.

2.2.1.2 <u>Carboxyhemoglobin Formation</u> Blood COHb accumulates when the concentration of carbon monoxide exceeds that which can be eliminated through the lungs (EPA 1985a). Since carbon monoxide is a metabolite of CH_2Cl_2, there was concern for potential carbon monoxide poisoning from occupational exposures to CH_2Cl_2 (Stewart et al. 1972a,b). Pulmonary elimination of carbon monoxide from carboxyhemoglobin is a first-order process that involves the exchange of hemoglobin-bound carbon monoxide with oxygen and the diffusion of

carbon monoxide into the alveolar spaces. The half-time for elimination of carbon monoxide is 4 to 5 hours in humans (NIOSH 1972, Lambertsen 1974) and is 1.8 to 2.5 hours in rats (McKenna et al. 1982, McKenna and Zempel 1981). The rate of pulmonary elimination is independent of the concentration of carboxyhemoglobin in the blood, but is shortened by increased alveolar ventilation or the inspired partial pressure of oxygen (Lambertsen 1974).

EPA (1985a) concluded that the kinetics of CH_2Cl_2 metabolism to carbon monoxide and carboxyhemoglobin are strikingly similar for humans and rats. Both species demonstrate Michaelis-Menten dose-dependent kinetics with similar estimates for VMAX (15 and 12.5% carboxyhemoglobin per time unit, respectively) and KM (200 and 170 ppm CH_2Cl_2, respectively). Comparison of the kinetic parameters estimated for the carbon monoxide pathway in the rat with those estimated by McKenna et al. (1982) for the total metabolism of CH_2Cl_2 by both pathways suggests that the overall KM is approximately equal to 400 ppm and is approximately twice that of the carbon monoxide pathway alone.

Methylene chloride-induced carboxyhemoglobin depends on the CH_2Cl_2 dose and on the duration of exposure, but usually does not exceed 10% to 12% in either humans or animals (EPA 1985a). This limit may be determined by the resultant first-order pulmonary elimination of carbon monoxide and zero-order hepatic CH_2Cl_2 metabolism to carbon monoxide. This limit may also be the result of direct effects by CH_2Cl_2 on hemoglobin. An early study indicated that CH_2Cl_2 can deform binding sites of myoglobin (Nunes and Schoenborn 1973). Settle (1975) observed that carbon monoxide binding was affected by the presence of CH_2Cl_2. Spectrophotometric studies have shown that CH_2Cl_2 decreases the affinity of heme for CO and oxygen, and the cooperativity of oxygen binding of

hemoglobin in solution (Harkey et al. 1979). Consequently, the kinetics of CH_2Cl_2 metabolism and direct effects by CH_2Cl_2 on hemoglobin may be responsible for the levels of carboxyhemoglobin attained in experimental studies.

2.2.1.3 Cytosolic pathway Methylene chloride is also metabolized to formaldehyde, formic acid, inorganic halide, and carbon dioxide (See Figure 1). The cytosolic enzyme system is a glutathione-S-transferase (GST) system that requires no cofactors other than glutathione. Ahmed and Anders (1976) proposed that the rate-limiting step of the reactions of CH_2Cl_2 with glutathione is displacement of the halogen to form a S-halomethylglutathione conjugate. This conjugate is postulated to undergo rapid nonenzymatic hydrolysis to S-hydroxymethylglutathione, which may subsequently break down to yield formaldehyde and regenerate glutathione. Alternatively, the S-hydroxymethylglutathione intermediate can be metabolized by formaldehyde dehydrogenase, a cytosolic liver enzyme, to S-formylglutathione, which can then be converted to formic acid and glutathione by the action of S-formylglutathione hydrolase.

Reitz et al. (1988) provided some in vitro data on the kinetics of GST in cytosol prepared from livers of several species. The CH_2Cl_2 concentrations used for all species varied from 12.5 to 100 mM, that is they were much higher than the concentrations used in the MFO studies (1 to 10 mM, see Section 2.2.1.1). Reaction rates from liver cytosol (nmoles/min/mg protein) were measured for hamster, mouse, rat and human tissue. The reaction rates were greatest in the mouse and least in the hamster. The relative rates of the human and rat tissue were dependant on the concentration chosen. The smallest

difference between the mouse and human reaction rates occurred at the lowest concentration tested, 12.5 mM, where the reaction rate for the mouse tissue (9.9) was 6.5 times the mean reaction rate for the human tissue (1.5). Reaction rates from lung cytosol were also measured in the mouse, rat, hamster, and human at the 40 mM CH_2Cl_2 exposure concentration. Although the data are extremely limited (apparently representing single experiments), the reaction rate from the mouse lung microsomes (7.3) was 20 times the reaction rate for the human lung microsomes (0.4); means and standard deviations were not presented. However, the report that human lung data exhibited GST activity while not exhibiting any P450 activity may be important in interpreting toxic effects that may result from GST metabolites, since this pathway may substantially predominate in human lung tissue. The GST pathway may predominate in the lung of mice as well. Kinetic constants were calculated for the male liver tissue of only mice and humans. The KM for the mouse data (137 mM) was 3-fold greater than the human value reported (44 mM). The VMAX for the mouse data (118 mmoles product/min/mg protein) was 17-fold greater than the value reported for humans (7 mmoles product/min/mg protein).

2.2.1.4 Carbon dioxide formation Oxidation of CH_2Cl_2 to CO_2 *in vivo* has been shown by several investigators (Angelo et al 1986a,b, Rodkey and Collison 1977a, DiVincenzo and Hamilton 1975, McKenna and Zemple 1981, McKenna et al 1982, and Yesair et al. 1977). Rodkey and Collison (1977a) studied the oxidation of CH_2Cl_2 to CO_2 in the rat. When exposed to 0.2 mmol $^{14}CH_2Cl_2$/kg animal (approximately 610 ppm for the 8 liter chamber), the evolution of $^{14}CO_2$ was measured to be 29% of the administered dose. Since the metabolic CO_2 produced in respiration greatly diluted (near 1000-fold) the CO_2 produced from CH_2Cl_2, proper measurement of CO_2 requires ^{14}C labeling.

There are two likely mechanisms of carbon dioxide formation from the metabolism of CH_2Cl_2. Studies in rats have shown that formaldehyde and formic acid produced by the cytosolic glutathione transferase system can be a source of carbon dioxide (McKenna et al. 1982). Gargas et al. (1986) hypothesize the possible production of CO_2 by the P450 pathway, but the study did not measure the metabolism of CH_2Cl_2 to CO_2 and data are not presented to substantiate the claim. An unpublished study (Green et al. 1987c) submitted to the Department implies that for mice exposed to low concentrations of CH_2Cl_2, CO_2 may be produced predominantly via the P450 pathway and not the GST pathway. Their conclusion is based on the assumption that GST "metabolism involves the breaking of C-Cl bond in the initial reaction with glutathione and hence the use of CD_2CCl_2 will not affect the rate of formation of carbon dioxide from this pathway." This is contrary to the results reported by Ahmed and Anders (1978) indicating that the use of a deuterated compound would decrease CO_2 production via the GST pathway. Specifically, Ahmed and Anders (1978) reported a primary isotope effect in the formation of formic acid that was dependant on NAD. As seen in Figure 1, the primary isotope effect would be expected to occur due to the oxidation of a C-H bond of S-hydroxymethyl glutathione by formaldehyde dehydrogenase to S-formyl glutathione. The results and conclusions of the Green et al. (1987c) study are discussed further in Section 2.2.2. Carbon monoxide may be metabolized to carbon dioxide by various animal tissues (Fenn 1970) by combining with the reduced form of tissue cytochrome oxidase at low oxygen levels (Coburn 1970. See Figure 1). Although the direct metabolism of carbon monoxide to carbon dioxide is thought to be a minor pathway, the metabolic conversion of CO to CO_2 appears to be a function of the body burden of CO in dogs (Luomanmaki and

Cobun, 1969), and thus the activity of the system may be inducible by increased body levels of carbon monoxide.

2.2.2 Dose-Dependent Metabolism: Saturation

There is evidence to suggest that the metabolism of CH_2Cl_2 is saturable under certain conditions. This evidence is based on increased expiration of unchanged CH_2Cl_2 at high dose levels, decreased expiration rates of CO and CO_2 at high dose levels, and the plateauing of carboxyhemoglobin production.

An early study on the extent of CH_2Cl_2 metabolism in the rat was conducted by DiVincenzo and Hamilton (1975). After injecting rats intraperitoneally with $^{14}CH_2Cl_2$ in corn oil, they determined the fate and disposition of radioactivity in exhaled air, urine, feces, and carcasses at 1, 8, and 24 hours after single doses (412 to 930 mg/kg). Approximately 90% of the administered CH_2Cl_2 was eliminated unchanged in the exhaled air, and 85% within two hours of administration. Two percent of the administered dose was metabolized to carbon monoxide, and approximately 3% was metabolized to a volatile compound that was not identified. Overall, less than 7% of the administered dose was metabolized. When rats were intravenously given a lower dose, 200 mg/kg CH_2Cl_2, they metabolized 6% of the dose to CO and 9% of the dose to CO_2 (Angelo et al. 1986b). At even lower doses of 50 and 10 mg/kg, slightly higher rates of CO and CO_2 production were reported (Angelo et al. 1986b). Rodkey and Collison (1977a) administered approximately 17 mg/kg $^{14}CH_2Cl_2$ by inhalation in an enclosed chamber. Approximately 47% of the administered ^{14}C was recovered as ^{14}CO and about 29% as $^{14}CO_2$. Thus, in this inhalation experiment a greater precentage of CH_2Cl_2 was metabolized. Only 2% of the $^{14}CH_2Cl_2$ remained unmetabolized in the chamber after 90 minutes.

TABLE 2-2

COMPARISON OF SATURATION DATA BASED ON THE PRODUCTION OF

CARBON MONOXIDE AND CARBON DIOXIDE

Species	Exposure Method	Dose or Concentration	Expired CO Level	Expired CO_2 Level	Study
Mouse	gavage	1 mg/kg	45%	> 50%	Yesair et al 1977
Mouse	i.v.[a]	10 mg/kg	15%	20%	Angelo et al 1986a
Mouse	i.v.	50 mg/kg	8%	14%	Angelo et al 1986a
Mouse	gavage	50 mg/kg	11%	18%	Angelo et al 1986a
Mouse	gavage	100 mg/kg	20%	25%	Yesair et al 1977
Mouse	gavage	500 mg/kg	9%	18%	Angelo et al 1986a
Mouse	gavage	1000 mg/kg	7%	19%	Angelo et al 1986a
Rat	i.p.[b]	412-881 mg/kg	2%	3%	DiVincenzo et al 1975
Rat	gavage	1 mg/kg	31%	35%	McKenna et al 1981
Rat	i.v.	10 mg/kg	12%	15%	Angelo et al 1986b
Rat	inhalation	17 mg/kg	47%	29%	Rodkey et al 1977a
Rat	i.v.	50 mg/kg	9%	10%	Angelo et al 1986b
Rat	gavage	50 mg/kg	6%	11%	McKenna et al 1981
Rat	gavage	50 mg/kg	16%	17%	Angelo et al 1986b
Rat	gavage	200 mg/kg	6%	9%	Angelo et al 1986b
Rat	inhalation	50 ppm	27%	26%	McKenna et al 1982
Rat	inhalation	500 ppm	18%	23%	McKenna et al 1982
Rat	inhalation	610 ppm	47%	29%	Rodkey et al 1977a
Rat	inhalation	1500 ppm	13%	10%	McKenna et al 1982

[a]The abbreviation i.v. refers to intravenous administration.

[b]The abbreviation i.p. refers to intraperitoneal administration.

McKenna and Zempel (1981) reported that rats dosed at 1 mg/kg metabolized 88% of the administered dose, whereas those given 50 mg/kg only metabolized 28%. In the same study, rats given 50 mg/kg CH_2Cl_2 exhibited a substantial decrease in the rate of CO and CO_2 production compared to the 1 mg/kg dose. The kinetics of pulmonary elimination of unmetabolized CH_2Cl_2 and of the metabolites carbon monoxide and carbon dioxide were reportedly first-order (essentially unaffected by the dose) despite large differences in the amounts of CH_2Cl_2 and its metabolites excreted. As summarized in Table 2-2, doses of 50 mg/kg or greater, administered to the rat, appear to be metabolized to a lesser extent than lower doses based on the decreased relative percentage of CO and CO_2 eliminated. This decreased elimination of CO and CO_2 implies saturation. Some indication of saturation of the P450 pathway was provided in studies of carboxyhemoglobin formation in rats following intraperitoneal injection (Kubic et al. 1974). Administration of 125, 250 and 500 mg/kg CH_2Cl_2 produced peak carboxyhemoglobin levels of 5, 8 and 9%, respectively. At the higher doses the peak levels came later, and the area under the dose-response curve indicates a greater response at the 250 and 500 mg/kg levels than the simple peak percent carboxyhemoglobin. Based on this study saturation would be expected at levels near 250 mg/kg for the rat.

As indicated in Table 2-2, the data for the mouse is similar to that produced for the rat, and similar trends are seen across routes. There is considerable variability from experiments on the concentration or dose saturating CO production. Furthermore, P450 saturation estimates based soley on CO production, instead of direct measurements of P450 activity, ignore the possibility of P450 inactivation by increasing production of reactive metabolites as the concentration increases. In a preliminary report, Yesair

et al. (1977) stated that the 100 mg/kg dose was metabolized by mice at 20 μmole/hr/kg, but the information in the abstract was never published as a complete report.

Evidence suggests that the CH_2Cl_2 metabolism may become saturated at inhalation exposure concentrations of 500 ppm and above (some have suggested levels as low as 200 ppm). When rats were exposed for 6 hours to 50, 500, and 1500 ppm CH_2Cl_2 there was a substantial decrease in CO and CO_2 production at the 1500 ppm level (McKenna et al. 1982). When rats were exposed for 6 hours to 500 or 1500 ppm CH_2Cl_2 the carboxyhemoglobin levels did not increase beyond the 500 ppm exposure level (McKenna et al. 1982). Rabbits do not exhibit signs of metabolic saturation of P450 at CH_2Cl_2 exposure levels up 11,000 ppm. A linear relationship between the change in % carboxyhemoglobin in blood resulting from increased CH_2Cl_2 inhalation was shown to occur in rabbits over the range tested of 1,270 to 11,520 ppm (Roth et al. 1975). Inhalation data for the mouse indicating percent CO and CO_2 production for the mouse has not been reported. However, an unpublished study by Green et al. (1987c), submitted to DHS, suggests saturation may occur near 500 ppm for the mouse.

Rodkey and Collison (1977b) investigated the relationship between administered dose and the extent of metabolism by the rat. Animals were exposed by inhalation to doses ranging from 80 to 800 μmol/kg (approximately 244 to 2440 ppm for the 8 liter chamber). They reported that the rate of CO production was virtually independent of CH_2Cl_2 dose, and that it suggested the metabolism of CH_2Cl_2 to carbon monoxide in rats was rate-limited by enzyme saturation The rate was approximated at the highest dose level to be 23 μmol/hour/kg body weight.

In another study, rats were exposed for 4 hours to concentrations up to 2000 ppm CH_2Cl_2 and some of the animals were pretreated with 2,3-EP, an hepatic glutathione depleting agent (Gargas et al. 1986). When naive animals were exposed to CH_2Cl_2, the carboxyhemoglobin level peaked with the 500 ppm exposure level. For rats pretreated with 2,3-EP, the carboxyhemoglobin levels did not peak until the 1000 ppm CH_2Cl_2 exposure level and the carbon monoxide yield attained was 43% greater than the level attained in the naive animals. That is, when the P450 pathway was supposedly saturated, addition of a cytosolic pathway inhibitor (2,3-EP), increased metabolism by the P450 pathway. One possible explanation for this result is that complete saturation of the MFO pathway had not occurred, but had simply reached some steady state.

Thus, although saturation of the P450 pathway is likely to occur at high doses, the precise exposure concentration has not been adequately defined. Information of this saturation concentration only exists for rats, and the saturation response based on carboxyhemoglobin formation may not be reliable based on the complicated mechanisms involved in carboxyhemoglobin formation and on the conflicting results produced in induction and inhibition studies discussed in Section 2.2.3.

DiVincenzo and Kaplan (1981a) evaluated the conversion of CH_2Cl_2 to carboxyhemoglobin (COHb) and CO in man for single exposure levels of 50, 100, 150, and 200 ppm for 7.5 hours and of 100, 150, and 200 ppm for 7.5 hours daily for 5 consecutive days. Exposure to 50, 100, 150, or 200 ppm of CH_2Cl_2 produced peak blood carboxyhemoglobin saturations of 1.9, 3.4, 5.3, and 6.8%, respectively, and produced net excretion levels of CO of 1.32, 2.66, 4.95, and 6.12 (mmol), respectively. The study concluded that CO concentrations were

directly proportional to the magnitude of CH_2Cl_2 exposure. Thus, the uptake of CH_2Cl_2, the exhalation of CO, the carboxyhemoglobin levels all increased proportionally with the dose, and saturation of the P450 pathway did not occur in concentrations up to 200 ppm in humans.

In an ealier human study volunteers were exposed to 213 or 514 ppm CH_2Cl_2 for 1.0 hour (Stewart et al., 1972b). The carboxyhemoglobin levels peaked at 2.4 in the one volunteer exposed to 213 ppm, while the levels ranged from 1.8 to 4.6 in the eight subjects exposed to 514 ppm. Based on this study it is difficult to determine whether saturation of the P450 metabolism occurred at 514 ppm, particularly since the carboxyhemoglobin levels are below those achieved by DiVincenzo and Kaplan (1981a) and humans exhibit considerable variability in this response.

In another study of healthy human male volunteers the kinetics of CH_2Cl_2 at exposures of 200 and 350 ppm during two 6-hour periods were compared (McKenna et al. 1980). No data were presented in the abstract but, the authors concluded that the metabolism of CH_2Cl_2 was dose-dependent, and that the data were consistent with metabolism by a Michaelis-Menten mechanism. Since the data were never reported in an abstract or in a subsequent publication, staff believe the conclusions made by the authors should not be considered in the evaluation of CH_2Cl_2 kinetics.

Some measurements of expired $^{14}CO_2$ have implied saturation of the cytosolic pathway at high doses in rats (McKenna and Zempel 1981, McKenna et al. 1982, Angelo et al. 1986b) and in mice (Angelo et al. 1986a, Yesair et al. 1977) following inhalation, oral and intravenous dosing. Evidence for the potential

saturation of CO_2 pathways is presented in Table 2-2. The expired level of CO_2 generally follows that of CO. Thus, based on production of CO_2, saturation of metabolic pathways producing CO_2 appears to follow the same trend as CO. Gargas et al. (1986), using male Fisher-344 rats in closed atmosphere gas uptake studies, measured formation of carboxyhemoglobin in the presence and absence of pyrazole to inhibit the microsomal pathway. Using the brominated methanes CH_2Br_2 and CH_2BrCl, they observed that the glutathione S-transferase pathway showed no indication of saturation at inhaled concentrations of up to 2,000 ppm based on plasma bromide concentrations. Since some carboxyhemoglobin was produced in the pyrazole-inhibited rats, another implication of the study is that CO may be produced to a small extent by the cytosolic or other non P450 pathway, since the study reported that after pyrazole was used, bromide ion was only released from the GSH-dependent pathway. However, since halide measurements were not made for CH_2Cl_2 and CO_2 was not measured in the study, conclusions regarding CO_2 production and cytosolic pathway activity are only hypothetical at this time. Furthermore, pyrazole not only inhibits MFO, but it also inhibits a variety of other metabolic systems including thyroid function and alcohol dehydrogenase (Szabo et al. 1978, Cornell et al. 1983). Thus, the decrease in production may have resulted from inhibition of numerous metabolic processes and not strictly MFO.

An unpublished study (Green et al. 1987c) submitted to DHS implies that for mice exposed to low concentrations of CH_2Cl_2, CO_2 may be produced predominantly via the P450 pathway and not the GST pathway. Deuterium isotope studies using CD_2Cl_2 reportedly produced CO_2 at a slower rate than CH_2Cl_2 at 100 and 500 ppm exposure concentrations. Green et al. assume that this slower rate is completely due to CO_2 metabolism via the P450 pathway, but literature

citations are not provided to substantiate this assumption. Since the rate of CO_2 production was not slowed as greatly as CO production, Green et al. calculated that 62% of the CO_2 production was via the P450 pathway at 100 ppm, 36% at 500 ppm and 10% at 4000 ppm. These calculations require that metabolism via the GST pathway would not be affected in any way by the use of CD_2Cl_2. This unpublished work is the only study that suggests the P450 pathway produced substantial quantities of CO_2 as well as CO. The results would be more convincing if glutathione depletion studies had been conducted which showed a substantial decrease in CO_2 production. The Green et al. (1987c) study disagrees with the published studies by Kubic and Anders (1978) which concluded that CO_2 production by P450 was minor, near 1%, when compared to the CO production. Furthermore, the basic assumption that the GST pathway would be unaffected by the use of a deuterated compound ignores the results reported by Ahmed and Anders (1978) which showed a primary isotope effect in the formation of formic acid that was dependant on NAD. This isotope efffect would be expected from the oxidation of a C-H bond of S-hydroxymethyl glutathione by formaldehyde dehydrogenase to S-formyl glutathione. Green et al. (1987c) conclude that the GST pathway is becoming saturated for rats in the 500 to 4000 ppm range because the 8-fold increase in concentration is only accompanied by a 2-fold increase in CO_2 production rate. However, the authors reject the possibility of GST pathway becoming saturated for mice even though an 8-fold increase in exposure concentration (500 to 4000 ppm) resulted in less than a 3-fold increase in CO_2 production rate. According to the authors, the less than 3-fold increase for mice shows "little or no evidence of saturation" while the 2-fold increase for rats indicates "it is becoming saturated." This distinction appears unwarranted. Although the studies are of great interest with regard to the underlying mechanism of CH_2Cl_2

carcinogenicity, their preliminary nature, their disagreement with other published results and the absence of impartial review of the methods, results and discussion make it difficult to place great weight on the study.

In summary, the metabolism of CH_2Cl_2 appears to be dose-dependent in the rat and mouse (EPA 1985a). In these species, saturation of metabolic capacity with zero-order kinetics appears to occur above 50 mg/kg orally and above 500 ppm from prolonged inhalation of CH_2Cl_2. At doses of 1 mg/kg orally (Yesair et al. 1977) or 50 ppm by inhalation (McKenna et al. 1982), 95% was metabolized. For humans, exposure to concentrations up to 200 ppm have not produced saturation, and at 200 ppm up to 95% of the absorbed dose may be metabolized (DiVincenzo and Kaplan 1981a).

2.2.3 Induction and Inhibition of Metabolism

Methylene chloride was shown to induce its own metabolism (based on the formation of carboxyhemoglobin) following repeated administration in rats (Kubic et al. 1974, Anders et al. 1977). An earlier study showed that repeated exposure of rats to CH_2BrCl had increased the rate of dehalogenation (Heppel and Porterfield 1948). Angelo et al. (1986a, b) noted an apparent increase from day 1 to day 14 in CH_2Cl_2 content in various tissues of rats and mice following repeated oral dosing. The authors concluded that neither pharmacokinetic nor metabolic induction occurred but a statistical analysis of the excretion data was not reported (Angelo et al. 1986a, b). Examination of the data by DHS staff indicate that for both rats and mice CO and CO_2 excretion rates were elevated at the doses up to 500 mg/kg. At the 1000 mg/kg

level for mice the CO and CO_2 excretion rates appeared to be substantially decreased, which may be indicative of metabolic inactivation.

The nature of the induction is unclear since short-term exposure of exposed rats to CH_2Cl_2 at 500 and 1,000 pm for 6 hours per day did not produce any "consistent changes" in P450 content of liver microsomes at day 5 and 10 of exposure (Kurppa and Vainio 1981). However, at day 5 the P450 content was elevated for the three exposure groups and the elevation of the 500 ppm group was statiscally significant compared to the control group. At day 10 the P450 content was elevated in all groups compared to the 5-day control group, but exposure groups were not different from the 10-day control group. The P450 content of kidney microsomes was elevated at day 5 and 10 of exposure and in two groups the elevation was statistically significant. In the same study, glutathione levels at day 5 or 10 of exposure were elevated in renal tissue but not in hepatic tissue.

Daily exposure to CH_2Cl_2 for short periods may influence other enzyme systems (EPA 1985a). When Kurppa and Vainio (1981) exposed rats to CH_2Cl_2 at 500 and 1,000 ppm for 6 hours per day for 5 and 10 days, they reported a 35% drop in NADPH-cytochrome C and a two-fold increase in UDP-glucuronosyltransferase. Heppel and Porterfield (1948) exposed adult male rats to an atmosphere of 1000 ppm CH_2BrCl for 6 to 7 hours per day, 5 days per week for 4 to 6 weeks. They reported an approximate 1.6-fold increase in the rate of enzymatic hydrolysis of the bromide ion from CH_2BrCl in a crude liver homogenate of exposed rats as compared to unexposed control rats. However, under their assay conditions, it is not possible to determine if enzymes of the MFO, glutathione, or both pathways were induced. Pritchard et al. (1982) administered CH_2Cl_2 to male

mice for 3 days by gavage (doses of 5, 50, 100, 250, 500, and 1,000 mg/kg in corn oil) and found no significant changes in hepatic weight, microsomal protein, P450, cytochrome b_5 content, or activities of aminopyrene N-demethylase or biphenyl 4-hydroxylase.

Pretreatment with 3-methylcholanthrene or phenobarbital, did not increase the formation of carboxyhemoglobin following CH_2Cl_2 dosing (Miller et al. 1973, Kubic et al. 1974, Hogan et al. 1976, Anders et al. 1977). Although Hogan et al. (1976) state that phenobarbital pretreatment induced CO production, and colbaltous chloride reduced CO production, the data were not provided in the abstract and a complete report has not been published. Roth et al. (1975) examined the metabolism of CH_2Cl_2 to carboxyhemoglobin in rabbits. Pretreatment with phenobarbital inhibited the formation of carboxyhemoglobin from CH_2Cl_2 exposure (1400 to 1500 ppm for 20 minutes); however, P450 activity itself was shown to be induced by the pretreatment. A study by Miller et al. (1973) also reported (as an abstract) no effect on carbon monoxide production from CH_2Cl_2 following phenobarbital pretreatment (Miller et al. 1973).

The administration of a known inhibitor of P450 metabolism, SKF 525-A, did not decrease carboxyhemoglobin formation significantly (Miller et al. 1973, Kubic et al. 1974). As noted above, phenobarbital inhibited carboxyhemoglobin formation in rabbits (Roth et al. 1975). One study, using a pyrazole pretreatment decreased carboxyhemoglobin formation in rats exposed to CH_2Cl_2 (Gargas et al. 1986). Pyrazole has been shown to inhibit thyroid function and alcohol dehydrogenase (Szabo et al. 1978, Cornell et al. 1983). Numerous experiments have shown that administration of ethanol blocks the formation of carboxyhemoglobin from CH_2Cl_2 exposure in guinea pigs, monkeys, and rats

(Balmer et al. 1976, Ciuchta et al. 1979, Glatzel et al. 1987, Pankow et al. 1985), but the currently suggested scheme for CH_2Cl_2 metabolism does not account for this interaction. However, blockage of the P450 pathway is likely to result in increased metabolism by the conjugation pathway. Thus, persons consuming alcohol may be at greater risk to the chronic effects of CH_2Cl_2 induced by metabolites of the conjugation pathway.

Human exposure studies (Divincenzo and Kaplan, 1981b) indicate that exercise increased carboxyhemoglobin levels and the amount of CO exhaled. In the one volunteer where CO and carboxyhemoglobin levels were reported, the extent of CO production increased from moderate to heavy exercise but the carboxyhemoglobin levels did not increase. This result indicates that although CH_2Cl_2 P450 metabolism increased carboxyhemoglobin levels did not reflect the increase in metabolism and therefore may not be a sensitive indicator of either CH_2Cl_2 metabolism or CO production in humans. Consequently, although CO production is consistent with P450 metabolism experiments, measuring only the formation of carboxyhemoglobin may not accurately reflect P450 metabolism. The results are consistent with CH_2Cl_2 acting directly on hemoglobin at high concentrations. Possibly at high concentrations CH_2Cl_2 decreases the affinity of heme for CO, and reduces the cooperativity of binding of hemoglobin subunits (Nunes and Schoenborn 1973, Settle 1975, Harkey et al. 1979); thus preventing greater formation of carboxyhemoglobin. Such a situation would explain the inability of inducers to increase carboxyhemoglobin levels in experimental animals exposed to CH_2Cl_2.

In contrast, for the related compound CH_2Br_2, the formation of microsomal cytochrome P450, and the *in vitro* production of carbon monoxide was found to be increased by *in vivo* pretreatment with pentobarbital or 3-methylcholanthrene (Kubic and Anders 1975). Another set of experiments where CH_2Br_2 was injected indicated that pretreatment of animals with phenobarbital or 3-methylcholanthrene resulted in significant increases in carboxyhemoglobin levels (Stevens et al. 1980). Pretreatment with SKF 525-A resulted in a significant decrease in carboxyhemoglobin levels (Stevens et al. 1980). And pretreatment with diethyl maleate decreased blood CO levels from CH_2Br_2 exposure. Using an isolated rat hepatocyte system, diethyl maleate, SKF 525-A and ethanol inhibited the production of CO from CH_2Br_2 (Stevens et al. 1980). Stevens et al. (1980) pointed out that their results with CH_2Cl_2 were in contrast to those obtained for CH_2Cl_2 in the literature. Cobaltous chloride has also been found to reduce microsomal carbon monoxide production from CH_2Br_2 exposure (Kubic and Anders 1975).

The cytosolic enzyme system is a glutathione transferase system that requires no cofactors other than glutathione. Metabolism of CH_2Cl_2 by this pathway was not induced by phenobarbital or by a 4-day repeated exposure to CH_2Cl_2 (Ahmed and Anders 1976). In contrast, for a related compound CH_2BrCl the cytosolic system appears to have been induced following a 4 to 6-week inhalation exposure (Heppel and Porterfield 1948).

In conclusion, the metabolic induction and inhibition studies of CH_2Cl_2 to CO by P450 do not fit the simplistic scheme presented in Figure 1. Standard inhibitors and inducers of P450 do not affect the production of CO from CH_2Cl_2 as one would expect. Metabolism of a compound similar to CH_2Cl_2, CH_2Br_2, does

respond to metabolic inhibitors and inducers as one would expect, that is, in a manner different from CH_2Cl_2. Thus, results for studies with CH_2Br_2 or other dihalomethanes may not reflect CH_2Cl_2 metabolism. The inability to account for the metabolic results reported for CH_2Cl_2 makes it difficult to conclude that the metabolic scheme has been completely elucidated.

2.3 Physiologically based pharmacokinetic model

Andersen et al. (1987) have proposed a physiologically-based pharmacokinetic model (PBPK) to describe the metabolism of methylene chloride. Using the information on the relative metabolic contributions of the two metabolic pathways, the PBPK model is supposed to offer an estimate of the potential target tissue dose in comparison to the conventional risk assessment methods based on total dose to the subject. Since conventional risk assessments often involve linear extrapolations of external doses (combined with inter-species factors based on body surface areas), Andersen et al. (1987) proposed that their PBPK model might permit the calculation of realistic internal doses for various chemicals through integration of known information on the administered doses, the physiological structure of the mammalian species, and the biochemical properties of the specific chemicals.

Andersen et al. (1987) recommend that their model be used to estimate "internal doses" of CH_2Cl_2 before calculating the possible risks of CH_2Cl_2 to humans. The general approach appears reasonable, although DHS staff express concern over the increasing use of extremely complex mathematical models in risk assessment. Such models are often surrounded by a sophisticated mathematical or computerized approach that may tend to hide key assumptions

made in the model. Furthermore, since data to satisfy all the needs of the model are usually lacking, numerous assumptions need to be made about the data, data representing a single experimental result are often incorporated in the models, and unpublished results are often used without external laboratory validation. And finally, data is continually being sought to "update" and "improve" such models. As a result, extensive review and reevaluations are required to assess the usefulness of the most current model. An intensive analysis of the model has been conducted by the U. S. Interagency Hazard/Risk Assessment Committee (HRAC) of the Integrated Chlorinated Solvents Project and a review draft report has been circulated by EPA (1987a). In addition to the comments made in the DHS health assessment the HRAC report could be consulted for further analysis. The Andersen et al. (1987) physiologically based-pharmacokinetic model is more fully described and discussed in Section 8 and Appendix E.

2.4 Covalent Binding to Cellular Macromolecules

The ability of a chemical to bind covalently to cellular macromolecules provides an indication of its potential genotoxicity and carcinogenicity. The likelihood of significant covalent binding of reactive metabolites of CH_2Cl_2 to cellular macromolecules depends on the postulated reactive intermediates (EPA 1985a): formyl chloride from microsomal oxidative metabolism and S-chloromethyl glutathione from cytosolic metabolism. These compounds would be expected to be capable of acylating cellular nucleophiles, and S-chloromethyl glutathione is structurally similar to the very reactive bis-halomethyl ethers, which are known carcinogens.

The extent and pattern of binding to microsomal lipid and protein after aerobic incubation of rat hepatic microsomes with $^{14}CH_2Cl_2$ were studied by Anders et al. (1977). They found that metabolites of CH_2Cl_2 bound covalently to microsomal protein and lipids under conditions optimized for metabolism of CH_2Cl_2 to carbon monoxide. Pretreatment of rats with phenobarbital to induce the mixed-function oxidase system resulted in increased binding by microsomes, suggesting that the formyl chloride intermediate may acylate macromolecules.

Cunningham et al. (1981) studied the comparative covalent binding of metabolites of CH_2Cl_2, carbon tetrachloride, and trichloroethylene to lipids and proteins in rat hepatocytes. They found that binding of CH_2Cl_2 metabolites was enhanced in the presence of oxygen, which is consistent with formation of formyl chloride, and that glutathione-depleted hepatocytes showed a marked drop in binding of labelled CH_2Cl_2 metabolites to both lipids and protein, suggesting inhibition of cytosolic glutathione conjugation with decreased production of S-chloromethyl glutathione and formaldehyde. Although they observed that <u>in vitro</u> administration of $^{14}CH_2Cl_2$ did not result in alkylation of RNA and DNA under the conditions of the experiment, they did not know the limits of detection of this assay system (Sipes 1987).

This work demonstrates that highly reactive intermediates are formed in the metabolism of CH_2Cl_2, from both the microsomal and cystolic pathways and is consistent with the proposed reaction pathways.

2.5 Summary and Conclusions

Limited data are available on the metabolism and pharmacokinetics of CH_2Cl_2 in humans, but there is extensive kinetic information in rats. Absorption from the gastrointestinal tract is rapid and complete, and occurs by first-order processes in the rat. Dermal absorption is very slow for CH_2Cl_2. Pulmonary absorption also occurs by first-order processes in humans and rats. CH_2Cl_2 is distributed throughout the body by virtue of its water solubility; it readily gains access to cells and accumulates in adipose tissues by virtue of its lipid solubility; and it is known to cross the blood-brain barrier and the placenta. The major processes of elimination of CH_2Cl_2 from the body are pulmonary exhalation of unchanged CH_2Cl_2, and pulmonary exhalation of metabolized CH_2Cl_2. Pulmonary elimination follows first-order kinetics, which suggests that there are at least three body-storage compartments.

Hepatic metabolism of CH_2Cl_2 occurs via two major enzymatic pathways. One is a cytochrome P450-mediated microsomal pathway where CH_2Cl_2 undergoes oxidative dehalogenation to carbon monoxide which can produce carboxyhemoglobin. Some evidence suggests possible production of CO_2 by this pathway as well. The other is a glutathione-S-transferase mediated dehalogenation cytosolic pathway, yielding formaldehyde and formic acid. These reaction products may be further metabolized to carbon dioxide. The relative utilization of the two pathways at low body burdens of CH_2Cl_2 is unclear, although some evidence indicates the P450-mediated pathway may be favored at low doses. Evidence indicates that CH_2Cl_2 metabolism is saturable at high concentrations. Both pathways appear saturable based on CO and CO_2 levels produced.

Carboxyhemoglobin levels in the blood of humans and animals increase as a consequence of metabolism of CH_2Cl_2 to carbon monoxide. The level of carboxyhemoglobin in the blood may be affected dose of CH_2Cl_2, first-order kinetics of pulmonary elimination of carbon monoxide, and direct effects of CH_2Cl_2 on hemoglobin. Experimental results indicate that the carbon monoxide pathway saturates above 500 ppm in rats and possibly in humans; comparable evidence in mice is not available. The glutathione pathway shows comparable evidence of saturation in rats based on CO_2 production. Some indirect evidence indicates an absence of saturation of the pathway.

Some studies indicate the CH_2Cl_2 may induce its own metabolism. However, traditional inducers such as phenobarbital and 3-methylcholanthrene do not induce any CH_2Cl_2 metabolism. Glutathione depleters decrease cytosolic metabolism, but tradition P450 inhibitors, such as SKF 525-A, do not inhibit P450 metabolism of CH_2Cl_2. Ethanol inhibits formation of carboxyhemoglobin from CH_2Cl_2. Consequently, the basic metabolic pathways have been established, but complex interactions within the pathways of metabolism of CH_2Cl_2 remain unclear due to unexpected (yet consistent) results obtained in induction and inhibition experiments.

3.0 ACUTE TOXICITY

3.1 Human Health Effects

Limited human data are available on the effects of acute exposures to CH_2Cl_2 vapors. A number of case reports have implicated exposures to exceedingly high levels of CH_2Cl_2 as one factor in human fatalities (Moskowitz and Shapiro 1952, Stewart and Hake 1976). The concentrations and durations of such exposures are not known and thus one cannot derive a dose-response relationship. Miller et al. (1985) reported a case of acute tubular necrosis in a 19-year-old male exposed to methylene chloride for two days in poorly ventilated rooms. Exposure estimates were not made. Acute renal failure, myoglobinuria, hypocomplementemia and elevations of liver enzymes were observed. The patient reportedly recovered after two weeks of hospitalization. Buie et al. (1986) described a case of diffuse pulmonary edema and pleural effusions in a 34-year-old male using furniture stripper containing methylene chloride. Horowitz (1986) reported a case of carboxyhemoglobinemia in a 35-year-old male who was a recurrent paint sniffer.

Experimental studies on human exposure to high concentrations of CH_2Cl_2 suggest that it depresses the central nervous system and alters behavior. Stewart et al. (1972b) reported that humans developed symptoms of "lightheadedness" and had difficulty with speech articulation following exposure to CH_2Cl_2 concentrations greater than 868 ppm for more than 1 hour. Subsequent experiments measured a decreased ability of human subjects exposed to concentrations greater than 300 ppm to perform a series of tasks, including

hand-eye coordination and auditory signal detection (Winneke 1974, Winneke and Fodor 1976, Fodor and Winneke 1971, Putz et al. 1976). Experimental and occupational exposure studies do not provide evidence that CH_2Cl_2 affects other organ systems following inhalation (Stewart et al. 1972 a,b, Gamberale et al. 1975, Ott et al. 1983 a,c,d). In comparison to other species, humans appear to be at least as sensitive, if not more sensitive, to the acutely toxic effects of CH_2Cl_2.

3.2 Animal Studies

Median lethal concentrations (LC_{50} values) for rats and guinea pigs exposed for 6 hours to CH_2Cl_2 vapors are approximately 16,000-18,000 ppm (Berger and Fodor 1968) and 11,500 ppm (Balmer et al. 1976), respectively. The LC_{50} values for mice exposed for 6 and 7 hours were 14,100 ppm (Gradiski et al. 1974) and 16,100 ppm (Svirbely et al. 1974), respectively. An LC_{50} value of 17,703 ppm was estimated for male B6C3F1 mice exposed for 4 hours (NTP 1986). A 30-minute LC_{50} for rats was reported to be approximately 25,400 ppm (Bakhishiev 1975).

Studies involving short-term exposures of experimental animals to CH_2Cl_2 vapors indicate that the central nervous system is the primary target organ. The nervous system appears to be affected at dose levels as low as 500 ppm.

Fodor and Winneke (1971) investigated the effects of CH_2Cl_2 inhalation on rapid eye movement (REM) sleep, sleeping time, and EEG patterns in rats during continuous sleep period exposure (approximately 13 hours). A concentration-dependent continuum of effects was observed. The duration of REM sleep was

reduced beginning at approximately 500 ppm. At 3,000 and 9,000 ppm, sleeping time increased, with a further decrease in REM sleep (Berger and Fodor 1968). Higher concentrations (27,000 ppm for 1.5 hours or 17,000 ppm for 6 hours) resulted in coma and subsequent brain death (Berger and Fodor 1968). Other investigators have reported that high airborne concentrations of CH_2Cl_2 (>1000 ppm) depress the nervous system and alter the behavior of laboratory animals (NTP 1986, Heppel and Neal 1944, Thomas et al. 1971, Weinstein et al. 1972).

Exposures to high levels of CH_2Cl_2 vapor also produce adverse effects on the liver, eyes, respiratory system, and cardiovascular system. Changes in cardiovascular function in rabbits and dogs (e.g., cardiac output and stroke volume) have been reported following short-term exposures (≥ 5 minutes) to concentrations of CH_2Cl_2 exceeding 5,000 ppm (Taylor et al. 1976, Aviado et al. 1977). Short-term exposure (for 5 minutes or less) of animals to more than 5,000 ppm CH_2Cl_2 caused cardiac arrhythmias (Aviado 1975, Aviado et al. 1977, Zakhari 1977).

Hepatic effects (e.g., increased liver triglycerides, changes in liver weight, fatty infiltration) have been reported in guinea pigs and mice following continuous inhalation exposure to approximately 5,000 ppm for 24 hours (Morris et al. 1979, Weinstein et al. 1972).

The reaction product of methylene chloride with GSH seems to be implicated as the causative factor for acute toxicity in mice. Chellman et al. (1986) showed that depletion of glutathione with GSH inhibitors diminished acute liver toxicity as well as kidney and central nervous system toxicity that would have been expected from a single 6-hour exposure to 1500 ppm methylene

chloride. Pretreatment with GSH inhibitors also increased the LC_{50} from 2200 to 3200 ppm in male mice. Berger and Sozeri (1987) examined the effects of methylene chloride and other halogenated hydrocarbons on isolated rat hepatocytes. Methylene chloride (20 mM 1-hour incubation) caused only a very modest increase in the release of an asparate aminotransferase from the hepatocytes, and a reversible depression in cellular oxygen utilization. The effects caused by methylene chloride were much less than those of the more hepatotoxic solvents such as chloroform and carbon tetrachloride (Berger and Sozeri 1987).

Aranyi et al. (1986) examined the effects of inhaled methylene chloride or murine lung host susceptibility to laboratory-induced respiratory infections. A single three-hour exposure to 100 ppm CH_2Cl_2 significantly ($p<0.001$) reduced pulmonary bacteriocidal activity. It was hypothesized that inhaled methylene chloride can prolong bacterial viability in the lung and enhance severeness of disease, in part because of reduced macrophage function (Aranyi et al., 1986).

3.3 Summary and Conclusions

Methylene chloride has a relatively low acute toxicity. The LC_{50} for mice, rats, and guinea pigs is greater than 10,000 ppm for 7-hour exposures. Animal and human studies suggest that the CNS is the principal target organ resulting in CNS depression.

Human studies have shown a decrease in hand-eye coordination from exposures to 300 ppm for up to 3 hours, while animal studies show a decrease in sleep as measured by EEG and rapid eye movement from a continuous 500 ppm exposure of

CH_2Cl_2 beginning at approximately 50 to 100 minutes in the sleep cycle. Because of these diverse endpoints, it is not possible to precisely define a toxicological threshold. However, based on the observed concentrations for these effects, no adverse effects are expected from ambient CH_2Cl_2 concentrations.

4.0 SUBCHRONIC AND CHRONIC TOXICITY

4.1 Effects on Humans

There have been relatively few reports of subchronic or chronic toxic effects resulting from exposure to CH_2Cl_2. It is often unclear whether the reported toxicities were due to the direct action of CH_2Cl_2, to the action of one or more of its major metabolites or to a combination of these.

In a series of studies designed to evaluate the effects of inhaling CH_2Cl_2 on carboxyhemoglobin levels in the blood of normal volunteers, Stewart and colleagues (Hake et al. 1974, Forster et al. 1974) and Peterson (1978) found carboxyhemoglobin levels ranging from control levels of 0.9% (males) and 1.4% (females) to 9.6% (males) and 10.1% (females) in subjects exposed to 250 ppm (869 mg/m^3) for 5 successive days. No other adverse effects were noted in a summary of the studies (EPA 1985a).

Several case reports of toxicity resulting from occupational exposure to CH_2Cl_2 exist. However, concurrent exposure to other chemicals make interpretation difficult. Reported effects included encephalopathy, eclampsia, and bilateral temporal lobe degeneration (Weiss 1967, Collier 1936, Barrowcliff 1978, Barrowcliff and Knell 1979).

Ott et al. (1983a,c) studied the mortality experience and did clinical laboratory evaluations of employees occupationally exposed to CH_2Cl_2. No mortality effects were attributed to time-weighted exposures of 60-475 ppm

(208-1650 mg/m^3). However, these studies had a low statistical power and suffered from several significant confounding factors. The mortality data are discussed in Section 8.2.2.

Ott et al. (1983a,c) studied six serum constituents that had the potential to detect possible liver injury in relation to CH_2Cl_2 exposures. The exposed group had 313 individuals; the reference group had 321. A dose-related rise in serum bilirubin was observed for both men and women. A consistent positive association between total bilirubin and CH_2Cl_2 exposure was found in three of four subgroups by exposure; however, the relationship of this endpoint to liver damage is unclear. Women in the subgroup exposed to 475 ppm (1650 mg/m^3) CH_2Cl_2 showed an increase in red cell counts, hemoglobin, and hematocrit, but men did not. These findings are suggestive of a hematopoietic effect. The carboxyhemoglobin concentration showed an increase and a possible association with CH_2Cl_2 exposure for all four subgroup exposures. Two standard liver function tests were not performed (SGOT, SGPT), and these might have proved to be more sensitive indicators of liver function.

4.2 Effects on Experimental Animals

Subchronic and chronic toxicity reported in animals have primarily involved weight loss and effects on the central nervous system, lungs, liver and kidneys. Many reports are only briefly summarized in this section because of the poor quality of the studies in general; more complete descriptions of chronic toxic effects reported in well-controlled, long-term studies are included in Section 8.3 and Appendix B, which report the results of rodent carcinogenicity studies.

4.2.1 Subchronic Exposures

Most of the data are qualitative and deal with neurobehavioral responses of various species to CH_2Cl_2. Based upon the observations reported, it is reasonable to assume that CH_2Cl_2 behaves similarly to other anesthetics (causing an initial excitatory phase followed by a progressive depression) (EPA 1985a, Heppel et al. 1944, Heppel and Neal 1944, Weinstein et al. 1972, Thomas et al. 1972, Savolainen et al. 1977). The lower threshold for excitatory central nervous system responses for subchronic exposures may be around 1,000 ppm (3,474 mg/m^3).

Kim and Carlson (1986) exposed both rats and mice to 200, 500 or 1000 ppm CH_2Cl_2 for either eight hours/day for five days or ten hours per day for four days. Carboxyhemoglobin levels were not significantly higher for the animals exposed for ten hours daily than for those exposed eight hours daily, although for both groups the levels were significantly greater than those of controls. The peak blood levels of CH_2Cl_2 were dependent on the duration of exposure, but the half-life was independent of both exposure duration and concentration. In extrapolating their findings to the human work place, the authors concluded "that the exposure limit for a chemical with a short biological half-life and readily reversible toxic effect may not need to be adjusted for a longer workshift" (Kim and Carlson 1986).

A three-month continuous exposure to 210 ppm CH_2Cl_2 was found to reduce DNA concentrations in the hippocampus of gerbil brains (Karlsson et al. 1987). No changes in protein concentrations in different brain regions were noted. The significance of these effects are not known. An earlier study had shown an

increase in concentrations of the astroglial proteins S-100 and GFA in the frontal and sensory motor cerebral cortex of gerbils following a ten-week continuous exposure to 350 ppm of CH_2Cl_2 (Rosengren et al. 1986).

The NTP (1986) 13-week study exposed groups of 10 rats and 10 mice of each sex to five concentrations of CH_2Cl_2 (525, 1050, 2100, 4200 and 8400 ppm) for 6 hours per day, 5 days per week. This study found that rats developed foreign body pneumonia (foci of mononuclear and multinucleated giant cells), although lung lesions were not reported in mice. Mortality of 10% in male and female rats (1/10) and depression of final mean body weight were also noted at the highest exposure level (8,400 ppm); mice exposed to the same concentration had 40% (4/10) mortality in males and 20% (2/10) in females. Final mean body weight of female mice at this exposure level was depressed compared to controls.

4.2.2 Chronic Exposures

The liver is a primary target organ of animals chronically exposed to high levels of CH_2Cl_2. Histopathological changes have been reported in the liver of animals chronically exposed to high levels of CH_2Cl_2. These changes may be related to deaths observed in various studies. For example, Heppel et al. (1944) reported moderate centrilobular congestion and fatty degeneration of the liver in dogs and guinea pigs exposed to 10,000 ppm (34,740 mg/m^3) for 4 hours per day, 5 days per week, for 8 weeks. Identical findings were reported by Weinstein et al. (1972) after mice were exposed to 5,000 ppm (17,370 mg/m^3) continuously for 7 days. At lower exposure levels, Weinstein and Diamond (1972) found elevated triglycerides, centrilobular fat accumulation, and

decreased liver glycogen in ICR mice exposed continuously from 3 days to 10 weeks to 100 ppm (347 mg/m^3) CH_2Cl_2.

Haun et al. (1972) continuously exposed mice, rats, dogs, and monkeys to 25 or 100 ppm (87 or 347 mg/m^3) CH_2Cl_2 for up to 100 days. Exposure to 100 ppm resulted in fatty change in the liver and, in rats, nonspecific tubular degeneration in the kidney with evidence of regeneration. No adverse effects were observed at 25 ppm. Other hepatic effects were noted in exposed rats and mice by Norpoth et al. (1974) and the NTP (1986).

Serota et al. (1986a) exposed male and female Fisher 344 rats for 104 weeks to CH_2Cl_2 in their drinking water at concentrations of 5, 50, and 125 mg/kg/day. They observed a positive dose-related trend in both sexes for liver histomorphological liver alterations (foci and area cellular alterations) and fatty changes in both sexes at levels of CH_2Cl_2 as low as 50 mg/kg/day. The authors suggested a no-observable-effect level (NOEL) for these effects at an oral dose level of 5 mg/kg/day.

4.2.3 Summary and Conclusions

Chronic and subchronic effects observed in humans and animals occurred at very high exposure levels. On the basis of the Ott et al. (1983a-e) epidemiological data, the EPA (1983) estimated that chronic inhalation exposures of up to about 100 ppm (347 mg/m^3) may represent a no-observed-effect level (NOEL) for humans. However, this estimate was made in the absence of a frank-effect adverse-effect level for mortality. The most

sensitive animal studies (Serota et al. 1986a) suggest an oral NOEL of 5 mg/kg/day. Assuming that the toxic effect is independent of exposure route, a NOEL for humans is 5 ppm. Since these concentrations are 3 to 4 orders of magnitude greater than those present in ambient air, the adverse health effects discussed in this chapter are not expected from current exposure levels.

5.0 REPRODUCTIVE EFFECTS

The potential reproductive toxicity of CH_2Cl_2 has not been extensively evaluated. In this section, developmental toxicity will be considered along with adverse effects on embryo development as a result of exposure before conception. Reproductive toxicity has not been reported for adult males or females.

Schwetz et al. (1975) exposed pregnant Swiss-Webster mice and Sprague-Dawley rats to 1,250 ppm CH_2Cl_2 (97.9% pure) by inhalation for 7 hours per day on days 6 through 15 of gestation. In both species there was a significant increase in liver weight and in carboxyhemoglobin content in exposed dams. Exposed mice exhibited a significant increase in body weight. The only statistically significant adverse effect on the mouse fetuses was on the number of litters in which fetuses exhibited an extra center of ossification in the sternum. This common anomaly may reflect the degree of embryonic development. Rat fetuses had significantly increased incidences of delayed ossification of lumbar ribs or spurs, of delayed ossification of sternebra, and of dilated renal pelvis, which may represent a slight, reversible delay in development. Due to the presence of maternal toxicity and the use of only one dose level of CH_2Cl_2, firm conclusions regarding the adverse effects of CH_2Cl_2 on fetal development cannot be made.

Hardin and Manson (1980) exposed female Long-Evans rats to 4,500 ppm CH_2Cl_2 (97% pure) by inhalation for 6 hours per day, 7 days per week, both before and during gestation. Increased liver weights and carboxyhemoglobin levels were

observed in the mothers, while fetuses exhibited lower body weights relative to controls. No other significant effects were observed. One or two individual animals from 10 of the 20 litters used in this study were evaluated for behavioral effects (Bornschein et al. 1980). Treatment-related effects were reported for the general activity tests. However, because of the small number of animals used, the presence of maternal toxicity, and the developing nature of the behavioral toxicology field, these results are inconclusive.

Nitschke et al. (1985) exposed male and female Fischer 344 rats to 0, 100, 500, or 1500 ppm CH_2Cl_2 for 6 hours/day, 5 days/week for 14 weeks. Male and female rats (F_0) from the same exposure group were mated, and they produced F_1 offspring. Selected F_1 rats were exposed to the same concentrations of CH_2Cl_2 for 17 weeks, at which point they were mated, and they gave birth to the F_2 offspring. No adverse effects on reproductive parameters, neonatal survival, or neonatal growth were noted in animals exposed to CH_2Cl_2 in either the F_0 or F_1 generations. Similarly, there were no treatment-related gross pathologic observations in F_0 and F_1 adults or in F_1 and F_2 weanlings at necropsy. Histopathologic examination of tissues from F_1 and F_2 weanlings did not reveal any lesions attributed to methylene chloride. These findings are consistent with those of Bornmann and Loeser (1967), who reported that ingestion of CH_2Cl_2 in the drinking water did not affect the reproductive performance of female rats. Thirty female Wistar rats were given 125 ppm CH_2Cl_2 in their drinking water for 3 months. An equal number of control rats were given drinking water alone. The estrous cycles of the females, evaluated by vaginal smear tests, were unaffected by treatment.

Fertility, litter size, neonatal growth and survival were evaluated in a two-generational study of F344 rats exposed to methylene chloride during the gestation period (Nitschke et al. 1988b). Maternal and paternal exposure to 0, 100, 500, or 1500 ppm CH_2Cl_2 (six hours/day, five days/week for fourteen weeks) caused no adverse reproductive or neonatal effects in either the F_1 or F_2 generation; histopathologic examination did not reveal any lesions attributable to CH_2Cl_2. The exposure to levels as high as 1500 ppm did not appear to significantly effect reproductive parameters in mice (Nitschke et al. 1988b).

Based on the available information, inhalation of high, maternally toxic concentrations (1250-4500 ppm) by pregnant rodents throughout pregnancy can result in fetotoxicity as evidenced by reduced fetal body weight and changes in ossification of the fetal skeleton. The results to date indicate that CH_2Cl_2 is of low teratogenic potential in rodents, but no firm conclusions as to the potential human health impact of CH_2Cl_2 exposure can be drawn. Experiments performed to date have been limited in that, except for rat studies, either they employed only one dose level, utilized relatively few numbers of test animals, or demonstrated signs of maternal toxicity possibly as a result of carboxyhemoglobin formation.

An epidemiological study conducted among women in the pharmaceutical industry found that with increasing frequency of exposure to CH_2Cl_2, the odds of having a spontaneous abortion were increased, although the statistical significance was borderline (Taskinen et al. 1987). No estimation of the range of exposure concentrations was given.

6.0 GENETIC TOXICITY

6.1 Introduction

CH_2Cl_2 has been tested in a variety of *in vitro* and *in vivo* systems designed to detect gene mutations, chromosomal aberrations, and other DNA binding or damage (see Table 6-1). This section summarizes the existing data and discusses what

conclusions may be drawn. Individual studies, with references, are summarized in Appendix A.

6.2 Mutagenicity

CH_2Cl_2 has been tested in bacteria, yeast, *Drosophila*, *Panagrellus*, and cultured mammalian cells. CH_2Cl_2 showed a positive, dose-related response in *Salmonella* strains TA1535, TA98, and TA100, both in the presence and absence of a metabolic activating system, when tested as a vapor in a sealed chamber in several laboratories (see Table A-1 for references). Cytotoxicity data (survival of bacteria) and the purity of the CH_2Cl_2 were not given in most of the published reports. (However, the most common contaminants in the ppm range are phosgene and cyclohexene, neither of which is genotoxic in the Ames test.) The addition of rat liver cytosol to the assay system slightly increased the mutagenicity of CH_2Cl_2 in *Salmonella*, which is consistent with formation of the electrophilic intermediate S-chloromethylglutathione following enzyme-mediated conjugation by the glutathione-S-transferases (see Section 3) (Green 1983, Jongen et al. 1982). The EPA (1985a) stated that on the basis of data obtained from Dr. Eugene Barber of the Eastman Kodak Company as a personal

communication, the mutagenic responses at the highest dose (115 μmoles/plate) for strains TA1535, TA98, and TA100 were 0.0006, 0.006, and 0.003 revertants/μmole, respectively. These determinations were made on the basis of GC/MS measurements of CH_2Cl_2 concentrations in the bacteria-containing aqueous phase of the petri dish and the vapor-phase head space of the closed incubation chamber, and as such, reflect the actual concentrations to which the bacteria were exposed (Barber et al. 1980). These results indicate that CH_2Cl_2 is a much weaker mutagen in Salmonella than benzo(a)pyrene or 3-(2-chloroethoxy)-1,2-dichloropropene. Benzo(a)pyrene induces 121,000 revertants/μmole in Salmonella (McCann and Ames 1977), and 3-(2-chloroethoxy)-1,2-dichloropropene, a volatile chloroallyl ether, induces 74,400 revertants/μmole, based on the amount incorporated into the assay and not accounting for volatilization (Distlerath et al. 1984).

Methylene chloride has exhibited mutagenic activity in two strains of E. coli, WU361089 (tyr^-) (tested for mutation to tyrosine prototrophy) and K49, a lamda lysogenic strain (tested for prophage induction). (However, as indicated by the authors, difference in solubility and volality made it difficult to estimate the doses and thus to compare these results to the mutagenicity of other substances [Osterman-Golkar et al. 1983]). CH_2Cl_2 was not mutagenic in the B. subtilis rec assay, with or without the addition of a microsomal enzyme-activating system (Kanada and Uyeta 1978).

CH_2Cl_2 was tested for gene conversion, mitotic recombination, and reverse mutation in two strains of the yeast Saccharomyces cerevisiae without the addition of exogenous enzymes (Callen et al. 1980, Simmon et al. 1977).

TABLE 6-1

SUMMARY OF TESTS OF THE GENETIC TOXICITY OF METHYLENE CHLORIDE

System	Results		
	Positive	Negative	Equivocal
Salmonella typhimurium (vapor exposure)	X		
E. coli tyrosine prototrophy (in desiccator)	X		
B. subtilis rec assay		X	
Saccharomyces cerevisiae			X
Drosophila sex-linked recessive lethal test			X
Mammalian cell mutation (V79, CHO, L5178Y cells)		X	
Mammalian cell transformation (C3H-10T-1/2, BALB/C-3T3, F1706 cells)			X
Syrian hamster embryo (adenovirus-transformed foci (vapor exposure))	X		
Unscheduled DNA synthesis		X	
Mammalian cell cytogenetics			
Chinese hamster ovary cell (chromatid gaps, chromatid breaks)	X		
Sister chromatid exchange	X		

CH_2Cl_2 had weak effects on mutation and recombination in one strain and no effects in the other. Callen et al. (1980) observed a greater than two-fold dose-related response in Saccharomyces cerevisiae for each of three endpoints, gene conversion, mitotic recombination, and reverse mutation. These yeast strains contain endogenous cytochrome P450 and hence are capable of metabolic activation. The differences in response between the two strains may be attributable to different levels of P450 or different experimental conditions. Extending the incubation period from 1 to 4 hours eliminated the genotoxic activity of CH_2Cl_2 in the one positive strain. Since an extended incubation time usually increases mutagenicity as a result of increased metabolic activation, the positive result may have been artifactual. Alternatively, the longer incubation time may have permitted detoxication and DNA repair.

Two feeding studies and an injection study were conducted with CH_2Cl_2 to detect sex-linked recessive lethal mutations in Drosophila (Abrahamson and Valencia 1980, Gocke et al. 1981). CH_2Cl_2 was negative in the injection study and in one of the feeding studies, but it was weakly mutagenic at very high doses in one of the three broods tested in the other feeding study. Several mammalian cell culture systems have been used to test the mutagenic potential of CH_2Cl_2. Jongen et al. (1981) exposed V79 and Chinese hamster ovary (CHO) cells to both liquid- and vapor-phase CH_2Cl_2 in a sealed container and obtained negative results for the induction of 6-thioguanine resistance over the dose range tested. Thilagar et al. (1984a) reported that CH_2Cl_2 was negative as a mutagen at the thymidine kinase locus of mouse lymphoma L5178Y cells.

6.3 Chromosomal Aberrations

Several studies to evaluate the ability of CH_2Cl_2 to cause chromosomal aberrations have been performed. Sprague-Dawley rats were exposed to CH_2Cl_2 via inhalation (Burek et al. 1984) and NMRI mice were exposed intraperitoneally (Gocke et al. 1981). Chromosomal preparations of bone marrow cells from the rats showed no increase in specific aberrations or in the frequency of abnormal cells. Bone marrow smears from the mice were examined for micronuclei, and at the two highest dose levels an increase in polychromatic erythrocytes was observed. Since the response was not dose-related and did not double the control value, the results are inconclusive although suggestive of a positive response.

Sheldon et al. (1987) also evaluated the ability of CH_2Cl_2 to induce micronuclei in mice. Bone marrow samples were taken 24, 36, 48 and 72 hours after oral dosing with 1250, 2500 or 4000 mg/kg of CH_2Cl_2. No significant increase in the incidence of micronuclei over control values were observed for any of the test groups (Sheldon et al. 1987).

Thilagar and Kumaroo (1983) exposed CHO cells <u>in vitro</u> to CH_2Cl_2 and found a replicable, dose-related positive response for specific aberrations, number of aberrations per cell, and total cells with chromosomal aberrations. A positive response was also reported for human peripheral lymphocytes and mouse lymphoma L5178Y cells in culture (Thilagar et al. 1984b).

6.3.1 Sister Chromatid Exchange

CH_2Cl_2 has been tested at concentrations of 0.5%, 1.0%, 2.0%, 3.0% and 4% with and without S9 for its ability to induce sister chromatid exchange *in vitro*, and it exhibited a weak positive response in Chinese hamster V79 cells (Jongen et al. 1981) and CHO cells (McCarroll et al. 1983). At lower doses in CHO cells (up to approximately 1.5%), the response was not significant (Thilagar and Kumaroo 1983).

6.3.2 Unscheduled DNA Synthesis

CH_2Cl_2 has been reported to be negative as an inducer of unscheduled DNA synthesis for V79 cells and primary human fibroblasts or lymphocytes with and without metabolic activation (Jongen et al. 1981, Perocco and Prodi 1981); it induced a "marginal positive response" in primary rat hepatocytes (Thilagar et al. 1984a). Oral exposure of rats or inhalational exposure of mice and rats to methylene chloride did not cause any unscheduled DNA synthesis in hepatocytes isolated from exposed animals (Trueman and Ashby 1987). Additional unpublished studies on unscheduled DNA synthesis have been reviewed in the recent draft EPA documents (EPA 1987a,b).

6.3.3 DNA Alkylation

The potential of CH_2Cl_2 to alkylate DNA *in vivo* has also been evaluated. DNA was isolated from the liver and salivary tissue of rats and hamsters that had been exposed to $^{14}CH_2Cl_2$ via inhalation (Schumann et al. 1983). Some labeling of DNA was detected, but it appeared to be associated only with normal bases

and nucleosides; no alkylated bases or nucleosides were detected above a detection level of <12 and 1 alkylation per 10^6 nucleotides for nucleosides and bases, respectively. Incorporation of ^{14}C may have occurred through normal biosynthetic pathways (one-carbon pool) or by unstable adduct formation. Another study examined the labeling of DNA in mouse and rat liver and lung tissue and allowed for a distinction between alkylation and labeling via the one-carbon pool; no evidence for DNA adduct formation was found (Green et al. 1988). The sensitivity of the assay was reported in terms of a covalent binding index (CBI), which is used as a measure of DNA alkylation in relation to dose (Lutz 1979). The minimum detectable CBI was 0.002 for the liver and 0.01 for the lung for mice. By comparison, the strong aklylating agent dimethylnitrosamine has CBIs of 5500 and 500 for mouse liver and lung DNA, respectively (Lutz 1979). CH_2Cl_2 was reported not to bind covalently to DNA following incubation with isolated rat hepatocytes (Cunningham et al. 1981); however, the limit of detection was not known (Sipes 1987).

Green et al. (1986a, 1988) studied the <u>in vivo</u> interaction of methylene chloride and its metabolites with F344 rats and B6C3F1 mouse lung and liver DNA following a three-hour exposure to 4000 ppm ^{14}C-methylene chloride. The DNA was isolated from the exposed animals either 6, 12 or 24 hours after the start of exposure, and was found to contain radioactivity at all time points. Higher radioactivity levels were obtained in the lung than in the liver, and levels in the mouse were higher than levels in the rat. The radioactivity associated with DNA in lung and liver tissue following methylene chloride exposure was compared to that produced by an intravenous dose of ^{14}C[formate]. Chromatographic comparison of hydrolyzed DNA indicated that the major nucleotide peaks were likely to be the result of radioactive incorporation via

the C-1 pool instead of direct alkylation. However, chromatographic analysis of hydrolyzed DNA from lungs and livers of mice exposed to CH_2Cl_2 exhibited a different radioactivity fingerprint and considerably more radioactive "noise" than that in animals given ^{14}C[formate]. This study is consistent with other genotoxicity studies indicating the mutagenic potential of CH_2Cl_2 is relatively weak.

6.4 Neoplastic Transformation

Purified, food-grade CH_2Cl_2 did not induce neoplastic transformation in BALB/c-3T3 or C3H-10T-1/2 cells (Sivak 1978, Thilagar et al. 1984a). Reagent-grade CH_2Cl_2, without further purification, was reported to elicit a positive response for transformation using an F1706 cell line (Price et al. 1978). The authors asserted that the positive response for reagent-grade CH_2Cl_2 was presumably due to a contaminant because no positive response was elicited when the purified sample that had been negative in the BALB/c-3T3 system was tested in the F1706 cell line. To be responsible for transformation of the F1706 cells, a contaminant of CH_2Cl_2 would have to be present in large quantities or be a very potent mutagen (i.e., similar to benzo(a)pyrene). DHS staff members have been told in telephone conversations with chemical supply companies that reagent-grade methylene chloride is 99% to 99.9% pure; the two primary contaminants are phosgene and cyclohexene neither of which is a potent mutagen. It should be noted that since reagent grade CH_2Cl_2 is as pure or purer than the high volume chemical used primarily in industrial operations, human exposures are likely to include exposure to any contaminants as well as CH_2Cl_2.

Roghani et al. (1987) demonstrated that CH_2Cl_2 as well as chloroform and carbon tetrachloride are potent activators of protein kinase C. This phospholipid-dependent kinase has a pivotal role in the transduction of various effectors triggering cell activation by polyphosphoinositide turnover and has been implicated in the tumor-promoting activity of phorbol esters (Sukla and Albra 1987, Castagna et al. 1982).

6.5 Conclusions

CH_2Cl_2 is weakly mutagenic in Salmonella, Saccharomyces, and E. coli using a variety of endpoints. In contrast, mutagenic activity has not been definitively demonstrated in mammalian systems. There is evidence that exposure of mammalian cells to CH_2Cl_2 in vitro can result in chromosomal aberrations and DNA damage. However, the evidence for DNA adduct formation in mammalian tissues is scant. In summary, methylene chloride is a mutagen in prokaryotes (Salmonella, E. coli) and lower eukaryotes (yeast). In mammalian cells, CH_2Cl_2 exhibits clastogenic (chromosome damaging) activity. Thus, methylene chloride should be classified as genotoxic.

7.0 CARCINOGENICITY

7.1 Introduction

This chapter reviews the epidemiological and experimental evaluations of the carcinogenicity of CH_2Cl_2. Two cohorts of workers exposed to CH_2Cl_2 have been studied, and several studies of rodents exposed orally or via inhalation have been reported. The animal studies are summarized in this section and discussed in greater detail in Appendices B and C.

7.2 Epidemiological Studies

7.2.1 Kodak Employees Friedlander and colleagues studied male workers employed by the Eastman Kodak Company at its film-making operation in Rochester, New York, where CH_2Cl_2 was used as the primary solvent (Friedlander et al. 1978, Hearne and Friedlander 1981, Friedlander et al. 1985 [a report issued by the Eastman Kodak Company], Friedlander et al. 1986 [a presentation at a toxicology forum], Hearne et al. 1987). Industrial hygiene surveys conducted there had found airborne levels of methylene chloride in the workroom ranging, in general, from 30 to 100 ppm. As control groups, the researchers considered (1) males in New York State not including New York City (this area was called "upstate New York" although it includes "downstate" areas such as Nassau and Suffolk Counties on Long Island) and (2) other males employed by Kodak in Rochester. Questionnaire data indicated that the prevalence tobacco use in the exposed cohort was about as extensive as in the control groups; however, the investigators did not note whether other relevant

variables, such as average duration of cigarette smoking and the number of cigarettes smoked per day, were similar (Hearne et al. 1987).

The major findings are contained in the papers of Friedlander et al. (1985) and Hearne et al. (1987), both of which are described below. In both reports, the investigators employed two tests to determine whether observed-expected differences were statistically significant: (1) a one-tailed test of significance at $p<0.05$ for outcomes that the investigators hypothesized might be associated with methylene chloride (such as lung and liver cancer and ischemic heart disease) and (2) a two-tailed test of significance at $p\leq0.01$ for nonhypothesized outcomes (including pancreatic cancer).

The 1985 report from Friedlander and colleagues noted that overall, significantly fewer deaths occurred during the follow-up period (January 1964 thru December 1984) than expected based on the New York State data (165 vs. 231.1) (Friedlander et al. 1985). However, the number of deaths among exposed workers was similar to that of the other Kodak employees (165 vs. 167.0). Thirty-nine deaths from malignant neoplasms were observed in the exposed cohort compared to 54.7 expected based on the New York State data or 43.2 expected on the basis of the data from other Kodak employees. The follow-up rate for this report was 94% for the exposed cohort (N=751). The above findings are consistent with the "healthy worker effect," in which working populations tend to experience lower mortality than the general population.

Using the statistical tests employed by the investigators, no cancer site displayed a significantly elevated death rate. Nevertheless, eight deaths from pancreatic cancer were observed compared to 3.0 expected based on the New

York data or 2.6 based on the other employees' data. Although this finding was not considered significant based on the two-tailed test described above, an exact one-sided Poisson test using the other Kodak employees as the control group yields a p-value of 0.0053 and suggests a possible relationship between exposure to CH_2Cl_2 and pancreatic cancer mortality. Somewhat smaller-than-expected death rates were observed for certain tumor sites, including the colon, and the genital and urinary organs; these observations did not reach statistical significance.

In the subset of the cohort which had been exposed for a minimum of 20 years by 1964 (N=252), fewer cancer deaths were observed than expected when compared to the New York State data (23 vs. 33.8) or to the data from other Kodak employees (23 vs. 27.0). A slight excess of deaths from pancreatic neoplasms was observed here (4 compared to 1.9 or 1.6 expected). None of these differences were statistically significant.

This report noted that the study had insufficient power to address mortality from rare disorders such as liver or male breast cancer. Assuming comparable smoking characteristics, the study had 97% power to detect a relative risk of 2.0 for lung cancer in the entire exposed cohort and 85% power in the 252-man subset with the upstate New York controls. With the Kodak employee controls, the corresponding power figures were 91% and 74%. To detect a relative risk of 1.5, the power figures were 62% or 41% with the upstate New York controls and 50% or 33% with the Kodak employee controls).

In the most recent report (Hearne et al. 1987), the investigators presented estimates of exposure and expanded the exposed cohort to 1,013 men who had at

least one year of experience in the methylene chloride operation between January 1964 and December 1970. The 1985 report analysed approximately 14,000 person-years of follow-up in the exposed cohort; this 1987 report presented data from 19,465 person-years, with a follow-up rate of 99% for the exposed cohort. Again, compared to either control group, no statistically significant difference was found between observed and expected deaths for respiratory or hepatic cancer mortality, based on a one-sided test, $p<0.05$. Among the nonhypothesized outcomes, workers exposed to CH_2Cl_2 still experienced more than a two-fold greater rate of mortality from pancreatic cancer (8 observed vs. 3.2 or 3.1 expected [New York State or Kodak controls]). The SMR for this site was 2.5 with 95% confidence limits of 1.1 to 4.9. DHS staff calculations indicate an exact one-sided Poisson test using the New York State data as a control yields a p-value of 0.017. Thus, the results still suggest a possible relationship between exposure to CH_2Cl_2 and pancreatic cancer mortality. The number of deaths remained somewhat smaller than expected for certain malignancies, including those of the large intestine (2 observed vs. 8.0 or 6.9 expected) and the genitourinary organs (3 observed vs. 8.0 or 7.6 expected). Overall cancer mortality in the exposed cohort was still low (41 observed vs. 59.3 or 52.7 expected). None of these differences was statistically significant at the 1% level ($p>0.01$) based on a two-tailed test.

Exposure estimates were based on over 1200 area and task-specific air samples collected between 1945 and 1986 and more than 900 full-shift personal samples collected between 1980 and 1986. Longstanding job progression patterns facilitated estimation of career methylene chloride exposure. The bulk of the cohort was exposed to eight-hour time-weighted average (TWA) CH_2Cl_2 concentrations averaging 10-100 ppm. Over 150 members of the cohort were

exposed to peak concentrations of 10,000 ppm an average of three times per day, 190 days per year. The majority of the cohort was exposed to peak concentrations of 500 ppm, an average of three times per day, 10 or 40 days per year (depending on occupational classification). Other solvents, including 1,2-dichloropropane and 1,2-dichloroethane were present at lower levels in the workroom. The sampling results, in combination with information on the amount of time each employee spent in each job, were used to develop a career exposure index in ppm-years. For the analysis, nine subgroups were created corresponding to three categories each of exposure (<350, 350-749 and >750 ppm-years) and latency, the time between initial exposure and the end of follow-up (<20, 20-29 and >30 years). Each person-year of observation for each worker was assigned to one of the nine cells (subgroups). The researchers found no evidence of a dose-response relationship according to career exposure, latency or both indexes for any of the hypothesized outcomes or total cancer mortality.

Hearne and colleagues also compared their observations of lung and liver cancer mortality with risk estimates based on the NTP (1986) animal bioassay. They estimated excess risk for the three career exposure categories using a unit risk estimate (0.014 for lifetime exposure to 1 ppm CH_2Cl_2) derived from the EPA (1985b) risk assessment. They then presented an animal-based prediction of cancer mortality for lung and liver (36.3, an excess of 14.5 over the New York State control rate) and noted that the observed 14 cases (all lung cancer) were significantly lower than the animal-based predictions. The investigators concluded that the projections based on animal data were "clearly inconsistent" with their observations (Hearne et al. 1987). Using data provided by Friedlander (personal communication), DHS staff members

concur with other researchers in finding such a conclusion is not warranted (Mirer et al., 1988, Tollefson et al., 1988). (See Appendix D).

Dr. Hearne has reported (November 1988) to DHS staff that preliminary findings of an update of mortality results in the Kodak cohort for an additional three years (through 1987) are consistent with the earlier data indicating fewer total cancer deaths than expected among exposed workers. Furthermore, there was no increase in pancreatic cancer deaths in the update, however, in these three years, 0.6 pancreatic cancer deaths were expected.

7.2.2 Employees at a Fiber Production Plant. In a series of reports, Ott and colleagues (Ott et al. 1983a-e) evaluated the health of employees working at a fiber production plant in Rock Hill, South Carolina, where CH_2Cl_2 was used as part of a solvent system. Workers in this plant were exposed to a mixture of methylene chloride and methanol from one process and to acetone from a second process. Median 8-hr TWA CH_2Cl_2 concentrations in the plant's two main work areas were 140 and 475 ppm. Health evaluations of these employees were compared to those of employees at a similar fiber production plant in Narrows, Virginia, where only acetone was used. One of the reports (Ott et al. 1983b) evaluated whether exposure to CH_2Cl_2 was associated with cancer deaths. Smoking habits were not considered in the analyses, although age was controlled for.

The target population was production and service employees who had worked for at least three months in the preparation or extrusion areas of either plant between January 1, 1954, and December 31, 1976. Mortality experience within the two cohorts was followed through June 1977. Few nonwhites were in either

cohort, so many of the analyses were restricted to the whites. The cohorts were characterized as follows:

COHORT	YEAR OF FIRST EXPOSURE[1]			YEARS OF EXPOSURE[1]			
	1954-59	1960-69	1970-76	<1	1-4	5-9	≥10
Exposed (N=1271)	45%	31%	24%	17%	39%	16%	28%
Unexposed (N=948)	36%	38%	26%	15%	32%	14%	39%

[1]For the unexposed cohort, "exposure" refers to work in the reference plant.

Compared with United States death rates, no excess mortality from any cause in either cohort was evident. A slight deficit in the observed deaths from cancer was noted among exposed white workers (7 observed vs. 12.4 expected). The cancer mortality experience of the methylene chloride-exposed workers was not significantly different from that of the reference cohort. One death from pancreatic cancer was reported in the exposed cohort. Of the seven cancer deaths in the reference cohort, one was from pancreatic cancer (Bond 1988).

Statistically significant risk ratios (RRs) for mortality from all causes (RR=2.2), circulatory diseases (RR=2.2), and external causes (including accidents and suicides, RR=2.5) were found among white men when comparing the two cohorts.

This study is of somewhat limited usefulness for evaluating the association between exposure to methylene chloride and cancer mortality. It had limited power to detect increases in malignancy rates in the exposed cohort. In that cohort, only 54 deaths, of which 7 were from cancer, were reported. There were no deaths observed among the 108 nonwhite women in the study. In addition, the follow-up period was probably insufficient for any carcinogenic effects from CH_2Cl_2 exposures to be manifested.

7.2.3 Conclusions Based on Epidemiological Studies

International Agency for Research on Cancer (1986) has concluded that no excess risk of death from malignancies was observed in these epidemiological studies, but noted that the studies had a limited power to detect excess risk. To date, epidemiological studies on methylene chloride do not provide sufficient evidence either to prove or disprove human carcinogencity.

7.3 Summary of Chronic Toxicity/Oncogenicity Studies in Rodents

The effects of long-term (2-year) exposure to methylene chloride by inhalation have been studied in rats, mice, and Syrian hamsters, and by ingestion of drinking water in rats and mice. These studies are evaluated in detail in Appendix B, and the results are summarized in this section. In Appendix C, the biological relevance of the exposure-related neoplasms is discussed.

There have been five long-term rodent bioassays examining the effects of inhaling CH_2Cl_2: two using an outbred stock of Spartan/Sprague-Dawley (SD) rats (Burek et al. 1984, EPA 1985a, Nitschke et al. 1988a), one with inbred Fischer 344 (F344) rats (NTP 1986), one using hybrid B6C3F1 mice (NTP 1986), and one with Ela:Eng (Syr) Syrian hamsters (Burek et al. 1984). The NTP (1986) studies have also been published by Mennear et al. 1988. Bioassays exposing animals orally to CH_2Cl_2 in drinking water were conducted in F344 rats and B6C3F1 mice (Serota et al. 1986a, 1986b). The experimental designs of these bioassays and the results obtained are summarized in Table 7-1. The NTP also conducted a bioassay in which CH_2Cl_2 was administered by gavage in

TABLE 7-1

SUMMARY OF CHRONIC TOXICITY/ONCOGENICITY STUDIES OF METHYLENE CHLORIDE IN RODENTS

Species/Strain	Sex	No. in Group	Route of Exposure	Duration of Exposure	Exposure Levels	Results	Reference
Rat/Spartan Sprague Dawley	M,F	129 (95 chronic; 34 interim sac. & ancillary studies)	Inhalation	6 hr/day, 5 day/wk, for 2 yr	0, 500, 1,500, 3,500 ppm	Neoplasms: Sarcomas of the salivary gland region in males. Increase in benign mammary tumors per tumor-bearing rat (both sexes). Nonneoplastic lesions: Hepatocellular vacuolization in males and females. Multinucleated hepatocytes in females; increased number of foci and areas of hepatocellular alteration in high dose females.	Burek et al. 1984, EPA 1985a (Dow 1980)
Hamster/ Ela:Eng (Syr)	M,F	107-109 (95 chronic; rest for interim sac. & ancillary studies)	Inhalation	6 hr/day, 5 day/wk, for 2 yr	0, 500, 1,500, 3,500 ppm	No exposure-related neoplasms or nonneoplastic lesions.	Burek et al. 1984, EPA 1985a (Dow 1980)
Rat/Spartan Sprague Dawley	M,F	90 (70 chronic) 500/0[c]:25F 0/500[c]:25F	Inhalation	6 hr/day, 5 day/wk, for 20 (males) or 24 (females) months	0, 50, 200, 500 ppm[b]	Neoplasms: Some increase in benign mammary tumors in tumor-bearing females. Nonneoplastic lesions: Hepatocellular vacuolization and multinucleated hepatocytes in females.	EPA 1985a (Dow 1982) (Nitschke et al. 1988a)
Rat/F344	M,F	85 C1[a] and treated; 50 C2[a] and 25 high dose recovery group	Oral/ drinking water	7 day/wk, for 2 yr; recovery group-- 78-wk treatment & 2- wk recovery	0, 5, 50, 125, 250 mkd[b]	Neoplasms: The EPA's Carcinogen Assessment Group reported borderline increase in liver tumors (combined neoplastic nodule and hepatocellular carcinomas) in females. Serota et al. considered this response not significant because incidence was within the laboratory's historical control range. Nonneoplastic lesions: Hepatocellular fatty change and foci of cellular alteration in males and females.	Serota (et al. 1986a)

TABLE 7-1 (continued)

Species/Strain	Sex	No. in Group	Route of Exposure	Duration of Exposure	Exposure Levels	Results	Reference
Mouse/B6C3F1	M	C1:60 C2:65 60 mkd :200 125 " :100 185 " :100 250 " :125 F 50/group	Oral/ drinking water	7 day/wk for 2 yr	0,60,125, 185,250 mkd	Neoplasms: Increased incidence of liver tumors (combined hepatocellular adenoma and carcinoma) in males at 125 and 185 mkd and borderline increase at 250 mkd compared to combined control groups. Nonneoplastic lesions: Hepatocellular fatty change in high dose males and females.	Serota et al. (1986b)
Rat/F344	M,F	50	Inhalation	6 hr/day, 5 day/wk, for 102 wk	0, 1,000, 2,000, 4,000 ppm	Neoplasms: Treatment-related increase in benign mammary tumors (fibroadenoma, adenoma, or fibroma in males; fibroadenoma or adenoma in females). Nonneoplastic lesions: Treatment - but not dose - related increase in hepatocytomegaly, cytoplasmic vacuolization, hemosiderosis, and bile duct fibrosis in both sexes.	NTP 1986 (Mennear et al. 1988)
Mouse/B6C3F1	M,F	50	Inhalation	6 hr/day, 5 day/wk, for 102 wk	0, 2,000, 4,000 ppm	Neoplasms: Dose-related increase in incidence and multiplicity of alveolar-bronchiolar lung tumors (adenoma and carcinoma) in both sexes. Elevated incidence and multiplicity of liver tumors (hepatocellular carcinoma or combined hepatocellular adenoma and carcinoma) in high dose males. Dose-related increase in incidence of hepatocellular adenoma and carcinoma and in multiplicity of liver tumors in females. Nonneoplastic lesions: Liver cytologic degeneration in both sexes. Atrophic and degenerative lesions in reproductive organs considered secondary to severe lung and liver cancer.	NTP 1986 (Mennear et al. 1988)

[a] C1 = control 1 group; C2 = control 2 group.
[b] mkd = mg/kg/day.
[c] Additional groups: 500/0 = 500 ppm for 12 months, air for 12 months; 0/500 = no exposure to methylene chloride for 12 months, then 500 ppm for 12 months.

corn oil to F344 rats and B6C3F1 mice. On July 23, 1983, the NTP announced that the results of these bioassays would not be published in final form. The NTP's decision was "based on some recently discovered significant discrepancies in experimental data that compromise a clear interpretation" (Fed. Reg. 1983). The NTP did not cite this study in its technical report (NTP 1986) when summarizing the results of two-year studies on CH_2Cl_2, however, it is expected that NTP will release the final results without a statistical evaluation.

7.3.1 Rats In the Dow (1980) study, male and female Sprague-Dawley rats were exposed to CH_2Cl_2 by inhalation for two years (EPA 1985a, Burek et al. 1984). (For concentrations of CH_2Cl_2 used, exposure durations, and other details of this and the other studies discussed, see Table 7-1.) The most notable increase in tumor incidence was in sarcomas in the salivary gland region of male rats. These tumors did not appear to arise from the salivary gland itself but were thought to involve the salivary gland.

Control and exposed female rats all had a high incidence (above 80%) of benign mammary tumors. However, female rats exhibited a dose-related increase in the number of benign mammary tumors per tumor-bearing animal. The proportion of male rats with mammary tumors increased somewhat at the highest exposure level, but the increase was not statistically significant. (For a more detailed description of the numbers and types of tumors found at various doses for this and the other studies discussed, see Appendix B).

In another 2-year inhalation study of CH_2Cl_2 (Dow 1982, Nitschke et al. 1988a) using male and female Sprague-Dawley rats, a slight increase in the number of

tumor-bearing female rats was observed (EPA 1985a). No other significant effect on tumor incidence was noted. However, the doses were considerably lower than in the first study.

In an NTP inhalation study (NTP 1986, Mennear et al. 1988), Fischer 344 rats of both sexes were exposed to CH_2Cl_2 for 2 years and exhibited a dose-related increase in the number of animals with benign mammary tumors (both sexes), which was statistically significant by life table tests ($p<0.001$) and also by incidental tumor tests in females ($p<0.001$).

A study in which CH_2Cl_2 was administered to F344 rats in drinking water for 2 years was conducted by the National Coffee Association (Serota et al. 1986a). Male rats showed no treatment-related tumor response. There was an increase in liver tumors in female rats compared to concurrent controls ($p<0.05$), particularly for the 50 and 250 mg/kg groups. However, the increase was not significant when compared to historical controls.

7.3.2 Mice Male and female B6C3F1 mice were exposed to CH_2Cl_2 by inhalation for 2 years (NTP 1986, Mennear et al. 1988). Both sexes of mice had statistically significant, dose-related increases in the incidence and multiplicity of adenomas ($p<0.001$) and carcinomas ($p<0.001$) of the lung. However, concurrent controls had lower incidences of such tumors than historical controls. Male mice had an elevated incidence of hepatocellular adenomas ($p<0.05$) and carcinomas of the liver at the high dose ($p=0.02$), while females had dose-related increases of both hepatocellular adenomas ($p<0.001$) and carcinomas ($p<0.001$) of the liver.

B6C3F1 mice were also exposed to CH_2Cl_2 in their drinking water for 2 years in the National Coffee Association study (Serota et al. 1986b). No treatment-related effect on tumor incidence was seen in female mice. Male mice had a slight increase in hepatic tumors of borderline statistical significance. However, the incidences fell within the range of historical controls. Compared to the rats exposed in a similar study (Serota et al. 1986a), mice appear to be as sensitive or less sensitive to the effects of CH_2Cl_2.

7.3.3 Hamsters An inhalation study in which Syrian golden hamsters were exposed to CH_2Cl_2 for 2 years was conducted by Dow (EPA 1985a, Burek et al. 1984). No exposure-related tumors were reported.

7.3.4 Conclusions The strongest evidence for the carcinogenicity of CH_2Cl_2 to rodents was provided by the NTP inhalation bioassays. Under the conditions of the study, benign mammary tumors were induced in F344 rats, and the female rats exhibited a dose-related response ($p<0.001$). Further evidence of exposure-related mammary tumorigenesis in female rats was provided by a dose-related increase in the number of benign mammary tumors per tumor-bearing rat in the Dow (1980) inhalation study, although the high spontaneous incidence of mammary tumors in control SD females obscured any dose-related response in the incidence, i.e., in the number of tumor-bearing animals.

Subcutaneous sarcomas of the ventral cervical-salivary gland region occurred in male SD rats exposed by inhalation to 1,500 and 3,500 ppm of CH_2Cl_2 but not in F344 rats exposed to 1,000, 2,000, and 4,000 ppm. In the Dow and the NTP studies, there was evidence that the rats had been infected by sialodacryoadenitis virus and rat-coronavirus based on clinical signs in SD

rats (Burek et al. 1984) and serological results in F344 rats (NTP 1986, Mennear et al. 1988). Burek et al. (1984) suggested that the combination of this viral infection and exposure to high concentrations of CH_2Cl_2 may have been associated with the tumor response in SD rats in the Dow study. However, F344 rats exposed to higher CH_2Cl_2 concentrations and the same viral agent did not develop similar tumors in the salivary gland region.

In B6C3F1 mice, exposure to CH_2Cl_2 by inhalation (NTP 1986, Mennear et al. 1988) was associated with an increased incidence and multiplicity of alveolar and bronchiolar tumors (adenoma and carcinoma) in the lungs of both sexes. The incidence and multiplicity of liver tumors were also increased in both sexes. Male mice had an increased incidence of hepatocellular carcinomas and of adenomas or carcinomas (combined) at the high exposure level, while female mice had dose-related increases in hepatocellular adenoma and hepatocellular carcinoma. The survival in both male and female high dose groups was significantly ($p<0.001$, trend test) decreased as compared to controls.

Treatment-related increases in the combined incidence of liver tumors, hepatocellular adenomas, and carcinomas occurred in B6C3F1 mice exposed orally (via drinking water) to lower doses (125 and 185 mg/kg/day) of CH_2Cl_2 but were not significant in the highest (250 mg/kg/day) exposure group.

8.0 RISK ASSESSMENT

8.1 Introduction

Qualitative evaluation of the carcinogenicity of an agent is an essential part of the assessment of the hazard posed by that agent. However, to quantify human risk, the observed animal cancer response must be extrapolated from high to low doses and, in some cases, from rodents to humans. Such extrapolations commonly involve the use of mathematical dose-response models.

Several different cancer dose-response models are discussed in this section. To demonstrate how different extrapolation models will affect low-dose risk, the risks from ambient exposures are calculated using both mechanistic and distribution models. Additionally, the Andersen et al. (1987) physiologically based pharmacokinetic (PBPK) model is used to estimate the delivered dose of CH_2Cl_2 to the mouse lung. The cancer risk is then estimated for humans using this calculated delivered dosage.

8.2 Mathematical Models

This section will briefly discuss some of the dose-response models used for low-dose extrapolation. A more detailed mathematical description of the different forms of the multistage models can be found in Appendix C.

8.2.1 Multistage Models

Most mathematical models of multistage carcinogenesis are simplifications of the original time-dependent model

developed by Armitage and Doll (1954; See Appendix C). More recently, Moolgavkar and Venzon (1979) suggested a generalized two-stage form of this model, which Thorslund et al. (1987) subsequently simplified to a time-independent form and modified to a dose-dependent form by incorporating the effect of environmental agents on cell transition rates and first-stage proliferation rates. These two-stage models are discussed in Appendix C.

8.2.1.1 Linearized multistage model (Global 82)

Guess and Crump (1977) generalized the multistage model as a time-independent form with a less restrictive polynomial form. This form of the multistage model is described in Appendix C.

8.2.1.2 Time-to-tumor model (WEIBULL 82) This model was originally intended to estimate the cancer risk when the death rate due to the agent is dependent on the exposure level and independent of the tumor of concern. Under this model the estimation of the tumor incidence requires the specification of whether the tumor caused death or was only found incidentally at necropsy. Few veterinary pathologists have reported this distinction. However, the model can be used for a modified lifetable analysis of the tumor rate when the agent of concern does not induce lethal tumors with very short latency periods. The Weibull 82 model is used to correct for mortality which does not have as its underlying cause the tumor(s) of concern. A mathematical description of the model is provided in Appendix C.

8.2.2 Other Mathematical Models A number of alternatives to the multistage model have been suggested as being potentially useful for estimating low-dose

cancer risk. Among them are the probit, logit, Weibull, and gamma multihit models. The computer program RISK 81 was used to estimate the parameters in these models (Kovar and Krewski 1981). Whittemore (1978) and Whittemore and Keller (1978) note that the underlying biological basis of each of these models is inconsistent with our present understanding of carcinogenesis. Even so, these models are useful in representing the potential range of estimates that might result due to the selection of a particular parametric form. It has been shown (Crump et al. 1976) that if background tumor rates are caused by factors that are dose-additive with the carcinogenic agent, low-level linearity is rapidly achieved as exposures are decreased from the high levels used in animal bioassays. In this case, the parametric form of the model has only a limited effect on low-dose risk estimates.

8.3 Estimates of Cancer Risk Based Upon Animal Bioassay Data

The tumor types used to obtain risk estimates were judged to be both biologically relevant and significantly different statistically from controls. Biological relevance was defined as a malignant tumor or a benign tumor with a reasonable chance of progressing to a malignant form. Statistical significance was measured by a Cochran-Armitage trend test that had a "p" value of less than 0.05 associated with it. The following tumor types met both of these criteria:

- Sarcomas of the cervical-salivary gland region in Sprague-Dawley male rats (Table B-1, Dow, 1980 inhalation study, reported by Burek et al. 1984),

- Alveolar and/or bronchiolar neoplasms of the lung in B6C3F1 male and female mice (Table B-9, NTP, 1986 inhalation study, Mennear et al. 1988), and

- Hepatocellular adenomas or carcinomas in female B6C3F1 mice (Table B-10, NTP, 1986 inhalation study, Mennear et al. 1988).

The tumor dose-response data that are used to estimate risk are shown in Tables B-1, B-9, B-10, and B-12 and are summarized in Table 8-1.

8.3.1 Linearized-Multistage (L-M), 95% Upper-Bound Estimates for All Significant Data Sets

Standard unit risk estimates (q_1^* in units ppb^{-1}) were obtained for these endpoints using the (L-M) 95% upper-bound approach as discussed in Appendix C. These results are shown in Table 8-1.

The lung tumors in mice are used as a basis for a more detailed, quantitative, risk-modeling effort. The rationale for this is:

- The lung is the organ of direct contact for inhalation exposures.

- The tumor data indicate a strong increasing response with dose ($p \leq 0.001$ by Cochran-Armitage trend test) and are consistent with the multistage models.

TABLE 8-1

TUMOR DOSE-RESPONSE DATA USED TO ESTIMATE RISK TO RODENTS

Exposure Level[a] (ppm CH_2Cl_2)	Sarcomas of the Cervical/Salivary Gland Region in SD Male Rats[b]	Alveolar and/or Bronchiolar Adenomas or Carcinomas in B6C3F1 Mice[c]		Hepatocellular Adenomas or Carcinomas in B6C3F1 Female Mice[c]
		Male	Female	
0	1/93	5/50	3/50	3/50
500	0/94	--	--	--
1,500	5/91	--	--	--
2,000	--	27/50	30/48	16/48
3,500	11/88	--	--	--
4,000	--	40/50	41/48	40/48
95% upper bound[e] linear term				
q_1^* in ppb^{-1}	2×10^{-7}	3×10^{-6}	3×10^{-6}	9×10^{-7}

[a] Animals were exposed 6 hours per day, 5 days per week.
[b] Dow 1980 inhalation study.
[c] NTP 1986 inhalation study, Mennear et al. 1988
[d] No exposure group included at this level.
[e] Lifetime excess risk for continous exposure. Adjusted for experimental conditions.

NOTE: All Cochran-Armitage trend tests are significant at $p<0.001$ level. All Fisher exact tests are significant at $p<0.001$ level except control versus 500 and 1,500 ppm in Dow study, which were not significant, i.e., $p>0.05$.

- The response is consistent between sexes.

- The largest 95% upper-bound linear coefficient (q_1^*) for the four data sets considered is associated with the lung tumor response in female mice.

8.3.2 Risk Estimates Based on Lung Tumors and Various Forms of the Multistage Model

A statistically significant, monotonically increasing dose-response relationship was obtained for both sexes of B6C3F1 mice in the NTP (1986) study (see Tables B-9 and B-10). The lung tumor response to be used in this analysis is alveolar and/or bronchiolar adenomas and carcinomas, since these stages of lung tumors are thought to be part of the same etiologic entity.

Stimulation of cell proliferation is believed to be a possible mechanism of tumor induction. However, a model based on this mechanism was rejected for both sexes in this case since the observed data did not exhibit sufficient curvature to be consistent with the hypothesis of preneoplastic cell proliferation.

Other two-stage models based on mechanistic descriptions of tumorigenesis adequately fit the data for both sexes. An exact fit is always obtained under certain conditions described in Appendix C. An exact fit was obtained for the lung tumor data sets from both male and female mice. As a result, the two-stage, different transition rate model (Equation 3, Appendix C) and the standard multistage model (Equation 7, Appendix C) give identical results.

However, a goodness-of-fit test is meaningless since the number of parameters estimated is equal to the number of exposure groups. The more restricted model, with identical transition rates, is also consistent with the observed data based on the chi-square goodness-of-fit test ($p>0.1$). The risks obtained from the various forms of the models are summarized in Table 8-2. The estimates obtained from the two-stage models are relatively consistent. The largest risk is associated with female mice using the upper-bound time-dependent multistage model with all tumors regarded as "incidental." This analysis uses the term "incidental" tumors for all tumors found at necropsy, including those discovered at terminal sacrifice. This terminology is used to distinguish between the "fatal" tumor analysis which excludes tumors found at terminal sacrifice. Using the female mice tumor data, the maximum likelihood estimates of risk and the 95% upper bounds are compared for the various models in the next section.

8.3.3 Comparison of Various Risk Models Using Female Mouse Lung Tumors.

The multistage time-dependent and time-independent models can be compared to a number of alternative models that have been used in risk assessment (Table 8-3). The risk values in Table 8-3 assume that human and animals exposed to equal doses of a carcinogen on a mg per surface area basis, for an equivalent proportion of a lifetime will encounter the same degree of risk. This is the surface area conversion for virtually completely absorbed gases. The basis for this conversion and methodology are shown in Appendix F. Since the observed background rates are not equal to zero, the various models give very comparable results as shown in Table 8-3. This is indicated by the ratio of

TABLE 8-2

MAXIMUM LIKELIHOOD ESTIMATES OF RISK FOR MICE BASED ON VARIOUS FORMS OF THE MULTISTAGE MODEL AND LUNG ALVEOLAR AND/OR BRONCHIOLAR ADENOMAS OR CARCINOMAS IN B6C3F1 MICE

		Lifetime Excess Risk from Exposure Under Experimental Conditions to 1 ppb CH_2Cl_2[a]	
Mathematical Model	Biological Hypothesis	Male	Female
Two-stage, dose-dependent, preneoplastic cell proliferation	Mitogenic stimulation of preneoplastic cells	Model rejected based on chi-square goodness-of-fit test, $p<.01$	
Two-stage, dose-dependent, different transition rates	Genotoxic at two different loci	2×10^{-6}	3×10^{-6}
Two-stage, dose-dependent, identical transition rates	Genotoxic at same locus on homologous chromosomes	9×10^{-7}	9×10^{-7}
(L-M) GLOBAL 82	Exposure additive with other factors causing background rates	2×10^{-6}	3×10^{-6}
Multistage time-to-tumor WEIBULL82 (incidental tumors)	Multiple linear unspecified events	2×10^{-3}	1×10^{-6}

[a]Linear term multiplied by age function evaluated at 104 weeks and the values were adjusted for continuous exposure.

TABLE 8-3

COMPARISON OF HUMAN RISKS OBTAINED USING DIFFERENT MODELS
ASSUMING CONSTANT LIFETIME INHALATION EXPOSURE
TO 1 ppb CH_2Cl_2[a,b]
(Risk from CH_2Cl_2 Minus Background Tumor Rate)

Model Used	Excess Risk from Continuous Exposure to 1 ppb in Air	
	Maximum Likelihood Estimate	95% Upper Confidence Limit
Multistage (Global 82)	7×10^{-6}	9×10^{-6}
Time-dependent multistage assuming all tumors are incidental (Weibull 82)	4×10^{-6}	10×10^{-6}
Probit	4×10^{-6}	8×10^{-6}
Logit	3×10^{-6}	7×10^{-6}
Weibull	7×10^{-7}	2×10^{-5}
Gamma multihit	4×10^{-6}	7×10^{-6}

[a]Crump multistage Global 82, Weibull 82, and Risk 81 (with additive background), surface area corrected for conversion from animals to humans.

[b]Based on female B6C3F1 mice with alveolar and/or bronchiolar adenomas or carcinomas of the lung observed in the NTP (NTP 1986, Mennear et al. 1988) inhalation bioassay.

the highest to the lowest value is only about a factor of two for the 95% upper-bound estimates.

Dose-additive forms for logit, probit, Weibull and gamma multihit models were used for the comparison. If a major portion of the background tumor rates were induced by a mechanism different from that of CH_2Cl_2, the dose-additive assumption would not be valid. Under the alternative assumption of independence, much greater variability in risk estimates would be expected.

8.4 Postulated Mechanisms of Action for Observed Tumor Responses Induced by Methylene Chloride

Several of the carcinogenicity studies of CH_2Cl_2 using both rats and mice yielded significantly increased incidences of tumors in organs of species and strains that have been reported to have relatively high spontaneous tumor incidences (Tarone et al. 1981). These included mammary tumors in F344 and Sprague-Dawley rats following inhalation exposure, liver tumors in B6C3F1 male mice exposed via ingestion and inhalation, and liver tumors in B6C3F1 female mice exposed via inhalation (see Table 7-1 in Chapter 7).

The mechanism of formation for tumors with high spontaneous incidence is controversial. Some authors have associated the increased incidence rates for these tumors with exposure to chemicals that have nongenotoxic mechanisms, i.e., do not interact directly with DNA. However, this does not imply that these tumors cannot also be caused by known genotoxic substances. Several potential nongenotoxic and genotoxic mechanisms might account for an increased rate of tumor formation specifically for the B6C3F1 mouse liver tumor.

The role of cell proliferation in tumorigenesis is not fully understood. It has been suggested that since the total number of spontaneous mutations in a tissue is proportional to the total number of mitotic divisions (Knudson 1985), cell proliferation results in more mutations, and in turn may yield more tumors.

The NTP (NTP 1986, Mennear et al. 1988) study in which B6C3F1 mice were exposed chronically to CH_2Cl_2 via inhalation revealed that hepatic "cytological degeneration" occurred as a result of treatment in both male and female mice. This lesion was not described further in the report and the NTP provided no dose-response information. Hepatocellular toxicity can be followed by regenerative hyperplasia, a common response when a tissue attempts to replace damaged cells by increasing the cell proliferation rates of the undamaged cells. Thus, CH_2Cl_2 could induce mouse liver tumor formation simply by stimulating cell proliferation in that organ in response to cytotoxicity. However, there are no experimental studies using CH_2Cl_2 that can be used to specifically document regenerative hyperplasia resulting in tumor formation.

Chemicals that are cytotoxic, i.e., that cause cell death and stimulate cellular regeneration, may damage DNA by virtue of cell lysis (Sina et al. 1983), perhaps through the release of lysosomal contents containing DNA hydrolases (Bradley 1985). Indirect cytotoxicity and genotoxicity may result from the stimulation of peroxisomal proliferation and concomitant overabundance of reactive oxygen species (Reddy et al. 1980). There is no direct evidence, however, that the carcinogenic effects of CH_2Cl_2 are due to any of these mechanisms.

CH_2Cl_2 may produce tumors via direct genotoxic action. The mutagenic activity of CH_2Cl_2 has been demonstrated in the Salmonella mutagenicity assay. DNA adducts have not been observed, although alkylation of DNA may have occurred below the detection limit in the experiments designed to detect adduct formation. The possibility of DNA binding is also suggested by covalent binding of CH_2Cl_2 metabolites to microsomal lipids and proteins, which demonstrates the formation of highly reactive intermediates. DNA alkylation may be significant for carcinogenesis at levels below the limit of detection for an experimental assay. Such alkylation might lead to mutation, so the possibility that CH_2Cl_2 interacts with DNA directly cannot be ruled out.

8.5 Estimation of Risk Adjusted for Pharmacokinetic Information

In addition to estimates derived under the standard "applied dose" approach, risk estimates for CH_2Cl_2 have been derived using variations of the physiologically-based pharmacokinetic model [PBPK] described by Andersen et al. (1987). The purpose of such modeling is to identify the dose of actual carcinogenic agent that will be present at the target tissue as accurately as possible. This type of approach requires the extensive use of data on the physiology of the species under study and on the pharmacokinetics of the substance being considered. The approach uses data available on the chemical in a quantitative manner whereas previously such data was only considered qualitatively. Thus, by using more information, it is the intention of the modelers to develop a more accurate picture of the potential for the chemical to induce cancer in humans. The limitations of the method basicly reflect the limitations of our knowledge of the chemical. Although DHS staff have not independently derived PBPK values for this risk assessment, staff of the EPA

(1987a,b) and the U.S. Consumer Product Safety Commission (Cohn 1987) have done so through the U.S. Interagency Hazard/Risk Assessment Committee of the Integrated Chlorinated Solvents Project (HRAC). The purpose of this subsection is to discuss application of the PBPK model to risk assessment and to indicate which values should be used in reporting the range of risks from CH_2Cl_2 exposure.

8.5.1 Identification of Carcinogenically Active Metabolites

Use of the PBPK model requires determination of the active species involved in CH_2Cl_2 carcinogenesis. Several hypotheses have been generated concerning the biologically active form of CH_2Cl_2 responsible for tumor induction in mice. One hypothesis is that the observed effects are related to the parent chemical. Due to the low reactivity of CH_2Cl_2 and evidence suggesting that it behaves like a weak alkylating agent, it is unlikely that CH_2Cl_2 functions as a direct-acting carcinogen. However, none of the available evidence precludes the possibility that the parent compound is carcinogenically active (EPA 1987a). As indicated in Section 6 (page 6-1) positive dose-related responses in Salmonella strains TA1535, TA98 and TA100 have been reported both in the presence and in the absence of a metabolic activating system.

As indicated in Section 2, metabolism of CH_2Cl_2 has been demonstrated to occur by two pathways (Ahmed and Anders 1978). One is catalyzed by mixed-function oxidase (MFO) cytochrome P450, while the other is catalyzed by glutathione S-transferase (GST). Each metabolic pathway involves formation of an active intermediate that is theoretically capable of irreversibly binding to cellular

macromolecules such as DNA (Ahmed and Anders 1976, Ahmed et al. 1980, Kubic and Anders 1978, EPA 1985b).

A second hypothesis is that the tumorigenicity of CH_2Cl_2 is related to the formation of metabolites derived from oxidative metabolism via the MFO pathway. Several lines of evidence suggest the production of genotoxic intermediates by this pathway. Jongen et al. (1982) reported that the addition of either microsomes (i.e., MFO) or cytosol (i.e., GST) increased the mutagenic response of CH_2Cl_2 in TA100 using a 6-hour incubation period. Snow et al. (1979) reported that with the addition of S9 (an MFO-containing fraction) from Syrian Golden Hamsters there was an increase in mutagenic response at all dose levels. These results are in contrast to Green T. (1983) who reported that the addition of cytosol increased the mutagenic response of CH_2Cl_2 in TA100, but the addition of an S9 fraction or of microsomes did not. However, Green T. (1983) incubated the assay for 3 days which is much longer than most other investigators. Consequently, the MFO pathway appears to exhibit mutagenic activity.

Compounds closely related to CH_2Cl_2 exhibit the production of mutagenic metabolites via the MFO pathway. The mutagenicity of chlorofluoromethane was increased in TA100 by the addition of S9 (Green T. 1983). There was a marked increase in mutagenicity of dibromoethane and diiodomethane when microsomes or cytosol were added to the medium and incubated 15 minutes (Van Bladeren et al. 1980). Thus, for three closely related compounds the MFO metabolism produced mutagenic metabolite(s).

As demonstrated in unpublished data of Green et al. (1987b) submitted to DHS, the nonciliated Clara cell of the bronchiolar epithelium of the mouse lung exhibited toxic effects and MFO damage following CH_2Cl_2 exposure. It appears that in this cell type the oxidative route produced metabolites which destroyed the enzymes from which they were formed, and caused cytotoxicity. That is, the MFO pathway produced reactive intermediates, which reacted with proteins and consequently may react with and damage DNA. Therefore there is a direct and indirect evidence that the metabolic intermediate(s) produced by the MFO pathway are mutagenic and possibly genotoxic.

Other data suggests that the MFO pathway is not important with regard to the formation carcinogenic metabolites. Cytochrome P450 appeared to be saturated at 500 ppm in rats (McKenna et al. 1982), while a dose-related increase in tumor incidence is observed in mice at higher concentrations. Indirect evidence has been presented (Andersen et al. 1987) which indicates that the MFO pathway may not be the primary carcinogenic pathway. This evidence consists of comparing the results of the NTP (1986) inhalation study, where tumors were clearly observed, with those of the National Coffee Association (NCA) drinking water study (Serota et al. 1986a,b) where there was a borderline increase in tumors (see Appendix B, page B-11). The PBPK target dose by the MFO pathway was higher in the NCA study, but the GST target dose was lower. Cohn (1987) of the CPSC concluded that the MFO pathway was saturated in both studies, and indicated that if the MFO pathway were of primary importance, similar carcinogenic responses would have occurred in the two studies. However, the basis for the conclusion is limited. First, the difference in carcinogenic response in the two studies can also be explained in terms of the applied dose since the dose in the NTP study was, on a mg/kg

basis, more than 10 times greater than the dose in the Serota et al. study. The EPA (1985b) stated that the response in the Serota et al. study was consistent with the calculated carcinogenic risk based on the potency estimated using data from the NTP study, without adjustments for pharmacokinetics. Second, this comparison does not imply that the MFO pathway is unimportant in terms of the carcinogenic response since it may still produce a significant amount of carcinogenic intermediates. Third, comparison of the two studies would be consistent with production of carcinogenic intermediates via both metabolic pathways. Fourth, Cohn's conclusion assumes that the calculation of the MFO target dose by the PBPK method is correct. This target dose has not been directly measured experimentally.

Cohn (1987) indicated that the "dose-response relationship for lung and liver would not be expected to be as pronounced as in the NTP bioassay if the MFO pathway was of primary importance," since this pathway is expected to be saturated based on the PBPK model. However, he states further that "this argument does not eliminate the MFO pathway from consideration with regard to a role in the carcinogenic response of methylene chloride, but it does indicate that some other pathway or chemical species is likely to be of greater importance." Although saturation of the total MFO pathway, as measured by CO production, and the PBPK analysis indicate overall MFO and GST activity they do not indicate the number of reactive intermediates produced by each pathway. Thus it is the opinion of DHS staff that the carcinogenicity data in animals do not indicate which pathway(s) produce carcinogenic metabolites. However, PBPK models have focused on the GST pathway as the primary producer of the carcinogenic metabolite, and have placed less weight on the metabolites produced via the MFO pathway, as is discussed below.

A third hypothesis suggests that CH_2Cl_2 metabolites formed by the GST system are responsible for tumorigenicity. This hypothesis is supported by several lines of evidence: One is that the increased production of GST pathway metabolites is consistent with the treatment-related tumor incidence, since the GST pathway was assumed to be not saturated at the exposure concentrations used in the inhalation bioassay. Second, the addition of a cytosolic fraction increased the yield of bacterial mutagens in a Salmonella assay system (Jongen et al. 1982, Green 1983). The latter observation also finds support from studies of the activation of two other halogenated hydrocarbons by glutathione (GSH), 1,2-dichloroethane (Guengerich et al. 1980) and 1,2-dibromoethane (Rannug et al. 1978). The third type of evidence implying that the GST pathway is important in carcinogenesis is the indication that the MFO pathway is unlikely to be fully responsible for the carcinogenic activity of CH_2Cl_2.

The observed tumorigenicity of CH_2Cl_2 appears to be related to the formation of GSH conjugates via the GST pathway. However, it is unclear as to how significant a role the GST pathway plays and whether the contribution of the MFO pathway is significant. That is, there is considerable uncertainty with regard to the identification of the methylene chloride-glutathione (CH_2Cl_2-GSH) conjugate as the sole metabolite responsible for carcinogenicity in laboratory animals and with regard to the exclusion MFO activity from the production of carcinogenic metabolites. The HRAC (EPA 1987a) indicated that "the mechanism of carcinogenic action of [methylene chloride] remains problematical..." and "...there remains some uncertainty as to whether or not the GST pathway is the sole path to carcinogenicity." The CPSC document (Cohn 1987) stated "again, of course, some or all of the intermediates of the various pathways, or the parent compound, may contribute to the carcinogenic

process." Although there is uncertainty in positively identifying the carcinogenic species, EPA and CPSC staff concluded that the MFO pathway only plays a minor role in the carcinogenic process in animals, and risk estimates were made using the PBPK model asssuming that the carcinogenic activity of CH_2Cl_2 is not dependant on the MFO pathway.

An extensive analysis of the PBPK model was presented in the federal interagency health/risk assessment committee (HRAC) draft report (EPA 1987a). The HRAC suggested that pharmacokinetic model adjustments consisted of two separate analyses (EPA 1987a). The first analysis adjusts for saturated metabolic pathways during the experimental exposure (referred to as a high-to-low-dose adjustment). The second adjusts for interspecies differences in the handling of the substance (referred to as the species-to-species adjustment). The HRAC analysis concluded that the PBPK model could be used to adjust for saturation of the MFO pathway in calculating the risk estimate for CH_2Cl_2. The EPA analysis (EPA 1987b) went further and suggested that an adjustment for interspecies differences could also be made using the PBPK model. The analyses discussed below will be based on the HRAC report (EPA 1987a), the CPSC report (Cohn 1987), and the EPA report (EPA 1987b). The PBPK model is itself described in Appendix E.

8.5.2 Adjustment for MFO Saturation: High-to-Low Dose Adjustment

The CPSC analysis (Cohn 1987) concluded that "based on the weight of evidence, some adjustment utilizing pharmacokinetic data would appear to be appropriate when extrapolating from high to low dose in the case of [methylene chloride]." Based on data discussed in Section 2.2.2 and the PBPK model, the MFO pathway

is expected to be saturated at all dose levels in mice in the NTP (1986) inhalation bioassay. CPSC viewed the PBPK model as generally able to estimate levels of CH_2Cl_2 in blood, lung and liver. The HRAC report (EPA 1987a) indicated that the PBPK model appeared sensitive to changes in the metabolic constants KF (the first order rate constant for the GST pathway), KM (the Michaelis-Menten constant for the MFO pathway), and VMAX (the maximum velocity of metabolism by MFO). Based upon data the CPSC obtained from the authors of the PBPK model for estimates of the three constants in humans (KF=0.53; KM=0.58; VMAX=118.9), the CPSC reported that consideration of the pharmacokinetic data required an adjustment of 2.2 for the lung tumor data (Cohn 1987). A sensitivity analysis to determine how large changes in the above three variables (KF, KM, and VMAX) would influence the adjustment factor was conducted. The adjustment was shown to be relatively insensitive (varying from 1.6 to 2.4) even if the three metabolic variables were in error by an order of magnitude. Consequently, consideration of the pharmacokinetic adjustments from high-to-low dose would reduce the estimated lung cancer potency by approximately 2.2-fold.

It is not possible to validate the appropriateness of this adjustment. As indicated in Table 8-1 the upper bound estimate of risk was based on the incidence of alveolar and/or bronchiolar adenomas or carcinomas in mice in the NTP (1986) inhalation study. The dose-response data are presented in Table B-10 in Appendix B. If the cancer dose-response model using an adjusted dose fit the data better, that would support the high-to-low dose adjustment. However, when the several carcinoma and adenoma endpoints in Table B-10 of Appendix B are examined, there is considerable variability in fit with the applied doses. For alveolar/broncheolar adenomas the doubling of applied dose

does not correlate well with the increase in tumor response (56 to 75%), and may indicate a saturating metabolism. However, for alveolar/bronchiolar carcinomas, the doubling of the applied dose correlates very well with the tumor response (27 to 60%). As indicated above, the carcinogenic response would not be expected to increase with dose if the MFO pathway was the only pathway producing carcinogenic intermediates and if the MFO pathway was saturated in the NTP (1986) study. However, the response would be expected to increase with dose if the MFO and GST pathways both contributed appreciably to the production of carcinogenic intermediates. Thus, considering the wide variability in dose-response, support for or against the high-to-low dose adjustment does not appear to be available based on an improved model fit of the tumor data.

8.5.3 Species-to-Species Adjustment

Extrapolation between species involves numerous factors, including metabolism and pharmacokinetics. As Cohn (1987) indicated "the ability to elucidate one component of a species difference does necessarily indicate what, if any, adjustments should be made; it does not provide more certainty than the empirical process currently used; in fact, making the necessary assumptions may introduce even more uncertainty than the present empirical procedure." As a result, the CPSC did not make a species-to-species adjustment for CH_2Cl_2. However, in addition to the high-to-low dose adjustment, the EPA did calculate an adjustment factor for species-to-species extrapolation (EPA 1987b), and the results are described below.

Two PBPK models were studied by HRAC (EPA 1987a). One was the model developed by Andersen et al. (Andersen et al. 1984, Andersen et al. 1987) which is based on inhalation of CH_2Cl_2. The other model was developed by Angelo et al. (Angelo et al. 1984, Angelo and Pritchard 1984, Angelo et al. 1986a,b) which is based on intravenous and oral exposure to CH_2Cl_2. The two models differ significantly in structure. EPA (1987b) chose to develop risk estimates based on the Andersen et al. (1987) approach and to use the Angelo et al. (1986a,b) model to obtain greater insight into the Andersen et al. model. The Anderson et al. model is briefly described in Appendix E and references to the "PBPK" model refer to the Anderson et al. model unless otherwise stated.

The Andersen et al. model estimates tissue-level doses for CH_2Cl_2 and its metabolites. Andersen et al. (1987) concluded that the estimate of risk from CH_2Cl_2 exposure to the human lung was 144-fold lower using the PBPK approach compared to the applied-dose approach. EPA (1987b) indicated that this difference was composed of an 11.3-fold difference based on the estimated dose adjustment, and a 12.7-fold factor used for surface area correction for dose when extrapolating across species. EPA further indicated that the difference between the two approaches was overstated for several reasons. Andersen et al. (1987) used a lower human breathing rate that represented a resting state (12.5 m^3/day) instead of a 24-hour average (20 m^3/day). Andersen et al. (1987) used a higher mouse breathing rate (0.084 m^3/day) than the standard EPA estimate (0.043 m^3/day). Furthermore, Andersen et al. compared their estimates using their breathing rates to the EPA estimates using EPA breathing rates. When the same breathing estimates are used in the applied-dose and PBPK approaches, the estimated PBPK dose is not 11.3-fold lower but

only 3.6-fold (using Andersen et al. rates) to 9.4-fold (using EPA rates) lower.

Using the PBPK model developed by Andersen et al. (1987), the average daily concentration of CH_2Cl_2 metabolites in the lung via the GST pathway was calculated for mice exposed to 2,000 or 4,000 ppm. The HRAC calculated the internal mouse and human dose levels as indicated in Table 8-4 based on the female mouse lung tumor data of the NTP (1986) bioassay. The internal dose calculated for humans in the lung from a 1 ppm exposure was 0.008386 mg/L/day. The unit risk reported by HRAC (EPA 1987a) was $1 \times 10^{-6}(ppb)^{-1}$ (or 3×10^{-7} $(\mu g/m^3)^{-1}$). The risks from CH_2Cl_2 calculated from the female mouse lung tumor data using various assumptions are shown in Table 8-5. Approximately a 9-fold reduction in estimated risk is achieved by the combined high-to-low dose and species-to-species adjustments. This reduction is mostly due to the assumptions concerning the species-to-species adjustment. As indicated in Section 8.5.2, accounting for saturation of CH_2Cl_2 metabolism (high-to-low dose adjustment) would account for about 28% of the total adjustment.

8.5.3.1 Use of a Surface Area Corection Factor.

The HRAC (EPA 1987a) analysis differed with the approach suggested by Andersen et al. (1987) regarding the surface area correction on applied dose. Andersen et al. (1987) assumed that the adjustment for metabolic differences across species in the PBPK model should replace the surface area adjustment (EPA 1987a). However, in this case the HRAC concluded that the surface area conversion factor (12.7) is still needed to account for interspecies differences in sensitivity of the tissues to the internal dose. Currently,

TABLE 8-4

HUMAN AND MOUSE CH_2Cl_2 INTERNAL DOSE LEVELS BASED UPON THE RESULTS OF A PHYSIOLOGICALLY BASED PHARMACOKINETIC MODEL[a]

Exposure Level (ppm)	Internal Lung Dose of CH_2Cl_2 (mg/liter blood)	
	Mice	Humans
4,000	240	
2,000	110	
1		0.008

[a]Based on the model of Andersen et al. (1987) as reported by EPA (1987a,b). The values were adjusted for continuous exposure. That is, the mouse exposure in ppm was multiplied by (6 hours/day)/(24 hours/day)x(5 days/week)/(7 days/week). The concentrations are calculated from the female mouse lung tumor data (NTP 1986).

TABLE 8-5

COMPARISON OF HUMAN CANCER RISK ESTIMATES FROM A 1 ppb CH_2Cl_2 EXPOSURE, BASED ON LUNG TUMORS IN FEMALE MICE, WITH AND WITHOUT ADJUSTING FOR METABOLISM[a]

Approach	Lifetime Risk	
	ppb^{-1}	$(\mu g/m^3)^{-1}$
Applied Dose	9×10^{-6}	3×10^{-6}
High-to-Low Dose Adjustment[b]	4×10^{-6}	1×10^{-6}
Total PBPK Adjustment[c]	1×10^{-6}	3×10^{-7}
Total PBPK Adjustment without Surface Area Correction[d]	9×10^{-8}	3×10^{-8}

[a]All values were adjusted for continuous exposure. That is, the mouse exposure in ppm was multiplied by (6 hours/day)/(24 hours/day) x (5 days/week)/(7 days/week). The concentrations are calculated from the female mouse lung tumor data (NTP 1986). The extrapolation was also corrected for surface area (a factor of 12.7) unless indicated otherwise. The risks are based on the linearized multistage model.

[b]High-to-low adjustment for saturation of MFO pathway based on the HRAC report (EPA 1987a).

[c]High-to-low adjustment for saturation of MFO pathway and the species-to-species adjustment based on the HRAC report which includes a 12.7 surface area correction (EPA 1987a).

[d]High-to-low adjustment for saturation of MFO pathway and the species adjustment based on the HRAC report (EPA 1987a), except without use of a surface area correction.

the PBPK model itself does not incorporate a susceptibility factor. The CPSC analysis (Cohn 1987) indicated that the surface area adjustment has been used to account for the many differences between species (e.g., metabolic activation rates, clearance, distribution, cell sensitivity, number of cells in exposed tissue, immunosurveillance, DNA longevity, efficiency of DNA repair, and cell proliferation rates). Knowledge of a single interspecies difference, such as the pharmacokinetics, does not shed light on the total interspecies correction since other factors may be compensating for the effect, such as the pharmacodynamics (i.e., the effect the chemical has on a living system).

There is likely to be difference in tissue susceptibility within species. Reitz et al. (1988) recently reported the PBPK internal dose estimates for lung and liver for the NTP (1986) mouse bioassay. The internal doses (in mg equivalent CH_2Cl_2 /liter volume of tissue) are reported in Table 8-6. As shown in the table, despite a higher PBPK dose delivered to the liver than to the lung, the tumor response is slightly lower in the liver than the lung. The potency of the PBPK dose was calculated for each tissue and the lung was found to be approximately 9-fold more sensitive to the PBPK dose than the liver. Thus, for this very homogeneous species, there was a substantial difference in tissue susceptibility to the PBPK dose. Consequently, the PBPK dose should not be considered the sole factor affecting intraspecies or interspecies differences.

There may be differences in susceptibility across species to the PBPK dose.At present an estimate of the difference cannot be made using published PBPK doses. One possible comparison would be production of lung tumors in female

mice to production of mammary tumors in female rats. This would compare the most sensitive sites of two species. However, the PBPK model is currently not able to calculate an internal dose for mammary tumors. A second possible comparison, would be of combined liver tumors in rats to that of mice based on the Serota et al. (1986a,b) studies. PBPK doses for all dose levels for these studies have not been published although they could be calculated. The difficulty in interpreting this comparison would be the borderline significant tumor response obtained in the studies.

In summary, the PBPK model assumes that sensivitity to the same target dose is identical in different species. However, based on the 9-fold difference between two tissues in the mouse NTP (1986) study, there is likely to be differences in susceptibility across species. Consequently, staff at DHS agree that the application of the surface area correction to account for sensitivity differences is justified, particularly in light of the numerous uncertainties involved in the PBPK procedure as discussed in Section 8.5.3.2, and questions regarding whether the carcinogenic pathway has been identified in humans. Furthermore, an uncertainty factor (such as the surface area correction) is needed to account for the potential variability in human response to CH_2Cl_2 exposure. Thus, DHS staff choose to use the uncertainty factor, (referred to as the surface area correction), adopted by EPA in the HRAC (EPA 1987a) document.

8.5.3.2 Concerns Regarding the Species-to-Species Adjustment.

There is a large number of variables and equations used in the PBPK models. As indicated above, a sensitivity analysis has been conducted on a few of the

parameters (Cohn 1987). A complete analysis of all the parameters would assist in identifying which parameters have the greatest impact on the final numerical outcome of the model. Once the most influential parameters have been identified, reporting that information would be very helpful in evaluating how such complex models operate.

The roles of either the CH_2Cl_2-GSH conjugates or MFO reactive metabolites have not been elucidated in human carcinogenesis. There is limited information onthe in vitro GST activity with CH_2Cl_2 in human and rodent liver (Rietz et al. 1988). Based on a per mg protein basis, mice produce 6- to 10-fold more reactive metabolite in vitro. However, the human liver (2198g) is more than 1000-fold larger than the mouse liver (1.84g) and would still be expected to produce 100-fold more of the putative metabolite per liver. The information for GST activity in the lung is more limited. Comparison of a single in vitro mouse tissue experiment to a single in vitro human tissue experiment (Rietz et al. 1988) indicated a 20-fold difference in GST activity on a per mg protein basis. The human lung (1065g) is more than 5000-fold larger than the mouse lung (0.2g) (Crosfill and Widdicombe 1961), and would be expected to produce 250-fold more of the putative metabolite per lung. The importance of this information to risk assessment probably depends on the relative contribution of other differences between species as discussed in Section 8.5.3.1.

Currently the PBPK model is restricted to considerating only the liver and the lung metabolism of CH_2Cl_2. Tumors at several sites have been associated with CH_2Cl_2 exposure in rodents. These tumors include salivary gland sarcomas in rats (Burek et al. 1984), mammary gland tumors in rats (Burek et al. 1984, NTP

1986), combined liver tumors in rats (Serota et al. 1986a), hepatocellular adenomas and carcinomas in mice (Serota et al. 1986b, NTP 1986), and alveolar/bronchiolar adenomas and carcinomas in mice (NTP 1986). Increases in tumors in a variety of organs indicates that the carcinogenic activity of CH_2Cl_2 is not tissue-specific. Humans may respond to an extent similar to other animals though at a different site. The relative distributions of GSH and GST throughout the body in laboratory animals versus humans have not been identified; thus, some human tissues may exhibit greater (or lesser) production of the CH_2Cl_2-GSH conjugate than their counterparts in rodents. Furthermore, more information needs to be developed regarding the relative activities of the MFO and GST pathways in various tissues across species.

PBPK models have to rely on information from the test species. Human estimates of enzymatic activity in vivo have been limited to methods relying on exhalation of CO or carboxyhemoglobin (COHb) levels. Measurements of enzyme activities and rates of formation of products in vitro are not available for all animal tissues and are only available for a few human liver and lung samples. Using 1,2-dibromoethane as a substrate to measure GST activity in the rat indicated that the highest activity occurred in the liver and kidneys and significant GST activity was reported in the lung, testis, spleen, and heart (Hill et al. 1978). Thus, based on GST activity levels, other tissues are possible sites of carcinogenicity for CH_2Cl_2. Studies by Black and Howerton (1984) and Mukhtar et al. (1981) suggest that both animal and human pancreatic tissue exhibit significant GST activity; as discussed in Sections 7.2.1 and 8.5, there is some suggestive evidence of increased pancreatic cancer in humans resulting from CH_2Cl_2 exposure (Hearne et al., 1987). However, it is not possible to estimate a PBPK dose for the pancreas

based on the data currently available. Although the primary site of GST and MFO activity is the liver, carcinogenicity is not restricted to that tissue. In extrahepatic organs, GST activity may predominate over MFO activity. Based on the limited data of Rietz et al. (1988), GST activity is more important in the human than the mouse relative to MFO activity (the ratio of GST/MFO activity is greater for humans). Another possibility is that simple formation of a toxic metabolite may not be the overriding factor in carcinogenesis. Instead, the relative toxifying and detoxifying activities in the specific organ may be more important.

In addition to the above general comments regarding the PBPK approach, DHS staff have some specific concerns regarding use of the Andersen et al. (1987) model in risk assessment. Several metabolic constants incorporated into the PBPK model (e.g., VMAX for MFO in the liver, KM for MFO, and KF for GST in the liver) were developed by an optimization procedure to obtain the best fit of the model to the data on the disappearance of the compound from the exposure chamber (Andersen et al. 1987). The model does not account for sequestering into lipid-rich compartments of some organs. In contrast to the sensitivity analysis for the high-to-low dose adjustment, the results for a species-to-species extrapolation is reportedly very sensitive to the value of KF (Cohn 1987, EPA 1987a). The human variability in KF has not been established.

There is uncertainty in the human variability of other physiological parameters, partition coefficients, and metabolic constants used in pharmacokinetic models. The catalytic affinity (KM) and maximum metabolic capability (VMAX) for a particular substrate may vary widely due to different MFO and GST isoenzymes predominanting in different individuals and tissues.

These isoenzymes may also differ in the responses of the genetic systems controlling their induction or repression to chronic exposure. GST is known to have at least 7 isoenzymes which differ in catalytic activity, in substrate specificity, and in both subcellular and tissue distribution. The specific activity of GSTs may vary by orders of magnitude for the same substrate when tested with different isoenzymes (Jakoby and Habig 1980). The isozymes of GST responsible for CH_2Cl_2 metabolism have not been identified. It is not clear if one or more isozymes is involved. The stability or variability of the isozymes in the human liver assay have not been verified. Up to 50% of the human population lack a specific GST isozyme present in the rest of the population (Seidergard and Pero 1985, Seidegard et al. 1985, 1986). Thus, if one or more of the pooled human livers lacked a potential CH_2Cl_2-specific isozyme, there would be a decreased GST activity in the sample. Since very few human livers have been assayed for GST activity, the inter-individual variability is not known. Furthermore, since the livers used may have been affected by the use of drugs or anesthetics in heroic measures to save trauma victims, it is unclear how representative the these measurements are of the general population.

The human internal dose estimates depend upon the parameter values selected for use in the PBPK model. Key parameters used in the PBPK model are the metabolic constants for the two biotransformation pathways proposed for the formation of specific metabolites in the liver and the lung. Due to the lack of quantitative data on the metabolism of CH_2Cl_2 itself, surrogate substrates were used to apportion the activity of the MFO and GST pathways of each tissue (Andersen et al. 1987, Gargas et al. 1986, Reitz et al. 1986) based on the study of Lorenz et al. (1984). To partition activities between the liver and

the lung, 2,4-dinitrochlorobenzene was used for the GST estimate, and 7-ethoxycoumarin was used for MFO estimate.

Partition coefficients incorporated into the model were obtained using homogenized tissues and a vial equilibration technique (Andersen et al. 1987). Homogenization alters the normal architecture of the tissue, destroys proteins that may interact with the compound, disrupts normal membranes, deteriorates the normal binding components and decreases the ability of the tissue to metabolize the compound. Applying such metabolic constants measured in acute experiments to chronic exposure may not take into account responses such as enzyme induction, tolerance, or other long-term adaptive or pathological tissue changes. Furthermore, due to the absence of available data, the PBPK model assumed that the tissue/air partition coefficients for liver, muscle and fat in humans was identical to that measured in the rat (Andersen et al. 1987).

As indicated in Andersen et al. (1987), there is no data to allow direct calculation of the first order rate constant KF for the GST pathway in humans. Based on an apparent allometric relationship of intrinsic clearance (range of 34 to 59 ml/hr/kg) between rodent species, the human value for the kinetic constant KF was derived by scaling from the rodent values based on body weight to the 0.7 power. However, Reitz et al. (1988) indicate that <u>in vitro</u> KF values are roughly equivalent to the scaled values.

Due to the use of test results from a variety of studies and procedures, there may be considerable variability in the parameters due to experimental error, systematic differences in assay methods, and tissue preparation which have not

been taken into account. For example, the KM values for the metabolism of CH_2Cl_2 to CO via the oxidative pathway reportedly vary by a factor of 58 (ECETOC, 1987: 0.86 mM; Ahmed and Anders 1976: 50.1 mM), and the VMAX values vary by a factor of 27 (ECETOC, 1987: 0.58 nmoles/min/mg protein; Ahmed and Anders, 1976: 15.5 nmoles/min/mg protein). The values for 2,4-nitrochlorobenzene metabolism (used to partition lung and liver activities) in rat liver cytosolic fractions for GST activity range over 8000-fold (Green et al. 1986c: 0.16 nmol/min/mg; Moron et al. (1979): 567 nmol/min/mg; Lorenz et al. (1984): 1380 nmol/min/mg). The values for 2,4-nitrochlorobenzene metabolism in rat lung cytosolic fractions for GST activity had over a 6000-fold range (Green et al. 1986c: 0.015 nmol/min/mg; Lorenz et al. (1984): 77 nmol/min/mg; Moron et al. (1979): 100 nmol/min/mg). Andersen et al. (1987) presented four estimates of the human VMAX for the MFO pathway that varied by a factor of 2 (82 to 159 mg/hr). Thus, there is considerable variation in the estimate of many of the parameters used in the PBPK model. Variation in the individual parameters may combine to increase the uncertainty of the overall outcome of the model.

References have been made to unpublished studies and draft reports throughout this section. Generally, DHS staff do not rely heavily on such information since unpublished data may be very preliminary in nature. Information that is unpublished or peer-reviewed may be useful for clarification of the issues discussed or to acknowledge those who have evaluated and analyzed the complex issues involved in using the PBPK model. In several instances in this document DHS staff have extensively evaluated the unpublished data. In some cases, the preliminary information has been found to be incorrect. For example, the initial unpublished report submitted by the Halogenated Solvents

Industry Alliance (HSIA) to DHS indicated that the activity of the human liver GST was zero (ECETOC 1987). This result, obtained from a small sample size, and an inadequate assay method, was reported to regulatory agencies and widely to the scientific community. However, the more recent submissions indicate some activity (Green et al. 1987a; Reitz et al. 1987, submission by DOW Chemical Co. to EPA; ECETOC 1988). A recent publication (Rietz et al. 1988) also reports GST activity in the liver.

In other instances, unpublished data may indicated results contrary to information already published. For example, Green et al. (1986b, 1987c) reported that mice exhaled more CO_2 than rats and that there was a resulting species difference. However the work of McKenna et al. (1982) and Angelo et al. (1986a,b) indicate the absence of a species difference. In such instances, the published information would be relied upon to draw conclusions. Another example is that Ahmed and Anders (1976) reported that the metabolism of CH_2Cl_2 to formaldehyde by rat liver cytosol was significantly greater than that reported in Green et al. (1986c). In another example, the values of 2,4-nitrochlorobenzene metabolism in liver and lung cytosolic fractions for GST activity were significantly lower in Green et al. (1986c) than in either Lorenz et al. (1984) or Moron et al (1979).

Other data in unpublished reports that appear contradictory often cannot be explained. For example, Green et al. (1986) describes exposure of mice for 6 hours to CH_2Cl_2. In this study blood levels of CH_2Cl_2 for the 2000 ppm exposure were lower at 6 hours (20 μg/ml) than at 1.5 hours (40 μg/ml). Such information may simply reflect the prelimary nature of the experiment and of scientific knowledge in this area.

However, the use of unpublished human data from studies conducted at DOW Chemical Co. (VMAX and KM for the MFO pathway in humans) in the PBPK models is of particular concern to DHS staff. Similar human data from other sources is not available for comparison. In Andersen et al. (1987) the source of the data is attributed to unpublished data by Nolan and McKenna at Dow Chemical Co. However, in a recent publication by Rietz et al. (1988) of Dow Chemical Co. the human data is cited by referencing Andersen et al. (1987) without mention of their unpublished nature. This circular referencing may lead to the misunderstanding that the data and study have been peer-reviewed and published.

In summary, while the general approach and structure of the PBPK model is attractive and seems reasonable, several issues regarding the ultimate accuracy of the PBPK model over the concentrations of interest remain unresolved. More information is needed with regard to validation of the predicted results of the PBPK model. The effects of some variations in model design on the final results needs to be quantified. Further research is needed to elucidate the relative importance of the methylene chloride-glutathione conjugates and the MFO pathway in human carcinogenesis. The accuracy of estimates of the individual parameters is highly variable. The sensitivity of the model to changes in parameter values should be further evaluated with regard to the uncertainty in the parameter values. In addition, sites other than the lung and liver should be assessed in terms of their contribution to metabolism and their susceptibility to carcinogenicity. On the other hand, the PBPK approach does allow for consideration of pertinent data that cannot be taken into account in the applied dose approach. Thus, despite the concerns enumerated above, cautious application of the PBPK model

to methylene chloride risk assessment is appropriate in conjunction with other more traditional approaches.

8.6 Conclusions

A variety of approaches were used to develop models to obtain estimates of cancer risk associated with exposure to CH_2Cl_2. The results obtained from those models, which DHS staff conclude are most relevant to the risk assessment of airborne CH_2Cl_2, are summarized in Table 8-7. The values shown represent the potential increase in cancer risk associated with continuous lifetime exposure to 1 ppm of CH_2Cl_2 in ambient air. The risks estimated for a 1 ppb lifetime exposure to CH_2Cl_2, based on induction of lung tumors, range from 1×10^{-6} to 10×10^{-6} (0.3 to 3×10^{-6} for 1 $\mu g/m^3$ for exposure). The State of New York reported a unit risk of 2.7×10^{-6} for a lifetime exposure to 1 $\mu g/m^3$ (Riano and Rest 1986). The PBPK-adjusted values required parameter estimates from a model as reported by HRAC (EPA 1987a), EPA (1987b) and the CPSC (Cohn 1987). The lower estimate (1×10^{-6}/ppb) incorporates a complete pharmacokinetic adjustment as calculated by EPA (1987b). DHS staff believe that the complete pharmacokinetic adjustment retains considerable uncertainty. In contrast, the applied dose value (10×10^{-6}/ppb) does not incorporate any of the available pharmacokinetic information, and thus ignores information regarding saturation of the MFO pathway in the NTP study; the likely result is that the applied dose value overestimates the risk. The high-to-low dose adjustment used by CPSC to generate a risk of 4×10^{-6}/ppb incorporates information regarding saturation of the MFO pathway in the NTP study. However, the high-to-low dose adjustment assumes a negligible contribution by the MFO pathway to the cancer risk of CH_2Cl_2, and thus may underestimate the

risk. EPA staff have indicated that incorporation of the MFO pathway would increase the value only slightly. DHS staff conclude that the full range of values presented above is scientifically plausible, however, after evaluation of the HRAC data (EPA 1987a) and other available evidence presented Sections 2 and 8, the CPSC adjusted value (Cohn 1987) of 4×10^{-6}/ppb appears to be the most likely estimate of the risk of CH_2Cl_2 exposure.

Air monitoring in several areas in California indicates that approximately 20.3 million people are exposed to a range of CH_2Cl_2 concentrations from 1.1 to 2.4 ppb weighted by population. This range of exposure would be associated with an upper 95% confidence interval of risk ranging from 1×10^{-6} (1.1 ppb x 1×10^{-6}/ppb) to 24×10^{-6} (2.4 ppb x 10×10^{-6}/ppb). With an estimated exposed population of 20.3×10^{6}, the 95% upper confidence limit range of estimates of excess cancer cases over a lifetime exposure to CH_2Cl_2 would be 20 to 500. This is the range of plausible upper bound estimates, and due to the uncertainties in the risk extrapolation process (over five orders of magnitude), the actual number of excess cancer cases may be as low as zero.

Air monitoring of CH_2Cl_2 in the South Coast basin has yielded an estimated range of ambient concentrations of 1.5 to 3.1 ppb weighted by population. This exposure would be associated with an upper 95% confidence interval of risk ranging from 2×10^{-6} (1.5 ppb x 1.2×10^{-6}/ppb) to 3.0×10^{-5} (3.1 ppb x 10×10^{-6}/ppb). With an estimated population of 10.09×10^{6}, the 95% upper confidence limit range of estimates of excess cancer cases over a lifetime due to exposure to CH_2Cl_2 would not likely exceed 20 to 300. Due to the uncertainties in the extrapolation process (five orders of magnitude) the actual number of cancer cases may be as low as zero.

Table 8-6

DIFFERENCES IN TISSUE SUSCEPTIBILITY TO THE PBPK DOSE

Exposure[a] (ppm)	LUNG		LIVER	
	PBPK Dose[b]	Tumors	PBPK Dose	Tumors
0	0	3/50	0	3/50
2000	321	30/48	785	16/48
4000	482	41/48	1670	40/48
Potency of PBPK dose[c] (per PBPK unit)	3.9×10^{-3}		4.5×10^{-4}	

[a]Exposure of B6C3F1 mice for 6 hours/day, 5 days/week.

[b]Internal dose calculated by Rietz et al. (1988) in average mg equivalents of CH_2Cl_2 metabolized by GST pathway per day per liter volume of tissue. The values were corrected for fraction of a lifetime exposed.

[c]Potency estimate using Global 86 per mg equivalent of CH_2Cl_2 metabolized by the GST pathway per day per liter volume of tissue.

TABLE 8-7

SUMMARY OF HUMAN CANCER RISKS ASSOCIATED WITH CONTINUOUS LIFETIME EXPOSURE TO 1 ppb CH_2Cl_2 BASED ON LUNG TUMORS IN FEMALE MICE USING VARIOUS MODELS AND ASSUMPTIONS[a]

Exposure Model	Mathematical Model	Endpoint	Increased Lifetime Cancer Risk Due to Continuous Lifetime Exposure to 1 ppb CH_2Cl in Ambient Air
Applied Dose	Linearized Multistage	Lung Tumors	9×10^{-6}
Applied Dose	Time-Dependent Multistage	Lung Tumors	10×10^{-6}
High-to-Low Dose[b]	Linearized Multistage	Lung Tumors	4×10^{-6}
High-to-Low Dose	Time-Dependent Multistage	Lung Tumors	5×10^{-6}
Total PBPK[c]	Linearized Multistage	Lung Tumors	1×10^{-6}

[a]Surface area conversion for rodent to human risks. Risk estimates are reported at 95% upper bound values.

[b]High-to-Low dose adjustment for saturation of MFO pathway based on the HRAC report (EPA 1987a).

[c]High-to-Low dose adjustment for saturation of MFO pathway and species-to-species adjustment based on the HRAC report (EPA 1987a).

9.0 CONCLUSIONS

The evidence characterizing acute, subchronic, and chronic health effects of exposure to CH_2Cl_2 may be summarized as follows. Acute toxicity studies conducted in laboratory animals indicate that the central nervous system is the primary target organ, with nervous system depression appearing at dose levels as low as 500 ppm. The available experimental studies of acute human exposure to CH_2Cl_2 suggest that it caused central nervous system depression and altered behavior at similar concentrations. The levels of CH_2Cl_2 required to elicit such effects are well above those which can be expected to be encountered in ambient air.

Subchronic or chronic exposure of experimental animals to 500 to 1,000 ppm or more of CH_2Cl_2 has been reported to cause behavioral changes, hepatic dysfunction, and, less frequently, lung lesions. There have been relatively few reports of subchronic or chronic toxic effects resulting from exposure of humans to CH_2Cl_2. Subchronic and chronic effects reported include alterations in carboxyhemoglobin levels, encephalosis, toxemia, and bilateral temporal lobe degeneration. In many cases, it is unclear whether the reported toxicities were caused by the direct action of CH_2Cl_2 or by the indirect action of carbon monoxide, a major metabolite of CH_2Cl_2. These effects are unlikely to occur at ambient concentrations.

The reproductive and teratogenic potential of CH_2Cl_2 cannot be adequately characterized because the data to evaluate toxicity to germ cells and potential teratogenicity are limited. The minor effects that have been

observed may have resulted from maternal toxicity due to carboxyhemoglobinemia.

Methylene chloride is a weak mutagen as indicated by positive responses in the Salmonella and E. coli bacteria test systems and in the yeast Saccharomyces. Methylene chloride induces cell transformation and chromosomal aberrations in vivo. Other in vitro and in vivo test systems designed to detect gene mutations and DNA binding have resulted in negative or equivocal results, although tests of clastogenic potential were positive.

There is sufficient evidence to regard CH_2Cl_2 as a probable human carcinogen based on responses in the B6C3F1 mouse liver and lung and the F344 rat mammary gland. Although a positive response was not obtained in the B6C3F1 mice exposed to CH_2Cl_2 in drinking water, the dose levels per unit bodyweight were lower than the levels resulting in positive effects in B6C3F1 mice exposed via inhalation. The available evidence from human epidemiology studies should be regarded as inadequate. Applying the EPA's criteria for evaluating the overall weight of evidence of carcinogenicity to humans places CH_2Cl_2 in Group B2. The EPA suggests that agents classified in Group B2 are "probable" human carcinogens. This category includes agents for which there is inadequate evidence from human epidemiologic studies and sufficient evidence from animal studies. An International Agency for Research on Cancer working group has recently evaluated the potential carcinogenicity of CH_2Cl_2 and also found sufficient evidence for carcinogenicity in animals, inadequate evidence in humans, and thus it recommended that CH_2Cl_2 be classified as a Group 2B agent (IARC 1986). International Agency for Research on Cancer suggests that agents classified in Group 2B are "possibly carcinogenic to humans."

There is no evidence from the known metabolic pathways for CH_2Cl_2 to suggest that there may be a biochemical threshold for carcinogenic action. Additionally, because the metabolism of CH_2Cl_2 results in mutagenic metabolites, a carcinogenic threshold dose cannot be estimated.

Mathematical models for the prediction of cancer risk associated with CH_2Cl_2 consistent with genotoxic and cell proliferation mechanisms of tumor induction were discussed. Cancer risks were estimated using models based on standard assumptions regarding biological mechanisms of carcinogenesis that fit the experimental data. Low-dose linear terms for the models were estimated and used as measures of cancer potency. The Andersen et al. (1987) PBPK model was also used and adjusted to account for exposure to a delivered dose of the putative biologically active CH_2Cl_2 metabolite. Estimation of delivered dose was based on several additional assumptions, so the potency developed in this manner should be viewed with caution. The DHS staff have presented the potency estimates developed by the EPA and the U. S. Consumer Product Safety Commission using a PBPK model. Thus, the range of risks presented include standard applied dose calculations and internal dose calculations.

Assuming that the airborne concentrations of CH_2Cl_2 to which animals were exposed can be extrapolated between species, potency estimates for CH_2Cl_2 were calculated using dose-response models that are linear at low doses. The 95% upper bound for unit risk estimates span the range 1×10^{-6} to 10×10^{-6} per ppb (or 3×10^{-7} to 3×10^{-6} per ($\mu g/m^3$)).

REFERENCES

ABRAHAMSON, S. and VALENCIA, R. 1980. Evaluation of substances of interest for genetic damage using Drosophila melanogaster. Prepared for FDA Contract No. 233-77-2119

AHMED, A.E. and ANDERS, M.W. 1976. Metabolism of dihalomethane to formaldehyde and inorganic chloride. Drug Metab. Dispos. 4:357-361

AHMED, A.E. and ANDERS, M.W. 1978. Metabolism of dihalomethanes to formaldehyde and inorganic halides. II. Studies on the mechanism of the reaction. Biochem. Pharmacol. 27:2021-2025

AHMED, A.E., KUBIC, V.L., STEVENS, J.L. and ANDERS. M.W. 1980. Halogenated methanes: Metabolism and toxicity. Feder. Proceed. 39, 1:3150-3155

ALTMAN, N.H. and GOODMAN, P.G. 1979. Neoplastic diseases. Chpt. 13. In: The Laboratory Rat. Vol. I. Biology and Diseases. Baker, H.J., Lindsey, J.R., and Weisbroth, S.H., eds., Academic Press, New York. pp. 339-340, 361-362

ANDERS, M.W. and SUNRAM, J.M. 1982. Transplacental passage of dichloromethane and carbon monoxide. Toxicol. Lett. 12:231-234

ANDERS, M.W., KUBIC, V.L., and AHMED, A.E. 1977. Metabolism of halogenated methanes and macromolecular binding. J. Environ. Pathol. Toxicol. 1:117-124

ANDERSEN, M.E., GARGAS, M.L., and RAMSEY J.C. 1984. Inhalation pharmacokinetics: Evaluating systemic extraction, total in vivo metabolism, and the time course of enzyme induction of inhaled styrene in rats based on arterial blood:inhaled air concentration ratios. Toxicol. Appl. Pharmacol. 73:176-187

ANDERSEN, M.E., CLEWELL, M.J., III, GARGAS, M.L., SMITH, F.A., and REITZ, R.H. 1987. Physiologically-based pharmacokinetics and the risk assessment process for methylene chloride. Toxicol. Appl. Pharmacol. 87:185-205

ANGELO, M.J., BISCHOFF, K.B., PRITCHARD, A.B. and PRESSER, M.A. 1984. A physiological model for the pharmacokinetics of methylene chloride in $B_6C_3F_1$ mice following i.v. administration. J. Pharmacokinet. Biopharm. 12, 4:413-436

ANGELO, M.J., PRITCHARD, A.B. 1984. Simulations of methylene chloride pharmacokinetics using a physiologically based model. Reg. Toxicol. Pharmacol. 4: 329-339.

ANGELO, M.J., PRITCHARD, A.B., HAWKINS, D.R., WALLER, A.R., and ROBERTS, A. 1986a. The pharmacokinetics of dichloromethane I. Disposition in $B6C3F_1$ mice following intravenous and oral administration. Food Chem. Toxicol. 24, 9:965-974

ANGELO, M.J., PRITCHARD, A.B., HAWKINS, D.R., WALLER, A.R., and ROBERTS, A. 1986b. The pharmacokinetics of dichloromethane II. Disposition in Fischer 344 rats following intravenous and oral administration. Food Chem. Toxicol. 24, 9:975-980

ARANYI, C., O'SHEA, W.J., GRAHAM, J.A. and MILLER, F.J. 1986. The effects of inhalation of organic chemical air contaminants on murine lung host defenses. Fundam. Appl. Toxicol. 6:713-720

ARMITAGE, P. and DOLL, R. 1957. A two-stage theory of carcinogenesis in relation to the age distribution of human cancer. Br. J. Cancer 11:161-169

ARMITAGE, P. and DOLL, R. 1954. The age distribution of human cancer and a multistage theory of carcinogenesis. Br. J. Cancer 8:1-12

AVIADO, D.M. 1975. Toxicity of aerosol propellants in the respiratory and circulatory systems: X. Proposed classification. Toxicol. 3:321-322

AVIADO, D.M, ZAKHARI, S., and WATANABE, T. 1977. Non-Fluorinated Propellants and Solvents for Aerosols. CRC Press, Cleveland

BAKHISHEV. G.N. 1975. Relationship between chemical structure and toxicity for some aliphatic halogenated series. Fiziologicheski Aktivnye Veshesta 7:35-36

BALMER, M.F., SMITH, F.A., LEACH, L.J., and YUILLE, C.L. 1976. Effects in the liver of methylene chloride inhaled alone and with ethyl alcohol. Am. Ind. Hyg. Assoc. J. 37:345-352

BARBER, E.D., DONISH, W.H., and MUELLER, K.R. 1980 (Abstract). Quantitative Measurement of the Mutagenicity of Volatile Liquids in the Ames Salmonella/Microsome Assay. P-39, Environmental Mutagen Society, 11th Annual Meeting (As cited in EPA 1985a)

BARROWCLIFF, D.F. 1978. Chronic carbon monoxide posioning caused by methylene chloride paint stripper. Med. Sci. Law 18:238

BARROWCLIFF, D.F. and KNELL, A.J. 1979. Cerebral damage due to endogenous chronic carbon monoxide poisoning caused by exposure to methylene chloride. J. Soc. Occup. Med. 29:12-14

BERGER, M., and FODOR, G.G. 1968. CNS disorders under the influence of air mixtures containing dichloromethane. Zentralbl. Bakteriol. (Ref.) 215:517

BERGER, M.L. and SOZERI, T. 1987. Rapid halogenated hydrocarbon toxicity in isolated hepatocytes is mediated by direct solvent effects. Toxicol. 45:319-330

BLACK, O. and HOWERTON, B.K. 1984. Glutathione S-transferase activity in rat pancreas. J. Natl. Cancer Inst. 72:121-123

BOND, G. 1988. Personal communication. Dow Chemical Company.

BORNMANN, G. and LOESER, A. 1967. Zur Frage einer cronish-toxischen Wirkung von Dischloromethan. Zeit. Lebenomit.-Untersuch. Forsch. 136:14-18

BORNSCHEIN, R.L., HASTINGS, L., and MANSON, L. 1980. Behavioral toxicology in the offspring of rats following maternal exposure to dichloromethane. Toxicol. Appl. Pharmacol. 52:29-37

BRADLEY, M.O. 1985. Cytotoxicity as a mechanism of carcinogenesis. In: Basic and Applied Mutagenesis. Muhammed, A. and von Borstel, R.C., eds., Plenum Press, New York, pp. 99-109

BUIE, S.E., PRATT, D.S. and MAY, J.J. 1986. Diffuse pulmonary injury following paint remover exposure. Am. J. Med 81:702-704

BUREK, J.D., NITSCHKE, K.D., BELL, T.J., WACKERLE, D.L., CHILDS, R.C., BEYER, J.E., DITTENBER, D.A., RAMPY, L.W., and McKENNA, M.J. 1984. Methylene chloride: A two year inhalation toxicity and oncogenicity study in rats and hamsters. Fundam. Appl. Toxicol. 4:30-47

CALLEN, D.F., WOLF, C.R., and PHILPOT, R.M. 1980. Cytochrome P450-mediated genetic activity and cytotoxicity of seven halogenated aliphatic hydrocarbons in Saccharomyces cerevisiae. Mutat. Res. 77:55-63

CARLSON G.P. and KIM, Y.C. 1986 (Abstract) Effect of forced exercise on dichloromethane blood levels and metabolism to carbon monoxide in rats. IRCS Journal of Medical Science 14:795

CASTAGNA, M., TAKAI, Y., KARBUCHI, K. et al. 1982. Direct activation of calcium-activated phoshalipid-dependent protein kinase by tumor-promoting pharbol esters. J. Biol. Chem. 257:7874-7851

CHELLMAN, et al. 1986. Inhibition of the acute toxicity of methylene chloride in male B6C3F1 mice by glutathione depletion. Toxicol. Appl. Pharmacol. 86:93-104

CIUCHTA, H.P., SAVELL G.M., SPIKER R.C. 1979. The effect of alcohols and toluene upon methylene chloride-induced carboxyhemoglobin in the rat and monkey. Toxicol. Appl. Pharmacol. 49:347-354

COBURN, R.E. 1970. The carbon monoxide body stores. In Biological effects of carbon monoxide. Proceedings of a Conference, New York Academy of Science, New York Academy of Science, New York, January 12-14, 1970 Ann. N.Y. Acad. Sci. 174:11-22

COHN, M.S. 1987. Updated risk assessment for methylene chloride (dichloromethane). In: Methylene chloride briefing package for U. S. Consumer Products Safety Commission, Washington, D.C.

COLLIER, H. 1936. Methylene dichloride intoxication in industry--a report of two cases. Lancet 1:594-595

CORNELL, N.W., HANSCH, C., KIM, K.H. and HENEGAR, K. 1983. The inhibition of alcohol dehydrogenase in vitro and in isolated hepatocytes by 4-substituted pyrazoles. Arch Biochem. Biophys. 227:81-90

CRAMER, H. 1946. Mathematical Methods of Statistics. Princeton University Press

CROSFILL, M.L. AND WIDDICOMBE, J.G. 1961. Physical characteristics of the chest and lungs and the work of breathing in different mammalian species. J. Physiol. 158:1-14

CRUMP, K.S., HOEL, D., LANGLEY, C., and PETO, R. 1976. Fundamental Carcinogenic processes and their implications for low-dose risk assessment. Cancer Res. 36:2973-9

CUNNINGHAM, M.L., GANDOLFI, A.J., BRENDEL, K., and SIPES, I.G. 1981. Covalent binding of halogenated volatile solvents to subcellular macromolecules in hepatocytes. Life Sci. 29:1207-1212

DISTLERATH, L.M., LOPER, J.C., and DEY, C.R.. 1984. Aliphatic halogenated hydrocarbons produce volatile Salmonella mutogens. Mutat. Res. 136:55-64

DiVINCENZO, G.D., YANNO, F.J., and ASTILL, B.D. 1972. Human and canine exposures to methylene chloride vapor. Am. Ind. Hyg. Assoc. J. 33:125-135

DiVINCENZO, G.D. and HAMILTON, M.L. 1975. Fate and disposition of 14C-methylene chloride in the rat. Toxicol. Appl. Pharmacol. 32:385-393

DiVINCENZO, G.D. and KAPLAN, C.J. 1981a. Uptake, metabolism, and elimination of methylene chloride vapor by humans. Toxicol. Appl. Pharmacol. 59:130-140

DiVINCENZO, G.D. and KAPLAN, C.J. 1981b. The effect of exercise or smoking on the uptake, metabolism and excretion of methylene chloride vapor. Toxicol. Appl. Pharmacol. 59:141-148

DOW CHEMICAL COMPANY. 1980. Methylene chloride: A two-year inhalation toxicity and oncogenicity study in rats and hamsters. FYI-OTS-0281-0097. Follow-up response A. Washington, D.C.: U.S. Environmental Protection Agency, Office of Toxic Substances

DOW CHEMICAL COMPANY. 1982. Nitschke, K.D., Bell, T.J., Rampy, L.W., and McKenna, M.J. Methylene chloride: A two-year inhalation toxicity and oncogenicity study in rats. Toxicology Research Laboratory, Health and Environmental Sciences, Dow Chemical Company, Midland, MI (October 11, 1983)

ECETOC Internal Document. 1987. The assessment of carcinogenic hazard for human beings exposed to methylene chloride. Technical Report No. 26. ISSN 07773-8072-26

ECETOC Internal Document. 1988. Methylene chloride (dichloromethane): Human risk assessment using experimental animal data. Technical Report No. 32. ISSN 07773-8072-32

ENVIRONMENTAL PROTECTION AGENCY (EPA). 1983. Reportable quantity document for dichloromethane. ECAO-CIN-R104

ENVIRONMENTAL PROTECTION AGENCY (EPA). 1985a. Health Assessment Document for Dichloromethane (Methylene Chloride). Final Report. EPA 600-8-82/004F

ENVIRONMENTAL PROTECTION AGENCY (EPA). 1985b. Addendum to the Health Assessment Document for Dichloromethane (Methylene Chloride). Updated Carcinogenicity Assessment of Dichloromethane (Methylene Chloride). EPA 600-8-82/004F

ENVIRONMENTAL PROTECTION AGENCY (EPA). 1987a. External Review Draft. Technical analysis of new methods and data regarding dichloromethane. EPA 600-8-8-7/029A.

ENVIRONMENTAL PROTECTION AGENCY (EPA). 1987b. External Review Draft. Update to the Health Assessment Document and Addendum for Dichloromethane (Methylene Chloride): Pharmacokinetics, Mechanism of Action, and Epidemiology. EPA 600-8-87/030A.

FEDERAL REGISTER, August 4, 1983 48(151):35508

FENN, W.O. 1970. The burning of CO in tissues. Ann. N.Y. Acad. Sci. 174:64-71

FODOR, G.G. and WINNEKE, G. 1971. Nervous system disturbances in men and animals experimentally exposed to industrial solvent vapors in England. In Proceedings of the 2nd International Clean Air Congress. Academic Press, New York. pp. 238-243

FOOD SOLVENTS WORKSHOP. 1984. I: Methylene chloride. 1984. Proceedings of Workshop March 8-9, 1984, Bethesda, Maryland

FORSTER, H.V., GRAFF, S., HAKE, C.L., SOTO, R., and STEWART, R.D. 1974. Pulmonary-hematological studies on humans during exposure to methylne chloride. Report No. NIOSH-MCOW-ENVM-MC-74-4. The Medical College of Wisconsin. Department of Environmental Medicine, Milwaukee

FRIEDLANDER, B.R. 1987. Personal communications. Eastman Kodak Company

FRIEDLANDER, B.R., HEARNE, T., HALL, S. 1978. Epidemiologic investigation of employees chronically exposed to methylene chloride. Mortality analysis. J. Occup. Med. 20:657-666

FRIEDLANDER, B.R., HEARNE, F.T., PIFER, J.W., and GROSE, F.H. 1986. Epidemiologic evidence regarding methylene chloride. Presented at the Winter Toxicology Forum, Washington, D.C., February 18

FRIEDLANDER, B.R., PIFER, J.W., and HEARNE, F.T. 1985. 1964 methylene chloride cohort mortality study. Update through 1984. Eastman Kodak Company. Rochester, N.Y.

GAMBERALE, F., ANNWALL, B.A., and HULTENGREN, M. 1975. Exposure to methylene chloride: II. Psychological functions. Scand. J. Work. Environ. Health 1:95-103

GARGAS, M.L., CLEWELL, H.J., and ANDERSEN, M.E. 1986. Metabolism of inhaled dihalomethanes in vivo: Differentiation of kinetic constants for two independent pathways. Toxicol. Appl. Pharmacol. 82:211-223

GLATZEL, W., TIETZE, K., GUTEWORT, R. and PANKOW, D. 1987. Interaction of dichloromethane and ethanol in rats. Toxicokinetics and nerve conduction velocity. Alcohol: Clin. Exper. Res. 11:450-452

GOCKE, E., KING, M.T., ECKHARDT, K., and WILD, D. 1981. Mutagenicity of cosmetics ingredients licensed by the European communities. Mutat. Res. 90:91-109

GRADISKI, D., MEGADUR, J.L., BAILLOT, M., DANIERE, M.C., and SCHUH, M.B. 1974. Comparative toxicity of the principle chlorinated aliphatic solvents. J. Eur. Toxicol. 7:247

GREEN, T. 1983. The metabolic activation of dichloromethane and chlorofluoromethane in a bacterial mutation assay using Salmonella typhimurium. Mutat. Res. 118:227-288

GREEN, T., PROVAN, W.M., COLLINGE, D.C., and GUEST, A.E. Internal ECETOC Document. 1986a. Methylene Chloride (Dichloromethane): Interaction with Rat and Mouse Liver and Lung DNA in vivo. Imperial Chemical Industries PLC Central Toxicology Laboratory Alderley Park Macclesfield Cheshire, United Kingdom. Report No. CTL/R/851

GREEN, T., PROVAN, W.M., GOWANS, N. Internal ECETOC Document. 1986b. Methylene Chloride (Dichloromethane): In vivo inhalation pharmacokinetics and metabolism in F344 rats and B6C3F1 mice Imperial Chemical Industries PLC Central Toxicology Laboratory Alderley Park Macclesfield Cheshire, United Kingdom. Report No. CTL/R/880

GREEN, T., NASH, J.A., and MAINWARDING., G. 1986c. Methylene Chloride (Dichloromethane): In vitro metabolism in rat, mouse, hamster liver and lung fractions and in human liver fractions. Imperial Chemical Industries PLC Central Toxicology Laboratory Alderley Park Macclesfield Cheshire, United Kingdom. Report No. CTL/R/879

GREEN, T., NASH, J.A., and HILL, S.J. Internal ECETOC Document. 1987a. Methylene Chloride (Dichloromethane): Glutathione-S-transferase metabolism in vitro in rat, mouse, hamster and human liver cytosol fractions. Imperial Chemical Industries PLC Central Toxicology Laboratory Alderley Park Macclesfield Cheshire, United Kingdom. Report No. CTL/R/934

GREEN, T., NASH, J.A., HILL, S.J., and FOSTER, J.R. Internal ECETOC Document. 1987b. Methylene Chloride (Dichloromethane): The effects of exposure to 4000 ppm on mouse lung enzymes. Imperial Chemical Industries PLC Central Toxicology Laboratory Alderley Park Macclesfield Cheshire, United Kingdom. Report No. CTL/R/935

GREEN, T., PROVAN, W.M., GOWANS, N. Internal ECETOC Document. 1987c. Methylene Chloride (Dichloromethane): In vivo inhalation pharmacokinetics in B6C3F1 mice (using stable isotpes) and F344 Rats. Imperial Chemical Industries PLC Central Toxicology Laboratory Alderley Park Macclesfield Cheshire, United Kingdom. Report No. CTL/R/931

GREEN, T., PROVAN, W.M., COLLINGE, D.C. and GUEST, A.E. 1988. Macromolecular interactions of inhaled methylene chloride in rats and mice. Toxicol. Appl. Pharmacol. 93:1-10

GUENGERICH, F.P., CRAWFORD, W.M., Jr., DOMORADZKI, J.Y., MACDONALD, T.L., and WATANABE, P.G. 1980. In vitro activation of 1,2-dichloroethane by microsomal and cytosolic enzymes. Toxicol. Appl. Pharmacol. 55:303-317

GUESS, H.A., CRUMP, K.S., and PETO, R. 1977. Uncertainty estimates for low-dose extrapolations of animal carcinogenicity data. Cancer Res. 37:3475-3483

HAKE, C.L, STEWART, R.D., FORSTER, H.V., LEBRUN, A.J., PETERSON, J.E., and WU, A. 1974. Results of the controlled exposure of human females to the vapor of methylene chloride. Report No. NIOSH-MCOW-ENVM-MC-74-3. Milwaukee, Wis. The Medical College of Wisconsin, Department of Environmental Medicine, Milwaukee

HALPERT, J.R., BALFOUR C., MILLER, N.E. and KAMINSKY, L.S. 1986. Dichloromethyl compounds as mechanism-based inactivators of rat liver cytochromes P-450 in vitro. Molec. Pharmacol. 30:19-24

HARDIN, B.D. and MANSON, J.M. 1980. Absence of dichloromethane teratogenicity with inhalation exposure in rats. Toxicol. Appl. Pharmacol. 52:22-28

HARKEY, M.R., SETTLE, W.L., JAMES, T.L., and TREVOR, A.J. 1979. Spectral studies on the interaction of dichloromethane with hemoglobin. Molec. Pharmacol. 16:1065-1074

HAUN, C.C., VERNOT, E.H., DARMER, K.I., and DIAMOND, S.S. 1972. Continuous animal exposure to low levels of dichloroemethane. AMRL-TR-130, No. 2. In Proceedings of the 3rd Conference on Environmental Toxicology, Wright-Patterson Air Force Base, OH, Aerospace Medical Research Laboratory, pp. 199-208

HEARNE, F.T. and FRIEDLANDER, B.R. 1981. Follow-up of methylene chloride study. J. Occup. Med. 23:660

HEARNE, F.T., GROSE, R., PIFER, J.W., FRIEDLANDER, B.R., and RALEIGH, R.L. 1987. Methylene Chloride Mortality Study: Dose response to characterization and animal model comparison. J. Occup. Med. 29:217-228

HEPPEL, L.A. and NEAL, P.A. 1944. Toxicology of dichloromethane (methylene chloride): II. Its effects on running activity in the male rat. J. Ind. Hyg. Toxicol. 26:17-21

HEPPEL, L.A., NEAL, P.A., PERRIN, T.L., ORR, M.L., and PORTERFIELD, V.T. 1944. Toxicology of dichloromethane (methylene chloride): I. Studies on effects of daily inhalation. J. Ind. Hyg. Toxicol. 26:8-16

HEPPEL, L.A. and PORTERFIELD. 1948. Enzymatic dehalogenation of certain brominated and chlorinated compounds. J. Biol. Chem. 76:763-769

HILL, D.L., SHIH, T.W., JOHNSTON, T.P., and STRUCK, R.F. 1978. Macromolecular binding and metabolism of the carcinogen 1,2-dibromoethane. Cancer Res. 38:2438-2442

HOGAN, G.K., SMITH, R.G., and CORNISH, H.H. 1976 (Abstract). Studies on the microsomal conversion of CH2Cl2 to CO. Toxicol. Appl. Pharmacol. 37:112

HOROWITZ, B.Z. 1986. Carboxyhemoglobinemia caused by inhalation of methylene chloride. Am. J. Emer. Med. 4(1):48-51

INTERNATIONAL AGENCY FOR RESEARCH ON CANCER (IARC). 1986. Monographs on the Evaluation of the Carcinogenic Risk of Chemicals to Humans. 41:43-85. IARC, Lyon, France.

JACOBY, R.O., BHATT, P.N., and JONAS, A.M. 1979. Viral diseases. Chpt. 11. In: The Laboratory Rat Vol. I. Biology and Diseases. Baker H.J., Lindsey, J.R., and Weisbroth, S.H., eds., Academic Press, New York. pp. 284-294

JAKOBY, W.B. and HABIG, W. 1980. Glutathione Transferases. Jakoby, W.B., ed. Enzymatic Basis of Detoxification, Vol. II. Academic Press, New York, pp. 63-64

JONGEN, W.M.F., ALINK, G.M., and KOEMAN, J.H. 1978. Mutagenic effect of dichloromethane on Salmonella typhimurium. Mutat. Res. 56:245-248

JONGEN, W.M.F., LOHMAN, P.H.M., KETTENHAGEN, M.J., ALINK, G.M., BERENDS, F., and KOEMAN, J.H. 1981. Mutagenicity testing of dichloromethane in short-term mammalian test systems. Mutat. Res. 81:203-213

JONGEN, W.M.F., HARMSON, E.G.M., ALINK, G.M., and KOEMAN, J.H. 1982. The effect of glutathione conjugation and microsomal oxidation on the mutagenicity of dichloromethane in S. typhimurium. Mutat. Res. 95:183-189

KALBFLEISCH, J.D., KREWSKI, D.R., and VAN RYZIN, J. 1983. Dose-response models for time-to-response toxicity data. Can. J. Stat. 11:25-49

KANADA, T. and UYETA, M. 1978 (Abstract). Mutagenic screening of organic solvents in microbial systems. Mutat. Res. 54:215

KARLSSON, J.E., ROSENGREN, L.E., KJELLSTRAND, P. and HAGLID, K. G. 1987. Effects of low-dose inhalation of three chlorinated aliphatic organic solvents of deoxyribonucleic acid in gerbil brain. Scand. J. Work Environ. Health 13:453-458

KIM, Y.C. and CARLSON, G.P. 1986. The effect on an unusual workshift on chemical toxicity. I. Studies on the exposure of rats and mice to dichloromethane. Fundam. Appl. Toxicol. 6:162-171

KLAASEN, C.D. 1986. Distribution, excretion and absorption of toxicants, In: Casarett and Doull's toxicology: the basic science of poisons. Klaassen, C.D., Amdur, M.O., and Doull, J. eds., Macmillan Publishing Co., NY pp. 56-57

KNUDSON, A.G. 1985. Hereditary cancer, oncogenes, and antioncogenes. Cancer Res. 45:1437-1443

KOVAR, J. and KREWSKI, D. 1981. Risk 81: a computer program for low-dose extrapolation of quantal-response toxicity data. Health & Welfare, Ottawa, Ontario

KUBIC, V.L., ANDERS, M.W., ENGEL, R.R., BARROW, C.H., and CAUGHEY, W.S. 1974. Metabolism of dihalomethanes to carbon monoxide. I. In vivo studies. Drug Metab. Dispos. 2:53-57

KUBIC, V.L. and ANDERS, M.W. 1975. Metabolism of dihalomethanes to carbon monoxide. II. In vitro studies. Drug Metab. Dispos. 3:104-112

KUBIC, V.L. and ANDERS, M.W. 1978. Metabolism in dihalomethanes to carbon monoxide. III. Studies on the mechanism of the reaction. Biochem. Pharmacol. 27:2349-2355

KURPPA, K. and VAINIO, H. 1981. Effects of intermittent dichloromethane inhalation on blod carboxyhemoglobin concentration and drug metabolizing enzymes in rat. Res. Comm. Chem. Path. Pharmacol. 32:535-544

LAMBERTSEN, C.J. 1974. Effects of excessive pressures of oxygen, nitrogen, carbon dioxide, and carbon monoxide: Implications in aerospace, undersea, and industrial environments. In Mountcastle, V.B., Vol. 2, 13th ed. C.V. Mosby Co., St. Louis, Missouri

LORENZ, J., GLATT, H.R., FLEISCHMANN, R., FERLINZ, R. and OESCH, F. 1984. Drug metabolism in man and its relationship to that in three rodent species: Mono-oxygena se, eposide hydrolase, and elutathione-s-transferase activities in subcellular fractions of lung and liver. Biochem. Med. 32:43-56

LUOMANMAKI, K., and COBURN, R.E. 1969. Effects of metabolism and distribution of carbon monoxide on blood and body stores. Am. J. Physiol. 271:354-363

LUTZ, W.K. 1979. In vivo covalent binding of organic chemicals to DNA as a quantitative indicator in the process of chemical carcinogenesis. Mutat. Res. 65:289-356

MACEWEN, J.D., VERNOT, E.H., and HAUN, C.C. 1972. Continuous animal exposure to dichloromethane. Report AMRL-TR-72-28. Aerospace Medical Research Lab. Wright-Patterson Air Force Base, Ohio, P 33

McCANN, J. and AMES, B.N. 1977. The Salmonella/microsome mutagenicity test: Predictive value for animal carcinogenicity. In: Origins of Human Cancer: Book C. Human Risk Assessment. Hiatt, H.H., Watson, J.D., and Winsten, J.A., eds., Cold Spring Harbor Laboratory, Cold Spring Harbor, New York. pp. 1431-1450

McCARROLL, N.E., CORTINA, T.A., ZITO, M.J., and FARROW, M.G. 1983. Evaluation of methylene chloride and vinylidene chloride in mutational assays. Environ. Mutagen 5:426-427

McGREGOR, D.G. 1979. Practical experience in testing unknowns in vitro. Chapter 4. In Paget, G.E., ed. Topics in Toxicology. Mutagenesis in submammalian systems: Status and Significance. University Park Press, Baltimore, Maryland. pp. 53-71

MCKENNA, M.J. and ZEMPEL, J.A. 1981. The dose-dependent metabolism of 14C-methylene chloride following oral administration to rats. Food. Cosmetic. Toxicol. 19:73-78

MCKENNA, M.J., ZEMPEL, J.A., BRAUN, W.H. 1982. The pharmacokinetics of inhaled methylene chloride in rats. Toxicol. Appl. Pharmacol. 65:1-10

MCKENNA, M.J., SAUNDEERS, J.H., BOECKLER, W.R., KARBOWSKI, R.J., NITSCHKE, K.D., and CHENOWETH, M.B. 1980 (Abstract). The pharmacokinetics of inhaled methylene chloride in human volunteers. Paper No. 176, 19th Annual Meeting of the Society of Toxicology, Washington, D.C., March 9-13

MENNEAR, J.H., MCCONNELL, E.E., HUFF, J.E. RENNE, R.A., AND GIDDENS, E. 1988. Inhalation toxicology and carcinogenesis studies of methylene chloride (Dichloromethane) in F344/N rats and B6C3F1 mice. Ann. New York Acad. Sci. Vol., 534 pp.343-351

MILLER, V.L., ENGEL, R.R., and ANDERS, M.W. 1973 (Abstract). In vivo metabolism of dihalomethanes to carbon monoxide. Pharmacologist 15:190

MILLER L., PATERAS, V., FRIEDERICI H., and ENGEL, G. 1985. Acute tubular necrosis after inhalation exposure to methylene chloride. Arch. Intern. Med. 145:145-146

MIRER F.E., SILVERSTEIN M., PARK R. 1988. Methylene chloride and cancer of the pancreas. J. Occup. Med. 210 (6):475-476

MOOLGAVKAR, S.H. and VENZON, D.J. 1979. Two-event models for carcinogenesis: Incidence curves for childhood and adult tumors. Math. Biosci. 47:55

MOOLGAVKAR, S.H. and KNUDSON, A.G. 1981. Mutation and cancer: A model for human carcinogenesis. JNCI 66:1037

MOOLGAVKAR, S.H. 1986. Carcinogenesis modeling: From molecular biology to epidemiology. Ann. Rev. Public Health 7:151-169

MORON, M.S., DEPIERRE, J.W., MANNERVIK, B. 1979. Levels of glutathione, glutathione reductase and glutathione S-transferase activities in rat lung and liver. Biochem. Biophys. Acta 582:67-78

MORRIS, J.B., SMITH, F.A., and GARMAN, R.H. 1979. Studies on methylene chloride induced fatty liver. Exp. Mol. Pathol. 30:386-393

MOSKOWITZ, S. and SHAPIRO, H. 1952. Fatal exposure to methylene chloride vapor. Arch. Ind. Hyg. Occp. Med. 6:116-123

MUKHTAR, H., ZOETEMELK, C.E.M., BAARS, A.J., WIJNEN, J.T., BLANKENSTEIN-WIJNEN, L.M.M., KHAN, P.M., and BREIMER, D.D. 1981. Glutathione S-transferase activity in human fetal and adult tissues. Pharmacol. 22:322-329

NATIONAL CANCER INSTITUTE (NCI). 1981. Surveillance Epidemiology End Results. Incidence and Mortality Data: 1973-77. Monograph 57. U.S. Dept. of Health and Human Services. NIH Publ. No. 81-2330

NATIONAL INSTITUTE FOR OCCUPATIONAL HEALTH (NIOSH). 1972. Criteria for a Recommended Standard for Occupational Exposure to Carbon Monoxide. HSM 73-11000

NATIONAL TOXICOLOGY PROGRAM (NTP). 1982. Draft technical report on the carcinogenesis bioassay of dichloromethane (methylene chloride), gavage study. Research Triangle Park, NC, and Bethesda, Maryland

NATIONAL TOXICOLOGY PROGRAM (NTP). 1986. Toxicology and Carcinogenesis Studies of Dichloromethane (Methylene Chloride) (CAS No. 75-09-2) in F344/N Rats and B6C3F1 Mice (Inhalation Studies). TR No. 306, NIH (USDHHS, PHS, NIH) Publication No. 86-2562

NESTMANN, E.R., LEE, E.G.H., MATULA, T.I., DOUGLAS, G.R., and MUELLER, J.C. 1980. Mutagenicity of constituents identified in pulp and paper mill effluents using the Salmonella/mammalian microsome assay. Mutat. Res. 79:203-212

NESTMANN, E.R., OTSON, R., WILLIAMS, D.T., and KOWBEL, D.J. 1981. Mutagenicity of paint removers containing dichloromethane. Cancer Lett. 11:295-302

NITSCHKE, K.D., EISENBRANDT, D.L., and LOMAX, L.G. 1985 (Unpublished Study). Methylene chloride: Two-generation inhalation reproduction study in Fischer 344 rats. Dow Chemical Co., Midland, Michigan

NITSCHKE, K.D., BUREK, J.D., BELL, T.J., KOCIBA, R.J., RAMPY, L.W., and MCKENNA, M.J. 1988a. Methylene chloride A two-year inhalation toxicity and oncogenicity study in rats. Fundam. Appl. Toxicol. 11:48-59.

NITSCHKE, K.D., EISENBRANDT, D.L., and LOMAX, L.G. and RAO, K.S., 1988b. Methylene chloride. Two generation inhalation reproductive study in rats. Fundam. Appl. Toxicol. 11:60-67

NORPOTH, K., WITTING, U., SPRINGORUM, M., and WITTING, C. 1974. Induction of microsomal enzymes in the rat liver by inhalation of hydrocarbon solvents. Int. Arch. Arbeitsmed. 33:315-321

NUNES, A.C. AND SCHOENBORN, B.P. 1973. Dicholoromethane and myoglobin function. Molec. Pharmocol. 9:835-839

OSTERMAN-GOLKAR. S., HUSSAIN, S., WALLES, S., ANDRSTAM, B., and SIGVARDSSON, K. 1983 Chemical reactivity and mutagenicity of some dihalomethanes. Chem-Biol Interactions 46:121-130

OTT, M.G., SKORY, L.K., HOLDER, B.B, BRONSON, J.M., and WILLIAMS, P.R. 1983a. Health evaluation of employees occupationally exposed to methylene chloride: General study design and environmental considerations. Scand. J. Work Environ. Health 9:1-7

OTT, M.G., SKORY, L.K., HOLDER, B.B., BRONSON, J.M., and WILLIAMS, P.R. 1983b. Health evaluation of employees occupationally exposed to Methylene chloride: Mortality. Scand. J. Work Environ. Health 9:8-16

OTT, M.G., SKORY, L.K., HOLDER, B.B, BRONSON, J.M., and WILLIAMS, P.R. 1983c. Health evaluation of employees occupationally exposed to methylene chloride: Clinical laboratory evaluation. Scand. J. Work Environ. Health 9:17-25

OTT, M.G., SKORY, L.K., HOLDER, B.B, BRONSON, J.M., and WILLIAMS, P.R. 1983d. Health evaluation of employees occupationally exposed to methylene chloride: Twenty-four hour electrocardiographic monitoring. Scand. J. Work Environ. Health 9:26-30

OTT, M.G., SKORY, L.K., HOLDER, B.B., BRONSON, J.M., and WILLIAMS, P.R. 1983e. Health evaluation of employees occupationally exposed to Methylene chloride: Metabolism data and oxygen half-satuaration pressures. Scand. J. Work Environ. Health 9:31-38

PANKOW, D., PONSOLD, W., and MORITZ R.-P. 1985. Zur sauerstofftransportkapazitat des blutes under einflutz von ethanol und dichlormethan. Z. Gesamte Hyg. 31:460-463

PEROCCO, P. and PRODI, G. 1981. DNA damage by haloalkanes in human lymphocytes cultured in vitro. Cancer Lett. 13:213-218

PETERSON, J. 1978. Modeling the uptake metabolism and excretion of dichloromethane by man. Am. Ind. Hyg. Assoc. 39:41-47

PETO, R. 1978. Carcinogenic effects of chronic exposure to very low levels of toxic substances. Environ. Health Perspec. 22:155-159

PRICE, P.J., HASSETT, C.M., and MASFIELD, J.I. 1978. Transforming activities of trichloroethylene and proposed industrial alternatives. In Vitro 14:290-293

PRITCHARD, A. and ANGELO, M.J. 1982. Internal NCA Study. Comparative pharmacokinetic studies of methylene chloride in oil versus drinking water. Report commissioned by the National Coffee Association of USA Inc. August 1982

PRITCHARD, A., DOONAN, B., and ROURKE, R. 1982. Internal NCA Study. Methylene chloride: Effects of microsomal enzymes in mice. Study commissioned by the National Coffee Association of USA Inc.

PUTZ, V.R., JOHNSON B.L., and SETZER, J.V. 1976. A comparative study of the effect of carbon monoxide and methylene chloride on human performance. J. Environ. Pathol. Toxicol. 2:97-112

RANNUG, V., SUUDVALL A., and RAMEL, C. 1978. The mutagenic effect of 1,2-dichloroethane on Salmonella typhimurium. I. Activation through glutathione in vitro. Chem. Biol. Interact. 20:1-16

REDDY, J.K., AZARNOFF, D.L., and HIGNITE, C.E. 1980. Hypolipidaemic hepatic peroxisomal proliferators form a novel class of chemical carcinogens. Nature 283:397

REITZ, R.H., MENDRALA, A.L., PARK C.N., ANDERSEN, M.E. and GUENGERICH, F.P. 1988. Incorporation of in vitro enzyme data into the PB-PK model for methylene chloride (CH_2CL_2): Implications for risk assessment. Toxicol. Lett. 43:97-116

REITZ, R.H., SMITH, F.A., and ANDERSEN, M.E. 1986. (Abstract). In vivo metabolism of 14C-methylene chloride (MEC). Toxicologist 6:260

REITZ, R.H., MENDRALA. A.L. and SCHUMANN, A.M. 1987 (Unpublished). Methylene chloride-glutathione-S-transferase studies: in vitro experiments in humans, mice, rats, and hamsters. For submission to the EPA Science Advisory Board, Dow Chemical Company, Midland, MI.

RIANO, M. and REST. V.M. 1986. Methylene chloride (dichloromethane): Summary of toxicologic data and cancer risk assessment. New York State Department of Environmental Conservation, Albany, NY

RILEY, E.C., FASSETT, D.W., and SUTTON, W.L. 1966. Methylene chloride vapor in expired air of human subjects. Am. Ind. Hyg. Assoc. J. 27:341-348

RODKEY, F.L. and COLLISON, H.R. 1977a. Biological oxidation of 14C-methylene chloride to carbon monoxide and carbon dioxide by the rat. Toxicol. Appl. Pharmacol. 40:33-38

RODKEY, F.L. and COLLISON, H.R. 1977b. Effect of dihalogenated methanes on the in vivo production of carbon momoxide and methane by rats. Toxicol. Appl. Pharmacol. 40:39-47

ROGHANI, M., DASILVA, C., and CASTOGNA, M. 1987. Tumor promotor chloroform is a potent protein kinase C activator. Biochem. Biophys. Res. Commun. 142:738-744

ROSENGREN, L.E., KJELLSTRAND, P., AURELL, A., and HAGLID, K.G. 1986. Irreversible effects of dichloromethane on the brain after long term exposure: A quantitiative study of DNA and the glial cell marker proteins S-100 and GFA. Br. J. Ind. Med. 43:291-299

ROTH, R.P., DREW, R.T., LO, R.J., and FOUTS, J.R. 1975. Dichloromethane inhalation, carboxyhemoglobin concentration, and drug metabolizing enzymes in rabbits. Toxicol. Appl. Pharmacol. 33:427-437

SAMOILOFF, M.R., SCHULZ, S., JORDAN, Y., DENICH, K., and ARNOTT, E. 1980. A rapid simple long-term toxicity assay for aquatic contaminants using the nematode Panagrellus redivivus. Can. J. Fish Aquat. Sci. 37:1167-1174

SAVOLAINEN, H., PFAFFLI, P., TENGEN, M., and VAINIO, H. 1977. Biochemical behavioral effects of inhalation exposure to tetrachloroethylene and dichloromethane. J. Neuropath. Exp. Neurol. 36:941-949

SCHUMANN, A.M., NITSCHKE, K.D., FOX, T.R., and WATANABE, P.G. 1983. (14C) Methylene Chloride: Pharmacokinetics and Interaction with Hamster and Rat Tissue Macromolecules. Report of the Toxicology Research Laboratory. Dow Chemical Company, Midland, Michigan

SCHWETZ, B.A., LEONG, B.K.J., and GEHRING, P.J. 1975. The effect of maternally inhaled trichloroethylene, perchloroethylene, methylchloroform, and methylene chloride on embryonal and fetal development in mice and rats. Toxicol. Appl. Pharmacol. 32:84-96

SEIDERGARD, J. and PERO, R.W. 1985. The hereditary transmission of high glutathione transferase activity towards transstilbene oxide in human mononuclear leukocytes. Hum. Genet. 69:66-68

SEIDERGARD, J., PERO, R.W., MILLER, D.G., and BEATTIE, E.J. 1985. A glutathione transferase in human leukocytes as a marker for the susceptibility to lung cancer. Carcinogenesis 7:751-753.

SEROTA, D.G., THAKAR, A.K., ULLAND, B.M., KIRSCHMAN, J.C., BROWN, N.M., COOTS, R.H. and MORGANEIDGE, K. 1986a. A two year drinking water study of dichloromethane on rodents: I. Rats Food Chem. Toxicol. 24:951-958

SEROTA, D.G., THAKAR, A.K., ULLAND, B.M., KIRSCHMAN, J.C., BROWN, N.W.M, COOTS, R.H. and MORGAREIGE, K. 1986b. A two year drinking water study of dichloromethane on rodents: II Mice. Food Chem. Toxicol. 24:959-963

SETTLE, W. 1975 (Abstract). Role of changes in carbon monoxide-hemoglobin finding in methylene chloride toxicity. Fed. Proc. 34:229

SHELDON, T., RICHARDSON, C.R., and ELLIOTT, B.M. 1987. Inactivity of methylene chloride in the mouse bone marrow micronucleus assay. Mutagen. 2(1):57-59

SHUKLA, R.R. and ALBRO, P.W. 1987. In vitro modulation of protein kinase C activity by environmental chemical pollutants. Biochem. Biophys. Res. Commun. 142:567-572

SIMMON, V.F., KAUHANEN, V., and TARDIFF, R.G. 1977. Mutagenic activity of chemicals identified in drinking water. Dev. Toxicol. Environ. Sci. 2:249-258

SIMMON, V.F. and KAUHANEN, V. 1978. In vitro microbological mutagenicity assays of 2-chloroethyl chloroformate. Final report, Contract No. 68-03-11-74. Prepared for U.S. Environmental Protection Agency, National Environmental Research Center, Water Supply Laboratory, Cincinnati, Ohio

SINA, J.F., BEAN, C.L., DYSART, G.R., TAYLOR, V.I., and BRADLEY, M.O. 1983. Evaluation of the alkaline elution/rat hepatocyte assay as a predictor of carcinogenic/mutagenic potential. Mutat. Res. 113:357-391

SIPES I.G. 1987. Personal communication. University of Arizona

SIVAK, A. 1978. BALBc/-3T3 Neoplastic Transformation Assay with Methylene Chloride (Food Grade Test Specification). Report to the National Coffee Association, Inc. Arthur D. Little, Inc., Cambridge, Massachusetts

SNOW, L., McNAIR, P., and CASTRO, B.C. 1979. Mutagenesis testing of methylene chloride and 1,1,1-trichloroethane in Salmonella strains TA-100 and TA-98. Personal Communication from Northrop Services, Inc., Research Triangle Park, North Carolina. September 19

STEVENS, J.L. and ANDERS, M.W. 1978. Studies on the mechanisms of metabolism of haloforms to carbon monoxide. Toxicol. Appl. Pharmacol. 45:297-298

STEVENS, J.L. and ANDERS, M.W. 1979. Metabolism of haloforms to carbon monoxide. III. Studies on the mechanism of the reaction. Biochem. Pharmacol. 28:3189-3194

STEVENS, J.L., RATNAYAKE, J.H., and ANDERS, M.W. 1980. Metabolism of dihalomethanes to carbon monoxide. IV. Studies in isolated rat hepatocytes. Appl. Pharmacol. 55:484-498

STEWART, R.D. and DODD, H.C. 1964. Absorption of carbon tetrachloride, trichloroethylene, tetrachloroethylene, methylene chloride, and 1,1,1-trichloroethane through the human skin. Am. Ind. Hyg. Assoc. J. 25:439-446

STEWART, R.D. and HAKE, C.L. 1976. Paint-remover hazard. JAMA J. Am. Med. Assoc. 235:398-401

STEWART, F.D., FISHER, T.N., HOSKO, M.J., PETERSON, J.E., BARETTA, E.D., and DODD, H.C. 1972a. Carboxyhemoglobin elevation after exposure to dichloromethane. Science 176:295-296

STEWART, R.D., FISHER, T.N., HOSKO, M.J., PETERSON, J.E., BARETTA, E.D., and DODD, H.C. 1972b. Experimental human exposure to methylene chloride. Arch. Environ. Health 25:342-348

SVIRBELY, J.L., HIGHMAN, B., ALFORD, W.C, and VON OETTINGEN, W.F. 1974. The toxicity and narcotic action of mono-chloro-mono-bromo-methane with special reference to inorganic and volatile bromide in blood, urine and brain. J. Ind. Hyg. Toxicol. 29:383-389

SZABO, S., HORBATH, E., KOVACS, K. and LARSEN, P.R. 1978. Pyrazole-induced thyroid narcosis; a distinct organ lesion. Science 199:1209-10

TAKANO, T. and MIYAZAKI, Y. 1988. Metabolism of dichloromethane and the subsequent binding of its product, carbon monoxide, to cytochrome P-450 in perfused rat liver. Toxicol. Let. 40:93-96

TARONE, R.E., CHU, K.C., and WARD, J.M. 1981. Variability in the rates of some common naturally occurring tumors in Fischer 344 rats and (C57BL/6NXC3H/HEN)F1 (B6C3F1) mice JNCI 66:1175-1181

TASKINEN, H., LINDBOHM, M.-L. and HEMMINKI, K. 1987. Spontaneous abortions among women working in the pharmaceutical industry. Brit. J. Ind. Med. 43:199-205

TAYLOR, G.J., DREW, R.T., LORES, E.M, and CLEMMER, T.A. 1976. Cardiac depression by haloalkane propellants, solvents, and inhalation anesthetics in rabbits. Toxicol. Appl. Pharmacol. 38:379-387

THILAGAR, A.K. and KUMAROO, P.V. 1983. Induction of chromosome damage by methylene chloride in CHO cells. Mut. Res. 116:361-367

THILAGAR, A.K., BACK, A.M., KIRBY, P.E., KUMAROO, P.V., PANT, K.J., and CLARKE, J.J. 1984a. Evaluation of dichloromethane in short-term in vitro genetic toxicity assays. Env. Mutat. 6:418-419

THILAGAR, A.K., KUMAROO, P.V., CLARKE, J.J., KOTT, S., BACK, A.M., and KIRBY, P.E. 1984b. Induction of chromosome damage by dichloromethane in cultured human peripheral lymphocytes, CHO cells, and mouse lymphoma L5178Y cells. Env. Mutat. 6:422

THILAGAR, A.K., BACK, A.M., KIRBY, P.E., KUMAROO, P.V., PANT, K.J., CLARKE, J.J., KNIGHT, R., and HAWORTH, S.R. 1984c (Abstract) Evaluation of dichloromethane in short term in vitro genetic toxicity assays. Journal of the Envir. Mutagen Soc. 6:418-419

THOMAS, A.A., PINKERTON, M.K., and WARDEN, J.A. 1971. Effects of methylene chloride exposure on the spontaneous activity of mice. In Proceedings of the 2nd Annual conference on Environmental Toxicology, Aerospace Medical Research Laboratory, Wright-Patterson Air Force Base, Ohio. AMRL-TR-H-120. Paper No. 13. pp. 185-189

THOMAS, A.A., PINKERTON, M.K., and WARDEN, J.A. 1972. Effects of low level dichloromethane exposure on the spontaneous activity of mice. In Proceedings of the 3rd Annual Conference on Environmental Toxicology, Aerospace Medical Research Laboratory, Wright-Patterson Air Force Base, Ohio. AMRL-TR72-130 Paper No. 14. pp. 223-227

THORSLUND, T.W., BROWN, C.C., and CHARNLEY, G. 1987. Biologically motivated cancer risk models. Risk Anal. 7:109-119

TOLLEFSON L., LORENTZEN R.J., SPRINGER J., BROWN R.N. 1988. Human and animal risk comparisons. J. Occup. Med. 30(1):18-19

TRUEMAN, R.W., and ASHBY, J. 1987. Lack of VDS activity in the livers of mice and rats exposed to dichloromethane. Environ. Molec. Mutagen. 10:189-195

VAN BLADEREN, P.J., BREIMER, D.D., ROTTEVEEL-SMIJS, G.M.T., and MOHN, G.R. 1980. Mutagenic activation of dibromomethane and diiodomethane by mammalian microsomes and glutathione S-transferases. Mutat. Res. 74:341-346

WEINSTEIN, R.S., and DIAMOND, S.S. 1972. Hepatotoxicity of dichloromethane (methylene chloride) with continuous inhalation exposure at a low-dose level. In Proceedings of the 3rd Annual Conference on Environmental Toxicology, Aerospace Medical Research Laboratories, Wright-Patterson Air Force Base, Ohio. AMRL-72-130. Paper No. 13. pp. 209-222.

WEINSTEIN, R.S., BOYD, D.D., and BACK, K.C. 1972. Effects of continuous inhalation of dechloromethane in the mouse. Morphologic and functional observations. Toxicol. Appl. Pharmacol. 23:660-679.

WEISS, G. 1967. Toxic encephalosis as an occupational hazard with methylene chloride. Zentrabl. Arbeitsmed. 17:282-285

WHITTEMORE, A. 1978. Quantitative theories of carcinogenesis. Adv. Cancer Res. 27:55-58.

WHITTEMORE, A. and KELLER, J.B. 1978. Quantitative theories of carcinogenesis. SIAM Rev. 20:1-30

WINNEKE, G. 1974. Behavioral effects of methylene chloride and carbon monoxide as assessed by sensory and psychomotor performance. In: National Institute for Occupational Safety and Health. Xintaras, C., Johnson, B.L., and DeGroot, eds., Publication DHEW (NIOSH) Publication No. 74-126. pp. 130-144

WINNEKE, G. 1981. The neurotoxicity of dichloromethane. Neurobehav. Toxicol. Teratol. 3:391-395

WINNEKE, G. and FODOR, G.G. 1976. Dichloromethane produces narcotic effects. Int. J. Occup. Health Safety 45:34-37

YESAIR, D.W., JAQUES, P., SHEPIS, P., and LISS, R.H. 1977 (Abstract). Dose-related pharmacokinetics of 14C-methylene chloride in mice. Fed. Proc. Am. Soc. Exp. Biol. 36:998

ZAKHARI, S. 1977. Methylene chloride. In: Non-Fluorinated Propellants and Solvents for Aerosols. Golberg, L., ed. CRC Press, Cleveland

APPENDIX A

RESULTS OF GENETIC TOXICITY TESTS OF METHYLENE CHLORIDE

The following table lists individual studies of different aspects of the genetic toxicity of CH_2Cl_2. These investigations include mutation in both prokaryotic and eukaryotic systems, mammalian cell transformation, and indications of DNA damage such as sister chromatid exchange and unscheduled DNA synthesis. Results of each study are summarized in the table, and discussed in the text in Section 6.0. The table is an updated and corrected version of that found in EPA (1985a).

TABLE A-1 Results of Genetic Toxicity Tests of Methylene Chloride
(Adapted from EPA 1985a)

Reference	Test System	Strain	Activation System	Concentration/Results				Comments
Simmon et al., 1977	Salmonella/S9 vapor exposure	TA1535 TA1537 TA1538 TA98 TA100	None	(Extrapolated from figure 17) 0, 50, 100, 200, 400, and 800 µl/9 liter desiccator	(Extrapolated from figure 17) TA100 Dose (µl) / Revertants/plate 0 / 170 50 / 210 100 / 300 200 / 400 400 / 650 800 / 1350			1. Toxicity not reported. 2. Number of revertants observed for TA100 not specified numerically 3. Data not presented for strains other than TA100 4. Purity and source of compound not provided 5. Positive response
Simmon and Kauhanen, 1978	Salmonella/S9 vapor exposure	TA100	Aroclor 1254 induced rat liver microsome S9 mix	0 and 1 ml/9 liter desiccator for 6.5 and 8 hours	TA100 revertants Dh / Met Act / Treated / Control 6.5 / - / 644 / 133 6.5 / + / 1344 / 130 8 / - / 810 / 174 8 / + / 917 / 158			1. Toxicity not reported 2. Purity and source of compound not provided 3. Used as a positive control in the testing of 2-chloroethyl-chloroformate. 4. Positive response

(continued on the following page)

TABLE A-1 (Continued)

Reference	Test System	Strain	Activation System	Concentration	Results	Comments
Kanada and Uyeta, 1978	Salmonella/S9 and B. subtilis rec assay testing	TA98 TA100	PCB induced rat liver microsome S9 mix	Not reported	CH_2Cl_2 reported negative for both strains in B. subtilis and positive for both in S. typhimurium	1. Results summarized in abstract form. 2. Positive results of "Ames" testing supports reports by other authors using same system.
Jongen et al., 1978	Salmonella/S9 vapor exposure	TA98 TA100	Phenobarbital induced rat liver microsome S9 mix	(ppm x 10^3)	TA100[a] +S-9 / -S9; TA98[a] +S9 / -S9	1. Testing conducted in gas tight perspex boxes. 2. Only highest dose exhibited less than 83% survival. 3. Purity of CH_2Cl_2 not reported. 4. Positive response.

(ppm x 10^3)	TA100[a] +S-9	TA100[a] -S9	TA98[a] +S9	TA98[a] -S9
0	152±19	129±12	21±4	19±5
5.7	379±37	248±32	54±5	44±8
11.4	515±76	407±47	74±4	56±10
14.1	757±82	582±56	93±9	66±12
22.0	865±82	653±89	123±10	96±11
57.0	1201±191	740±94	149±42	110±42

[a]Results from three experiments, five plates/dose.

Reference	Test System	PPM Vapor	µmoles/plate[a]	Revertants/Plate TA1535 -S9	TA1535 +S9	TA98 -S9	TA98 +S9	TA100 -S9	TA100 +S9	Comments
E.D. Barber et al. 1980	Salmonella/S9 vapor exposure	0	0	23	28	23	39	254	264	1. Tested redistilled sample of CH_2Cl_2 >99.9% pure. 2. Revertants/mole at highest dose for TA1535, TA98, and TA100 were 0.0006, 0.006, and 0.03, respectively. 3. Data shown for testing in gas tight chamber. 4. Negative response in standard test; positive response in gas tight chamber.
		3,600	38	40	36	259	288	752	1152	
		7,200	76	59	51	441	297	1440	960	
		9,100	96	78	78	459	322	2640	1096	
		10,900	115	64	50	741	479	3060	3240	

[a]CH_2Cl_2 concentration measured by GC/MS in aqueous phase of petri dish

(continued on the following page)

TABLE A-1 (Continued)

Reference	Test System	Strain	Activation System	Concentration/Results				Comments
McGregor, 1979	Salmonella/S9 vapor exposure	TA1535	None	Atmospheric Concentration% Theoretical	Actual	Plate Concentration µg	Revertants	1. Purity of CH_2Cl_2 not reported 2. Positive response
				0	nd	nd	15	
				0.5	0.14	245	20	
				1.0	0.33	600, 595, 530	25	
				2.0	0.67	1400	50	
				4.0	1.60	2475	75	
				10	nd	nd	80	
Nestmann et al., 1980	Salmonella/S9 vapor exposure	TA1535 TA1537 TA1538 TA98 TA100	Aroclor-induced rat liver S9					1. Data not presented. 2. Negative response in standard test. 3. Positive response in gas tight chamber. Doubling in revertant counts for TA1535; 6-fold increase for TA100.

Reference	Test System	Strain	Activation System	Dose (µl/Chamber)	Results TA100** +S9	TA100** -S9	TA98 +S9	TA98 -S9	Comments
Snow et al., 1979	Salmonella/S9 vapor exposure	TA98 TA100	Methylene chloride induced Syrian Golden Hamster liver S9 microsome mix	0	66	63	38	39	1. Purity of CH_2Cl_2 not reported 2. No information about variability of results. 3. Positive response.
				100	177	142	47	31	
				300	463	274	69	46	
				500	642	468	92	61	
				1000	972	632	39	72	

(continued on the following page)

**Mean calculated from three plates/dose.

TABLE A-1 (Continued)

Reference	Test System	Strain	Activation System	Dose	Results		Comments
Green, T. 1983	Salmonella/S9 vapor exposure	TA1535 TA100	Rat liver fractions	Dose (% in air)	TA 100 +S9	-S9	1. Preliminary results presented in abstract form. 2. Metabolic studies conducted in rat tissue and TA100. Similar metabolism in both systems. Radiolabel reported to bind to bacterial DNA but not to rat liver DNA. 3. Purity of DCM not reported. 4. Positive response. Author thinks this is due to close proximity of cytoplasmic enzymes and intermediates to DNA in bacteria and that negative responses would be obtained in higher organisms. Positive responses in other tests argue against this. See discussion in text.
				0	69±3	-	
				1.4	283±10	267±20	
				2.8	506±27	462±28	
				5.5	825±34	872±27	
				8.3	1050±88	997±88	

(continued on the following page)

TABLE A-1 (Continued)

Reference	Test System	Strain	Activation System	Concentration/Results	Comments
Westmann et al., 1981	Salmonella/S9 vapor exposure	TA1535 TA1537 TA1538 TA98 TA100	Aroclor 1254-induced rat liver S9		1. Levels of CH_2Cl_2 in exposure chambe related directly to the mutational dose-effect curves of 3 paint removers. 2. Data shown for one paint remover only. Other 2 gave similar response. 3. Purity of CH_2Cl_2 not reported. 4. Positive response for paint removers likely due to CH_2Cl_2

Material Type	Material Weight (mg) Added	Material Weight (mg) Vaporized	his+ Revertants/Plate[a] TA1535	TA100	TA98	CH_2Cl_2 Time Averaged[b]	CH_2Cl_2 Max 6h Calculated[c]	Exposure Level (mg/l) CH_2Cl_2 Measured	Exposure Level (mg/l) Methanol	Exposure Level (mg/l) Ethanol[d]
Paint remover	0	--	16	144	25	---	---	---	---	---
	203	144	22	310	31	12.7	15.5	13.0	<0.5	---
	370	241	14	433	42	21.9	25.5	23.0	<0.5	---
	790	469	23	563	76	40.1	49.9	45.0	0.7	---
	1435	903	31	785	60	80.2	95.4	86.0	1.9	---
		(ml)								
Mix [90:5:5 v/v/v CH_2Cl_2 methanol/ ethanol]	0	---	13	154	32	---	---	---	---	---
	0.1	0.1	15	268	43	12.2	12.8	11.5	<0.5	<0.9
	0.2	0.2	24	401	73	26.9	25.3	27.5	<0.5	0.7
	0.4	0.4	25	789	138	50.6	50.8	50.0	0.9	1.1
	0.8	0.8	34	1084	164	94.1	101.0	94.5	2.6	2.7
4NQO (Average values from triplicate plates in 4 experiments	0.001	---	28	878	162	---	---	---	---	---

(a) Average values from triplicate plates. (b). Determined from an area under curve for concentration against time. (c). Calculated from amount vaporized assuming only CH_2Cl_2 vaporized, in 9l chamber. (d). Maximum measured.

(continued on the following page)

TABLE A-1 (Continued)

Reference	Test System	Strain	Activation System	Dose	Results			Comments
Green, 1983	Salmonella/S9 vapor exposure	TA100	Aroclor-1254 induced rat liver S9, microsomes, and cytosol		Revertants			1. Bacterial and mammalian metabolism similar. 2. Cytosol and glutathione catalyze CH_2Cl_2 to formaldehyde and CO_2. S-chloromethylglutathione is a putative intermediate. 3. CH_2Cl_2 onverted to carbon monoxide in the presence of microsomes. Formyl chloride is a putative intermediate. 4. Purity of CH_2Cl_2 not given. 5. Positive response. See entry for Green (1980).
				% Vapor	+S9	-S9	+ Cytosol	
				0	100	100	—	
				2.8	458	386	490	
				5.0	700	720	—	
				8.4	950	900	—	
Gocke et al., 1981	Salmonella/S9 vapor exposure	TA1535 TA1537 TA1538 TA98 TA100	Aroclor-1254 rat liver S9		Revertants			1. Spontaneous revertants for TA100 too low. 2. No information presented for toxicity. 3. Purity of CH_2Cl_2 not given. 4. Equivocal positive response.
				µl/desiccator	+S9	-S9		
				0	30±0	40±0		
				125	54±7	85±7		
				250	68±32	110±14		
				500	105±7	195±21		
				750	203±32	295±7		
Jongen et al., 1982	Salmonella/S9 vapor exposure	TA100	Aroclor-1254 rat liver S9, microsomes, and cytosol		% CH_2Cl_2			1. Purity of CH_2Cl_2 not given. 2. Positive response.
				Activation	0	0.35	0.7	1.4
				---	150	210	350	550
				S9	150	240	410	730
				Cytosol	150	220	420	810
				Microsomes	150	215	380	610

TABLE A-1 (Continued)

Reference	Test System	Strain	Dose (mM)	% survival	Response/10^6 Survivors: trp-5 Conversion	Recombination	Total Genetic Alterations	ilv-1 Revertants	Comments
Callen et al., 1980	Saccharomyces cerevisiae	D7	0	100	18	310	3300	2.7	1. Positive response 2. Active metabolites produced by this system are made intracellularly rather than by an exogenously employed activation system.
			104	77	28	190	3900	4.4	
			157	42	107	4490	14000	5.8	
			209	--	--	--	--	--	
Simmon et al., 1977	Saccharomyces cerevisiae suspension test	D3							1. Data not provided but reported negative for mitotic recombination. 2. Strain differences and differences in treatment conditions (i.e., time and temperature) may be the cause of differences between this study and that of Callen et al. (1980) 3. Cytochrome P450 concentration not known. Callen et al. (1980) report different yeast strains have different levels.

TABLE A-1 (Continued)

Reference	Test System	Strain	Chemical	Route	# Chromosomes Tested	# Lethals	Corrected Lethals (%)	Comments
Abrahamson and Valencia, 1980	Drosophila sex-linked recessive lethal test	FM6 females, Canton S males	EMS	Fed	773	44	5.69	1. No precaution taken to design exposure chambers to prevent evaporation of the compound for feeding experiment.
			Tris	Fed	2442	35	1.43	
			Neg. Controls	Fed or Inj.	94491	230	0.233	2. No concurrent negative controls reported for the injection experiment.
			CH_2Cl_2	Fed	14682	34	0.204	
				Inj	8262	18	0.157	3. Negative response at dose tested (224 mM).
Gocke et al., 1981	Drosophila sex-linked recessive lethal test	Basc females, Berlin K males	CH_2Cl_2 (mM)	Lethals/Brood (Percent) 1	2	3		1. Positive response for Brood 1 indicating CH_2Cl_2 is mutagenic to sperm in Drosophila.
			0	19/7130 (0.27)	8/5525 (0.14)	13/3416 (0.38)		2. Higher dose used than in test by Abrahamson and Valencia (1980).
			125	16/3632 (0.44)	2/2579 (0.08)	6/1310 (0.46)		
			620	8/1213 (0.66)	3/735 (0.41)	5/1005 (0.50)		
			Total treated	24/4845 (0.50)	5/3114 (0.16)	11/2315 (0.47)		
				P < 0.05				

(continued on the following page)

TABLE A-1 (Continued)

Reference	Test System	Concentration (mol/L)	Mutation Frequency (Lethal mutations/10^5 loci)	Toxicity (Survival rel. to controls)				Comments
				L2 Juveniles	L2-L3 Molt	L3-L4 Molt	L4 Adult Molt	
Samoiloff et al., 1980	Panagrelus redivivus sex-linked recessive lethal test	CH_2Cl_2						1. Equivocal positive response. 2. Not dose-related. 3. Some positive controls gave negative (e.g. EMS) or only marginally positive response (e.g. 3-methylcholanthrene). 4. Test system not validated.
		10^{-8}	6.0	1.02	0.99	0.97	0.46	
		10^{-6}	10.0	1.02	1.00	0.86	0.15	
		10^{-4}	9.8	1.00	1.00	0.88	0.17	
		Proflavine						
		10^{-8}	12.5					
		10^{-6}	10.0					
		10^{-4}	28.6					

TABLE A-1 (Continued)

Reference	Test System	Concentration (%)		V79 Mutants/10^5 Survivors	V79 Survival (%)	CHO Mutants/10^5 Survivors	CHO Survival (%)	Comments
Jongen et al., 1981	6-thioguanine resistance in V79 and CHO cells	DCM	0	2	100	1.9	100	1. Equivocal negative response.
			1	1.8	98	1.8	90	2. Highest dose only resulted in 20% decrease in survival. Higher doses up to about 80% toxicity should be tested.
			2	2	95	1.2	85	
			3	1.7	85	0.9	80	
			4	1.6	80	2.1	73	
			5			2.5	76	
		EMS	0	2				
			2	13				
			4	33				
Thilagar et al. 1984a	Mutation at thymidine kinase locus in mouse lymphoma C5178Y cells and neoplastic transformation in C3H10T1/2 cells							1. Reported negative in meeting abstract. 2. Experimental details not given.

TABLE A-1 (Continued)

		Concentration (%)		Foci/Plate	Survival (%)	Comments
Sivak 1978	Neoplastic transformation in BALB/C-3T3 cells	CH_2Cl_2	0	0.11 ± 0.07	100	1. Food grade purity CH_2Cl_2
			$1x10^{-5}$	0.28 ± 0.14	60	
			$1x10^{-4}$	0.47 ± 0.15	58	
			$1x10^{-3}$	0.11 ± 0.08	71	
			$1x10^{-2}$	0.40 ± 0.13	66	
		3-MC	0	0.11 ± 0.07	100	
			$2x10^{-4}$	3.69 ± 0.52	29	
Price et al. 1978	Neoplastic transformation in F1076 cells		0	0		1. Reagent grade purity CH_2Cl_2 was used.
			.16	7		
			1.6	9		

TABLE A-1 (Continued)

Reference	Strain/Tissue	Route of Exposure	Dose	Breaks Chromatid	Breaks Chromosome	Dicentrics	Rings	Exchanges	Comments
Burek et al. 1984	Male and female Sprague-Dawley rat/bone marrow	Inhalation	0	0.9 ± 0.99	0.2 ± 0.42	0	0	0	1. 5 animals/sex/dose.
			500	0.5 ± 0.71	0.2 ± 0.42	0	0	0	2. 200 cells/animals.
			1500	0.5 ± 0.97	0.1 ± 0.32	0	0	0.1 ± 0.32	3. Dose to bone marrow cells may have been low.
			3500	0.7 ± 0.48	0.2 ± 0.42	0	0.2 ± 0.42	0	4. Negative response.

Reference	Test System	Dose	RCG[a]	Breaks Chromatid	Breaks Isochromatid	Exchange	Number of Aberrations/Cell	Cells w/ Aberration (%)	Comments
Thilagar and Kumaroo, 1983	Cultured CHO cells	CH_2Cl_2 (µl/ml)							1. Positive response.
		0	100	2	0	0	0.02	2	2. Four experiments yielded similar response.
		2	98.4	4	0	2	0.06	6	
		5	75.3	8	14	8	0.34	26	
		10	66.7	12	34	10	0.56	38	
		TEM 1 µg	N.D.	22	30	42	0.96	66	

[a]RCG = Relative cell growth.

Reference	Strain/Tissue	Route of Exposure	Dose ppm	Breaks Chromatid	Breaks Chromosome	Dicentrics	Rings	Exchanges	Comments
Gocke et al., 1981	Male and female NMRI mice/bone marrow	i.p. Injection	No. injection x mg/kg		Micronucleated Polychromatic Erythrocytes (%)				1. Probable positive response; authors indicate negative response.
			0		0.19				2. Dose to bone marrow cells may have been low.
			2 x 425		0.19				
			2 x 850		0.35				
			2 x 1700		0.28				

TABLE A-1 (Continued)

Reference	Test System	Dose	Results		Comments
		% CH_2Cl_2	SCE/Cell Experiment # 1	7	
Jongen et al., 1981	SCE/V79 cells in culture	0	0.26 ± 0.02	0.30 ± 0.03	1. Exposure time 1 hour. 2. Positive response. Significant increases in SCEs ($P < 0.001$). 3. Same type of dose-response observed in experiments 2-6 (data not shown). 4. DMSO did not increase incidence of SCE.
		0.5	0.40 ± 0.02	---	
		1.0	0.46 ± 0.02	0.47 ± 0.02	
		2.0	0.45 ± 0.03	0.51 ± 0.03	
		3.0	---	0.58 ± 0.03	
		4.0	0.51 ± 0.03	0.61 ± 0.03	

Reference	Test System	Dose	SCE/Cell (X ± SD)	Range of SCE's	M_1	M_2	M_3[a]	Comments
Thilagar and Kumaroo, 1983	Cultured CHO cells	CH_2Cl_2 (µl/ml) 0	10.28 ± 3.17	5-17	0	3	97	1. Marginal, but not significant increas in SCEs. 2. Three other experiments yielded simil responses. 3. Results not inconsistent with test by Jongen et al. (1981) where highest dose was three times greater (i.e., 471 mM vs 156 mM).
		2	11.36 ± 3.09	3-19	0	16	84	
		5	12.56 ± 2.95	7-18	6	54	40	
		10	12.36 ± 3.35	7-21	4	56	40	
		Triethylene melamine 0.025 µg/ml	47.74 ± 4.76	39-61	4	49	47	
McCarroll et al. 1983	Cultured CHO cells							1. A dose-related increase in SCE's was observed over the dose range 1.8, 3.6, 5.4, and 7.0% CH_2Cl_2 following a 24-hour exposure in the atmosphere. 2. Reported as a meeting abstract; actual results not given.

[a] M_1 - Percentage of cells at first mitosis.
M_2 - Percentage of cells at second mitosis.
M_3 - Percent of cells between first and second mitosis.

TABLE A-1 (Continued)

Reference	Test System	Dose *	Unscheduled DNA Synthesis	Comments
Jongen et al. 1981	V79 cells	0 (%) 0.5 2.0 3.0 4.0		1. Negative results reported. 2. Positive results obtained for 4NQO as a positive control.
Thilager et al. 1984c	Primary rat hepatocytes			1. Reported negative in meeting abstract. 2. Experimental details not given.

Reference	Test System	Dose	S9 Mix	^{3}H-TdR Uptake	
Perocco and Prodi 1981	Primary human lymphocytes	0	-	715±26 (dpm)	1. CH_2Cl_2 was negative as an inducer of unscheduled DNA synthesis.
		2.5	-	593±24	
		5	-	491±27	
		10	-	532±31	
		0	+	612±26	
		2.5	+	573±35	
		5	+	510±40	
		10	+	537±39	

* - % CH_2Cl_2

TABLE A-1 (Continued)

Reference	Test System	Dose	Results	Comments
Osterman-Golkar et al. 1983	E. Coli K39 (λ), prophage induction	10 μl	positive	1. Qualitative Test Procedure 2. Obtained strong positive response (+++ on a scale of + to ++++)

APPENDIX B

DETAILS OF CARCINOGENICITY BIOASSAYS AND DESCRIPTION OF TUMOR TYPES

The following section describes in detail the results of the chronic bioassays performed using CH_2Cl_2.

B.1 Dow 1980 Inhalation Study in Sprague-Dawley Rats (EPA 1985a and Burek et al. 1984)

Rats were housed no more than three per cage in 8x8x8-foot rooms. During nonexposure periods, animals remained in the chamber rooms with filtered air. Controls were continuously exposed to filtered air.

During the first 2 months of the study, there was an outbreak of sialodacryoadenitis virus infection involving control and exposed rats. This virus has been associated with acute inflammation and diffuse coagulative necrosis of the parotid and submandibular salivary glands and Harderian gland. Inflammatory edema and inflammatory cells are prominent in salivary and Harderian glandular interstitial and periglandular connective tissue. Squamous metaplasia of glandular epithelium occurs in the reparative phase of the disease. High morbidity may occur during outbreaks of disease, but mortality is negligible (Jacoby et al. 1979). Burek et al. (1984) suggested that the combination of viral infection and high exposures to CH_2Cl_2 may have been associated with the salivary gland tumors.

Toward the end of the study, there was a statistically significant increase in mortality in males exposed to 1,500 and 3,500 ppm at 21-22 months and increased mortality in females exposed to 3,500 ppm between 18-24 months. Mortality was high; however, in control groups of both sexes, with 85% mortality in males and 78% in females at 24 months (EPA 1985a). No data were published on the reasons for the poor survival of controls.

Carboxyhemoglobin blood levels were increased in exposed animals, but this elevation was not dose-related.

Neoplasms. As noted in Table B-1, the carcinogenic end points in this study were sarcomas arising in the ventral cervical/salivary gland area in males and benign mammary tumors.

TABLE B-1

SARCOMAS OF THE CERVICAL/SALIVARY GLAND REGION IN SD MALE RATS (Burek et al. 1984)

CH_2Cl_2 (ppm)	Incidence
0	1/93 (1%)
500	0/94
1,500	5/91 (5.5%)
3,500	11/88 (12.5%)*
Historical laboratory control	0-2%

* $p < 0.05$, Fisher's Exact Probability Test

The sarcomas were composed of round to spindle cells with high mitotic activity; local invasion of tissue by tumor cells and necrosis within the tumor were frequent. Areas of different histologic types (fibrosarcoma, neurofibrosarcoma) often occurred within a single tumor; other sarcomas were anaplastic (undifferentiated). All sarcomas involved the salivary glands but did not appear to arise from glandular components. Ultrastructural examination revealed no evidence of epithelial origin (absence of desmosomes or secretory products).

Incidences of benign mammary tumors in both sexes of rats were as follows:

TABLE B-2

BENIGN MAMMARY TUMORS IN SD RATS
(Burek et al. 1984)

	Males		Females		
CH_2Cl_2 (ppm)	Rats with Tumors	No. Tumors	Rats with Tumors	No. Tumors	Avg. No. Tumor/Rat
0	7/95 (7%)	8/95	79/96 (82%)	165/96	1.7
500	3/95 (3%)	6/95	81/95 (85%)	218/95	2.3
1,500	7/95 (7%)	11/95	80/96 (83%)	245/96	2.6
3,500	14/95 (15%)	17/95	83/97 (86%)	287/97	3.0
Historical laboratory control	10%		80%		

High-dose males had a slight increase in the number of benign mammary tumors compared to concurrent controls and a slight increase in the number of tumors per tumor-bearing rat. Incidences of benign mammary tumors in females were similar in control and exposed rats and comparable to historical laboratory controls. However, the total number of mammary tumors increased in exposed females in a dose-related manner. There was no exposure-related increase in the number of animals with malignant mammary tumors in either sex.

As was the case for all rat and mouse studies listed in Table 8-1, exposure-related nonneoplastic lesions were present in the liver. The primary exposure- and dose-related lesion was hepatocellular vacuolization consistent with fatty change (Table B-3).

TABLE B-3

HEPATOCELLULAR VACUOLIZATION IN SD RATS
(Burek et al. 1984)

CH_2Cl_2 (ppm)	Male	Female
0	17%	34%
500	38%*	52%*
1,500	45%*	59%*
3,500	54%*	65%*

$p < 0.05$ using Fisher's Probability Test

B.2 Dow 1980 Inhalation Study in Syrian Hamsters (EPA 1985a, Burek et al. 1984)

Animal husbandry and conditions of experimental exposure were similar to those of the Dow 1980 inhalation study in rats. Hamsters were housed three or four to a cage during the first year, then individually for the remainder of the study. An infestation of mange mite (Demodex) occurred during the study in control and treated animals but did not result in increased mortality.

There were no exposure-related increases in mortality in males, although mortality was high at 24 months (82% in control males, 85% in high-dose males). In females, controls had 100% mortality at 24 months, a value higher than any of the exposed groups (95.7%--500 ppm, 89.5%--1,500 ppm, 90.3%--3,500 ppm).

Blood carboxyhemoglobin levels were elevated in all exposed groups at 22 months with a slight dose-related trend in females.

Neoplasms. No exposure-related neoplasms occurred.

Nonneoplastic Lesions. No exposure-related nonneoplastic lesions occurred. Systemic amyloidosis was common in control animals and occurred at a decreased incidence (the decrease was dose-related) in exposed animals.

B.3 Dow 1982 Inhalation Study in Sprague-Dawley Rats (EPA 1985a).

The toxicology report was not available for review; therefore, this discussion is excerpted from secondary sources (EPA 1985a). This study has recently been published in the literature by Nitschke et al. (1988a).

As the study was conducted at Dow, the majority of animal husbandry and experimental exposure conditions presumably were similar to the previous rat study (Section B.2). However, the study pathologist noted that rats were kept in chambers during the exposure period, then housed in conventional animal rooms overnight and during the weekends. He noted that female rats housed under these conditions often had lower incidences of mammary tumors than rats kept in chambers 100% of the time (Food Solvents Workshop 1984).

A total of 360 male and 492 female Sprague-Dawley rats (Spartan substrain, 6 to 8 weeks old) were used in this study. Groups of 90 rats/sex were exposed by inhalation to 0 (control), 50, 200, and 500 ppm (0, 173, 692, and 1,735 mg/m^3) CH_2Cl_2 (technical grade, lot #TA 05038, with purity of at least 99.5 percent) 6 hr/day, 5 days/week, for 20 (males) or 24 months (females). In addition, 30 extra female rats, identified as 500/0, were exposed to 500 ppm CH_2Cl_2 for the first 12 months of the study and were housed as control rats for the duration of the study (last 12 months). Another 30 female rats, identified as 0/500, were housed in the same manner as control rats for the first 12 months of the study and were exposed to 500 ppm CH_2Cl_2 for the remaining 12 months of the study. To determine the rate of DNA synthesis in the liver, 18 female rats were included in each group. After 6, 12, 15, and 18 months of exposure, five rats of each sex at each exposure level were

sacrificed. In addition, five female rats from each of the 500/0 and 0/500 groups were sacrificed at the 18-month interim necropsy.

Exposure-related increases in mortality did not occur during the study. Mortality in males at 20 months was: control--70%, 50 ppm--70%, 200 ppm--79%, and 500 ppm--66%. Because of the high mortality rate, males were terminated at 21 months of the study. Mortality in females at 24 months was: control--64%, 50 ppm--76%, 200 ppm--67%, 500 ppm--61%, 500/0--72%, and 0/500--52%.

Neoplasms. In females, there was a nonsignificant increase in the number of benign mammary tumors per tumor-bearing rat (Table B-4).

Rats exposed to 500 ppm during the first 12 months of the study ("500/0") had a higher average number of tumors per animal than did the rats exposed to 500 ppm only after 12 months ("0/500").

Nonneoplastic Lesions. An outbreak of sialodacryoadenitis infection (based on clinical signs) occurred after 2 months in exposed and control groups, and lasted approximately 3 weeks. Blood carboxyhemoglobin levels were elevated in all exposed groups. High-dose females had hepatocellular vacuolization (control--4/45 (9%), 500 ppm--11/42 (26%)); high-dose and 500/0 females had an elevated incidence of multinucleated hepatocytes (control--8/70 (11%), 500 ppm--27/70 (39%; $p = 0.0002$ by Fischer Exact Test), 500/0--9/25 (36%; $p = 0.009$ by Fischer Exact Test))

TABLE B-4

BENIGN MAMMARY TUMORS IN SD FEMALE RATS
Dow 1982 Inhalation Study

CH_2Cl_2 (ppm)	No. of rats with tumors/ Total No. of Rats	Total No. of Tumors/ Total No. of Rats	Average No. Tumors per Tumor-Bearing Rat
0	52/70	105/70	2.0
50	58/70	133/70	2.3
200	61/70	135/70	2.2
500	55/70	147/70	2.7
500/0	23/25	60/25	2.6
0/500	23/25	50/25	2.2

B.4 National Coffee Association 1982 Drinking Water Study in Fischer 344 Rats (EPA 1985a, Serota et al. 1986a)

Rats were caged individually in stainless steel hanging wire mesh cages in a Bioclean laminar flow facility and given the compound in deionized drinking water in water bottles at doses of 5, 50, 125 and 250 mg CH_2Cl_2/kg/day for 104 weeks. An additional group, the recovery group, received 250 mg CH_2Cl_2/kg/day for 78 weeks followed by a 26-week recovery period. Control animals and the recovery group received only dionized water. Complete histopathology was performed on the control groups, high-dose group, and recovery group; liver (target organ) and tumors were examined in the low-dose groups.

Administration of CH_2Cl_2 did not appear to effect mortality; survival was more than 70% in control animals of both sexes at the end of 2 years.

Neoplasms. Exposure-related neoplasms did not occur in male rats. In females, there was an increase in liver tumors (combined neoplastic nodules and hepatocellular carcinomas) when compared to concurrent controls. However, concurrent controls had a lower tumor incidence (zero) than historical laboratory controls (Table B-5).

TABLE B-5

LIVER TUMORS IN FEMALE F344 RATS
Drinking Water Study (EPA 1985a)

CH_2Cl_2 (mg/kg/day)	Neoplastic Nodule	Hepatocellular Carcinoma	Combined
0	0/85	0/85	0/85
0	0/49	0/49	0/49
5	1/85 (1.2%)	0/85	1/85 (1.2%)
50	2/83 (2.4%)	2/83 (2.4%)	4/83 (4.8%)a
125	1/85 (1.2%)	0/85	1/85 (1.2%)
250	4/85 (4.7%)	2/85 (2.4)	6/85 (7.1%)b
250/recovery[c]	2/25 (8%)	0/25	2/25 (8%)
Historical control	19/419 (4.5%)	5/419 (1.2%)	24/419 (5.7%)

[a] $p < 0.05$ Fischer's Exact Test.
[b] $p < 0.01$ Fischer's Exact Test.
[c] Exposed to methylene chloride for 78 weeks and allowed to recover.

Based on concurrent controls, EPA's Carcinogen Assessment Group (CAG) considered the liver tumor data to represent a borderline neoplastic response (EPA 1985a). However, Serota et al. (1986a) considered the findings not significant based on historical control liver tumor incidence, which was higher than concurrent controls (Serota et al. 1986a).

Nonneoplastic Lesions. There were statistically significant ($p < 0.05$ or $p < 0.01$) increased incidences of hepatocellular fatty change (125 and 250 mg/kg) and foci of hepatocellular alteration (50, 125, and 250 mg/kg and 250 mg/kg recovery group) in both sexes. These incidiences were analyzed using Fischer's Exact Test.

B.5 National Coffee Association 1982 Drinking Water Study in B6C3F1 Mice (EPA 1985a, Serota et al. 1986b)

Animal husbandry and conditions of experimental exposure were the same as for rats (see Section B.4).

There was no effect of CH_2Cl_2 exposure on mortality, and survival was good in all dosed groups of mice. During the study, control and treated mice developed clinical signs of convulsions, particularly when the mice were weighed. An etiologic agent was not identified.

At necropsy, there was a dose-related increase in gross lung masses, but these were not identified histologically. This finding could indicate that lung masses were not trimmed in from wet tissue; however, the study received an intensive external audit, which did not note the presence of "missed" lung masses (Food Solvents Workshop 1984).

Neoplasms. Exposure-related neoplasms did not occur in female mice. In exposed male mice, there was an increased incidence of liver tumors (Table B-6).

These results were considered borderline evidence of chemically induced neoplasia by the EPA Carcinogen Assessment Group (EPA 1985a). The results were deemed not significant by the National Coffee Association because incidences in exposed groups were within the National Toxicology Program's historical control range as of 1981 (mean of 32%, Tarone et al. 1981). Incidences in historical NTP male controls as of 1986 also were similar: 540/1,784 (30% ± 8%) (NTP 1986).

Nonneoplastic Lesions. Fatty change in liver cells occurred in males and females receiving 250 mg/kg/day (high dose).

TABLE B-6

LIVER TUMORS IN MALE B6C3F1 MICE

CH_2Cl_2 (mg/kg-day)	Hepatocellular Adenoma	Hepatocellular Carcinoma	Combined[d]
0	6/60 (10%)	5/60 (8%)	11/60 (18%)
0	4/65 (6%)	9/65 (14%)	13/65 (20%)
60	20/200 (10%)	33/200 (17%)	51/200 (26%)
125	14/100 (14%)	18/100 (18%)	30/100 (30%)[a]
185	14/99 (14%)	17/99 (17%)	31/99 (31%)[a]
250	15/125 (12%)	23/125 (18%)[c]	35/125 (28%)[b]

[a]EPA (1985a) -- Statistically significant using combined control incidence of 24/125 ($p < .05$).
[b]Borderline (EPA 1985a).
[c]Fischer's Exact Test, $p = 0.0114$ compared to the first control group (Serota et al. 1986b)
[d]By trend test, $p = 0.03$.

B.6 National Toxicology Program 1986 Inhalation Study in Fischer 344 Rats (NTP 1986, Mennear et al. 1988)

Rats were housed individually in stainless-steel mesh cages in exposure chambers and remained in the chambers during nonexposure periods.

Sialodacryoadenitis virus/rat corona virus infection occurred during the study as indicated by positive serologic titer results at 19 and 24 months on study. Rats also had titers to a number of other infectious agents: Sendai virus, pneumonia virus of mice (PVM), Kilham rat virus (KRV), and *Mycoplasma pulmonis*. Of the latter agents, all but KRV are primarily respiratory system pathogens.

Mortality was high in all dose groups of males (66%-82%), with the majority of deaths occurring during the final 16 weeks of the study. There was an unusually high incidence of advanced mononuclear cell leukemia in all groups of male rats. Mortality was significantly increased in high-dose females after 100 weeks on study. Otherwise, there was no correlation between exposure and mortality.

Because of the aberrant elevated incidence of leukemia in control males (Table B-7), NTP could not make an unequivocal conclusion about elevated leukemia incidence in 2,000- and 4,000-ppm females (23/50, 46%, for both exposed groups.)

TABLE B-7

MONONUCLEAR CELL LEUKEMIA IN CONTROL F344/N RATS
NTP (1986)

	Male	Female
Concurrent control	34/50 (68%)	17/50 (34%)
Laboratory historical control	36/100 (36%)	27/99 (27%)
NTP historical control	458/1,727 (27% ± 9%)	307/1,772 (17% ± 6%)

Neoplasms. Male and female rats exposed to CH_2Cl_2 had dose-related increases in incidence of benign mammary tumors (Table B-8), which NTP interpreted as "clear evidence of carcinogenicity" in female rats and "some evidence of carcinogenicity" in male rats.

Dose-related multiplicity of mammary tumors was not discussed in the NTP technical report. Incidence of benign mammary tumors in concurrent control females was lower than in historical laboratory or NTP controls. However, the incidence observed in the high dose female is statistically significant when compared with incidence in either historical laboratory ($p = 0.0003$) or historical NTP ($p = 0.01$) controls.

TABLE B-8

BENIGN MAMMARY TUMORS IN F344/N RATS

NTP (1986)

	CH_2Cl_2 (ppm)			
	0	1,000	2,000	4,000
MALES				
Fibroadenoma, adenoma, or fibroma	1/50 (2%)[d]	1/50 (2%)	4/50 (8%)	9/50 (18%)[a,b,c]
Historical controls:				
Fibroadenoma:				
Laboratory	0/100			
NTP	51/1,727 (3% ± 3%)			
Fibroma:				
Laboratory	6/100 (6%)			
NTP	91/1,727 (5% ± 3%)			
FEMALES				
Fibroadenoma or adenoma	5/50 (10%)	11/50 (22%)	13/50 (26%)	23/50 (46%)
Life Table Test	p<0.001	p = 0.045	p = 0.022	p < 0.001
Incidental Tumor Test	p < 0.001	p = 0.092	p = 0.083	p < 0.001
Cochran- Armitage Trend Test	p < 0.001			
Fisher Exact Test		p = 0.105	p = 0.070	p < 0.001
Historical controls, fibroadenoma:				
Laboratory	16/99 (16%)			
NTP	492/1,772 (28% ± 10%)			

[a]Life Table Test p=0.002
[b]Incidental Tumor Test p=0.008
[c]Fischer Exact Test p=0.008
[d]Cochran-Armitage Trend Test p ≤ 0.001

Nonneoplastic Lesions. Hepatocytomegaly, hemosiderosis, cytoplasmic vacuolization, and bile duct fibrosis were more frequent in treated than control rats of both sexes, but the incidence did not increase in a dose-related manner.

B.7 National Toxicology Program 1986 Inhalation Study in B6C3F1 Mice (NTP 1986, Mennear et al. 1988)

Animal husbandry procedures and conditions of experimental exposure were the same as for the CH_2Cl_2 rat inhalation study performed at Battelle Pacific Northwest Laboratories (see Section B.6).

Based on serologic titers at the terminal kill, mice had concurrent infections with pneumonia virus of mice (PVM) and mouse hepatitis virus (MHV) during the course of the study.

Mortality was significantly greater than controls in exposed male mice: control--11/50 (22%), 2,000 ppm--26/50 (52%), 4,000 ppm--41/50 (82%). Female high-dose mice had significantly greater mortality (84%) than control (50%) or 2,000 ppm (50%) animals ($p < 0.001$ for life table trend test for a decreasing survival of male mice with increasing dose; and $p = 0.002$ for the same test for female mice (NTP 1986)).

Neoplasms. Both sexes of mice had dose-related increases in incidence and multiplicity of alveolar-bronchiolar tumors (adenomas, carcinomas) of the lung (Tables B-9, B-10). Male mice exposed to 4,000 ppm had an elevated incidence

of liver tumors--hepatocellular carcinoma and combined adenoma or carcinoma (Tables B-11, B-12). Female mice had dose-related increases in both hepatocellular adenoma and carcinoma. These findings were interpreted by NTP as "clear evidence of carcinogenicity" in B6C3F1 mice. As noted in the NTP technical report, the incidences of alveolar/bronchiolar neoplasms in the concurrent control groups were lower than the historical laboratory control groups from Battelle and, particularly for the males, lower than historical NTP controls.

Nonneoplastic Lesions. A treatment-related increase in cytologic degeneration occurred in the livers of both sexes of mice. Increased incidences of testicular atrophy in exposed males and ovarian and uterine atrophy in exposed females were considered by NTP to be secondary to the extensive lung and liver neoplasia.

APPENDIX C

MATHEMATICAL MODELS FOR LOW-DOSE EXTRAPOLATION

Multistage Models

Armitage and Doll (1954) derived a mathematical model for carcinogenesis that assumes a complex, time-dependent process. This model assumes that a cell must undergo a total of k ordered transformations to evolve into a neoplastic cell. If the transformation rates are constant over time and linearly related to exposure, the probability of a tumor response P(d) may be expressed as

$$P(d) = 1-\exp -\left[\sum_{i=1}^{k} (a_i+b_i d)\right]$$

where k is the number of stages, $a_i > 0$ and is proportional to the background transition rate of the ith stage, $b_i \geq 0$ and is proportional to the transition rate for the ith stage per unit of exposure, and d is a constant exposure rate. The number of stages, k, that have transition rates that are exposure-dependent (i.e. $b_i > 0$) defines the degree of the polynomial relationship. The exponent of the multistage model in this form is a constant times the product of k linear equations. In the resulting polynomial, each of the coefficients associated with terms of order m or less exist (i.e. are greater than zero) and are subject to certain nonlinear constraints. For example, if the coefficient corresponding to the jth power is denoted as C_j and two stages are exposure-dependent, it follows that $C_j > 0$ for j = 0, 1, 2 and $C_1 \geq 2(C_0C_2)^{1/2}$ (Kalbfleish et al. 1983).

Two-Stage Models. Since the original multistage model did not account for the proliferation of preneoplastic cells and only two stages are required to explain the age-dependence of tumor rates, Armitage and Doll (1957) proposed an alternative two-stage model. Their updated model of carcinogenesis is based on the assumption that stem cells become premalignant cells that divide at a constant rate, producing an exponential growth of preneoplastic clones. A preneoplastic cell is then transformed into a cancerous cell, which may ultimately develop into a tumor.

More recently, Moolgavkar and Venzon (1979) generalized the two-stage model by allowing for a variable number of cells at risk over time and a stochastic rather than deterministic growth of preneoplastic cells. The evolving biological basis for this model is discussed in detail in Moolgavkar and Knudson (1981) and Moolgavkar (1986). Thorslund et al. (1987) have extended this form of the two-stage model to incorporate the effects of environmental agents on cell transition rates and first-stage cell proliferation rates. A number of models can be generated from these extensions in which the parameters have a biological basis. Three simple forms of this model that can be used to estimate cancer risk with the limited bioassay data usually available are briefly presented here.

(1) The first form is based upon the assumption that both transition rates are exposure-dependent. This form may be expressed as

$$P(d) = 1-\exp-\left[(a_1+b_1d)(a_2+b_2d)\right]$$

where the terms are defined as for the multistage model. This form may be reparameterized so that the resulting parameters are estimable and have a biological interpretation. The reparameterized model may be expressed as:

$$P(d) = 1\text{-}\exp\text{-}\left[A[1+(S+I)d](1+Sd)\right],$$

where 1-exp-A > 0 and is the background tumor rate, S > 0 and is the smallest of the relative exposure-dependent transition rates, and I ≥ 0 and is the incremental change between the largest and smallest relative transition rates.

(2) The second model is a specific case of the first, which assumes that both transition rates are the same linear function of dose d. As pointed out by Moolgavkar (1986), this situation would occur when two genes on homologous chromosomes that regulate cell growth experience critical point mutations, which would reduce the control on growth. Knudson (1985) lists a number of human cancer types that are consistent with this hypothesis. If these point mutations are linear functions of dose, the dose-response relationship may be expressed as

$$P(d) = \left[1\text{-}\exp\text{-}A(1+Sd)^2\right].$$

(3) The third simple form of the two-stage model can be used when it is reasonable to hypothesize that preneoplastic cell proliferation occurs. It is based on the assumption that the carcinogenic agent stimulates the growth rate G of transformed first-stage cells proportional to dose. Under

this assumption, the dose-response model has the form

$$P(d) = 1\text{-}\exp\text{-}\left[2A[\exp(Gd)-1-Gd]/(Gd)^2\right],$$

where 1-exp-A is the background tumor rate and G is the change in the preneoplastic cell growth rate per unit of exposure.

All of the multistage models discussed above have the common property that they are linear in form at low doses, and as a result give conservative estimates of cancer risk.

The multistage model can also be generalized to account for age or duration of exposure. One such formulation for the multistage model may be expressed as

$$P(d,t) = 1\text{-}\exp\text{-}\sum_{i=1}^{k}\left[(a_i+b_i d)(t-t_o)^k\right],$$

where t is age or duration of exposure and t_o is the latency period. Other formulations are possible, but computer programs to implement their use are not readily available at this time.

Based upon the various multistage models, a number of algorithms or approaches to bound or predict cancer risk can be employed. A brief discussion of some of these approaches, with an indication of the strengths and weaknesses of each, is given in the following sections.

Linearized multistage model (GLOBAL 82). To avoid computational problems posed by the original multistage model, Guess and Crump (1977) "generalized" the multistage model by giving it a less restrictive polynomial form:

$$P(d) = 1-\exp-[\sum_{i=0}^{k} C_i d^i],$$

where k is the number of exposure levels used in a bioassay and $C_i \geq 0$ for i = 0, 1,..., k.

As a first step when using this approach, the maximum likelihood estimates for the C's are obtained. Next, the unique polynomial is derived that gives the maximum risk at a specified level of exposure, subject to a goodness-of-fit constraint. The linear term in this polynomial times an environmental exposure level is, to a very close approximation, the statistical upper bound on cancer risk at low doses.

The advantage of this method is that a unique upper-bound estimate can be routinely obtained. The disadvantages are: (1) the algorithm cannot be used to obtain a stable point estimate of risk for the MLE; (2) only a modified goodness-of-fit test exists for the method; and (3) the mathematical form is based upon an extraneous arbitrary factor, the number of exposure levels used in the bioassay.

Time-to-tumor model (WEIBULL 82). A time-to-tumor model of the product form:

$$P(d,t) = 1-\exp-\left[\sum_{i=0}^{m} C_i d^i (t-t_o)^k\right]$$

can be used to estimate cancer risk when the ages at which a tumor is observed are available for individual animals. Such an approach is critical when the death rate due to the agent, independent of the tumor of concern, is exposure level-dependent. For the specific case where $k \geq 1$ and is not restricted to integers and m is equal to the number of exposure levels, the "WEIBULL 82" computer program can be used to obtain cancer risk estimates and their upper bounds. To use this program, one must specify whether a tumor was incidental or the proximal cause of death. The advantages of this approach are:

(1) it supplies a method for adjusting for differential, exposure-induced mortality, and

(2) it gives estimates that are a time-dependent extension of the linearized multistage upper-bound approach.

A number of disadvantages also exist. Among them are:

(1) the model does not have an underlying biological rationale;

(2) it is necessary to classify tumors as incidental or fatal, which in many cases is difficult;

(3) when the presence of a tumor influences the death rate due to a competing cause, a bias of unknown magnitude is created; and

(4) it must be assumed that the competing risk-adjusted rate at the end of the observation period is a reasonable measure of the lifetime risk.

Direct use of the two-stage model. It is possible to obtain estimates of risk directly using one of the three specific forms of the two-stage model. The advantages of this approach are:

(1) At low doses, the models converge to a linear no-threshold form so that conservative estimates are obtained.

(2) A stable point estimate of risk can be obtained directly.

(3) Several forms of the models have only two parameters that have to be estimated, so that goodness-of-fit tests can be run on the data from the standard bioassay with one control and two exposure groups.

(4) The mathematical form of the model follows directly from one current hypothesis about the mechanisms of cancer induction.

(5) Effects of cell proliferation can be taken into account.

(6) Steep dose-response data can be fitted with the model.

(7) The parameter estimates are biologically meaningful.

It should also be noted that when only two exposure levels exist, the two-stage transition rate model and the linearized multistage model give identical results if the parameter estimates of the latter approach are consistent with the constraints imposed by the multistage theory.

The disadvantages of using the two-stage model are that:

(1) only approximate maximum likelihood estimates can be obtained for the parameters until extensive computer programs that are being developed are completed;

(2) no method exists to adjust for time-to-tumor data; and

(3) a positive estimate of the background tumor rate is required.

APPENDIX D.

HUMAN EPIDEMIOLOGICAL DATA AND RISK ASSESSMENT

As noted in Section 7.2.1, suggestive evidence of an elevated pancreatic cancer mortality rate associated with CH_2Cl_2 exposure was reported in an epidemiological study of Kodak workers (Hearne et al. 1987). These data were not used in a formal risk assessment because (1) the association between CH_2Cl_2 exposure and excess cancer was not statistically significant at a site for which an a priori hypothesis from animal data existed, and (2) a dose-related trend was not observed, (3) confounding factors may have been responsible for the apparent association and (4) the result could be an artifact of multiple statistical testing. Nevertheless, even epidemiological studies which yield negative results in a hypothesis test may be useful in evaluating the plausibility of risk estimates derived from animal studies.

DHS staff undertook two approaches to evaluate the compatibility of the animal and human results. First, a prediction derived by fitting the linearized multistage model to animal data was compared to observed cancer mortality. Second, one of the authors of the epidemiological study supplied additional data that enabled quantitative estimation of risk (Friedlander, 1987). A relative risk model was fitted to these data and the resulting unit risk was compared to the animal-based unit risk.

Comparison of Animal-Based Risk Predictions with Human Epidemiological Data

Hearne et al. (1987) reported that the occupational data in their study were inconsistent with an animal-based risk assessment. Using the EPA's unit risk, they calculated that 14.5 excess lung and liver tumors would be predicted among the exposed workers. However, there is no reason to assume that the most sensitive site in animals will be the most sensitive site in humans.

Therefore, DHS staff compared the predictions based on the lung tumors in mice to the cancer site having the greatest relative risk among workers occupationally exposed: the pancreas. The expected number of pancreatic cancer deaths in this cohort was 3.2 based on rates in the New York State population outside of New York City. Using the upper 95% confidence limit on the unit risk derived (in this document) by fitting the linearized multi-stage model to lung tumor data for female mice in the NTP (1986) study, 9.7 excess cancers were predicted for this cohort (see Table D-1 for calculations). If the 9.7 predicted from exposure are added to the 3.2 expected background pancreatic cancer deaths, a total of 12.9 such deaths would be predicted for this cohort.

Considering that the animal-to-human extrapolation is over two orders of magnitude of exposure, and that the animal and human exposures were temporally dissimilar, this prediction (12.9) is quite close to the observed mortality: eight pancreatic cancer deaths 95% confidence interval = (3.5, 15.8).

In general, DHS staff do not assume that the most sensitive target site is the same organ in humans as it is in the test species used for extrapolation. Nevertheless, staff note that the conclusion here would remain the same even using the same target site: if the 14.5 excess cancer deaths predicted by Hearne et al. (1987) are added to the 21.8 background expected lung and liver cancer deaths, the resulting prediction is also within a factor of 3 of the observed 14 deaths due to cancers at these sites. Thus, given the confidence limits about the human data, DHS staff believe that the human data could be consistent with animal data, contrary to the assertion of Hearne et al. (1987) that they are inconsistent. Other investigators have reached conclusions similar to those of DHS staff (Mirer et al., 1988, Tollefson et al., 1988).

Calculation of Risk Based on Human Epidemiological Data

The second approach to utilizing the human epidemiological study was to fit a model to the data relating methylene chloride exposure and pancreatic cancer deaths. The data used for this analysis are in Table D-2. A relative risk model was fitted to these data: this model assumes that the excess cancer rate due to the exposure is proportional to the background cancer rate. An absolute risk model was not used because in extrapolating from adult workers to the general population, this model assumes the excess rate to be independent of age; the relative risk model assumes excess risk will follow the same distribution as the background risk, a more plausible assumption.

Details of the model fitting are shown below. The slope was estimated as 0.584 with a variance of 0.1323. The pancreatic cancer death rate was significantly elevated when compared to the control population, however the trend with dose was not, resulting in a large variance for the slope.

Using the background incidence of 0.008 pancreatic cancer deaths based on U.S. 1981 Vital Statistics, the lifetime exposure to 1 ppb CH_2Cl_2 is 9×10^{-6}. This value can be compared to unit risks for the same exposure shown in Table 8.7 for the purpose of ascertaining the general consistency between the data sets.

Methods for Fitting Model and Deriving Excess Risk

The relative risk model assumes that the background cancer rate is increased by a proportional amount R per unit of exposure. Under this model

$$E[O_{ij}] = e_{ij} + Rx_i e_{ij} = e_{ij}(1+Rx_i),$$

where $E[\cdot]$ denotes the expectation of a random variable, subscripts i and j denote dose-level and age respectively (i=0 for the control group); and:

x_i = the lifetime continuous equivalent (LCE) in ppm for exposure group i,

e_{ij} = the expected number of cases based upon Kodak controls,

O_{ij} = the observed number of cases, and

R = unknown slope parameter to be estimated from the data.

The observed number of cases, O_{ij}, is assumed to follow a Poisson distribution. The estimate of R and its asymptotic variance were obtained using the maximum likelihood method [see Cramer (1946) for a discussion of the general approach]. The excess risk for an individual with continuous lifetime exposure to x ppm is then:

$$P(x) = P_o Rx$$

where P_o denotes the average lifetime background risk of pancreatic cancer. In the context of the model, P_o can be interpreted as the expected number of cases for the average individual. Values for P_o are obtained from NCI (1981) vital statistics and are based upon the sum of risks up to age 75 for the total U.S. population (Table D-3). A 95% confidence interval is obtained for the risk by substituting $R \pm 1.64[V(R)]^{1/2}$ for R in the risk equations.

Conclusions

In summary, the human epidemiological data are inadequate either to establish or to rule out carcinogenicity of methylene chloride in humans. Nevertheless, several investigators have compared the human epidemiological data with that of the animal risk assessment data to ascertain if they are generally consistent with each other. In this report two procedures were used to compare the human epidemiological data and the animal risk assessment data. Based on these comparisons, DHS staff conclude that the human data could be consistent with animal data.

TABLE D-1

CALCULATION OF PREDICTED EXCESS CANCER DEATHS

AMONG KODAK EMPLOYEES USING LINEARIZED-MULTISTAGE

MODEL FITTED TO LUNG TUMOR DATA IN NTP INHALATION BIOASSAY

1	Lifetime continuous exposure	650 ppb	1790 ppb	3810 ppb	
	95% UCL of slope L-M (ppb^{-1})	9×10^{-6}	9×10^{-6}	9×10^{-6}	
2	Individual excess risk	6×10^{-6}	17×10^{-6}	36×10^{-6}	
3A	Number of workers	350	353	310	
3B	Number excess deaths predicted to age 70 if all workers live	2.1	5.9	11.1	
4C	Adjustment for less than lifetime follow-up (from Hearne et al. 1987)	0.21	0.42	0.61	
Total					
5	Number excess deaths	.4	2.5	6.8	9.7

To use any other estimate of slope, take numbers in row 5, divide by L-M slope, multiply by choice of (alternate) slope, e.g., using 1.1×10^{-6}, row 5 has: 0.05, 0.2, 0.7.

The only difference between these numbers and those Hearne et al. (1987) is that the DHS unit risk (slope = 9×10^{-6}) is based on lung tumors only while EPA's (14×10^{-6}) is based on combined liver and lung tumors.

TABLE D-2

PERSON-YEARS, OBSERVED AND EXPECTED DEATHS FROM PANCREATIC CANCER FOR KODAK EMPLOYEES EXPOSED TO CH_2CL_2 WITH FOLLOW-UP THROUGH 1984

Cohort	Age Group							
	15-24	25-34	35-44	45-54	55-64	65-74	75+	Total
<350 ppm-years ($LCE=x_j=0.65$):								
Person-years	1,109.9	3,381.7	2,808.3	1,047.4	612.8	306.2	88.2	--
Pancreatic cancer deaths:								
Observed o_{1j}	0	0	0	0	1	0	1	2
Expected e_{1j}	.000	.030	.134	.163	.226	.107	.058	.718
350-749 ppm-years ($LCE=x_j=1.79$):								
Person-years	0	135	1,737.1	1,986.9	1,334.0	683.8	132.0	--
Pancreatic cancer deaths:								
Observed o_{2j}	0	0	0	1	0	1	0	2
Expected e_{2j}	.000	.001	.083	.310	.493	.239	.087	1.213
750+ ppm-years ($LCE=x_j=3.81$):								
Person-years	0	0	165.8	1,112.3	1,643.5	1,005.2	174.8	--
Pancreatic cancer deaths:								
Observed o_{3j}	0	0	0	0	1	3	0	4
Expected e_{3j}	.000	.000	.008	.174	.607	.351	.116	1.256

TABLE D-3

PERSON-YEARS, OBSERVED PANCREATIC CANCER CASES AND

RATES FOR KODAK EMPLOYEE CONTROL GROUP FOLLOW-UP THROUGH 1984

		Pancreatic Cancer	
		Age-Specific	
Age Group	Person-Years	Deaths	Rate
15-24	56,887	0	0
25-34	113,789	1	0.88×10^{-5}
35-44	83,627	4	4.78×10^{-5}
45-54	76,934	12	15.60×10^{-5}
55-64	67,713	25	36.92×10^{-5}
65-74	42,910	15	34.96×10^{-5}
75+	22,689	15	66.11×10^{-5}

APPENDIX E

PHYSIOLOGICALLY-BASED PHARMACOKINETIC MODEL

The Andersen et al. (1987) physiologically-based pharmacokinetic model (PBPK) consists of a series of mass balance differential equations describing the concentration of CH_2Cl_2 in five compartments, organs or tissue groups, i.e., the lungs, the liver, fat tissue, richly perfused tissues (e.g., kidney, brain and viscera), and slowly perfused tissue (e.g., skin and muscle) and a sixth compartment for gas exchange. The lung compartment is placed between the gas exchange compartment and the systemic arterial blood supply (Figure E-1). This model provides a mathematical description for the absorption, concentration, metabolism and elimination of CH_2Cl_2 to determine the effective dose at the animal target organs.

With this model CH_2Cl_2 may enter the body by inhalation in the gas exchange compartment, or by ingestion with absorption directly into the liver compartment. It is assumed that absorption of ingested CH_2Cl_2 occurs by a zero-order process entering the liver at a constant hourly rate.

This PBPK model requires three types of biochemical and physiological data: (1) partition coefficients for the different types of tissues, (2) physiological constants for blood flow and tissue volumes, and (3) biochemical constants for the critical biotransformation pathways.

The partition coefficients for liver, muscle and fat tissues were experimentally determined <u>in vitro</u> by measuring the distribution of CH_2Cl_2 using a tissue homogenate. The tissues were homogenized as a 1:3 w/v

mixture using 0.9% saline and then partition coefficients determined with a closed vial equilibrium technique for various tissues including blood. The authors took the ratio of the air/blood to air/tissue coefficients to derive the tissue/blood partition coefficients. Blood/air partition coefficients were measured for mouse, rat, and human blood. Tissue/air partition coefficients for mouse and human tissue were not determined and were assumed to be equal to those measured in the rat. Physiological constants for tissue volume and blood flow were obtained from the published literature, and enzyme activities were obtained either from the literature, from in vitro studies, or through computer optimization of results from closed inhalation chambers. For apportioning the the enzymatic activities between liver and lung, the authors selected values from Lorenz (1984) using 7-ethoxycoumarin for the MFO pathway and 2,5-dinitrochlorobenzene for the gluthathione S-transferase (GST) pathway. Andersen et al. (1987) used a computer curve-fitting technique in the optimization procedure to obtain the "whole tissue" kinetic constants for KM, VMAX and KF. However, since there was no human data for KF, the cytosolic GST rate constant, allometric scaling of body weight to the 0.7 power from mouse, hamster and rat data was used to obtain a human value. A cross-species comparison is conducted with this model.

The model is designed to estimate the tissue concentration of CH_2Cl_2 (or metabolites) in lung and liver for a given animal or human exposure. Liver and lung are assumed to be able to metabolize CH_2Cl_2 by both the cytochrome P450 (MFO) and cytosolic GST pathways. The P450 pathway is considered to be saturated at the experimental doses of the NTP (1986) bioassay, while the GST pathway was presumed not saturated, but first order. In this model, the

GST-pathway is assumed to be the only source of a carcinogenic intermediate.

DHS staff are concerned about the method used to derive the tissue partition coefficients since these values may substantially influence the final result. The use of tissue homogenate destroys the integrity of critical cell membranes and may significantly distort the true partitioning of materials. In a review of this model, the EPA concluded that the model overestimates postexposure blood concentration of CH_2Cl_2 by inhalation and suggested that this may be due to the data from the in vitro equilibrium studies (EPA 1985b). Additionally, the EPA noted that the MFO and the GST activities selected by Andersen et al. may not reliably reflect the appropriate rates or specific activity of CH_2Cl_2 in each tissue. For example, the specific activity of GST varies significantly depending on the substrate used for the assay, there is approximately a 15-fold difference between specific activities of 1-chloro-2,4-dinitrobenzene and 1,2-dichloro-4-nitrobenzene.

The DHS staff believe that this model requires experimental validation. The authors could have used noncarcinogenic endpoints, e.g., acute toxicity, to demonstrate the model's ability to predict biological responses. The computer optimization procedures, based on the loss of CH_2Cl_2 from closed chamber inhalation studies, used to derive the whole tissue kinetic constants is inexact. The fit of the model to the experimental data is a circular argument, since the fit was optimized to the experimental data, and it does not accurately reflect the model's ability to predict the experimental data.

DHS staff are aware that in vitro studies of CH_2Cl_2 GST studies in humans, mice, rats and hamsters are in progress at several different laboratories. This may help to clarify the role of the GST pathway in humans as compared to mice. DHS staff believe that the MFO pathway also produces reactive metabolites that may contribute to the observed tumorigenic response, and the focus on the GST pathway may be inappropriate. However, the basic purpose of the PBPK model, to incorporate additional data to improve the exposure assessment, is sound. The model represents a scientifically plausible estimate of human exposure. Consequently, a risk estimate, based on the PBPK dose is presented in Section 8.

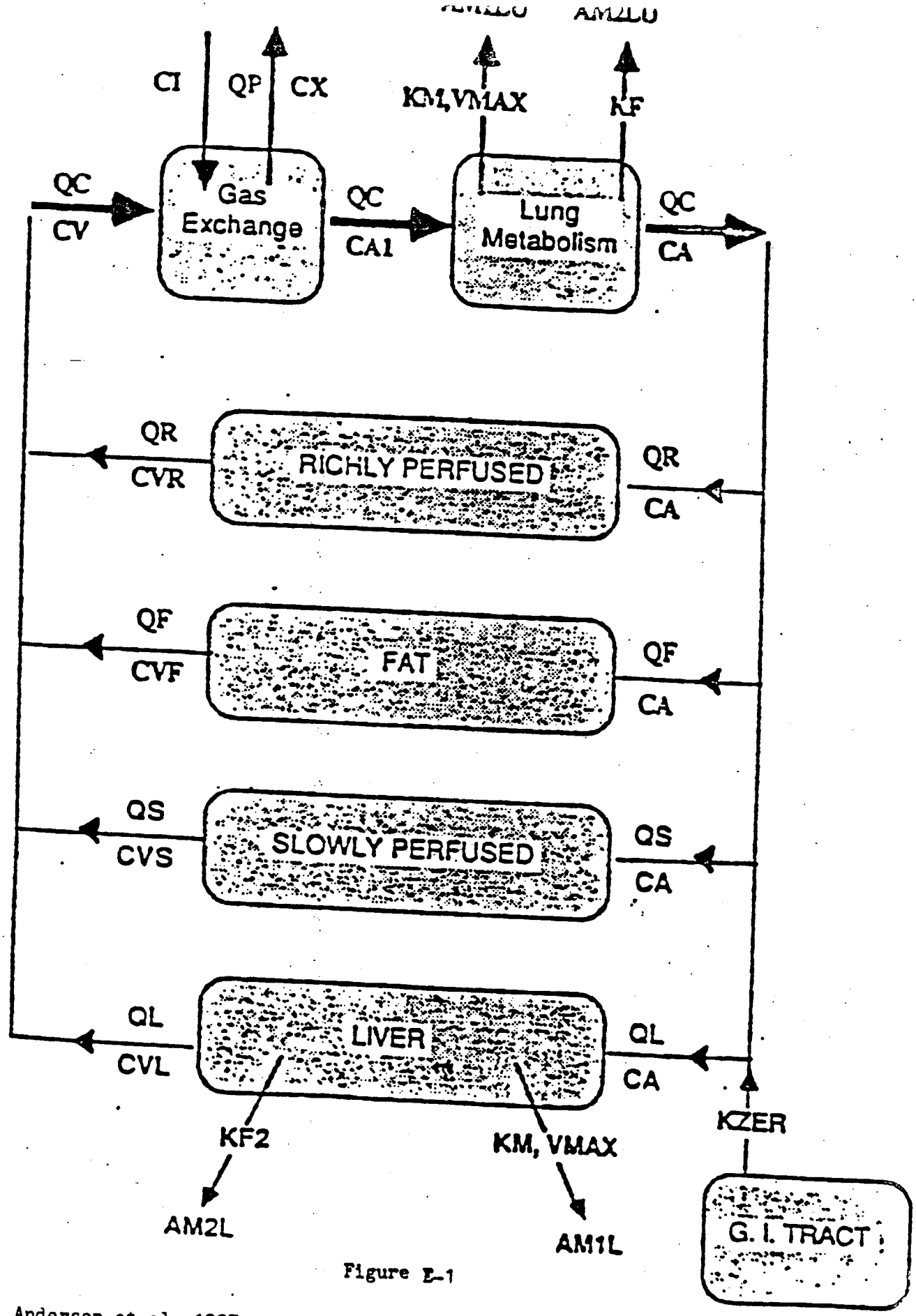


Figure E-1

After: Andersen et al. 1987

LEGEND

AM1L - Amount of CH_2Cl_2 metabolized by MFO in liver.

AM2L - Amount of CH_2Cl_2 metabolized by gluthathione S-transferase (GST) in liver.

AM1LU - Amount of CH_2Cl_2 metabolized by MFO in lung.

AM2LU - Amount of CH_2Cl_2 metabolized by GST in lung.

CA - Concentration of (mg/l) CH_2Cl_2 (mg/l) in arterial blood.

CA1 - Concentration of (mg/l) CH_2Cl_2 (mg/l) in blood leaving gas exchange compartment.

CI - Concentration of CH_2Cl_2 (mg/l) in inhaled air.

CV - Concentration (mg/l) of CH_2Cl_2 in mixed venous blood.

CVL - Concentration (mg/l) of CH_2Cl_2 in venous blood leaving liver.

CVF - Concentration (mg/l) of CH_2Cl_2 in venous blood leaving fat compartment.

CVS - Concentration (mg/l) of CH_2Cl_2 in venous blood leaving slowly perfused tissues.

CVR - Concentration (mg/l) of CH_2Cl_2 in venous blood leaving richly perfused tissues.

CX - Concentration (mg/l) of CH_2Cl_2 in alveolar air.

KF - First-order rate constant for lung GST pathway.

KF2 - First-order rate constant for liver GST pathway.

K_m - Michaelis-Menten constant for MFO pathway.

KZER - Zero-order rate of input of CH_2Cl_2 to liver.

QC - Cardiac output (l/hr).

QF - Blood flow through fat compartment (l/hr).

QL - Blood flow through liver compartment (l/hr).

QR - Blood flow through richly perfused compartment (l/hr).

QS - Blood flow through slowly perfused compartment (l/hr).

V_{max} - Maximum velocity of metabolism by MFO.

(Andersen et al. 1987)

TABLE E-1

QUALITATIVE EVALUATION OF PARAMETERS AND ASSUMPTIONS

IN THE PHYSIOLOGICALLY-BASED PHARMACOKINETIC MODEL[a]

Assumptions	Mouse Data[b]	Human Data[c]
Model structure is reasonable	B[d]	C
MFO saturates at high concentrations	A	C
MFO saturation concentration identified	B	D
Identification of ultimate carcinogen(s)	D	F
Restriction of metabolism to lung and liver	C	F
Restriction of lung and liver as target organs	B	F

Input Parameters	Mouse Data	Human Data
Partition coefficients	D	D
Concentration of CH_2Cl_2 in alveolar air	B	B
First-order rate constant for lung GST pathway (KF)	C	F
First-order rate constant for liver GST pathway	C	F
Michaelis-Menten constant for MFO pathway (KM)	C	D
Maximum velocity of metabolism by MFO	C	D
Zero-order rate of input of CH_2Cl_2 from GI tract to liver	C	D
Cardiac output.	B	B
Ventilation rate	B	B
Tissue weights	B	B
Tissue volumes	B	B
Blood flow through fat compartment	C	C

TABLE E-1 (Continued)

Calculated Parameters	Mouse Data	Human Data
Blood flow through liver compartment	C	C
Blood flow through richly perfused compartment	C	C
Blood flow through slowly perfused compartment	C	C
Amount of CH_2Cl_2 metabolized by MFO in liver	B	D
Amount of CH_2Cl_2 metabolized by GST in liver	B	D
Amount of CH_2Cl_2 metabolized by MFO in lung	C	F
Amount of CH_2Cl_2 metabolized by GST in lung	C	C
Concentration of CH_2Cl_2 in arterial blood	B	C
Concentration of CH_2Cl_2 in blood leaving gas exchange compartment	C	D
Concentration of CH_2Cl_2 in inhaled air	A	A
Concentration of CH_2Cl_2 in mixed venous blood	B	D
Concentration of CH_2Cl_2 in venous blood leaving liver	C	F
Concentration of CH_2Cl_2 in venous blood leaving fat compartment	D	F
Concentration CH_2Cl_2 of in venous blood leaving slowly perfused tissues	D	F
Concentration of CH_2Cl_2 in venous blood leaving richly perfused tissues	C	D

[a]This table reflects a subjective rating of the confidence DHS staff have in the values, parameters and assumptions used in the PBPK model of Andersen et al. 1987 as applied by EPA (1987a,b).

TABLE E-1 (Continued)

[b]Mouse data refers to test data or assumptions used by CPSC and EPA in applying the PBPK model. This evaluation refers to the high-to-low dose adjustment used by CPSC (Cohn 1987, EPA 1987a).

[c]Human data refers to test data, extrapolated data or assumption used by EPA (1987a,b) in applying the PBPK model for their estimate of risk from CH_2Cl_2 exposure.

[d]A indicates high confidence in value or assumption, F indicates low confidence in value or assumption.

APPENDIX F

CALCULATION OF HUMAN EQUIVALENT DOSAGES

To extrapolate the absorption of an inhaled substance across species, there are two alternate baseline assumptions: (1) the carcinogenic agent is a water-soluble gas and is absorbed in proportion to the amount of air inhaled; or (2) the agent is not water-soluble; thus, after equilibrium is reached the rate of absorption is proportional to the metabolic rate (EPA 1985a). Methylene chloride is moderately water-soluble (2 g/100 ml); thus, it is likely to be absorbed by the lungs to a high degree in both humans and rodents. Andersen et al. (1987) directly measured the blood/air partition coefficient in B6C3F1 mice and in humans as 8.29 and 9.7, respectively. Klassen (1986) considers a blood-gas solubility of about 1.2 as the demarcation between the two solubility cases defined by low solubility, thus perfusion limited, and gases with high solubility and hence ventilation limited. Thus, CH_2Cl_2 should be considered as a very soluble gas at low concentrations and the application of an interspecies correction based on surface area appears appropriate. The EPA treated CH_2Cl_2 as a water-soluble agent and estimated the risks to humans at low doses using the interspecies conversion formula based on surface area (EPA 1985b). The draft update (EPA 1987b) also used the surface area correction although it is used primarily to account for interspecies differences in CH_2Cl_2 pharmacodynamics.

F.1 Conversion of Rodent Exposures to Lifetime mg/kg/day

The inhalation rates for the rodents were estimated using the following formulas (EPA 1985b):

For mice: $I = 0.0345\ [wt(kg)/0.025(kg)]^{2/3}\ m^3/day$

For rats: $I = 0.105\ [wt(kg)/0.113(kg)]^{2/3}\ m^3/day$

Inhalation rates were calculated using the NTP (1985) average weights for mice and rats (both sexes) at the midpoint (51 weeks) of the bioassay, since the NTP report does not give the average animal weight over the whole study period (Table F-1).

TABLE F-1

ESTIMATED INHALATION RATES FOR NTP (1986) TEST ANIMALS (EPA, 1985b)

	Weight at Bioassy Midpoint (kg)	Estimated Inhalation Rate (m^3/day)
Male Rat	0.462	0.268
Female Rat	0.278	0.191
Male Mouse	0.037	0.0448
Female Mouse	0.032	0.0407

Dose conversion factors can be calculated between the NTP dosing schedule (6 hr/day, 5 days/week) and a continuous mg/kg/day exposure assuming a lifetime exposure:

Continuous exposure

= (Experimental Dose) x (6 expt hours/day + 24 hours/day) x (5 expt days/week + 7 days/week)

= (Experimental Dose) x (0.17857)

An inhalation exposure in ppm can be converted to an estimated inhaled dose in mg/kg (assuming 1 ppm = 3.47 mg/m^3) as shown in the following example:

Average inhaled dose for mouse breathing 2000 ppm CH_2Cl_2

= (2000 ppm) x (3.47 mg/m^3/ppm) x (0.0407m^3/day) x (0.17857) ÷ (0.032kg)

= 1576 mg/kg-day

F.2 Calculation of Human Equivalent Doses

Equivalent human CH_2Cl_2 doses are calculated using the Carcinogen Assessment Group (CAG) methodology for well-absorbed vapors. The CAG assumed that humans and animals exposed to equal doses of a carcinogen on a $(mg/kg)^{2/3}$ basis over equivalent proportions of a lifetime will encounter the same degree of cancer risk. This implies that a rodent with weight W_R, exposed to a dose of D mg/kg/day and a human with weight W_H, exposed to a dose of $D \left(W_H/W_R \right)^{-1/3}$ mg/kg/day encounter the same lifetime cancer risks (EPA 1985b).

Using this information, the surface area correction, based on an inhaled rodent dose can be calculated by the following formula where C is the exposure concentration and BR refers to the breathing rate of humans (H) or rodents (R).

Estimated Human Dose Equivalent

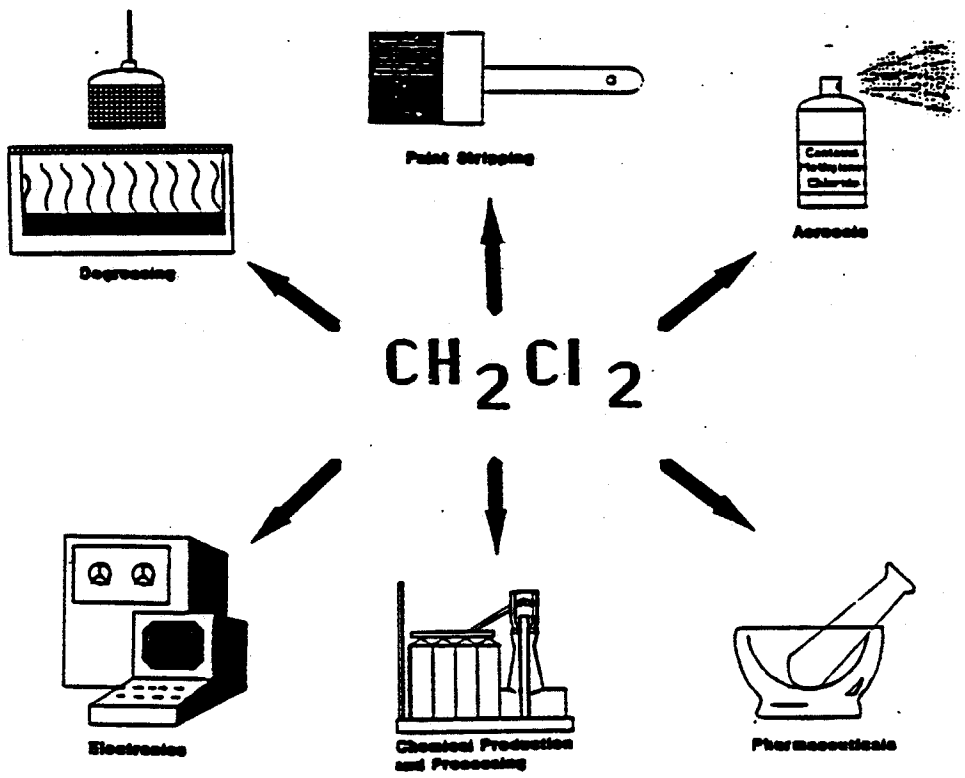
$$= C \times \left(BR_R/W_R \right) \times \left(W_H/W_R \right)^{-1/3} \times \left(W_H/BR_H \right)$$

$$= C \left(BR_R/BR_H \right) \times \left(W_H \right)^{2/3} / \left(W_R \right)^{2/3}$$

TECHNICAL SUPPORT DOCUMENT

PROPOSED IDENTIFICATION OF METHYLENE CHLORIDE AS A TOXIC AIR CONTAMINANT

Part C Report



State of California
Air Resources Board
Stationary Source Division

May 1989

FINAL DRAFT

PART C - PUBLIC COMMENTS AND RESPONSES TO THE DRAFT PART A AND B METHYLENE CHLORIDE REPORT

Prepared by the Staffs of the Air Resources Board
and the Department of Health Services

May 1989

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A. Comments from the Halogenated Solvents Industry Alliance

HSIA HALOGENATED SOLVENTS INDUSTRY ALLIANCE
1225 19th Street, N.W., Suite 300, Washington, D.C. 20036 • (202) 223-5890

January 14, 1988

Mr. Robert Barham, Chief
Toxic Air Contaminant Identification Branch
Air Resources Board
Attn: Methylene Chloride
P. O. Box 2815
Sacramento, CA 95812

Dear Mr. Barham:

The Halogenated Solvents Industry Alliance (HSIA) offers the enclosed comments on the preliminary draft Report to the Air Resources Board on Methylene Chloride (Parts A & B).

HSIA is an association of producers, distributors, importers, and users of halogenated solvents, including methylene chloride. Our members, as well as other users of methylene chloride, have a vital interest in the accuracy and scientific validity of the Report.

Sincerely,

Paul A. Cammer

Paul A. Cammer, Ph.D.
President

Enclosure

BEFORE THE CALIFORNIA AIR
RESOURCES BOARD

Comments of the Halogenated Solvents Industry Alliance on the Preliminary Draft Report to the Air Resources Board on Methylene Chloride (Parts A & B)

Paul A. Cammer, Ph.D.
President
January 14, 1988

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INTRODUCTION AND SUMMARY

The Air Resources Board is considering whether methylene chloride should be identified as a toxic air contaminant. Such an identification requires a finding that methylene chloride "may cause or contribute to an increase in mortality or an increase in serious illness, or may pose a present or potential hazard to human health" under Section 39655 of California's Health and Safety Code.

The Halogenated Solvents Industry Alliance (HSIA) is an association of producers, distributors, and users of halogenated solvents, including methylene chloride. The comments that follow provide HSIA's views on both parts of the preliminary draft Report to the Air Resources Board on Methylene Chloride (The "draft Report"), dated November 1987. Our members, as well as other users of methylene chloride, have a vital interest in the accuracy and scientific validity of the Report.

Biological data on methylene chloride are extensive. When the bioassay results are interpreted in light of these data, described in detail below, the weight of the evidence strongly suggests that levels of methylene chloride in the California environment do not pose a human cancer risk.

First, well-conducted epidemiology studies show no evidence of an increased overall cancer risk or risk at the primary sites associated with lesions found in animal studies. This situation differs significantly from one in which only animal evidence is available and a regulatory agency has little choice other than to treat the substance as if it were a human carcinogen.

Second, methylene chloride does not appear to be acting by a direct genotoxic mechanism. In light of the overall negative results in genetic toxicity studies in higher order animals, including several important DNA-binding studies, genotoxicity would not appear to be a significant factor in inducing tumorigenic effects.

Third, the evidence for induction of lung and liver tumors in mice was obtained at toxic doses. Studies suggest that cytotoxic damage in the lungs of the mice exposed at the dose levels of the NTP study played a significant role in the enhanced lung tumor response observed. Cytotoxic effects were not observed in the rats. This difference is consistent with the known metabolic differences between lung tissue of mice, rats, and humans.

Fourth, available metabolic data indicate that substantial quantitative differences between species exist. Moreover, in vitro metabolism data are available to explain the observed sensitivity of the mouse. These data, coupled with the

high spontaneous lung and liver tumor incidence normally found in B6C3F1 mice, suggest that the mouse is a less appropriate model than the rat or hamster for predicting potential human response to this chemical.

The final version of the Report should provide the staff's reasoning in its hazard identification. It is not adequate simply to state, as the draft Report does, that (IARC) the International Agency for Research on Cancer and EPA have found the animal evidence to be sufficient and consider methylene chloride to be a probable human carcinogen. Indeed, it is incorrect to state that IARC considers methylene chloride to be a probable human carcinogen. At the same time that IARC decided to place methylene chloride in its Group 2B, it changed the terminology describing that category from "probably carcinogenic to humans" to "possibly carcinogenic to humans." This change reflects increasing awareness of the fact that classification decisions (such as IARC's) based strictly on a scoring of the bioassay evidence for carcinogenicity cannot adequately serve to characterize the overall likelihood of a substance to cause cancer in humans.

In addition, EPA's most recent assessment (the draft HAD Update) is currently being reviewed by their Science Advisory Board, which may take issue with EPA's classification of methylene chloride. Moreover, EPA is expected in the near future to reassess its Guidelines for Carcinogen Risk Assessment, in

order to take into account developments since they were proposed in 1984 and published in 1986. These include scientific advances enabling toxicological evidence to be better taken into account in the hazard identification stage and the changes in the IARC terminology.

PART A

Part A of the draft Report concerns public exposure to, and sources of, atmospheric methylene chloride in California. HSIA does not have detailed information on current ambient exposures to methylene chloride in California and cannot offer a thorough review of Part A at this time. We note that the draft Report states (page III-3) that methylene chloride use "is expected to decline due to health concerns and the possibility of governmental regulation." In summarizing methylene chloride use in Table III-1, however, estimates of current U.S. use are based on information provided by HSIA for calendar year 1983. A more realistic estimate of current U.S. use would be 497 million pounds for 1987 from an estimate by Dow Chemical Company (USA) based on current government data.

The Air Resources Board used emissions and meteorological information to estimate the ambient concentration of methylene chloride associated with operations at three different industrial facilities. The Report states that the Industrial Source Complex

Short Term (ISCST) model was used to estimate the annual average concentrations. The ISCST model is designed to estimate short-term (one to twenty-four hour) averages, not annual averages. Annual average concentrations should be estimated using a statistical summary of recorded meteorological data rather than sequential data and the long-term version of the model (ISCLT).

Hourly meteorological data should be used when short term maximum concentrations are being estimated. The hourly data allow the user to estimate short term peaks in concentrations associated with specific meteorological phenomena. If an annual average is being sought, use of a statistical summary of meteorological data is preferred as this format allows the model to consider multiple years of data (summaries are usually made from five or ten years of data) in determining the annual average concentrations.

The ISC model series includes two separate models; one for estimating short term concentrations (ISCST) and the other for use in estimating annual averages (ISCLT). ISCLT differs from ISCST on two very important points:

(1) estimated concentrations are averaged over the sector in question and "smoothed" between sectors to avoid discontinuity, and

(2) a maximum of 16 wind direction sectors is allowed rather than the 36 sectors used by the ISCST model.

The effect of both of these points is to reduce the estimated concentration in most cases. The first point recognizes the normalizing effect that the wind direction variability throughout the year would have on the annual average concentration. Secondly, since concentrations calculated by ISCST using 36 sectors (10 degrees each) can be no more than 5 degrees off the maximum centerline concentration, most calculated concentrations would be greater than if the emissions were spread over a 22.5 degree sector (the smallest allowed by ISCLT's requirement that no more than 16 sectors be considered) following the algorithm in the ISCLT model.

The Report also states that the emissions from the automobile assembly plant were modeled as an area source even though methylene chloride is actually emitted from a series of stacks. Modeling the emissions as though they emanated from an area source rather than stacks does not recognize the benefits of enhanced dispersion due to the velocity and temperature of the exhaust as it exits the stack. The ISCST model does allow the user to input an effective emissions height to account for the "plume rise" due to the momentum and/or buoyancy of the exhaust but the Report does not say if an effective emission height was calculated and used in this analysis.

The draft Report (pages II-4 and II-5) uses Gleit's method to estimate mean concentrations of methylene chloride below the level of quantification of the monitoring stations.[1] Although Gleit's method has been shown to outperform the current EPA policy of replacing monitored values below the detection limit with the detection limit, the method was designed to determine a mean for comparison with a specific value (e.g. a standard). The Air Resources Board has no specific value with which it can compare the data; rather, the Air Resources Board conducted the monitoring study to assess the potential health hazard that may be posed by existing levels of methylene chloride in the ambient air. For this purpose, a summary of the data, including the percent of data below the dection limit and a range of possible average concentrations (assuming all missing data are equal to zero for the lower limit of the range and all missing data are equal to the detection limit for the upper limit of the average) would be more informative.

The statement in the draft Report that water chlorination is a source of environmental methylene chloride seems in error. The kinetics of reaction between chlorine and organic material in water is such that only chloroform is released to any appreciable extent.[2]

The draft Report should integrate the pharmacokinetic information addressed in Part B with the exposure information in Part A. Health effects information suggests that the effects of methylene chloride at high exposure levels are unlike the effects after chronic low exposure. An analysis of the pharmacokinetics of methylene chloride provides an explanation for this difference.

Appendix A contains some detailed technical comments on Part A of the draft Report.

PART B

Part B of the draft Report concerns the health effects of methylene chloride. The Executive Summary concludes that environmental levels of methylene chloride in California are well below any known acute and noncarcinogenic chronic levels that may cause adverse health effects. This conclusion is adequately supported by the available data and by reviews by other scientific panels, including EPA's Science Advisory Board.

Because methylene chloride exposures are widespread, albeit very low, it is appropriate for the draft Report to assess the possibility of human carcinogenic effects. However, because it

concludes that methylene chloride may pose a cancer risk to humans at ambient concentrations, and presents estimates of human cancer risk derived by applying an essentially linear (multistage) model to the positive results from a single animal bioassay, the draft Report is seriously flawed. The draft Report wrongly interprets available human data, incorrectly assumes that methylene chloride is genetically active with mammalian cells, and fails to make use of available data on metabolism, pharmacokinetics, and mechanism. Most significantly, it fails to address the overall weight of the evidence as to carcinogenicity, and fails to use more biologically motivated extrapolation models. These points are addressed in turn below. If all the available data were taken into account, the draft Report would not conclude that methylene chloride is a probable human carcinogen at ambient environmental (or workplace) concentrations.

Epidemiology

The best scientific evidence addressing the potential carcinogenicity of a substance in humans is provided by studies of human populations actually exposed to the substance (i.e., epidemiology studies). Two well-conducted epidemiology studies have been completed on worker populations exposed to methylene chloride. They show no evidence of an increased overall cancer risk. The draft Report is seriously deficient in failing to

recognize that these studies provide no indication that methylene chloride has had a carcinogenic effect in workers exposed for many years on a daily basis to concentrations from 3,000 to 100,000 times higher than the three parts per billion cited in the draft Report (page i-2) as the average ambient concentration in California.

The first of these studies, of employees at an Eastman Kodak Company plant in Rochester, New York, compares mortality rates of over 1000 employees with identified methylene chloride exposure to mortality rates for an unexposed worker population, as well as to the overall mortality of upstate New York males.[3] This study indicates that workers exposed to methylene chloride for an average of 22 years actually had a decreased incidence of cancer mortality when compared to either of the comparison groups. The study demonstrates no unusual mortality patterns for such hypothesized causes as lung and liver malignancy, and concludes that there is no evidence of an increased mortality risk for lung cancer. As to mammary gland and liver tumors, the authors acknowledged that there are power limitations. With respect to lung tumors, however, they concluded that the study is sufficiently powerful that it would detect relative risks of increased lung cancer mortality of at least 1.6. Thus, the Eastman Kodak study would adequately detect any statistically meaningful increase in realtive risk caused by a carcinogen, although power limitations obviously would prevent detection of a very weak carcinogen.

Significantly, the authors compared the number of excess lung and liver cancer deaths observed in their study with the number predicted from the results of the NTP inhalation study using a mathematical extrapolation model very similar to that used in the draft Report.[4] They found that:

> [a]lthough 36.3 deaths were predicted, only 14 were observed, a highly significant difference . . . . Thus, the projections based on animal data were clearly inconsistent with human experience.

Rather than acknowledging that the Kodak study is most plausibly interpreted as indicating that exposure to methylene chloride at workplace levels does not pose an increased risk of lung cancer, the draft Report interprets it as a positive study. The draft Report states (page 7-3) that the Kodak study suggests a relationship between exposure to methylene chloride and pancreatic cancer mortality. Accordingly, it concludes (page 7-5) that risk estimates based on positive results in the animal studies are consistent with the human data. This analysis is flatly inconsistent with that of the study authors and, indeed, that of EPA, which concluded in a recent draft Update to the Health Assessment Report and Addendum for Dichloromethane (the "draft HAD Update")[5] (page 94) that

> the increase in pancreatic tumors may merely reflect the fact that a few apparent increases, even statistically significant increases, can be expected (even when no excess exists), due to chance alone. The increase in pancreatic tumors cannot be considered an unequivocal positive response and should not be interpreted as evidence that methylene chloride is a human carcinogen.

The draft Report uses the pancreatic tumor findings in a scientifically inappropriate way. An epidemiologic study that examines a large number of end points can be expected to produce false positives. In the Kodak study, the pancreas was the only site for which there was an increase in mortality above projected values. At sites where increases were hypothesized, such as lung and liver, mortality was below that expected. Moreover, deficits comparable in magnitude to the increase of pancreatic lesions were reported for two sites, colon-rectum and genito-urinary organs. It should also be noted that of the 34 deaths that have occurred in the cohort during the past two years (1985-1986), none has been due to pancreatic cancer.

A detailed discussion of this epidemiology study prepared by Eastman Kodak is contained in Appendix B.

The other epidemiology study of methylene chloride examined the health and mortality of over 1200 employees exposed to a mixture of methylene chloride, ethanol, and acetone in a fiber production plant.[6] The study showed no excess mortality from cancer in the workers exposed to methylene chloride, even though exposures had ranged up to 475 parts per million and over 300 of the workers had been followed at least 17-1/2 years after exposure. Some of the workers were exposed for over 30 years.

This study is currently being updated by Epidemiology Resources, Inc. (the New England Epidemiology Institute) to address certain weaknesses: analysis of exposure categories, reference population, number of employees lost to follow-up and length of the follow-up period.

Completion of the updated study will address the deficiencies outlined in the draft Report and will provide additional information that should be taken into account in assessing whether methylene chloride poses a cancer risk.

Genotoxicity

The draft Report correctly states that methylene chloride is weakly mutagenic in bacteria. It further states that methylene chloride has been shown to damage chromosomes in mammalian cells, and that methylene chloride should be classified as genotoxic.

Recently completed studies, however, have shown no DNA alkylation in lung and liver tissues from mice and rats exposed to high concentrations of methylene chloride. These results suggest that methylene chloride does not act directly on mammalian DNA. The genetic potential of methylene chloride is addressed extensively below.

The only consistent unequivocal positive responses across laboratories for methylene chloride genotoxicity are in genetically altered Salmonella bacteria used in the Ames assay. The positive response appears to be due to (1) the ability of the bacteria to metabolize methylene chloride to a transient reactive intermediate(s), (2) the close proximity of the unprotected DNA to the transient intermediate(s) (lack of a nuclear membrane), (3) the lack of effective DNA repair processes, and (4) the enhanced permeability of the Salmonella bacterial cell wall. These factors combine to provide an indicator of biologically reactive intermediate formation that is not predictive of effects in higher animals. The proposed reactive intermediates would not be likely to affect the protected mammalian genome due to their transient nature.[7]

As one moves up the phylogenetic tree to mammalian cells or whole animals, consistent positive responses disappear and, overall, methylene chloride appears to have little if any toxicologically relevant genotoxic activity. In yeast, methylene chloride was found to be negative in Saccharomyces cerevisiae

strain D3 and positive in strain D7.[8,9] The positive response was only observed after one hour of incubation at a test concentration (157 mM) which was lethal to 58 percent of the yeast cells. A 34 percent reduction in dose to 104 mM was devoid of significant genetic activity in this assay, thus stressing the steepness of the apparent dose-responsive curve. Interestingly, four hours of incubation with strain D7 (rather than one hour) resulted in only marginal effects. Considering all of these uncertainties (high dose, toxicity, narrow window of apparent activity, apparent lack of activity in other yeast strains), methylene chloride is not likely to be mutagenic for yeast.

Progressing to insects, two studies have been reported in Drosophila melanogaster fruit flies. Abrahamson and Valencia evaluated methylene chloride in a sex-linked recessive lethal assay either by feeding or by injection.[10] In the feeding study the flies may not have been adequately challenged because of the volatility of methylene chloride. However, injection of methylene chloride resulted in a dose-related increased mortality (up to 30 percent) indicating that this element of the study did provide an adequate challenge. Methylene chloride did not produce a mutagenic response.

In a second study, Gocke et al. exposed Drosophila to either 125 or 620 mM methylene chloride by feeding in 2 percent DMSO and 5 percent saccharose.[11] The highest dose reported was near the LD_{50}. A statistically significant increase in the

frequency of sex-linked recessive lethal mutations was reported in this study in one of three broods, after combining the two treatment groups. Neither individual dose group differed statistically from the control value.

Several factors impinge on the interpretation of the Gocke study. It is not clear whether the experiment was adequately controlled. The control value to which the treatment values were compared represented accumulated values from all of the experiments conducted (on many different compounds).. At least four different solvents (vehicles) were used from which the control values were tabulated. There is no way to tell from the data, as presented, what contribution these different solvents made to the final control values. Thus, the overall control value may have underestimated the true value which might have arisen for a particular solvent and therefore overestimated the test chemical response. Furthermore, the cumulative control values for broods 1, 2 and 3 showed enough variation (0.14-0.39 percent) that the biological significance of the brood 1 response, which was the only significant response, becomes questionable. Only by combining both treatment groups in brood 1 (125 and 620 mM) was a significant response attainable; that is, neither individual group differed statistically from the cumulative control value. Thus, this study provides only suggestive evidence for genotoxic activity of methylene chloride,

and coupled with the negative study by Abrahamson and Valencia, suggests the true biological relevance of the reported positive result to be low.

With respect to mammalian cells, methylene chloride did not induce a genotoxic response in several assay systems, including neoplastic transformation in studies in BALB/C-3T3 and C3H-10T1/2 cells, respectively.[12] While reagent grade methylene chloride gave positive transformation results in a F1076 cell line, this effect was not repeatable with purified food grade methylene chloride.[13] Methylene chloride did not induce unscheduled DNA synthesis (UDS) based on the results of Jongen et al., Andrae and Wolff, and Trueman et al.[14] The study by Trueman et al. is particularly significant since UDS was not induced either in vitro, in rat hepatocytes or in an in vivo/in vitro assay in hepatocytes from mice and rats exposed to high concentrations of methylene chloride. The "marginal positive" response reported by Thilagar et al. reflects a low but statistically significant increase in unscheduled DNA synthesis. The biological significance of such a low response is debatable. Methylene chloride has been found to be negative in CHO V79 and mouse lymphoma mammalian cell gene mutation assays.[15] Most recently, methylene chloride was evaluated and found to be negative in the mouse micronucleus test.[16]

One other system with seemingly consistent positive

responses to methylene chloride is in vitro cytogenetics.[17] At an Environmental Mutagen Society meeting in the spring of 1985, a symposium was conducted which indicated that acid, salts, and sugar can give positive in vitro chromosomal aberration results by affecting the osmolality and pH of the media (even when neutralized). In addition, cell toxicity or pharmacologic effects may be significant for the induction of chromosomal aberrations in vitro via peroxide or free radical formation. A study by Dutton and Bowden describes the concept of releasing "clastogenic factors" within the cell.[18]

The implication of these observations is that induction of chromosomal aberrations in vitro may in some cases be caused by a secondary effect on DNA through the release of intracellular factors rather than a direct chemical-DNA interaction. Thus, lowering the dose, which would preclude the events leading to the secondary effect should preclude effects on the genome. It is likely that such secondary events occurred with methylene chloride in some of the in vitro chromosomal aberration systems. Importantly, the results of Burek et al. and Gocke et al. show no induction of chromosomal effects when methylene chloride is evaluated in whole animals (in vivo cytogenetics and a micronucleus assay, respectively).[11, 19]

Finally, the potential for methylene chloride labelled with radioactive carbon to alkylate target tissue DNA has been evaluated by Schumann and coworkers.[20] Rats and hamsters were

exposed to 3,500 ppm of methylene chloride labelled with radioactive carbon for 1.5 to 3 hours and the DNA from liver and salivary tissue was isolated and purified. While radioactive carbon activity was associated with DNA in these tissues (more in the hamster, which was negative in a chronic toxicity bioassay), it appeared associated only with normal bases and nucleosides, indicating radioactive carbon incorporation by normal biosynthetic pathways (one carbon pathway pool labeling). Importantly, no alkylated bases or nucleosides were identified at low levels of detection (12 and 1 alkylation(s) per 10-6 nucleotides per fraction for nucleosides and bases, respectively). Similar in vivo experiments have now been conducted by Green and coworkers, using mouse and rat lung and liver tissue.[21] This study allowed for distinction between incorporation derived from alkylation of DNA by methylene chloride or its metabolites as opposed to incorporation via the normal C-1 metabolic pool. Again, there was no evidence for the formation of DNA adducts in either species, but the incorporation of the radioactive carbon activity via the single carbon pool was evident.

These results suggest that neither methylene chloride nor any of its metabolites act directly on mammalian DNA. This is consistent with the absence of a clear carcinogenic effect in the lungs and livers of the rats and hamsters that have been tested, and with the hypothesis, supported by experimental data, that the positive results in the mice are related to the

peculiar mechanism and metabolism of methylene chloride in that animal species.

Metabolism and Pharmacokinetics

While it is encouraging that the state recognizes the potential of physiological-pharmacokinetic (PB-PK) models for improving the accuracy of risk assessments, it is obvious that the department does not yet have a good understanding of this technique.

Many of the objections raised by DHS (which were cited by EPA in their 1985 document) as limiting the use of PB-PK have been addressed in recent studies sponsored by Dow Chemical and CEFIC and are no longer pertinent. EPA has summarized and intepreted these data in their recent draft HAD update.[5] Furthermore, significant misunderstandings of the PB-PK model are evident in DHS's statements about the role of metabolism of methylene chloride. It is not true that both pathways are equally utilized at low concentrations as stated in section 2 (page 19), and the fact that tumors increase with dose at high concentrations is completely consistent with a protective effect of the saturable MFO pathway at low doses. It is noteworthy that

the USEPA, the Canadian government, and several European governments have recently incorporated the technique into their own hazard evaluations for methylene chloride.

The draft Report fails to make use of available information on animal and human metabolism, particularly in hazard identification. A casual reader of the draft Report would not suspect that methylene chloride has been more widely studied in terms of metabolism and pharmacokinetics than virtually any other industrial chemical. The draft Report also indicates a misunderstanding of metabolism and pharmacokinetics at several points. Although the Executive Summary (page ii-2) indicates that the glutathione-S-transferase pathway is responsible for the carcinogenic response in mice, the supporting text rejects this concept. Using new studies from Dow Chemical and Imperial Chemical Industries P.L.C., the available PB-PK models show a correlation between glutathione-S-transferase activity in various animal species and tumorigenesis. Further, human glutathione-S-transferase enzyme activity now has been measured with methylene chloride as a substrate in these same laboratories. Since this is cited as one of the reasons that California did not choose the PB-PK model, these data need to be integrated and utilized in determining whether methylene chloride poses a cancer risk to humans at low concentrations. EPA has recognized in the draft HAD Update (page 29) that a PB-PK model should be used as part of any quantitative risk assessment for methylene chloride.[5]

One reason for the draft Report's inadequacy is its failure to make use of the results of current research efforts sponsored by the European Council of Chemical Manufacturer's Federation (CEFIC) and Dow Chemical Company (USA). These research programs began in late 1985, and draft and final reports have been provided to the Air Resources Board staff as they have become available.[22] The CEFIC work provided much of the data evaluated in EPA's draft HAD Update and was recently cited by members of EPA's Science Advisory Board as "indicat[ing] that methylene chloride is probably not a human carcinogen."[23] Yet these studies are not used or cited in the draft Report.

The results of the latest research strongly support earlier indications that the National Toxicology Program (NTP) mouse bioassay (discussed below) is not appropriate for evaluating the human health effects of methylene chloride, due to the peculiar metabolism of the mouse. Because of important species differences in metabolism, the mouse does not accurately predict toxic responses in rats, hamsters, or humans.

Methylene chloride is metabolized via two pathways: (1) an oxidative pathway (MFO) involving cytochrome P-450 that appears to yield carbon monoxide (CO) as well as considerable amounts of carbon dioxide (CO_2), and (2) a glutathione dependent pathway (GSH) that produces CO_2 but not CO. In vitro metabolism studies conducted to date utilizing human, mouse, rat, and

hamster liver fractions have indicated that the metabolic rate of the GSH pathway is significantly higher in the mouse than in the rat and that metabolism by this pathway was lower still in either the hamster or man.[24]

Further in vitro metabolism studies across species have been conducted using radiotracer techniques in an attempt to lower the detection limits of the earlier work. These studies detected measurable levels of glutathione-S-transferase enzyme activity (GST) that utilizes methylene chloride as a substate in mouse, rat, hamster, and human tissues. Measurements indicate that the highest level of enzyme activity is found in mouse tissue, a significantly lower level is found in rat tissue, and very low levels of activity are found in hamster and human tissue. This is consistent with the earlier findings of significant species differences in the metabolism of methylene chloride. The levels of GST found in human tissues are in substantial agreement with those estimated from allometric scaling by Anderson and coworkers.[25]

CEFIC studies completed in late 1986 determined the in vivo pharmacokinetics of methylene chloride and its major metabolites, CO and CO_2, in rats and mice both during and immediately after inhalation exposure to 500, 1,000, 2,000 and 4,000 ppm methylene chloride, the dose levels in the NTP study.[26] Saturation of the MFO pathway occurred at 500 ppm exposure. There was evidence for significant metabolism of methylene chloride in the mouse at

higher dose levels (exceeding 500 ppm) by the GSH pathway, which produces CO_2. Measurements after 4,000 ppm exposure for six hours showed almost an order of magnitude more CO_2 produced per kg body weight in the mouse than the rat, even allowing for increased MFO contribution. Overall, saturation of the MFO pathway occurred at similar levels in both species, but significantly more methylene chloride was metabolized by the GSH pathway in the mouse when assessed either from the blood levels of methylene chloride or by CO_2 formation at high dose levels. These data add to the weight of evidence indicating that, in view of its metabolic differences, the mouse is not a good model for predicting methylene chloride's effect on humans.

The extent of species differences is apparent upon application of a PB-PK model, which, by taking into account known physiological and biochemical factors, allows accurate estimation of the "internal" target tissue dose of methylene chloride or metabolic products.[25] Such models allow comparison of the "internal dose" across species (e.g., mice, rats, hamsters, man) as well as across routes of exposure (drinking water versus inhalation) and therefore allow a more informed comparative evaluation as to the relative hazard posed by exposure to methylene chloride.

A significant aspect of PB-PK modeling, in terms of hazard evaluation for humans, is its indication that the MFO pathway does not correlate with tumorigenicity. In mice,

approximately equal amounts of MFO metabolites are produced in the lung and liver at the nontumorigenic dose of 250 mg/kg and at 2,000 ppm. Very small amounts of GSH metabolites are produced at 250 mg/kg of methylene chloride in the drinking water, but significant (100-fold) lower amounts are produced at 2,000 ppm. As long as the MFO pathway is not saturated, so that there is relatively little metabolism of methylene chloride via the GSH pathway, the tumor incidence in mice does not appear to increase above background levels.

A PB-PK model also has been used to compare internal dose estimates of methylene chloride metabolites in the lung and liver of mice and humans over an exposure concentration range of 4,000 to 1 ppm methylene chloride for six hours. There is significantly less MFO metabolism in humans. Moreover, the target tissue concentration of metabolites via the biologically relevant GSH pathway was found to be significantly lower in humans at non-saturating exposure concentrations. This is in contrast to the body surface area procedure used by the Department of Health Services, which would predict that humans are most sensitive to a given concentration of methylene chloride than mice.

Thus, there are important, known differences between mice and humans with respect to the bioactivation of methylene chloride. These differences indicate that the tumorigenic

effects observed in the NTP study in B6C3F1 mice would not occur in humans exposed to ambient environmental or workplace levels of methylene chloride.

In addition to the value of the metabolic and pharmacokinetic data in predicting whether environmental or workplace concentrations of methylene chloride would pose a cancer risk to humans, a PB-PK model provides a way to incorporate these data into quantitative risk assessment. Use of a PB-PK model allows for a more realistic estimate of upper-bound risk, where such a calculation is desirable to assess the potential risk from methylene chloride if it were carcinogenic to humans. Any use of such estimates should make clear that the weight of the evidence does not support a conclusion that environmental concentrations of methylene chloride in California pose a carcinogenic risk, and that the risk, if any, could be anywhere between zero and the calculated upper-bound value. The use of upper-bound estimates is inconclusive, precisely because the risk could be zero.

It is generally agreed that, where metabolic and pharmacokinetic information is available, a low-dose extrapolation model that takes these data into account is more appropriate biologically than the linearized multistage procedure. While the draft Report (page E-4) acknowledges that use of the PB-PK model "may substantially improve the basis of risk assessment," it rejects use of the model for several

reasons, the most significant being absence of validation. None of the mathematical models used to generate risk estimates in Table 8-8 have been biologically validated, and those models incorporate a greater range of assumptions than the PB-PK model. The PB-PK model of Anderson and coworkers was validated with four sets of data from B6C3F1 mice, F344 rats and human volunteers.[25] None of these data sets were used in the derivation of model parameters, as erroneously stated in the draft Report. Consequently, rejection of the PB-PK model on these grounds seems totally unwarranted. Moreover, data from the CEFIC research program, including three reports issued in October 1987, are providing additional experimental validation in several critical areas. As indicated above, preliminary and final reports of this research are being provided to the Air Resources Board as they become available.

The draft Report indicates (page i-1 and elsewhere) that both MFO and GST pathways "theoretically" produce metabolites that may interact with DNA. There is no experimental evidence for this view. As described above, neither pathway led to observable DNA alkylation in intact animals. The draft Report also states page ii-2 that a PB-PK model is not needed because there is no evidence for "saturation" in the tumor incidence data. This statement misses the point of the need for a PB-PK model to account for the higher affinity, lower capacity MFO pathway which protects animals until it becomes saturated. In addition, this statement misinterprets the tumor incidence

record. For example, for combined adenomas or carcinomas among female mice in the NTP bioassay, 22-23 mice with tumors would be expected at the intermediate dose, based on an assumption of strict linearity, whereas 27 were found. In effect, the dose-response data form an asymptotic curve which is consistent with saturation. However, the differences are small and a better explanation for a lack of strict linearity is random statistical fluctuation. Biochemical data suggest that these levels are not saturating. Conclusions based on tumor incidence do not seem appropriate.

In its draft HAD Update, EPA used the PB-PK model to extrapolate risk across species and from high to low doses, based on the amount of metabolism of methylene chloride by the GSH pathway. In making use of the PB-PK model, EPA stated (page 29):[5]

> [I]t is clear that the model used by Andersen and Reitz may be improved by additional data and validation. Nevertheless, EPA believes that the structure of the model is sufficiently well developed at present to provide a means of considering the available knowledge of methylene chloride metabolism and pharmacokinetics, as it relates to risk assessment, in a way that is not possible

through the applied-dose method. While confidence in the results of the model are expected to increase upon further model validation, the development of preliminary estimates using the results of the model as currently developed provides insight into the effect on risk estimates of metabolism and pharmacokinetic information.

Use of the PB-PK model in the draft EPA HAD Update resulted (page 84) in a unit risk estimate of 4.7 x 10-7, as opposed to the applied-dose extrapolation in the draft Report (page i-5) of 2.6 - 3.4 x 10-6. EPA indicated (p. 85) that use of body weight rather than surface area as a scaling factor would further reduce the unit risk estimate to 3.7 x 10-8.

In its August 13, 1987 review of the draft HAD Update, EPA's Science Advisory Board indicated that it approved of EPA's use of the PB-PK model. Hence, the risk estimate contained in the draft Report maybe 10 to 100-fold higher than the estimate that EPA will be using as an upper bound on the carcinogenic potency of methylene chloride. The draft Report should be revised to incorporate the more recent scientific data.

Mechanism Data

The draft Report briefly describes postulated mechanisms of action for chemicals that are not genotoxic. It acknowledges (page 8-11) that treatment-related cytological degeneration occurred in both the male and female mice in the NTP study. It concludes (page 8-11) that methylene chloride "could induce mouse liver tumor formation simply by stimulating cell proliferation in that organ in response to cytotoxicity," but states that "there are no experimental studies using [methylene chloride] that can be used to specifically report regenerative hyperplasia resulting in tumor formation." The draft Report goes on to dismiss this plausible mechanism, based on speculation that DNA alkylation may have occurred. However, methylene chloride has not been shown to bind DNA in mammalian cells, whereas data on stimulation of S-phase in liver cells are available.[20, 21, 27]

The tumor formation observed in the NTP study is consistent with a mechanism by which methylene chloride accelerates the appearance of tumors that normally occur spontaneously later in the life of the mouse, due to toxic effects on cells in the two target organs (lung and liver) and accompanied by an accelerated cellular turnover. A 10-day inhalation toxicity study from CEFIC shows toxic effects of methylene chloride on Clara cells in the mouse lung and on the mouse liver.[28] These effects (histopathologic damage to Clara cells and statistically significant changes in liver weight) were noted at the same concentrations used in the NTP study. While it is true that increased liver size does not invariably lead to toxicity, these

observations form part of a pattern in the two target organs of the mouse. Methylene chloride caused a change in liver homeostasis, not degeneration, in a tissue prone to high spontaneous tumor incidence.

The study indicates that the appearance of lung tumors correlates with the doses at which cytotoxic effects occur in the mouse, but not other species. Studies have shown substantial acute pulmonary injury in B6C3F1 mice after only a single exposure to methylene chloride.[28] The mice suffered extensive injury to Clara cells after six-hour exposure to 4,000 or 2,000 ppm, the doses used in the NTP bioassay. Twenty-four hours after exposure, Clara cells were vacuolated and swollen, and numerous necrotic cells were present. Although some apparent accommodation was evident after two to three weeks of exposure, it seems likely that the homeostasis of the mouse lung would remain altered with continued high exposure. It seems likely that the apparent histopathological accommodation conceals an increased turnover of Clara cells in the lung and/or an increased turnover of metabolic enzymes in the Clara cell. Effects on Clara cells did not occur in Fischer 344 rats tested at the same doses.

Mouse lung differs significantly from the lung of man or other animals in the number, distribution and ultrastructural morphology of Clara cells. For example, mice have Clara cells throughout the tracheobronchial tree, whereas most human Clara cells occur in bronchioles. Clara cells in the mouse primarily

have smooth endoplasmic reticulum with associated mixed function oxidase activity. In contrast, human Clara cells have primarily rough endoplasmic reticulum, which is associated with protein synthesis. Also, the mitochondria in Clara cells of the mouse are large. These locational, ultrastructual, and metabolic differences help to explain the unusual sensitivity of the mouse.

Results from several bioassays show that the association between methylene chloride exposure and increased lung tumors is unique to mice. The Fischer 344 rats used in the NTP and Serota bioassays (discussed below) did not have a tumorigenic response in the lungs. (Indeed, the B6C3F1 mice tested in the Serota study did not show an increased incidence of lung tumors.) Similarly, hamsters in one bioassay and Sprague-Dawley rats in two bioassays did not have a statistical increase in the incidence of lung tumors. Rats and hamsters have a very low incidence of spontaneous lung tumors. There is an obvious association between the high spontaneous background incidence of lung tumors in the mouse and the observation of an increase in tumors upon exposure to high levels of methylene chloride.

There are multiple reasons to regard the doses used in the available chronic bioassays of methylene chloride as excessive. This interpretation of concurrent toxicity is consistent with a mechanism by which methylene chloride accelerates the appearance of tumors that normally occur spontaneously later in the life of

the mouse, due to toxic effects on cells in the two target organs (lung and liver) and accompanied by an accelerated cellular turnover.

NTP noted toxic effects in its chronic study as follows:

MORTALITY (NON-ACCIDENTAL DEATH)

	Control	2,000 ppm	4,000 ppm
Male	11/50	24/50	38/50
Female	24/50	22/50	40/50

CYTOLOGIC DEGENERATION OF LIVER

	Control	2,000 ppm	4,000 ppm
Male	0/50	0/50	22/50
Female	0/50	23/48	21/48

GLANDULAR STOMACH DILATION

	Control	2,000 ppm	4,000 ppm
Male	3/49	7/47	9/49
Female	1/49	2/47	10/48

KIDNEY TUBULE CASTS

	Control	2,000 ppm	4,000 ppm
Male	6/50	11/49	20/50
Female	8/49	23/48	23/47

Further, the Technical Report of the NTP bioassay (page 62) states that methylene chloride produced liver degeneration in both male and female mice.[4] Final mean body weights of high-dose male mice and high and low-dose female mice were 10 - 17% lower than chamber controls. These effects occurred late during the treatment period.

One interpretation of these observations is that tumors arose and shortened the lifetime of the tumor-bearing animals. The NTP study does not attempt direct biological observations of the cause of mortality ("attribution of death"). Mice in the treated groups could have died from either tumors (fatal tumors) or effects of methylene chloride unrelated to the occurrence of

tumors (incidental tumors). However, there are two sources of information on which an inference can be based. The first is to see if there are reasons to expect that toxicity to the whole animal that occurs independently of tumor formation is the cause of tumor formation. Alternatively, treatment with methylene chloride could be "nontoxic" except for the induction of tumors. The information in the appendix to the Technical Report supports the former interpretation. Observations independent from the bioassay suggest strongly that mortality will arise from toxicity causes unrelated to tumor formation. (Indeed, the prime candidate for a mechanism of tumor formation is recurrent organ toxicity in lung and liver, leading to selection of a previously initiated cell or earlier appearance of normally occurring tumors.)

The second source of information is statistical observations on the bioassay results. EPA carried out such an analysis in the final version of The Addendum to the Health Assessment Document for Methylene Chloride (September, 1985) and concluded that "a simple comparison demonstrates that the observed tumors may reasonably have produced this mortality." To reach this conclusion, two types of evidence were provided: (a) a table of mortality (and tumors) within time intervals in the mouse study, and (b) a comparison of risk estimates using a time-to-tumor model. In fact, neither observation is helpful, since they both assume <u>independence</u> of treatment-induced mortality from treatment-induced tumors, whereas the best hypothesis for

tumorigenesis in the mouse is that treatment-induced organ toxicity accelerates cell turnover, leading to earlier appearance of spontaneous tumors. Therefore, a more useful interpretation may be gained from observation of the mice that died earliest, since these treatment-related deaths would occur before sufficient time has elapsed for toxicity to induce tumors. For the male mice, the first five deaths occurred earlier in the treated groups.

AVERAGE WEEKS OF AGE AT DEATH

CONTROL	79
2,000 ppm	45
4,000 ppm	53

There were only small differences between the average age of death for female mice, consistent with the lower overall mortality seen with the female mice.

In the Serota study, liver histopathology was noted at the highest dose (250 mg/kg/day). A no-effect level of 185 mg/kg/day was determined. MacEwen and coworkers observed toxic effects in mice continuously exposed to 1000 ppm for 14 weeks.[29] The exposure differed somewhat from that in the NTP study, but the dose was similar to the 2,000 or 4,000 ppm used by NTP. Weinstein and Diamond observed increases in triglyceride levels, centrolobular fat accumulation, and decreased liver glycogen

levels in ICR mice exposed to 100 ppm of methylene chloride.[30] While these effects are not toxic per se, they support the observation of MacEwen and coworkers at the higher "environmental" exposure level. Haun and coworkers confirmed liver fat accumulation, noted the appearance of hepatocyte vacuoles, and found a decrease in the liver cytochrome P450 content of mice exposed to 100 ppm.[31] While these are not permanently toxic changes, they support the conclusion of toxicity at the higher levels.

Half-maximal saturation of the mouse's metabolic capacity for methylene chloride occurs at about 125 ppm. Bioassays conducted at levels above 125 ppm will be difficult to use in interpreting risks (and therefore weight of the evidence) for environmental exposures. At higher levels, such as the 2000 and 4000 ppm used by NTP, metabolism of methylene chloride does not resemble that occurring at low doses. Low capacity pathways become saturated, low affinity metabolic systems may produce metabolites not seen at environmental exposure levels and co-factors become depleted.

Methylene chloride induced S-phase in hepatocytes of mice in vivo after one or two inhalation exposures to 4000 ppm for two hours.[27] The incidence of S-phase hepatocytes increased variably, and the increases were statistically significant as compared to concurrent (air) controls. These

findings suggest rapid liver cell turnover of a small fraction of cells, while the bulk of liver cells increase in size but not number.

In sum, cell proliferation resulting from cytotoxicity is a plausible explanation for the mouse lung and liver tumors observed in the NTP study. The draft Report states (page 8-6) that "a model based on this mechanism was rejected for both sexes in this case since the observed data did not exhibit sufficient curvature to be consistent with the hypothesis of preneoplastic cell proliferation." The meaning of this statement is unclear. The curve of a data set is not necessarily inconsistent with, and cannot negate, experimental data that warrant a different approach to low-dose extrapolation. Further, as demonstrated in the metabolism section above, the number of observations in a chronic bioassay are sufficiently small that they are not amenable to precise interpretations of this kind.

Overall Weight of Evidence

While the available bioassay evidence is discussed in the draft Report (page 7-8 to 7-14, Appendix B), the draft Report fails to provide a weighing of negative and positive results, along with analysis of the other available toxicological evidence and human data, in an effort to characterize the likelihood that methylene chloride concentrations in the California environment

would pose a cancer risk to humans. As recognized by the National Academy of Sciences and the Office of Science and Technology Policy, this is an essential first step in any assessment of carcinogenic risk.[32]

The overall weight of the evidence overwhelmingly indicates that levels of methylene chloride found in the California environment are not likely to cause cancer. The bioassay results are discussed below, along with a summary of other relevant information that should be used in a weight-of-evidence evaluation.

1. The NTP Bioassay

Male and female mice in the NTP study showed an increase in lung and liver cancer which was, for the most part, statistically significant and dose-related.[4] This indicates that methylene chloride has the potential to increase the incidence of certain naturally-occurring tumors in mice. However, these tumors occurred at doses above a reasonable metabolic dose (saturated metabolism) and were accompanied by signs of overt toxicity in the lung. In addition, as discussed below, there is considerable question as to the significance of these tumors in the mouse for the assessment of cancer potential in humans.

The results of the NTP rat bioassay were quite

different from the mouse results; no increase in malignant tumors was observed. There was a statistically significant increase over the concurrent control group in benign mammary gland tumors in female rats at the two highest dose levels and in males at the highest dose level, representing an enhancement in spontaneously-occurring tumors. However, the statistical comparison disregards historical rates of benign mammary tumors in Fischer 344 rats, which average approximately 28 percent and range up to above 40 percent.[33] The response at the lower dose levels in the NTP rat study was within the mid-range of historical control data. The response at the top dose level was barely elevated above the highest incidences observed in the historical controls. The past presidents of the Society of Toxicology have determined that where incidence rates in treated groups are within historical control ranges, differences between treated and concurrent control groups may not be biologically significant.[34]

Moreover, the benign mammary gland tumors observed did not progress to malignant tumors.[35] While the technical report of the NTP bioassay indicates that there was "clear evidence of carcinogenicity" in rats as that category is defined by NTP, it emphasizes that this is based on an increased incidence of benign tumors. In April 1986, NTP changed the definition of "clear evidence of carcinogenic activity" to include an increase of benign neoplasms (assuming they are not combined with malignant neoplasms) only if "there is an indication from this

or other studies of the ability of such tumors to progress to malignancy".[36] During the review of the NTP study, it was accepted that the benign tumors observed did not progress to malignancy, and there are no other studies showing such progression.

2. Burek Study

Inhalation studies performed at Dow Chemical Company on Sprague-Dawley rats resulted in an increase in benign mammary tumors per tumor bearing rat in female (all doses) and male (high dose only) rats. Exposures were 0, 500, 1,500, and 3,500 ppm.[37] A low but statistically significant increase in sarcomas in the ventral neck region and in and around the salivary glands in male rats was observed at the high exposure and a slight elevation was observed at the 1,500 ppm dose. These results were previously reviewed by EPA's Science Advisory Board, which concluded that it would be inappropriate to use them as a basis for estimating cancer risk from methylene chloride.[38] This conclusion was based on:

- the biology of the tumors, which some Board members thought were surprising (that is, they appeared to be of connective tissue origin rather than parenchrymal cell origin) and which might be manifestations of virus

infections,

- appearance in one sex of rat only,
- lack of appearance in similarly-exposed hamsters,
- an apparent lack of reproducibility in subsequent studies in rats,
- the metabolic properties of methylene chloride, and
- mechanistic considerations.

The overall lack of appearance of the ventral neck region tumors in the NTP bioassay would appear to support this conclusion as to the irreproducibility and lack of significance of these results.

Burek, et al. also studied the effects of methylene chloride on Syrian Golden hamsters at exposures of 0, 500, 1,500, and 3,500 ppm. There was no increase in tumors, even at the highest exposure level.

3. <u>Nitschke Study</u>

A second inhalation study on Sprague-Dawley rats was conducted at Dow to explore the toxicity of methylene chloride at concentrations below those that cause saturation of the metabolic processes.[39] Exposures were 0, 50, 200, and 500 ppm for two years. No increased incidence of tumors was observed, except an increase in the spontaneous incidence of benign mammary tumors in the female rats at 500 ppm. A no-observed-effect level for the rat following lifetime exposure was established at 200 ppm in this study.[40]

4. Serota Study

Other important studies of methylene chloride, sponsored by the National Coffee Association, also showed no carcinogenic response.[41] Reviewers at a Workshop sponsored by the Nutrition Foundation concluded that methylene chloride in drinking water at doses up to 250 mg/kg/day did not cause a tumorigenic response in either rats (Fischer 344) or mice (B6C3F1).[42] These studies are considered state-of-the-art, and their results must be given careful consideration in any assessment of the potential carcinogenicity of methylene chloride. The negative results cast considerable doubt on linear extrapolation from the NTP mouse data even within the same species.

5. Significance of Mouse Results

Results from several bioassays show that the association between methylene chloride exposure and increased lung tumors is unique to mice. Rats and hamsters do not exhibit a tumorigenic response in the lung. Rats and hamsters have a very low incidence of spontaneous lung tumors. There is an obvious association between the high spontaneous background incidence of lung tumors in the mouse and the observation of an increase in tumors upon exposure to high levels of methylene chloride.

The spontaneous nature of the mouse tumors and the likelihood that they signal a promotional event should be taken into account. Lung and liver tumors in B6C3F1 mice are widely recognized as having limited relevance to cancer potential in man. The risk assessment principles adopted by the Office of Science and Technology Policy recognize that evaluation of data from experimental animals that ordinarily have high incidences of certain tumors poses a number of special problems. OSTP Principle 9 states that "the interpretation of cancer incidence in some strains of rats with testicular or mammary tumors, or in some strains of mice with lung or liver tumors, must be approached carefully in the light of other biological evidence bearing on potential carcinogenicity."[43] The EPA Guidelines for Carcinogen Risk Assessment also provide for classification of mouse liver tumors as limited evidence, where certain conditions are met.[44]

EPA's Science Advisory Board has indicated that data

showing an increased incidence of mouse liver tumors alone do not meet the criterion of sufficient evidence.[45] This determination is supported by a substantial body of scientific literature.[46] A panel of distinguished scientists has urged that NTP give serious consideration to "replacement of the B6C3F1 mouse with a strain having an established lower and less variable spontaneous incidence of important tumors that are induced by chemicals."[47] The recent identification of an oncogene in B6C3F1 mouse liver tumors casts further doubt on the value of mouse liver tumors as an end-point in assessing human risk.[48] As for lung tumors, they occur spontaneously in B6C3F1 mice at an average rate of approximately 14 percent.[49]

Quantitative Evaluation

The draft Report should acknowledge, as EPA has recognized, that

> the linearized multistage procedure tends to a plausible upper limit to the risk that is consistent with some proposed mechanisms of carcinogenesis. Such an estimate, however, does not necessarily give a realistic prediction of the risk. The true value of the risk is unknown, and may be as low as zero. The range of risks, defined by the upper limit given by the chosen model and the lower limit which may be as

low as zero, should be explicitly stated.[50]

EPA's statement recognizes that linear estimates would give accurate projections for a few carcinogens but are not likely to give realistic estimates for most substances, which will have much lower low-dose risk.

Current methodology for quantitative risk assessment is dominated by two policy assumptions: (1) that carcinogens do not have practical thresholds; and (2) that carcinogenic risk is a linear function of dose at low doses.[51] Given these policy choices, California has chosen a mathematical model that performs well in estimating a maximum linear slope that is not inconsistent with bioassay data. The model, referred to in the draft Report as the "linearized multistage," takes incidence data obtained at all doses into account, whereas "straight-line" or "single-hit" (Poisson) models may not fit the data when more than one response point is present.[52] The linearized multistage model operates with the data from a bioassay as follows:

- A version of the multistage model is developed that mathematically resembles a true multistage model, with the number of stages constrained by the number of non-zero doses used in the bioassay.

- A maximum likelihood fit of this specific model to

the bioassay data is developed with the exponential values for each stage constrained to give only positive and increasing risks.

- All exponential values higher than the single-hit (linear) component are held fixed and the magnitude of the single-hit exponent is enlarged in the direction of increasing risk to obtain a maximum value compatible with the data in a 95% confidence limit sense. The value of the linear exponent ("q_1^*") is used for risk estimation purposes.

There are serious problems associated with use of the linearized multistage model, which has changed sufficiently from the original model of Armitage and Doll in that it no longer retains a biological rationale.[53] The stages in the linearized multistage model do not relate to discrete modifications of a cell line in the pathway to an observable tumor, and the number of stages is not related to the number of stages in the carcinogenic process. The exponents do not relate to the times between these discrete cell variants, and the overall set of exponent values do not relate to the time-to-tumor. The constraint on non-negative exponents rules out use of models in which a substance lengthens the time of one stage. However, this biological effect has been observed experimentally. In short, a linearized multistage model for a substance is not derived from

an underlying biological theory of carcinogenesis or from knowledge of relevant biological effects of the substance in question. Instead, it is a curve fitting mechanism.

Such curve-fitting exercises entail computer capabilities. Point values are produced which tend to be insensitive to changes in the shape of the dose-response curve. The confidence limit-driven slope is more sensitive to the number of animals the investigator may choose for the bioassay, which is an irrelevant variable for a model of the carcinogenic effect of a substance. This can best be visualized by applying the model to the NTP study data assuming that no effect occurred at all. If the control incidence is substituted for the actual 2,000 and 4,000 ppm values, a q_1^* value will result that does not differ drastically from the current estimate. A linearized multistage model does not readily accept pharmacokinetic or time-to-tumor data. Information on age-specific cancer incidence, background rates, including cell-turnover or cell population kinetics, and lack of mutagenicity cannot be used at all unless the model maker arbitrarily alters the parameters.

The final Report should use GST pathway target tissue dose estimates derived from the PB-PK model which then can be utilized in any risk model, including the linearized multistage model. Doses derived from the PB-PK analysis and utilized in a linear model retain the desired policy positions of linearity and no threshold, but gives a more realistic estimate for hypothetical

"what-if" risk. In addition, the PB-PK dose adjustment should be coupled with other models. The final Report might also include estimates derived from a Moolgavkar-Knudson model.[54] Moolgavkar-Knudson models describe cancer induction as a filtered Poisson process with deterministic and stochastic elements that account for the dynamics of a cell population that is intermediate between two stages, transition from normal cells and transition to malignant cells. Biologically, these two transitions are characterized as rare and irreversible in practice. Use of a Moolgavkar-Knudson model for methylene chloride offers many advantages.[55] A Moolgavkar-Knudson model can describe some of the pharmacodynamic factors involved in dose adjustment of methylene chloride between species, interrelating background rates and age-specific incidence. Many of the advantages of the Moolgavkar-Knudson model over the linearized multistage model are directly relevant to the specific properties of methylene chloride, such as (1) high background tumor rates in the rodent species used as bioassay subjects, (2) strong suggestions of action by a non-genotoxic mechanism, and (3) pharmacokinetics data that have nonlinearities. Data on liver weight changes, cell number and cells in S-phase are available for mice at tumorigenic methylene chloride doses. While use of the linearized multistage model is questionable with non-genotoxic materials, Moolgavkar-Knudson models are appropriate with either genotoxic materials or a substance like methylene chloride that appears to induce tumors at high doses through subtle, organ-specific cytotoxicity.

A Moolgavkar-Knudson model is not, however, necessarily an optimal description of methylene chloride effects. Neither absence of a threshold nor low-dose linearity necessarily applies to methylene chloride, although both assumptions are built into currently available Moolgavkar-Knudson models.

The linearized multistage model used in the draft Report incorporates dose adjustment on the basis of body surface area. Dose conversion by body weight provides a better basis for dose adjustment between species. EPA recently co-sponsored a comparison of carcinogenic potency of various substances in humans and rodents. Body weight proved far superior to body surface area for prediction of the potency value.[56] In addition, two groups of investigators have tested the ability of mice to predict results in rats, and vice-versa. Both Wilson and Crouch[57] and Gaylor and Chen[58] have found body weight superior to body surface area in predicting interspecies dose adjustment.

Reproductive and Developmental Toxicology

In addition to its shortcomings in assessing the potential carcinogenicity of methylene chloride' the draft Report does not adequately describe available data on reproductive effects. While acknowledging that methylene chloride has low teratogenic potential in rodents, the draft Report states (page i-2) that

"experimental data are inadequate to make inferences about effects on human reproduction." This is inconsistent with a determination made by EPA in 1984 to withdraw a proposed reproductive effects test rule for methylene chloride because a two-generation reproductive study in rats undertaken by HSIA "is expected to provide sufficient data to reasonably determine or predict the effects [of methylene chloride] on human reproduction". (49 Fed. Reg. 25009, June 19, 1984) A two-generation reproduction study by Nitschke and coworkers, which was submitted to EPA, showed no treatment-related reproductive effects.[59]

The conclusion that no information exists about reproductive and developmental effects is in error. First, several chronic bioassays of rodents at very high methylene chloride levels have failed to demonstrate changes in reproductive organ weights. These results have been confirmed by Bornmann and Loeser for female rats in a study of methylene chloride administered in drinking water.[60] Second, several developmental studies are available. Schwetz and coworkers directly observed development in mice and rats exposed to high methylene chloride concentrations and found no conclusive effects.[61] In the case of mice, maternal toxicity was noted at the same dose tested. Third, Nitschke and coworkers performed a two-generation inhalation reproduction study in rats exposed to methylene chloride and found no significant effects.[59] Hardin and Manson showed similar results in a one-generation design study.[62]

The draft Report concludes (page 5-3) from some of these data that methylene chloride has low teratogenic potential, but that experimental data are inadequate to make inferences about man. In fact, the data are quite extensive. It is always appropriate to question whether animal tests will be predictive of human effects. However, HSIA is aware of no medical reports suggesting a need for more exhaustive testing. The doses used in the animal studies, in relation to the approximately 10,000-fold lower ambient concentrations in California, appear to merit a more straightforward scientific conclusion that human reproductive and developmental effects are not expected.

CONCLUSIONS

The Department of Health Services, under Section 39660 of the California Health and Safety Code, is required to provide an evaluation of the health effects of substances that may be determined to be toxic air contaminants. Section 39660 requires this evaluation to assess the availability and quality of data on health effects, including potency, mode of action, and other relevant biological factors, and to estimate levels of exposure that may cause or contribute to adverse health effects. Part B of the draft Report requires substantial revision in order to provide an adequate assessment of available biological data on methylene chloride and more realistic estimates of exposure that may cause or contribute to adverse health effects. HSIA would be pleased to assist in this process by providing additional information or amplifying these comments.

CITATIONS

1 Gleit, A., Estimation for Small Normal Data Sets with Detection Limits, Environmental Science and Technology 19: 1201-1206 (1985).

2 Analysis of Organic Micropollutants in Water, Third Edition, 1979, Volume II, EEC publication X11/992, 2/79-EN OMP 7/79, page 132-135.

3 Hearne, F.T., Grose, F., Pifer, J.W., Friedlander, B.R., and Raleigh, R.L., Methylene Chloride Mortality Study: Dose Response Characterization and Animal Model Comparison, J. Occup. Med. 29: 217-228 (1987).

4 NTP Technical Document on the Toxicology and Carcinogenesis Studies of Dichloromethane (Methylene Chloride) (CAS No. 75-09-2) in F344/N Rats and $B_6C_3F_1$ Mice (Inhalation Studies), NTP TR 306 (1986). NTP has announced the initiation of a two-year inhalation study in female mice that will provide information on the dose-responsive curve beneath the low dose used in this study. Chemical Regulation Reporter 10: 1141 (Nov. 28, 1986).

5 Update to the Health Assessment Document and Addendum for Dichloromethane (Methylene Chloride): Pharmacokinetics, Mechanism of Action, and Epidemiology. External Review Draft, EPA/600 18-87/030A; July, 1987.

6 Ott, M.G., Skory, L.G., Holder, B.B., Bronson, J.M. and Williams, P.R., Health Evaluation of Employees Occupationally Exposed to Methylene Chloride: Mortality, Scand. J. Work Environ. Health 8-16 (1983).

7 Green, T., The Metabolic Activation of Dichloromethane and Chloroflurometh ane in a Bacterial Mutation Assay Using Salmonella typhimurium, Mut. Res. 118: 277-88 (1983).

8 Simmon, V.F., Kauhanen, V., and Tardiff, R.G., Mutagenic Activity of Chemicals Identified in Drinking Water, Dev. Toxicol. Environ. Sci. 2: 249-258 (1977).

[9] Callen, D.F., Wolf, C.R., and Philpot, R.M., Cytochrome P-450-Mediated Genetic Activity and Cytotoxicity of Seven Halogenated Aliphatic Hydrocarbons in Saccharomyces cerevisiae, Mutat. Res. 77: 55-63 (1980).

[10] Abrahamson, S., and Valencia, R., Evaluation of Substance of Interest for Genetic Damage Using Drosophila Melanogaster (1980), prepared for the U.S. Food and Drug Administration, Contract No. 233-77-2119.

[11] Gocke, E., King, M.T., Eckhardt, K., and Wild, D., Mutagenicity of Cosmetics Ingredients Licensed by the European Communities, Mutat. Res. 90: 91-109 (1981).

[12] Sivak, A., Balb/C-3T3 Neoplastic Transformation Assay with Methylene Chloride (Food Grade Test Specification), Document to National Coffee Association, Inc., 1978; Also see Thilagar, A.K., Back, A.M., Kirby, P.E., Kumaroo, P.V., Pant, K.J., Clarke, J.E., Knight, R., and Haworth, S.R., Evaluation of Dichloromethane in Short-term In Vitro Genetic Toxicity Assays, Environ. Mutagenesis 6: 418-419 (1984).

[13] Price, P.J., Hassett, C.M., and Mansfield, J.I., Transforming Activities of Methylene Chloride and Proposed Industrial Alternatives, In Vitro 14: 290-293 (1978).

[14] Jongen, W.M.F., Lohman, P.H.M., Kettenhagen, J.J., Alink, G.M., Berends, F., and Koeman, J.H., Mutagenicity Testing of Dichloromethane in Short-Term Mammalian Test Systems, Mutat. Res. 81(2): 203-213 (1981); Andrae, U., and Wolff, T., Dichloromethane is Not Genotoxic in Isolated Rat Hepatocytes, Arch. Toxicol. 52: 287-89 (1983); Trueman, R.W., Ashby, J., Millward, S.W., and Marsh, J.R., Methylene Chloride (Dichloromethane): In Vivo and In Vitro Unscheduled DNA Synthesis Studies in the Mouse and the Rat. CEFIC Document No. CTL/P/1444 (1986).

[15] Thilager, A.K., Back, A., Kirby, P.E., Kumaroo, P.V., Pant, J.J., Clarke, J.E., Knight, R., and Haworth, S.R., Evaluation of Dichloromethane in Short-Term In Vitro Genetic Toxicity Assays, Environ. Mutagenesis 6: 418-419 (1984).

[16] Sheldon, T., Richardson, C.R., Hamilton, K., Randall, V., Hart, D., and Hollis, K., Methylene Chloride (Dichloromethane): An Evaluation in the Mouse Micronucleus Test, European Council of Chemical Manufacturers' Federation (CEFIC) Document No. CTL/P/1603 (1986).

[17] Thilagar, A.K., and Kumaroo, P.V., Induction of Chromosome Damage by Methylene Chloride in CHO Cells, Mutation Research 116: 361-367 (1983); Thilagar, A.K., Kumaroo, P.V., Clarke, J.J., Kott, S., Back, A.M., and Kirby, P.E., Induction of Chromosome Damage by Dichloromethane in Cultured Human Peripheral Lymphocytes, CHO Cells and Mouse Lymphoma L5178Y Cells, Environ. Mutagenesis 6: 422 (1984).

[18] Dutton, D.R., and Bowden, G.T., Indirect Induction of a Clastogenic Effect on Epidermal Calls by a Tumor Promoter, Carcinogenesis 6: 1279-1284 (1985).

[19] Burek, J.D., et al., Methylene Chloride: A Two-Year Inhalation Toxocity and Oncogenicity Study in Rats an Hamsters, Fund. Appl. Toxicol. 4: 30-47 (1984);

[20] Schumann, A.M., Nitschke, K.D., Fox, T.R., and Watanabe, P.G., (^{14}C) Methylene Chloride: Pharmacokinetics and Interaction with Hamster and Rat Tissue Macromolecules, Document of the Toxicology Research Laboratory, Dow Chemical Company, Midland, Michigan; Also see The Toxicologist, Vol. 4 (1984).

[21] Green, T., Provan, W.M., Collinge, D.C., and Guest, A.E., Methylene Chloride (Dichloromethane): Interaction with Rat and Mouse Liver and Lung DNA In Vitro. CEFIC Document No. CTL/R/851 (1986).

[22] Letters of March 4 and October 8, 1986 to William V. Loscutoff from Dr. M. R. Harris submitting various reports from CEFIC.

[23] "New Data Indicate Methylene Chloride Not Carcinogenic, Some SAB Members Say," Chem. Req. Rep. 11: 862 (Aug. 14, 1987).

[24] Green, T., Nash, J.A., and Mainwaring, G., Methylene Chloride (Dichloromethane): In Vitro Metabolism in Rat, Mouse, and Hamster Liver and Lung Fractions and in Human Liver Fractions (1986), European Council of Chemical Manufacturers Federation (CEFIC) Document No: CTL/R/879; Also see Green, T., and Reitz, R.H., Measurement of Methylene Chloride/Glutathione-S-Transferase Enzymes in Various Species, Update (May 6, 1987).

[25] Andersen, M.E., Clewell, H.J., III, Gargas, M.L., Smith, F.A., and Reitz, R.H., Physiologically Based Pharmacokinetics and the Risk Assessment Process for Methylene Chloride, Tox. Appl. Pharm. 87: 185-205 (1987).

[26] Green, T., Provan, W.M., Nash, J.A., and Gowans, N., Methylene Chloride (Dichloromethane): In Vivo Inhalation Pharmacokinetics and Metabolism in F344 and $B_6C_3F_1$ Mice, European Council of Chemical Manufacturers Federation (CEFIC), Document No. CTL/R/880 (1986).

[27] Lefevre, P. A., and Ashby, J., Methylene Chloride: Induction of S-phase Hepatocytes in the Mouse After In Vivo Exposure, European Council of Chemical Manufacturer's Federation (CEFIC), Document No. CTL/R/885 (1986).

[28] Hext, P.M., Foster, J., and Millward, S. W., Methylene Chloride: 10 Day Inhalation Toxicity Study to Investigate the Effects on Rat and Mouse Liver and Lungs, European Council of Chemical Manufacturer's Federations (CEFIC) Document No. CTL/P/1432 (1986).

[29] MacEwen, J.D., Vernot, E.H. and Haun, C.C. (1972). Continuous animal exposure to dichloromethane. Document AMRL-TR-72-28. Aerospace Medical Research Laboratory, Wright-Patterson Air Force Base, Ohio, p. 33.

[30] Weinstein, R.S. and Diamond, S.S. (1972). Hepatotoxicity of dichloromethane (methylene chloride) with continuous inhalation exposure at low-dose level. In: Proceedings of the 3rd Annual Conference on Environmental Toxicology, Wright-Patterson Air Force Base, Aerospace Medical Research Laboratories, Ohio (AMRL-72-130, Paper No. 3), pp. 209-222.

[31] Haun, C.C., Vernot, E.H., Darmer, K.I. and Diamond, S.S. (1972). Continuous animal exposure to low levels of dichloromethane. In: Proceedings of the 3rd Annual Conference on Environmental Toxicology, Wright-Patterson Air Force Base, Aerospace Medical Research Laboratory, Ohio (AMRL-TR-130, Paper No. 12), page 199-208.

[32] National Research Council, Risk Assessment in the Federal Government: Managing the Process, National Academy Press (1983); Office of Science and Technology Policy, Chemical Carcinogens; Review of the Science and its Associated Principles, Fed. Reg. 50: 10372, 10378 (Mar. 14, 1985)

[33] Transcript, NTP Board of Scientific Counselors Review of Draft Technical Document of Long-Term Toxicology and Carcinogenesis Studies of Dichloromethane (March 29, 1985) at 172-73.

[34] "If the incidence rate in the concurrent control group is lower than in the historical control groups, but the incidence rates in the treated groups are within historical control ranges, the differences between treated and control groups are not biologically significant." Task Force of Past Presidents, Society of Toxicology, Animal Data in Hazard Evaluation: Paths and Pitfalls, Fund. Appl. Toxicol. 2: 101-107 (1982). See also OSTP Principle 14(a), Fed. Reg. 50: 10377 (1985).

[35] Transcipt, NTP Board of Scientific Counselors Review of Draft Technical Document of Long-Term Toxicology and Carcinogenesis Studies in Dichloromethane (March 29, 1985) at 140-141, 160-161.

[36] Fed. Reg. 51: 11844 (Apr. 7, 1986).

[37] Burek, J.D., Nitschke, K.D., Bell, T.J., Wackerle, D.L., Childs, R.C. Beyer, J.E., Dittenber, D.A., Rampy, L.W., and McKenna, M.J., Methylene Chloride: A Two-Year Inhalation Toxicity and Oncogenicity Study in Rats and Hamsters, Fund. Appl. Toxicol. 4: 30-47 (1984).

[38] U.S. Environmental Protection Agency, Science Advisory Board, Environmental Health Committee, Letter Report of July 18, 1984 to William D. Ruckelshaus, concerning the EPA Health Assessment Document for Methylene Chloride. Washington, D. C.

[39] Nitschke, K.D., et al., Methylene Chloride: A Two-Year Inhalation Toxicity and Oncogenicity Study in Rats, Dow Chemical Company Midland, MI. (1982). This study has been submitted to Fundamental and Applied Toxicology.

[40] A subsequent study by Breslin and Landry has shown a statistically significant increase (2.3x) in serum prolactin levels in female Sprague-Dawley rats exposed to 3500 ppm of methylene chloride for 15-19 days. It is hypothesized that elevated prolactin levels, if maintained over the course of long-term methylene chloride exposure, may play a role in the enhanced spontaneous benign mammary gland tumor response in chronically exposed female rats. Breslin, W.J., and Landry, T.D., Methylene Chloride: Effects on Estrous Cycling and Serum Prolactin in Sprague-Dawley Rats, Mammalian and Environmental Toxicology Research Laboratory, Dow Chemical Company, Midland, MI. (1986).

[41] Serota, D.G., Thakar, A.K., Ulland, B.M., Kirschman, J.C., Brown, N.M., Coots, R.H. and Morgareide, K. A Two-Year Drinking Water Study of Dichloromethane on Rodents, Food Chem. Tox. 24:951-963 (1986).

[42] Nutrition Foundation, Proceedings of Food Solvents Workshop I: Methylene Chloride (Mar. 8-9, 1984) at 119-120.

[43] Fed. Reg. 50: 10377 (March 14, 1985).

[44] Fed. Reg. 51: 33999 (September 24, 1986).

[45] U.S. Environmental Protection Agency, Science Advisory Board, Letter Report of Jan. 29, 1987 to Lee Thomas, concerning the Addendum to the Health Assessment Document for Tetrachloroethylene ("the Subcommittee concludes that the animal evidence of carcinogenicity is 'limited' because of positive results in only one strain of mouse of a type of tumor that is common and difficult to interpret"); Letters of December 17, 1984 and January 4, 1985 to William D. Ruckelshaus, concerning the Health Assessment Documents for Trichloroethylene and Tetrachloroethylene, respectively.

[46] International Expert Committee to the Nutrition Foundation, The Relevance of Mouse Liver Hepatoma to Human Carcinogenic Risk (1983); Schach von Wittenau, M., and Estes, P.C., The Redundancy of the Mouse Carcinogenicity Bioassay, Fund Appl. Toxicol. 3:631-39 (1983); Butler, W.H., and Newberne, P.M. (ed.), Mouse Hepatic Neoplasia, Elsevier (Amsterdam, 1975); Tomatis, L., Partenshy, C., and Montesano, R., The Predictive Value of Mouse Liver Tumor Induction in Carcinogenicity Testing -- A Literature Survey, Int. J. Cancer 12:1-20 (1973); Grasso, P., and Crampton, R.F., The Value of the Mouse in Carcinogenicity Testing, Food Cosmet. Toxicol. 10:418-26 (1972).

[47] Board of Scientific Counselors, National Toxicology Program, "Document of the NTP Ad Hoc Panel on Chemical Carcinogenesis Testing and Evaluation" (1984) at 116. Reviews of a number of bioassays involving different chemicals have found mouse liver tumors to be the most prevalent cancers observed. Hamm (CIIT), The Occurrence of Neoplasms in Long-Term In Vivo Studies, Env. Sci. Res. 29:9 (1983); Haseman et al. (NIEHS), Results from 86 Two-Year Carcinogenicity Studies conducted by the NTP, J. Toxic. & Environ. Health 14:621 (1984).

[48] Fox, T.R., and Watanabe, P.G., Detection of a Cellular Oncogene in Spontaneous Liver Tumors in $B_6C_3F_1$ Mice, Science 228:596 (1985). Dr. Ian Munro has stated that this "may account in whole or in part for [the mouse's] unique susceptibility to hydrocarbon induced carcinogenesis." Munro, Comments on EPA Health Assessment Document for Chloroform, Feb. 1986.

[49] Chu, K.C., Cueto, C., and Ward, J., Factors in the Evaluation of 200 National Cancer Institute Carcinogen Bioassays, J. Tox. Env. Health 8:251 (1981). While the Science Advisory Board has not had occasion to provide an exhaustive evaluation of the significance of such tumors, one member expressed serious concerns about the significance of the lung tumor results from the NTP study during a review of a draft Addendum to the Health Assessment Document for methylene chloride. Transcript, SAB Evironmental Health Committee Review of Draft Addendum to the Health Assessment Document for Methylene Chloride (May 23, 1985) at 92-94.

[50] Fed. Reg. 51: 33998.

[51] Anderson, E., and the Carcinogen Assessment Group of the U.S. Environmental Protection Agency, Quantitative Approaches in Use to Assess Cancer Risk. Risk Analysis 3:277 (1983).

[52] Crump, K. S., An Improved Procedure for Low-Dose Carcinogenic Risk Assessment from Animal Data. J. Environ. Pathol. Toxicol. 5: 675 (1981).

[53] Armitage, P. and Doll, R., The Age Distribution of Cancer and a Multistage Theory of Carcinogenesis, Br. J. Cancer 8: 1 (1954).

[54] Moolgavkar, S. H., and Knudson, A. G., Mutation and Cancer: A Model for Human Carcinogenesis, J. Natl. Cancer Inst. 66: 1037-1052 (1981).

[55] Thorslund, T. W., Brown, C. C., and Charnley, G., Biologically Motivated Cancer Risk Models, Risk Analysis, 7: 109-119 (1987).

[56] Allen, B.C., Shipp, A.M., Crump, K.S., Kilion, B., Hogg, M.L., Tudor, J. and Keller, B., Investigation of Cancer Risk Assessment Methods. U.S. Environmental Protection Agency Contract #68-01-6807; December, 1988; Washington, D.C.

[57] Crouch, E. and Wilson, R., Interspecies Comparison of Carcinogenic Potency. J. Toxicol. Environ. Health 5: 1095 (1979). Crouch, E. and Wilson, R., Regulation of Carcinogens. Risk Analysis 1: 47 (1981); Crouch, E., Uncertainties in Interspecies Extrapolations of Carcinogenicity. Environ. Health Perspectives 50: 321 (1983).

[58] Gaylor, D. W., and Chen, J. J., Relative Potency of Chemical Carcinogens in Rodents, Risk Analysis, 6: (1986) Gaylor, D. W., and Chen, J. J., Carcinogenic Risk Assessment: Comparison of Estimated Safe Doses for Rats and Mice, Environ. Health Perspect. 72: (1987).

[59] Nitschke, K.D., Eisenbrandt, D.L., and Lomax, L.G., Methylene Chloride: Two-Generation Inhalation Reproduction Study in Fisher 344 Rats, Dow Chemical Company, 1985.

[60] Bornmann, G. and Loeser, A., Zur Frage einer Cronish-toxischen Wirkung von Dischloromethan. Zeit. Lebenomit.-Untersuch. Forsch. 136: 14-18 (1967).

[61] Schwetz, B.A., Leong, B.K.J. and Gehring, P.J., The Effect of Maternally Inhaled Trichloroethylene, Perchloroethylene, Methyl Chloroform and Methylene Chloride on Embryonal and Fetal Development in Mice and Rats. Toxicol. Appl. Pharmacol. 32: 84-96 (1975).

[62] Hardin, B.D. and Manson, J.M., Absence of Dichloromethane Teratogenicity with Inhalation Exposure in Rats. Toxicol. Appl. Pharmacol. 52: 22-28 (1980).

APPENDIX A

Page III-5	Methylene chloride use has been declining at approximately 10% per year since 1984. 1987 production estimates are approximately 500 million pounds. In 1984 the market was over 600 million pounds. Aerosols and certain formulated products have shown the biggest reduction in methylene chloride use.
Page III-4 (1)	The potential for accounts receiving shipments of methylene chloride in California and then reshipping outside the state is not restricted to chemical distributors. Examples include aerosol fillers and paint stripper formulators. This volume could be more than a small fraction.
Page III-6 line 2 under Paint Removers	We question whether 7,500 tons of methylene chloride were used in paint removers in California.
Page III-8 2nd & 3rd lines from end	The percentage of methylene chloride in the total aerosol formulation depends on what the product is. Aerosol paint strippers contain 85% methylene chloride.
Page III-19 last line, 2nd to last paragraph	Voluntary labeling will begin in 1988, not 1986.
Page III-20 last few lines	Air stripping would be adequate for this volatile compound. Stream stripping would not be necessary.
Page III-9 last paragraph	Some methylene chloride is typically retained in the foam. Methylene chloride is also used as a flush for urethane foam nozzles and for cleanup purposes in polyester molding operations.

Page III-10 Degresing Operations	Few degreasers use methylene chloride. The estimates given in this paragraph appear to be for stripping and circuit board stripping.
Page III-11 Photographic Film Processing	To our knowledge, film <u>cleaning</u> is done instead with 1,1,1-trichloroethane.
Page III-11 Pesticide Mfg. 3rd line	800 tons seems high.
Page III-11 & first paragraph Page III-12	This section is confusing. Is there one facility that used 800 tons in 1983 as a solvent for process use plus 90 more tons for extraction, phase separation, purification, crystallization, and as a general transport solvent? The first page intimates there was only one facility.
Page III-12 Chemical Processing	Some of this methylene chloride could be part of the final product, which may be used (emitted) outside of California.

APPENDIX B

RESPONSE TO THE CALIFORNIA DEPARTMENT OF HEALTH SERVICES PRELIMINARY DRAFT, TECHNICAL SUPPORT DOCUMENT, PART B - HEALTH EFFECTS OF METHYLENE CHLORIDE.

Epidemiology Section
Health and Environment Laboratories
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January 1988

Introduction

This document has been prepared in response to the preliminary draft of the California Department of Health Services (DHS) entitled, "Part B: Health Effects of Methylene Chloride", dated November 1987. It addresses certain issues concerning the Eastman Kodak Company epidemiologic studies, including a comparison of animal model predictions with human findings.

Epidemiological Studies (Section 7.2)

The DHS report summarized the results of the Kodak mortality studies of two populations of workers exposed to methylene chloride: (1) a 1964 cohort of about 750 men followed through 1976[1], 1980[2], and 1984[3], and (2) a 1964-1970 cohort of approximately 1,000 men (the 751 employees originally studied as well as 262 men hired between 1965 and 1970) followed through 1984. There were two references to the second study, a presentation of preliminary findings,[4] and a description of the final results,[5] which incorporated detailed exposure history data, dose-response analyses, and comparison with animal model extrapolations.

The information, as presented, is extremely confusing. Rather than describe each of the the studies in chronological sequence, selected data (statistical results, exposure measurements, power, etc.) from the five articles were intermixed. For example, exposure estimates (p 7-5) from a preliminary analysis were shown instead of updated information from the final published report. The statement that "tobacco smoking was not considered in these analyses..." (p 7-1) may be misinterpreted as applying to all studies when, in fact, cigarette smoking was considered in the 1987 article only. Also, the follow-up rate of 99% (p 7-2) was observed only for the most recent study. As a final comment, we note that it is often unclear as to which study the observed-expected values refer.

In presenting the epidemiologic results, it would have been more appropriate to emphasize the latest study findings,[5] which provided the most complete follow-up and exposure information for an expanded cohort of

methylene chloride-exposed workers. (See Appendix A for summary.)

Risk Assessment (Section 8.5)

To evaluate the validity of animal-based risk estimates, the DHS staff used two analytic techniques: 1) a relative risk model fit to the human data, and (2) a comparison of the observed cancer mortality with predictions derived from the linearized multistage model applied to the animal data. From these analyses, they concluded "...that the quantitative extrapolation from mice to humans yields plausible estimates of cancer risk...." (p 8-15)

1. Relative Risk Model

In this approach, a 95% upper bound on risk generated from the nonsignificant human pancreatic finding was compared with a unit risk estimate derived from the female mouse lung cancer data. Specific criteria included: (1) pancreatic cancer, (2) all age groups, (3) Kodak Rochester controls, and (4) summary statistics for expected deaths and estimated dose. From the relative (multiplicative) model, the 95% upper bound on the estimated human lifetime incremental risk for 1 ppm of continuous methylene chloride exposure was estimated to be 7.90×10^{-3} (see Note below) compared with a unit risk of 3.25×10^{-3} from the mouse data.

The relatively simplistic modeling procedure used by the DHS, which was based solely on summary data, allowed only linear changes with dose while forcing the line through the origin, and thus it is not unexpected that the results would be consistent with linearized multistage model bounds. It is likely that other models utilizing more detailed dosage information would give entirely different results.

In order to examine the sensitivity of the multiplicative model, we calculated estimates of lifetime incremental risk based on various

Note. This value is incorrect. A DHS staff member (IH) has concurred with our calculated value of 9.4×10^{-3}.

scenarios of data outcome (Table). As noted by the DHS staff, the upper bounds for Scenarios 1 (linearized multistage model applied to NTP female mouse lung adenomas and carcinomas) and 2 (multiplicative model applied to epidemiologic data for pancreatic cancer) were comparable. On the other hand, the human lower bound is negative, indicating that the data are also consistent with no excess risk. Scenario 3, in addition to using the same factors as the DHS staff, assumed that there were half as many observed pancreatic malignancy deaths in each of the three career dose categories. The results showed that the upper 95% bounds were marginally changed compared with the DHS parameters (4.2 vs. 9.4 x 10^{-3}), and still consistent with the animal unit risk value (3.3 x 10^{-3}). In Scenario 4 it was assumed that only one observed death occurred in each exposure group (a total of three deaths), which resulted in an SMR of 94. The upper 95% bound (2.1 x 10^{-3}) remained about the same as the animal unit risk value, albeit the maximum likelihood estimate was negative. The human lung cancer data, applied in Scenario 5, showed that even though the observed-expected ratio decreased with dose and the overall SMR was below 100, the results (1.7 x 10^{-3}) were still comparable to the upper bound risk derived from the mouse data.

It is thus evident that the upper bound risk generated from this model is highly insensitive to differences in both the number of observed deaths and the dose-response relationship within the cohort. Reducing the number of pancreatic cancer deaths from eight to four to three (Scenarios 2-4) did not materially affect the upper bound. Additionally, for those scenarios showing a decrease in risk with career dose and an SMR at or below unity (Scenarios 4 and 5), the upper bound remained essentially unchanged vs. the linearized multistage model.

To summarize, the draft document's risk assessment approach (1) selected the pancreatic cancer results even though they may not be related to

Scenario	Exposure Group (ppm-Years)	No. of Observed/ Expected Deaths	Parameter Estimate of b	Asymptotic Standard Error of b	Two-Tailed p Value[1]	Estimated Lifetime Incremental Risk of 1 ppm of Continuous Methylene Chloride Exposure: 95% Lower Bound	Maximum Likelihood Estimate	95% Upper Bound
Scenario 1:[2] NIH Female Mouse Data						0	2.5×10^{-9}	3.3×10^{-9}
Scenario 2: Human Pancreatic Cancer SMR[3] - 251								
	< 350	2/0.718 = 279						
	350-749	2/1.213 = 165						
	750+	4/1.256 = 318						
	Total	8/3.187 = 251	0.583	0.358	0.10	-4.7×10^{-9}	4.7×10^{-9}	9.4×10^{-9}
Scenario 3: One Half Pancreatic Cancer Deaths SMR - 126								
	< 350	1/0.718 = 139						
	350-749	1/1.213 = 82						
	750+	2/1.256 = 159						
	Total	4/3.187 = 126	0.113	0.248	0.65	-2.4×10^{-9}	9.0×10^{-4}	4.2×10^{-9}
Scenario 4: Three Pancreatic Cancer Deaths SMR - 94								
	< 350	1/0.718 = 139						
	350-749	1/1.213 = 82						
	750+	1/1.256 = 80						
	Total	3/3.187 = 94	-0.053	0.191	0.78	-2.9×10^{-9}	-4.2×10^{-4}	2.1×10^{-9}
Scenario 5: Human Lung Cancer SMR - 85								
	< 350	4/ 3.255 = 123						
	350-749	5/ 6.121 = 82						
	750+	5/ 7.181 = 70						
	Total	14/16.557 = 85	-0.079	0.076	0.30	-7.5×10^{-9}	-2.9×10^{-9}	1.7×10^{-9}

1 Two-tailed test of linear model parameter difference from 0.

2 Scenario 1: NIH female mouse data, lung adenomas and carcinomas, linearized multistage model.
Scenario 2: Human pancreatic cancer, occupational controls, and summary expected deaths and dose.
Scenario 3: Same as Scenario 2, except the number of pancreatic cancer deaths is reduced by one-half in each career exposure group.
Scenario 4: Same as Scenario 2, except there is one pancreatic cancer death in each career exposure group.
Scenario [illegible] Human lung cancer, occupational controls, and summary expected deaths and dose.

3 Standardized Mortality Ratio.

methylene chloride exposure, (2) used a strictly linear model forced through the origin, and (3) ignored information contained in the lower bound estimate. Such a strategy virtually insures compatibility with the animal bioassay extrapolation findings. Under these circumstances, it is difficult to imagine any epidemiologic study which would provide sufficient evidence to contradict conclusions drawn from the animal studies. In our judgment the draft document should have evaluated data for all primary cancers rather than restrict the analysis to the only site for which there was a suggestive increase, which may have been due to chance.

2. Predicted Deaths from Linearized Multistage Model

For this analysis, the number of excess deaths predicted in the Kodak cohort from the application of the linearized multistage model to the female mouse lung tumor results were compared with the pancreatic cancer findings. The total of 12.5 deaths (9.3 excess plus 3.2 background) was reported to be "...quite close to the observed (eight pancreatic cancer deaths)." (p 8-14) In addition, DHS noted that the 36.3 lung/liver deaths predicted (14.5 excess plus 21.8 background) were "...within a factor of 3 of the observed 14 deaths...," (p 8-15), and thus concluded that ". .the animal-based risk assessment should be viewed as consistent with the human data." (8-14)

Contrary to the DHS statement that the numbers of observed and expected pancreatic cancers were "quite close", it should be emphasized that the probability of observing eight or fewer deaths, given expectation of 12.5, is relatively low (0.125). Similarly, although the observed and expected lung/liver cancer deaths were "within a factor of 3", it is highly unlikely (probability less than 0.0001) that 14 or fewer deaths would have been observed if the animal model predictions were correct. We, therefore, disagree with the DHS staff concerning their interpretation of the difference between the animal model predictions and the human findings.

Biologic Plausibility of Pancreatic Cancer

The DHS staff's selection of the pancreas for their animal model vs. human comparison was based on the observation that this site exhibited the "...greatest relative risk among workers occupationally exposed..." (p 8-14) As further justification, they stated that "...there is no reason to assume that the most sensitive site in animals will be the most sensitive site in humans." (p 8-13)

In assessing the risk associated with exposure to a chemical, it is essential that the issue of biologic plausibility be carefully examined. With respect to pancreatic cancer, numerous studies have been conducted that clearly demonstrate that such tumors are found in association with chemical exposures in the rat, e.g., bis(chloromethyl)ether[6], 4-hydroxyamino-quinoline-1-oxide[7], the diazoketone azaserine[8], the methylnitrosourea-containing amino acid N-(N-methyl-N-nitroso carbamoyl)-L-ornithine[9], and more recently, hypolipidemic drugs such as nafenopin[9] and clofibrate.[9] However, chronic oral[10] and inhalation[11-13] studies of methylene chloride in rats have not demonstrated the pancreas to be the site of toxic action. Similarly, chronic inhalation studies in hamsters[11,12] and mice[12,13] have not shown this chemical to be a pancreatic carcinogen. (Hamsters, in addition to rats, have been extensively used as a model for experimental pancreatic cancer induction.[9])

Since pancreatic cancer has not been observed in chronic studies with rats and mice dosed orally, or in rats, mice, and hamsters dosed by inhalation, there is no evidence that methylene chloride is a pancreatic carcinogen in animal species widely accepted as models for this disease. Assessing these results within the context of the Kodak pancreatic findings (e.g., marginally-elevated risk, no dose response, the presence of confounding variables, and the probability of a chance finding), it is unlikely that methylene chloride is a human pancreatic carcinogen.

Conclusions

We disagree with the the DHS staff's statement "...that human risk estimates based on animal studies are consistent with the results of this (Kodak) epidemiological study." (p i-3) In arriving at this conclusion, the DHS staff failed to adequately consider the overall mortality results, including total cancer and such hypothesized tumor sites as lung and liver. Instead, they restricted their analysis to the only site with an elevated SMR (pancreas) for which current evidence of an association with methylene chloride is not supported by animal model studies.

There are at least four reasons for rejecting this approach. First, it is likely that the observed-expected difference was due to chance. (This possibility has been strengthened by recent data which indicate that no new cases have occurred since the last reported follow-up in 1984.) Second, no dose-related effect was observed in the mortality study. Third, the results may have been confounded by personal (smoking, alcohol consumption, diabetes, etc.) and occupational (other chemical exposures) risk factors. And lastly, there is no apparent evidence of biologic plausibility for pancreatic cancer in man.

With reference to the DHS modeling technique, the use of an insensitive upper bound from a linear model for any cancer site with an increased relative risk is likely to demonstrate consistency with extrapolations from animal bioassay data. Finally, in interpreting the differences between animal model predictions and actual cohort mortality, it is important to recognize that the DHS staff's use of such terms as "quite close" or "within a factor of 3" is misleading since the probability of observing the cohort results (given that the predictions are valid) is small. Based on the totality of information available, we therefore conclude that the overall weight of the evidence does <u>not</u> support the allegation that methylene chloride is a human carcinogen.

References

1. Friedlander BR, Hearne T, Hall S: Epidemiologic investigation of employees chronically exposed to methylene chloride: Mortality analysis. J Occup Med 1978;20:657-666.

2. Hearne FT, Friedlander BR: Follow-up of methylene chloride study (letter) J Occup Med 1981;20:660.

3. Friedlander BR, Pifer JW, Hearne FT: 1964 Methylene Chloride Cohort Mortality Study: Update Through 1984. Eastman Kodak Company, Rochester, New York, 1985.

4. Friedlander BR, Hearne FT, Pifer JW, Grose FH: Epidemiologic Evidence Regarding Methylene Chloride. Presented at the Winter Toxicology Forum, Washington DC, February 18, 1986.

5. Hearne FT, Grose F, Pifer JW, Friedlander BR, Raleigh RL: Methylene chloride mortality study: Dose-response characterization and animal model comparison. J Occup Med 1987;29:217-228.

6. Boorman GA: Naturally occurring lesions of the exocrine pancreas in the rat. In: Scarpelli DG, Reddy JK, Longnecker DS, eds. Experimental Pancreatic Carcinogenesis. Boca Raton: CRC Press, Inc, 1987:145-157.

7. Kokkinakis DM, Scarpelli DG, Hollenberg PF: Metabolism and activation of nitrosamine pancreatic carcinogens. In: Scarpelli DG, Reddy JK, Longnecker DS, eds. Experimental Pancreatic Carcinogenesis. Boca Raton: CRC Press, Inc, 1987:3-20.

8. Flaks B: Sequential microscopic and ultrastructural morphological changes during the development of ductal adenocarcinoma of the hamster pancreas induced by BHP. In: Scarpelli DG, Reddy JK, Longnecker DS, eds. Experimental Pancreatic Carcinogenesis. Boca Raton: CRC Press, Inc, 1987:65-103.

9. Scarpelli DG, Rao MS: Experimental carcinogenesis of exocrine pancreas in the Syrian golden hamster (mesocricetus auratus). In: Scarpelli DG, Reddy JK, Longnecker DS, eds. Experimental Pancreatic Carcinogenesis. Boca Raton: CRC Press, Inc, 1987:45-64.

10. Serota DG, Thakur AK, Ulland BM, Kirschman JC, Brown NM, Coots RG, Morgareidge K: A two-year drinking water study of dichloromethane on rodents: I. Rats. Food Chem Toxicol 1986;24:951-958.

11. Burek JD, Nitschke KD, Bell TJ, et al: Methylene chloride: A two-year inhalation toxicity and oncogenicity study in rats and hamsters. Fundam Appl Toxicol 1984;4:30-47.

12. US Environmental Protection Agency: Health Assessment Document for Dichloromethane (Methylene Chloride). Final Report (EPA-600/8-82-004F), Washington DC, Office of Health and Environmental Assessment, 1985.

13. National Toxicology Program: NTP Technical Report on the Toxicology and Carcinogenesis Studies of Dichloromethane (Methylene Chloride) (CAS No. 75-09-2) in F344/N Rats and B6C3F$_1$ Mice (Inhalation Studies). US Department of Health and Human Services, NIH publication No. 86-2562, NTP-TR 306, 1986.

APPENDIX

SUMMARY OF HEALTH EFFECTS DATA
METHYLENE CHLORIDE MORTALITY STUDY

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January 1988

Introduction

This summary report addresses the issue of the potential carcinogenic risk associated with chronic occupational exposure to methylene chloride as evaluated in a recently published epidemiologic mortality investigation of Eastman Kodak Company employees.[1]

Methodology

The population at risk in this historical cohort study included a cohort of 1,013 hourly men who were (1) employed in film coating operations (methylene chloride area) at any time between January 1964 and December 1970 and (2) had worked in this area at least one year during their Kodak careers. Employees, including retirees, were followed for a maximum of 21 years, from 1964 to 1984. Cause of death statistics were compared with mortality data for both the general population (upstate New York men, 1965-1980) and an employed population (Kodak Rochester hourly men, 1964-1984).

Standard methodological procedures were applied to calculate person-years and the expected number of deaths by major cause and cancer subcategory. The Standardized Mortality Ratio (SMR) was used to summarize the cohort's mortality experience. Based on the results of an NTP animal bioassay,[2] the hypothesized outcomes of respiratory and liver malignancy were assessed by a one-tailed test for significance at an alpha level of 5%. In contrast, nonhypothesized causes were evaluated using a two-tailed test with an alpha of 1%.

Estimates of career methylene chloride exposure were derived from extensive workplace monitoring, including > 1,200 area samples collected during the past four decades (1945-1986) and > 900 personal samples obtained since 1980. Analyses of the data by lifetime exposure (ppm-years) and latency were performed to assess dose-response relationships. Finally, to address the issue of the consistency of toxicologic and epidemiologic evidence, the number of excess lung and liver cancer deaths predicted from the NTP animal model were computed, and the results were compared with the actual number observed in the cohort.

Results

1. Cohort Description and Follow-up

The 1,013 study subjects (mean tenure: ~ 26 years) contributed more than 19,000 person-years during the 21-year observation period. There were 176 deaths (17%).

The follow-up rate was 99%. Nine persons remained untraced after a search of company and social security records and completion of a mail survey among disabled and retired employees.

2. Exposure and Latency

The mean methylene chloride concentration, adjusted for seasonal variation, was 26 ppm as an 8-hour time-weighted average. Exposures were characterized according to seven occupational categories selected on the basis of personal dosimetry results. The mean 8-hour TWA averages ranged

from < 1 ppm (polyester film worker and electroplater) to 114 ppm (group leader). Methylene chloride concentrations for other jobs were: film coater, 48 ppm; coater's assistant, 40 ppm; and cleaner, mechanic, chemical worker, 23 ppm. The maximum peak exposure estimates ranged from 500 ppm to 10,000 ppm. On average, the study population was employed in film casting operations 22 years since methylene chloride was introduced in approximately 1944.

In order to assess dose-response, the exposure data were classified into three categories of approximately the same number of employees: < 350 ppm-years, 350-749 ppm-years, and 750 or more ppm-years. The mean exposure rates were, respectively, 16, 22, and 42 ppm as 8-hour TWAs while median latencies were 17, 31, and 37 years (overall 30 years). There was approximately an 8-fold difference in career exposure levels between the highest and lowest groups (1,200 vs. 150 ppm-years).

3. Cancer Mortality

The total number of malignancy deaths (41) did not differ statistically from the number predicted based on New York State (59.3) and Kodak Rochester (52.7) death rates; the SMRs were 69 and 78, respectively. In addition, there were no significant differences between the number of observed and expected deaths for the hypothesized target organs (lung and liver). Whereas no primary hepatic neoplasms were reported, the 14 respiratory cancer deaths observed compared with expectation of 21.0 (NY State, SMR 67) and 16.6 (Kodak Rochester, SMR 84). Among non-hypothesized sites there was a suggestive excess vs. state data for pancreatic malignancy (8 observed vs. 3.2 expected) as well as comparable deficits for neoplasms of the genito-urinary organs (3 vs. 8.0) and colon-rectum (2 vs. 8.0).

4. Dose-Response

There was no evidence of a dose-response relationship with respect to ppm-years for lung and liver cancer even though, as indicated, the mean methylene chloride concentration in the highest dosage category was approximately eight times greater than that in the lowest. The chi square tests for both homogenity and trend were not statistically significant. SMRs (state rates) for lung carcinoma were, respectively, 98, 64, and 54 for the three career exposure classes. Similar results were reported for latency: < 20 years (SMR 163), 20-29 years (SMR 26), and 30 years or more (SMR 74).

5. Toxicologic vs. Epidemiologic Results

A comparison of the number of combined lung-liver cancer deaths observed in the cohort (14) with the number predicted from the animal model (36.3) demonstrated a highly significant difference ($p < 0.0001$). Furthermore, this discrepancy was apparent at even the highest career dosage level: 5 observed vs. 19.6 predicted ($p < 0.001$). Thus, the predictions from the animal bioassay were clearly inconsistent with human experience.

Discussion

1. Strengths of Study

a. Methylene Chloride Exposure

Accurate and complete characterization of exposure is an essential element for an assessment of dose-response. In the current investigation, career methylene chloride exposure estimates were derived from well-documented job history records and environmental sampling results. Factors contributing to the quality of data were:

(1) The company's industrial hygiene database included a substantial amount of both historical and current information concerning solvent levels in this department. Included were 1,220 area samples collected during the past 40 years from a wide spectrum of operating conditions, job functions, and work sites. In addition, 944 full-shift personal samples, specifically chosen to quantify exposures according to job function, were analyzed from 1980 to 1986.

(2) The personal dosimetry data, collected during usual working conditions, were considered to be representative of historical solvent levels. Although operational changes have been made during the past four decades, the technology used to manufacture film base as well the tasks associated with specific production processes have remained relatively constant.

(3) Estimates of career exposure were based on analysis of extensive occupational history information, including review of more than 4,900 individual job assignments from 1944 to 1984. The quality of the data was further enhanced by the fact that the department has traditionally adhered to a rigid organizational and job progression structure.

(4) The validity of the exposure estimates was supported by (a) mass spectrometry and/or gas chromatography analyses, (b) biologic monitoring findings, and (c) historical engineering data collected from the continuous process monitoring of solvent concentrations within the film casting machines.

b. Control Groups

The study design was strengthened by the selection of both general population and occupational referents. The primary advantages of using the New York State controls were rate stability (large population base) and geographic similarity. The choice of an industrial comparison group mitigated the bias associated with the "healthy worker effect" and assured comparability for such socio-economic and personal characteristics as pay class, education, cigarette smoking, health insurance coverage, and access to medical care.

c. Case-Finding

The study was also enhanced by the essentially complete follow-up and death certificate ascertainment.

d. Cohort Size and Follow-up

The study population of more than 1,000 men was relatively large, and the observation period (21 years) of reasonable length. In addition, the median follow-up from first exposure was 30 years.

e. Duration of Exposure

On average, cohort members worked in the methylene chloride environment 22 years; for those with the highest career exposure, the mean was 29 years.

f. Dose-Response

The relationship between methylene chloride dose and cancer mortality was assessed in terms of career exposure (ppm-years), latency (number of years elapsing between first documented exposure and last follow-up), and both indices. There was no evidence of a dose-response relationship.

g. Latency

The median latency period of 30 years was sufficient to allow expression of the postulated lung and liver cancer effects. An analysis of observed-expected differences demonstrated no consistent pattern.

h. Statistical Power

The power of a study, the probability of detecting a given increased risk if it actually exists, is important in interpreting epidemiologic findings. Power may be used to (1) detect a fixed relative risk (RR), or (2) determine the RR for a given power.

In this investigation, there was adequate power (63%, 98%, and > 99%) to detect an increased risk of respiratory carcinoma of 1.5, 2.0, and 2.5, respectively. Conversely, RRs of 1.6, 1.7, and 1.8 would be identified with 80%, 85%, and 90% power.

i. Cigarette Smoking

The principal confounding variable for lung cancer was cigarette smoking. The results of a mail questionnaire survey showed that tobacco usage patterns among surviving cohort members, including retirees, were similar to those reported for other groups of employed persons and the population at large. In addition, a review of decedents' medical records indicated that 84% were "ever smokers".

2. Limitations of Study

a. Power for Rare Forms of Cancer

With fewer than 20,000 person-years, there was clearly inadequate power to detect an excess of liver cancer. In this case, a RR of 5.7 or greater would be needed in order to achieve 80% power; however, as indicated, no liver malignancies were observed.

b. Other Exposures

Although methylene chloride has been the primary solvent in film casting operations at Kodak since approximately 1944, 1,2-dichloropropane (DCP) and 1,2-dichloroethane (DCE), both suspected animal carcinogens, were also present at similar concentrations during certain manufacturing processes from 1930 to 1966. Other potential exposures included acetone (prior to 1950), and methanol, ethanol, isopropanol, and butanol (in lesser amounts). In addition, some maintenance employees may have worked with asbestos and chromium. Since historical air sampling measurements for these materials, including the identification of potentially-exposed workers, are unavailable, it has not been possible to measure the impact of such exposures on cancer mortality.

c. Cancer Incidence Not Evaluated

Because the cohort's illness-absence experience was not studied, some malignancy sites with favorable survival rates may not have been completely assessed. On the other hand, it is likely that all lung and liver cancers were identified due to their extremely high case-fatality rates.

Conclusions

Although the study demonstrated no statistically significant observed-expected differences for total or site-specific cancers, further assessment of the pancreatic findings appears warranted.

It is important that the results of this study be incorporated in the risk-assessment process since the toxicologic findings differed from the epidemiologic experience. A possible explanation for this inconsistency may relate to the metabolic variation between the mouse and man as demonstrated in recent pharmacokinetic studies. Finally, it should be noted that one other epidemiologic investigation, an update of mortality in a South Carolina fiber manufacturing facility, may provide important evidence concerning the potential carcinogenic effects of this chemical.

Bibliography

1. Hearne FT, Grose F, Pifer JW, Friedlander BR, Raleigh RL: Methylene chloride mortality study: Dose-response characterization and animal model comparison. J Occup Med 1987;29:217-228.

2. NTP Technical Report on the Toxicology and Carcinogenesis Studies of Dichloromethane in F344/N Rats and B6C3F$_1$ Mice (Inhalation Studies). National Toxicology Program. US Dept of Health and Human Services, NIH publication No. 86-2562, NTP-TR 306, 1986.

APPENDIX C

HSIA submitted extensive references to ARB concerning methylene chloride on March 27, 1986. Enclosed are the following additional references:

Transcript of EPA's Science Advisory Board (SAB) August 13, 1987 review of methylene chloride.

Update to the Health Assessment Document and Addendum for Dichloromethane (Methylene Chloride): Pharmacokinetics, Mechanism of Action, and Epidemiology. External Review Draft, EPA/600 18-87/030A; July, 1987.

CEFIC Statement on Research into Species Differences.

Dow Chemical Statement on Research into Species Differences.

Hearne, F. T., Grose, F., Pifer, J. W., Friedlander, B. R., and Raleigh, R. L., Methylene Chloride Mortality Study: Dose Response Characterization and Animal Model Comparison, J. Occup. Med. 29: 217-228 (1987).

Anderson, M. E., Clewell, H. J., III, Gargas, M. L., Smith, F. A., and Reitz, R. H., Physiologically Based Pharmacokinetics and the Risk Assessment Process for Methylene Chloride, Tox. Appl. Pharm. 87: 185-205 (1987).

Green, T., Provan, W. M., and Gowans, N., Methylene Chloride: In Vivo Inhalation Pharmacokinetics in $B_6C_3F_1$ Mice (using stable isotopes) and F344 rats. CEFIC Report No. CTL/R/931 (1987)

Green, T., Nash, J. A., and Hill, S. J., Methylene Chloride: Glutathione-S-transferase Metabolism In Vitro in Rat, Mouse, Hamster and Human Liver Cytosol Fractions. CEFIC Report No. CTL/R/934 (1987)

Green, T., Nash, J. A., Hill, S. J., and Foster, J. R., Methylene Chloride: The Effects of Exposure To 4000 ppm On Mouse Lung Enzymes. CEFIC Report No. CTL/R/935 (1987)

B. Comments from the National Association of Photographic Manufacturers, Inc.

NATIONAL ASSOCIATION OF PHOTOGRAPHIC MANUFACTURERS, INC.

600 Mamaroneck Avenue, Harrison, New York 10528

Telephone 914 • 698-7603

January 7, 1988

Mr. Robert Barham, Chief
Toxic Air Contaminant Identification Branch
Air Resources Board
Attention: Methylene Chloride
P. O. Box 2815
Sacramento, CA 95812

Re: Preliminary Draft - Report to the Air Resources Board on Methylene Chloride -- November 1987

Dear Mr. Barham:

The National Association of Photographic Manufacturers, Inc. (NAPM) is a voluntary trade association composed of companies involved in the manufacture of photographic equipment and supplies. On the supply side our members manufacture photographic film and photographic paper.

Our comments on the captioned report are directed specifically to Chapter III, Production, Uses and Emissions; Part A - Public Exposure to, and Sources of, Atmospheric Methylene Chloride in California.

In this chapter, the staff of the California Air Resources Board estimated uses of methylene chloride by the photographic industry in California, citing several sources. Included were (1) "Methylene Chloride: Initiation of Regulatory Investigation," 50 FR 42037; and (2) "Survey of Methylene Chloride Emission Sources," EPA-450/3-85-015. These EPA documents contain significant errors of fact concerning the use of methylene chloride in the photographic industry. In comments to the Environmental Protection Agency (December 16, 1985) and the Occupational Safety and Health Administration (February 19, 1987), Eastman Kodak Company, a member of NAPM, provided information to correct these factual errors. Copies of these letters (without attachments) are enclosed for your information.

Methylene chloride is used in the manufacture of photographic film. It is the principal solvent in the manufacture of cellulose triacetate photographic film base. It is also used as a solvent in the manufacture of specialized chemicals for incorporation in photographic film emulsions and for coating operations. None of these manufacturing operations are located in California.

We are not aware of any use of methylene chloride in the chemical formulations used in the processing of exposed photographic film. Photographic film is spliced into larger units for processing by most photoprocessing laboratories. All splices used in the processing of exposed photographic film, however, are mechanical tape splices. Therefore, the Draft Report is incorrect in identifying "photographic film processing" as a use category in California.

Splicing cement is used when processed film is edited during the production of motion pictures. Methylene chloride has a minor use as an ingredient in the splicing cement. Kodak informed OSHA that it processed less than 2 metric tons of methylene chloride for use in film cements for both domestic and export sale and estimated the total industry usage as less than 5 metric tons in 1984 (see enclosed letter). Using the methodology of Appendix II (11% of US population) and even assuming all domestic sales, the usage in California would be about 0.5 metric tons; therefore, the amount of methylene chloride involved in motion picture film splicing annually in California is small in relation to other emissions. It should be included as another example under "Miscellaneous Uses" (p. III-15).

The following corrections should be made in Chapter III:

Page III-2, Figure III-2 - Remove the category and data for "photo film processing" from the chart.

Page III-3, Section B - Remove "photographic film processing" from the list of uses.

Page III-5, Table III-1 - Change "Photographic Film Processing" to "Photographic Film Manufacturing" and change the estimated California use to zero (0).

Page III-7, Table III-2 - Remove "Photographic Film Proc." and associated data from this table.

Page III-11 - Remove section on "Photographic Film Processing" entirely.

Page III-15 - Under "Miscellaneous Uses," insert another example as follows: "a constituent in photographic film cement."

Appendix II, Page 1 - Remove "photographic film processing" from entry "Paint removers, aerosols, photographic film processing and miscellaneous."

Appendix II, Page IV - Add section on photographic film manufacturing as follows:

Photographic Film Manufacturing

Methylene chloride is used as a solvent in the manufacture of cellulose triacetate photographic film base. It is also used in the manufacture of specialized chemicals for photographic emulsions and for certain coating operations. Methylene chloride is not used in the above manner in California.

We appreciate the opportunity to comment and feel confident that our remarks and suggestions will be given due consideration. If there are any questions, please feel free to get in touch with me.

Sincerely,

Thomas J. Bufficy

Thomas J. Bufficy
Executive Vice President

TJD/jb
ENclosures

December 16, 1985

Document Control Officer (TS-793)
Environmental Protection Agency
Office of Toxic Substances
401 M Street, SW; Room E-209
Washington, DC 20460

Dear Sir:

Subject: Methylene Chloride: Initiation of Regulatory Investigation; 50 FR 42037; October 17, 1985; Docket No. OPTS-62045

Eastman Kodak Company is a manufacturer of imaging products, chemicals, fibers, and plastics, with major manufacturing facilities in nine states. Kodak uses methylene chloride in the manufacture of certain photographic products and has made significant capital investments in process and recyling equipment. Although Kodak does not manufacture methylene chloride, we are concerned about the potential impact on Kodak of any regulatory action on methylene chloride.

I. Introduction

Kodak is submitting these comments on the above referenced ANPR to call EPA's attention to three areas that merit consideration in EPA's evaluation of the potential risks of human exposure to methylene chloride and of regulatory measures that may be taken to ameliorate any such identified risks.

A. Kodak is concerned, based on EPA and CPSC criticisms of Kodak's epidemiologic study of methylene chloride, that EPA will discount these data, and rely exclusively on the animal toxicologic data for developing estimates of risks to humans exposed to methylene chloride. Kodak is submitting, with these comments, a detailed discussion that responds to each of the specific EPA or CPSC criticisms of the epidemiologic study. This discussion also provides additional support for Kodak's contention that the data from the epidemiologic study and follow-up reports should be incorporated into EPA's assessment of the potential risks to humans from exposure to methylene chloride.

B. Kodak is providing data to clarify, add to or correct some aspects of the information presented by EPA in the ANPR and support documents relating to the use of methylene chloride by the photographic industry and to the emissions and exposures associated with these uses.

C. Kodak is concerned that EPA is continuing to evaluate chemicals as potential substitutes for methylene chloride that may have known or relatively uncharacterized hazards of a greater magnitude than those that have been demonstrated to be associated with the use of methylene chloride. Kodak urges that EPA carefully evaluate both the identified adverse effects of the substitutes, and the significance of any data gaps relating to potential hazards of the substitutes.

II. Epidemiologic Study of Workers Exposed to Methylene Chloride

Kodak has conducted an epidemiology study on a cohort of Kodak workers who have been or are currently involved in the manufacture of cellulose triacetate film base using a solvent which incorporates methylene chloride. The initial report and first update on this study were critized by EPA as lacking sufficient latency period, cohort size, exposure data or statistical power.

Kodak recently completed a second update of this study, extending the follow-up by four years and thereby increasing the latency period and statistical power of the study. The results of this update were furnished to EPA on June 28, 1985. We are concerned that the Agency's comments in the ANPR and support documents continue to discount the use of this study and do not address the scientifically valid conclusions contained in the study. Dr. Symons, of EPA's Science Advisory Board's Environmental Health Committee, urged EPA to consider alternate conclusions that can be based on the epidemiology work. He characterized the study as "...an important piece of information that we often don't have."[3]

In the enclosed paper, Kodak Response to EPA and CPSC Comments Concerning Kodak's Epidemiologic Study of Methylene Chloride Employees, Kodak responds in detail to each of EPA's and CPSC's specific criticisms of the Kodak epidemiologic study. Kodak concludes that the size of the study cohort was adequate; the latency period in the study was sufficient to allow the hypothesized effects to be expressed; the exposure levels were sufficiently well characterized for epidemiologic risk assessment purposes; and the statistical power of the study was adequate to detect excess deaths from certain hypothesized causes at low to moderate levels of relative risk. Kodak is also presenting in the enclosed paper a brief discussion of an analysis comparing the results actually observed in the epidemiologic study with the results predicted from models extrapolating from the animal bioassay data.

Significant conclusions from the epidemiologic study (and updates) include:

1. There is "no evidence of an increased mortality risk for lung cancer" resulting from worker exposures to methylene chloride; and

2. The study cohort showed an approximate 30% deficit in both total mortality and total cancer deaths compared to the general population, and showed no significant differences in total mortality or total cancer deaths when compared to an industrial control group.

Kodak urges EPA to reconsider its evaluation of the Kodak epidemiologic study, and to incorporate the significant data and conclusions from this study into its risk assessment of methylene chloride.

III. Uses of Methylene Chloride in the Photographic Industry

A. Photographic Manufacturing

The ANPR contains several factual errors relating to the use of methylene chloride in the photographic industry. The major use by Kodak of methylene chloride is in the manufacture of certain photographic products.

Methylene chloride is used by Kodak in the manufacture of clear plastic sheet and some photographic products, not in the processing of photographic films and papers. The principal use for methylene chloride by Kodak is as a solvent in the manufacture of cellulose triacetate plastic film base. We believe that certain non-U.S. manufacturers of photographic products also use methylene chloride in the production of film base. Other uses in the manufacture of photographic products include use as a solvent for applying surface coatings to photographic film base and lithographic plates. It is also used as a solvent in the production of certain chemicals used in the manufacture of photographic films and papers.

The major Kodak manufacturing processes are designed to recycle substantially all of the methylene chloride used in the processes. Typically, the recovery rate for methylene chloride exceeds 90%. Projecting total industry use and emissions based on the use experience with methylene chloride by Eastman Kodak Company, we estimate that total ambient emissions of methylene chloride from use in manufacture of photographic products would be about 5,000 metric tons per year.

Methylene chloride ambient emissions and exposures in the photographic manufacturing industry occur at two Kodak sites, and, perhaps, at a limited number of additional sites in the industry. Potential population exposure, therefore, is not nationwide and must be substantially less than 227 million people. Potential exposure during use of methylene chloride by Kodak involves less than 1,500 workers.

We suggest EPA add a second job category of "production worker" under "photographic applications" in Table 1 to reflect the workers involved in the manufacture of film base and other photographic products. We also suggest Table 2 be changed by adding a source category of "photographic manufacturing" with two known sites and annual emissions of 5000 metric tons.

B. Other Uses of Methylene Chloride in the Photographic Industry

Section II of the ANPR contains the statement that "other major uses of methylene chloride include use in ... photographic film processing ... ." (50 FR 42038). Table 2 lists "photo processing" as a source category, with emissions of 8,100 metric tons (MT/yr) and general population exposure of 227 million people. (50 FR 42045) The ANPR is based on the support document, which lists use of methylene chloride in film splicing cements and assumes total consumption and emission of 8,100 metric tons (Mg) in 1983[1]. The ANPR and the support document do not contain any explanation of the derivation of the general population exposure estimate of 227 million people. As discussed in the following sections, methylene chloride is not used in photo processing operations and the quantity of methylene chloride used in and emitted during film splicing is less than 5 metric tons, which is considerably lower than the estimates given by EPA in the ANPR.

1. Photo processing

Kodak does not include methylene chloride as an ingredient in any photochemicals used in the processing of photographic films or papers. Moreover, we are not aware of any photoprocessing chemicals of other companies that utilize methylene chloride. The statement in the ANPR that photographic film processing is a major use of methylene chloride is wrong and should be deleted. Table 2 in the ANPR should be amended to delete "photo processing" as a major source category for methylene chloride.

As indicated in Table 1 (50 FR 42045), liquid film cement containing methylene chloride is used in splicing certain photographic films. EPA states this use involves less than 1400 workers who are exposed at a maximum 8-hour TWA exposure level of 3 milligrams per cubic meter. It should be noted that this is the lowest exposure level for any entry indicated in Table 1. However, the data in Table 2, "Methylene Chloride Ambient Releases and Exposures" relating to potential emissions from the use of methylene chloride in photographic processing are not correct. (50 FR 42045).

In 1984, Kodak processed less than 2 metric tons of methylene chloride for incorporation in film cement products for both domestic and export sale in containers of one gallon or less. These Kodak products are used only for splicing motion picture films, which is done in about 500 U. S. motion picture laboratories.[2] While other companies also sell motion picture film cements, we estimate the total quantity (inclusive of Kodak's use) will be less than 5 metric tons of methylene chloride.

Only an extremely small amount of this total of methylene chloride is used in the cement involved in any one splice, resulting in an exposure level that EPA estimated to be 3 milligrams per cubic meter. Moreover, no methylene chloride is used in the processing of photographic films or papers. The emissions from this source, therefore, cannot conceivably result in the exposure of the entire national population (227 million) to methylene chloride as indicated in this Table.

Table 2 in the ANPR should be corrected as follows:

a. The identity of the source category should be amended to "photographic film splicing;"

b. The quantity of emissions from this source should be amended from 8,100 metric tons per year to 5 metric tons;

c. The population estimate should be revised to reflect the limited number of sites and the small quantity of methylene chloride used in film splicing operations.

In summary, Tables 1 and 2 should be revised as follows to reflect accurate data on the use of methylene chloride by Kodak in the photographic industry:

Table 1

Industry Category	Job Category	Estimated No. of Workers
Photographic Applications	Splicer	1376
	Production Worker	1500

Table 2

Source Category	No. of Sites	Emissions (MT/yr)
Photographic Film Splicing	500	5
Photographic Manufacture	2	5,000
Photoprocessing (delete)	(delete)	(delete)

IV. Comments on Potential Substitutes for Methylene Chloride

A. Potential Substitutes for Methylene Chloride in Photographic Manufacture

Each photographic film product is designed to provide a combination of photographic and physical properties required for proper customer use of the photographic films and for customer satisfaction with product quality. Selection of the appropriate film base material is important in order to achieve the desired product properties. There are no suitable substitutes for the cellulose triacetate film base which is manufactured by Kodak using methylene chloride. Moreover, photographic products available from non U.S. manufacturers also utilize cellulose triacetate film base manufactured, we believe, with methylene chloride.

Kodak has explored the use of alternate solvents in manufacturing cellulose triacetate film base. No adequate substitutes have been found because of the solubility characteristics of cellulose triacetate or the known or potential toxicity and physical hazards of other solvents, such as flammability.

B. Comments on Potential Substitutes Suggested in the ANPR

The use of several of the materials suggested in the ANPR (50 FR 42042) as possible substitutes for methylene chloride has been reduced or discontinued because of known or potential toxicity and physical hazards. For example, the Recommended Maximum Contaminant Level (RMCL) established by EPA under the Safe Drinking Water Act for trichloroethylene and carbon tetrachloride is zero (50 FR 46380). This RMCL was established on the basis of suspected carcinogenicity. We are not aware of any report that demonstrates the proposed substitutes have been as carefully evaluated by human epidemiological studies during industrial use as has been methylene chloride. EPA should not continue consideration of use of these substances as alternatives for methylene chloride.

V. Conclusion

Kodak urges EPA to give careful consideration to the epidemiological data that have been developed on methylene chloride, and strongly suggests that EPA incorporate these data into its assessment of the risks of methylene chloride exposures to humans. Kodak requests that EPA correct

its data on the uses of, and exposures to, methylene chloride in the photographic industry, as described in these comments, and that EPA incorporate the new information on exposures associated with photographic manufacturing into its data base. Furthermore, Kodak suggests that EPA give particular consideration to the known or potential hazards associated with the suggested substitutes for methylene chloride.

Kodak is continuing to study the use of methylene chloride, the data on occupational exposure, and the epidemiological studies of Kodak workers exposed to methylene chloride. Kodak expects to share any further conclusions or new information developed during these studies with EPA. Kodak is available to discuss these comments with the Agency at any time.

Very truly yours,

Robert P. Brothers
Director, Regulatory Affairs

RFB:nls
1909e
Enclosure

cc: Dr. P. A. Cammer,
Halogenated Solvents Industry Alliance

Mr. T. J. Dufficy
National Association of Photographic Manufacturers

References

1. Source Assessment of Methylene Chloride Emissions; August 1985; DCN No. 231-020-19-06-02.

2. 1984-1985, Wolfman Report on the Photographic and Imaging Industry in the United States, Pg. 92, ABC Leisure Magazines, Inc.

3. Dr. Michael Symons, Transcript, Pg. 76, June 7, 1984, Science Advisory Board Environmental Health Committee

February 19, 1987

Docket Office
Occupational Safety and Health Administration
Docket No. H-71; Room N-3670
U.S. Department Of Labor
200 Constitution Ave. N.W.
Washington, D.C. 20201

Dear Sirs:

Subject: Occupational Exposure to Methylene Chloride: 51 FR 42257; November 24, 1986

Eastman Kodak Company (Kodak) is a manufacturer of photographic products with major manufacturing facilities in nine states. As a multi-state employer, we share the interest of the public, Congress and OSHA in a safe workplace.

Kodak uses methylene chloride in the manufacture of certain photographic products and has made significant capital investments in process and recycling equipment. In addition, Kodak has conducted epidemiology studies on employees with job assignments in areas where this solvent is used. We have reviewed the referenced Advanced Notice of Proposed Rulemaking and submit the following comments and information on Sections A, B, D, F, I, and M.

A. Health Effects:

Kodak has conducted an epidemiologic mortality study on a cohort of Kodak workers who have been or are currently involved in the manufacture of cellulose triacetate film base using a solvent containing methylene chloride. Specifically the study addresses the potential health risks, including the risk of carcinogenicity, associated with chronic occupational exposure to this chemical.

The initial study report based on a 1964 cohort, was published in 1978 followed by an update in 1981. (OSHA Exs. 4-027 & 4-030) A second update was conducted through 1984, extending the follow-up period by four years, thereby increasing the latency period and statistical power of the study. The update was submitted to OSHA and other government agencies in 1985. (OSHA Ex. 7-044)

Kodak continued work on the epidemiology study and presented preliminary findings at the Winter Toxicology Forum in February 1986. The study cohort was expanded to include workers employed in film coating operations during 1964-70. A dose response assessment using an index of career exposure was also performed. An initial analysis of these findings was provided to OSHA, EPA, FDA and CPSC in meetings with representatives of these agencies during 1986. (OSHA Ex. 7-040)

A report of the expanded epidemiology study has been completed and is attached as Appendix 1. This report is scheduled for publication in 1987, although minor editorial changes may be made prior to publication. Appendix 2 is a summary of the report, prepared by its authors, and a discussion of its relevance to assessment of potential human health risks.

Significant conclusions from the epidemiology report include:

> This epidemiologic investigation of more than 1000 employees chronically exposed to methylene chloride demonstrated no unusual mortality patterns for such hypothesized causes as lung and liver malignancy and ischemic heart disease. There was, in addition, no evidence of a dose-response relationship with respect to career exposure and latency. (Appendix 1, pg. 35)

OSHA has cited an evaluation conducted by CAG/EPA in 1985 of the Kodak reports published in 1978 and 81. After reviewing that evaluation, OSHA stated "based on current published data, the essentially negative Friedlander studies do not appear to have the power to rule out an overall cancer risk or the lung cancer risk that is predicted from the NTP mice study." ((51 FR 42262)

The CAG/EPA assessment was based on the study report published in 1978 of a cohort of 252 long-term workers. The most recent Kodak study reports on an expanded cohort of 1,013 workers, as well as covering a longer latency period and including more extensive exposure data (see Appendix 1). Appendix 2 includes a discussion of risk assessment and power calculations based on the most recent data and states:

> We therefore conclude from the 1986 study results that the power to identify total cancer and respiratory malignancy, predicted from the animal bioassay, was substantially greater (0.48 - 0.63) than the lower estimate reported by CAG (0.07 and 0.09, respectively, for total malignancy and lung cancer deaths).

Kodak urges OSHA to evaluate the most recent epidemiology information, submitted with these comments, and to incorporate the significant data and conclusions from this human epidemiology study as OSHA develops its own assessment of occupational exposures and risks.

B. Permissible Exposure Levels

The Kodak Health and Environment Laboratories review available health effects data on chemicals of interest to Kodak. The Laboratories reviewed the American Conference of Government and Industrial Hygienists (ACGIH) decision to reduce its recommended threshold limit value (TLV) from 500 ppm to 100 ppm. The laboratories concurred and established an exposure guideline of 100 ppm for Kodak operations.

In 1986, the ACGIH proposed to further lower the TLV for methylene chloride from 100 ppm to 50 ppm. However, the most recent update and expansion of the Kodak epidemiology study of workers involved in manufacturing operations using methylene chloride demonstrated no unusual mortality patterns for hypothesized causes of death and no evidence of a dose-response relationship with respect to career exposure and latency. (see Appendices 1 and 2) New information, including the latest epidemiologic findings, confirms Kodak's conclusion that continued use of this solvent at current occupational exposure levels in Kodak's operations does not present a significant risk of serious human health effects.

D. Substitution Availability:

The major use by Kodak of methylene chloride is in the manufacture of certain photographic products. Principally this involves use as a solvent in the manufacture of cellulose triacetate plastic film base. Each photographic film product is designed to provide a combination of photographic and physical properties required for proper customer use of the film and for customer satisfaction with product quality. For certain products, cellulose triacetate exhibits a unique combination of the necessary characteristics, which are described in Appendix 3. For the reasons discussed in Appendix 3, we are not aware of any satisfactory replacement film base material, for those products now using cellulose triacetate, that can achieve the desired product properties and meet customer needs.

Kodak has reviewed potential substitutes for methylene chloride as a solvent in the manufacture of cellulose triacetate film base. Appendix 3 also contains a discussion of the necessary solubility characteristics of any potential solvent and demonstrates that most potential solvents do not possess the necessary characteristics to be effective substitutes. Appendix 4 contains a review of the physical and health hazards of four possible alternative solvents. In addition to the undesirable characteristics cited in Appendix 4, these chemicals have not been as carefully evaluated for possible human health effects, particularly by epidemiological studies during industrial use in Kodak's operations, as has been methylene chloride. Based on this information, Kodak has concluded methylene chloride is necessary as a solvent for cellulose triacetate film support production because no other potential coating solvent is known to be as safe as methylene chloride.

In addition to the potential physical and health hazards associated with substitutes, replacement of methylene chloride would be a major change in the manufacturing process for production of cellulose triacetate film base. Each photographic product must pass a battery of quality assurance tests designed to measure the photographic quality and stability of the product. These tests are rigorous, costly and time-consuming and must be satisfactorily completed before a product is introduced into the marketplace. Changing from methylene chloride as the base-making solvent would require photographic revalidation of all of the products that would be coated on the base material made by the new process. This would require at a minimum several years to complete and would involve significant expenses in addition to any capital investments required by substitution.

Based on our review of the solvent characteristics, physical properties and health hazards of possible substitutes, Kodak has concluded that there are no solvents currently available that would be acceptable as alternatives to the continued use of methylene chloride in the manufacture of cellulose triacetate film base.

F. Workers exposure and monitoring:

OSHA has cited the report prepared for EPA by Pedco Environmental, Inc. as its "primary source of occupational exposure data." (51 FR 42260) This report contains factual errors relating to the use of methylene chloride in the photographic industry. These errors were pointed out to EPA in our comments dated December 16, 1985. (OSHA Reference 4-096)

The Pedco report contained no information on the major use by Kodak in the manufacture of certain photographic products. Principally, this involves use as a solvent in the manufacture of cellulose triacetate plastic film base. Extensive data on job categories, exposure and monitoring methods associated with this use can be found in the attached epidemiology report. (see Appendix 1)

Other uses in the manufacture of photographic products include use as a solvent for applying surface coatings to photographic film base and lithographic plates. The chemical is also used as a solvent in the production of certain chemicals used in the manufacture of photographic films and papers.

The only job category cited in the Pedco report for photographic applications is splicer. Liquid film cement containing methylene chloride is used in splicing certain photographic films. In 1984, Kodak processed less than 2 metric tons of methylene chloride for incorporation in film cement products for both domestic and export sale in containers of one gallon or less. These products are used only for splicing motion picture films, which is done in about 500 motion picture laboratories. Only an extremely small amount of methylene chloride is used in the cement involved in any one splice, resulting in an exposure level that EPA estimated to be 3 milligrams per cubic meter.

I. Control measures and benefits:

Kodak has reviewed its manufacturing operations and considered the costs of implementing engineering controls to reduce current occupational exposure levels. A capital investment program of as much as $100 million would probably be required to meet the ACGIH proposed reduction. Achievement of significantly lower exposure levels would require a substantially larger financial investment, including development of new manufacturing technology and the design and construction of new manufacturing facilities. No identifiable benefit would result in an occupational setting for which the latest epidemiology study "demonstrated no unusual mortality patterns" and "no evidence of a dose-response relationship with respect to career exposure and latency." (see Appendix 1, p. 35)

M. Financial and Economic Profile:

Cellulose triacetate film base is a photographic support material manufactured by Kodak and used for many Kodak motion picture, graphic arts, and amateur roll and movie film products. Kodak is currently the only domestic manufacturer of cellulose triacetate film base. This base material is manufactured by other companies at sites outside the U. S. and is used as the support material for competitive photographic products. Unreasonable regulatory requirements that apply only to domestic manufacture of this film base may adversely affect the U. S. trade balance in view of the availability of competitive film products on the same base material provided by non-domestic manufacturers.

Conclusion

Kodak urges OSHA to give careful consideration to the new data included in the attached epidemiology report and to incorporate it into its assessment of occupational exposures and the risk of human health effects. Furthermore, OSHA should give particular consideration to the known physical and health hazards associated with any potential substitutes for methylene chloride as well as to the infeasibility of substitution for technical reasons. Finally, OSHA must carefully review the potential economic impact of imposing excessive regulatory requirements on the domestic photographic industry in view of the new epidemiologic findings based on this industry.

We appreciate the opportunity to provide this information to OSHA. We will submit a copy of the paper on the epidemiology study when it has been published.

Very truly yours,

Robert F. Brothers

Robert F. Brothers
Director, Regulatory Affairs

RFB:nls
Enclosures

C. Comments from the Allied Corporation

Allied Corporation
Department of Toxicology
P.O. Box 1021R
Morristown, New Jersey 07960

December 16, 1987

Mr. Robert Barham, Chief
Toxic Air Contaminant Identification Branch
Air Resources Board
Attn.: Methylene Chloride
P.O. Box 2815
Sacramento, CA 95812

Dear Mr. Barham:

On November 23, 1987, Peter D. Venturini, Chief of the Stationary Source Division of the State of California Air Resources Board announced that the preliminary draft report on methylene chloride was available for review and comment.

We appreciate the opportunity to comment on the preliminary draft report which includes: Part A - Public Exposure to, and Sources, of Atmospheric Methylene Chloride in California, and, Part B - Health Effects of Methylene Chloride.

In Part B - Health effects of Methylene Chloride, we are in basic agreement with your conclusion that "the environmental concentrations in California of 3 ppb are well below any known acute and non-carcinogenic chronic levels that may cause adverse health effects". However, we would extend this further to include the contention that no carcinogenic effects or cancer-related mortality would be expected to occur at current ambient levels. At the present time, compelling scientific evidence to refute this latter contention simply does not exist. Liver tumors associated with oral exposure of laboratory animals to methylene chloride or other agents (e.g., carbon tetrachloride) are always preceded by clinically observable hepatic damage which is considered to be a preprequisite to tumor formation. In the preliminary draft of the technical support document Part B - Health Effects of Methylene Chloride, a calculated no observable adverse effect level (NOAEL) for adverse health effects, other than cancer, for humans is suggested to be 5 ppm, based on the assumption of equivalency of toxicity by the oral and inhalation exposure routes for methylene chloride. We would agree this is a reasonable and scientifically-sound assumption.

In the 1982 National Coffee Association drinking water study in rats, in which the lowest oral dose used was 5 mg/kg/day (equivalent to the suggested human NOAEL of 5 ppm), zero hepatocellular carcinomas were observed. This result is clearly indicative of a NOAEL for a carcinogenic effect. At the high dose of 250 mg/kg/day, only one male and two female rats were observed with hepatocellular carcinomas. Furthermore, rats exposed to 250 mg/kg/day for 78 weeks and then allowed

to recover, did not develop hepatocellular carcinomas. This implies some type of repair mechanism being operative during the recovery phase following liver insult from methylene chloride exposure. This same repair mechanism appears to be overwhelmed during high-dose exposure in the absence of a recovery period. At low dose where there is no observable hepatotoxicity, there should be no cause for concern for carcinogenicity. If one accepts the validity of this statement and the supporting animal data, then the concept of mathematical modeling used for low-dose extrapolation of human carcinogenic risk clearly does not apply in the case of methylene chloride. Other carcinogenicity studies, outlined in Part B, also suffer from either exceedingly high dose exposures which lose relevance when viewed in the context of current ambient levels and cancer incidence, or tend to lose statistical significance when data are compared to concurrent or historical control data. In addition to liver tumors, other types of tumors observed from animal experimentation were not seen in human epidemiological studies. Moreover, from the epidemiological study [Friedlander, et al. (1986) and Hearne et al. (1987)] of Eastman Kodak employees, no statistically significant differences were found between observed and expected deaths for respiratory and hepatic cancers which were hypothesized by the authors based upon results from animal studies. The possibility of causation of pancreatic cancer in humans by exposure to methylene chloride (8 observed versus 3.2 expected) is at best only suggestive. Apparent disagreement exists on the statistical significance of this finding which is definitely not consistent with tumor profiles obtained through laboratory animal studies. One would not expect adverse non-carcinogenic <u>or</u> carcinogenic health effects to occur at current California ambient air levels of methylene chloride which are 3 to 4 orders of magnitude less than the 5 ppm NOAEL suggested for humans.

Allied-Signal Inc. endorses the Halogenated Solvents Industry Alliance (HSIA) belief that methylene chloride does not cause adverse health effects when used according to current industrial hygiene guidelines or labeled instructions and firmly believes that methylene chloride should not be identified as a toxic air contaminant.

We thank you for the opportunity to provide comment on the preliminary draft report on methylene chloride.

Sincerely yours,

Roger W. Reinhold

Roger W. Reinhold, Ph.D., D.A.B.T.
Manager, Department of Toxicology

RWR:rb

Allied Corporation
Department of Toxicology
P.O. Box 1021R
Morristown, New Jersey 07960

December 17, 1987

Dr. Paul A. Cammer
Executive Director
Halogenated Solvents Industry Alliance
2315 M Street, N.W., Third Floor
Washington, D.C. 20037

Dear Paul,

It was nice talking with you last week relative to methylene chloride. Thank you for your time and thoughts.

Per your request, enclosed is a copy of a rather hastily prepared (though hopefully well-thought out) response to the California Air Resources Board's request for review and comment on the preliminary draft report on methylene chloride.

Thank you, once again, for allowing us to emphasize HSIA's belief that methylene chloride does not merit identification as a toxic air contaminant.

Sincerely yours,

Roger W. Reinhold

Roger W. Reinhold, Ph.D., D.A.B.T.
Manager, Department of Toxicology

RWR:rb
Enc.

cc: G. Loewengart
G.M. Rusch
File

D. Comments from Bendix Environmental Research, Inc.

ENDIX ENVIRONMENTAL RESEARCH, INC.

FOX PLAZA, SUITE 902 • 1390 MARKET STREET • SAN FRANCISCO, CA 94102 • TELEPHONE (415) 861-8484

30 November 1987

Robert Barham, Chief
Toxic Air Contaminant Identification Branch
Air Resources Board
ATTN: methylene chloride
P.O. Box 2815
Sacramento, CA 95812

RE: Preliminary Draft Report to the Air Resources Board on Methylene Chloride, Part A

Dear Mr. Barham:

I would like to call your attention to a growing use of methylene chloride to degrease sampling tubes used for soil and sediment samples at contaminated or potentially contaminated sites. Although undoubtedly a small use in the spectrum of total use of methylene chloride, I am concerned that this methodology is being recommended by the Environmental Protection Agency. I feel that, in view of the questions about the safety of methylene chloride, it is unwise to encourage new uses for this substance. Geotechnical field personnel are not commonly trained in the handling of carcinogens and there is a potential for substantial exposure to geotechnical personnel and of improper disposal of the used methylene chloride. I believe that regulatory agencies should encourage the use of safer solvents in procedures designed to evaluate and abate existing environmental problems.

Sincerely,

Selina Bendix, Ph.D.
President

SB:bdw

E. Comments from Roger Atkinson and Arthur Winer, University of California, Riverside

UNIVERSITY OF CALIFORNIA, RIVERSIDE

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STATEWIDE AIR POLLUTION RESEARCH CENTER-6

RIVERSIDE, CALIFORNIA 92521-0522

December 22, 1987

Dr. Robert Barham
Toxic Air Contaminant Identification Branch
Air Resources Board
1102 Q Street
P.O. Box 2815
Sacramento, CA 95812

Dear Dr. Barham:

Thank you for your letter of November 23, 1987, enclosing the preliminary draft methylene chloride identification report for our comments. As you requested, our comments deal only with the section concerning the atmospheric fate of methylene chloride.

Since it is clear that CH_2Cl_2 reacts relatively slowly with the OH radical, it will undergo long range transport and dispersion. Hence the use of a regionally or globally averaged OH radical concentration is required to calculate the atmospheric lifetime of CH_2Cl_2 due to this reaction. Despite the uncertainities in the ambient atmospheric OH radical concentrations, the annually and diurnally averaged OH radical concentration is, based upon the measured lifetimes of various tracers and computer modeling studies, approximately 5×10^5 molecule cm^{-3}. For tropospheric temperatures of 265-298 K this leads to a calculated lifetime of CH_2Cl_2 of 150-250 days.

As stated on page IV-5, the initial reaction with the OH radical does form the $CHCl_2$ radical (reaction 2). The subsequent reactions of this radical are as follows. The $CHCl_2$ radical will rapidly react with O_2 to form a peroxy radical.

$$CHCl_2 + O_2 = OOCHCl_2$$

This peroxy radical will then react with NO (if present), HO_2 radicals or other peroxy (RO_2) radicals

$$OOCHCl_2 + NO = OCHCl_2 + NO_2$$

$$OOCHCl_2 + HO_2 = HOOCHCl_2 + O_2 \quad \text{(forming dichloromethylhydroperoxide)}$$

$$OOCHCl_2 + RO_2 = OCHCl_2 + \text{other products}$$

These reactions have not been experimentally studied; rather they are expected to occur based on our knowledge of the atmospheric reactions of other simple alkyl peroxy and haloalkyl peroxy radicals (see, for example,

DeMore et al., 1985, and D. L. Baulch, R. A. Cox, R. F. Hampson, Jr., J. A. Kerr, J. Troe and R. T. Watson, J. Phys. Chem. ref. Data, 13, 1259-1380, 1984). The $CHCl_2O$ radical is known (H. Niki, P. D. Maker, C. M. Savage and L. P. Breitenbach, Int. J. Chem. Kinet., 12, 1001-1012, 1981) to eliminate a chlorine atom to form formyl chloride

$$OCHCl_2 = HC(O)Cl + Cl$$

The Cl atom will react with whichever organics are present (in the cleaner troposphere this will be mainly with methane) to yield HCl.

The above paragraph should replace the last 4 lines of page IV-5 and all of page IV-6.

The following additional points may be helpful in revising the present draft:

- It would be helpful to the reader to reference the five groups cited at the bottom of page IV-2, in which case the sentence stating the years in which they published their data could be eliminated.
- In the first paragraph on page IV-3, it is not clear what the phrase "included to illustrate the variation among the different studies" refers to. Also in this paragraph it should be "authors'" and not "author's".
- At the bottom of page IV-3 you may want to state explicitly for the uninitiated reader that OH radicals are only present during daylight.
- In the last sentence of the first paragraph on page IV-5, it is not clear which OH radical concentration "this" refers to.

We hope that this will be of help to you; if you have any further questions regarding this draft report, please feel free to contact us.

Yours sincerely,

Roger Atkinson
Research Chemist

Arthur M. Winer
Research Chemist

II. Air Resources Board Responses to Part A - Related Comments

AIR RESOURCES BOARD STAFF RESPONSES TO PUBLIC COMMENTS

ON THE DRAFT PART A REPORT ON METHYLENE CHLORIDE

1) The Halogenated Solvents Industry Alliance, January 14, 1988.

a) Comment: In summarizing methylene chloride use in Table III-1, estimates of current U.S. use are based on information provided by HSIA for calendar year 1983. A more realistic estimate of current U.S. use would be 497 million pounds (248,500 tons) for 1987 from an estimate by Dow Chemical (USA) based on current governmental data.

Response: As stated in Table III-1, the U.S. use estimate for methylene chloride is 241,000 tons for 1983. Staff acknowledge that this estimate does not represent current U.S. methylene chloride use. Data for 1983 were used because 1983 was the most recent year where methylene chloride consumption by use category was available. However, in response to your comment, the U.S. use estimate listed in Table III-1 has been footnoted to indicate the U.S. use estimate for 1987 as estimated by Dow Chemical.

b) Comment: The report states that the Industrial Source Complex Short Term (ISCST) model was used to estimate annual average concentrations. The ISCST model is designed to estimate short-term (one to 24 hour) averages, not annual averages. Annual average concentrations should be estimated using a statistical summary of recorded meteorological data rather than sequential data and the long-term version of the model (ISCLT).

Response: This comment concerns the belief that ISCLT is more appropriate than ISCST for estimating annual average concentrations. However, we believe this to be untrue. ISCLT uses sector averaging which can lead to under-predictions of peak concentrations. Also, ISCLT uses average mixing heights and ambient temperatures. Again, the use of average values leads to under-prediction of peak concentrations. For these reasons, we do not recommend the use of ISCLT.

The South Coast Air Quality Management District has selected the ISCST model for use in new source review for carcinogenic air contaminants (see SCAQMD proposed Rules 223 and 1401). In addition, the "Air Toxics Assessment Manual" (1987) prepared by the Interagency Working Group has omitted the ISCLT model from the list of models recommended for use in toxic modeling studies. In this document, the ISCST model is recommended for all averaging times. The Agencies participating in the review and release of this document include the Environmental Protection Agency, California Air Resources Board, South Coast AQMD, San Diego APCD, Bay Area AQMD, and Engineering-Science, Inc.

c) Comment: Hourly meteorological data should be used when short-term maximums are being estimated.

Response: See response to comment (b).

d) Comment: For estimating an annual average, use of a statistical summary of meteorological data is preferred to hourly meteorological data as this format allows the model to consider multiple years of data (summaries are usually made from five to ten years of data) in determining the annual average concentrations.

Response: See response to comment (b).

e) Comment: ISCLT differs from ISCST on two very important points:

1) estimated concentrations are averaged over the sector in question and "smoothed" between sectors to avoid discontinuity.

2) a maximum of 16 wind direction sectors is allowed rather than the 36 sectors used by the ISCST model.

The effect of both of these points is to reduce the estimated concentration in most cases.

Response: See response to comment (b).

f) Comment: The report states that the emissions from an automobile assembly plant were modeled as an area source even though methylene chloride was emitted from a series of stacks. Modeling the emissions as though they emanated from an area source rather than stacks does not recognize enhanced dispersion due to the velocity and temperature of the exhaust as it exits the stack.

Response: The stacks at the Automobile assembly plant were modeled as an area source with an effective release height of approximately 289 feet, which accounts for both the buoyancy and momentum of the exhaust under neutral atmospheric conditions.

g) Comment: The report does not say if an effective emission height was calculated and used in the analysis.

Response: The report has been revised to state that the effective emission release height is approximately 289 feet.

h) Comment: The draft report (pages II-4 and II-5) uses Gleit's method to estimate mean concentrations of methylene chloride below the level of quantitation of the monitoring stations. The method was designed to determine a mean for comparison with a specific value (e.g. a standard). The Air Resources Board has no specific value with which to compare the data.

Response: The use of Gleit's statistical method for estimating the "expected value" for data below the detection limit does not involve comparison with any specific value. The statistical method used in our estimates has been compared with other commonly used estimators and was found to outperform all available techniques.

i) Comment: A summary of the data including the percent of data below detection and a possible range of average concentrations (assuming all missing data are equal to zero for the lower limit of the range and all missing data are

equal to the detection limit for the upper limit of the average) would be more informative.

Response: Table II-1 currently reports the number of samples for each station and percent of values below detection.

The use of zero and the detection limit do not give an actual lower and upper limit of annual mean concentrations. The limited sample size and uncertainties encountered in sample collection and analysis outweigh the uncertainty caused by the use of Gleit's statistical methods for estimating values for censored data. The estimates reported by ARB use both a 20% uncertainty in sampling and analysis and two standard errors to estimate the expected range of the mean concentrations.

j) Comment: The statement in the draft report that water chlorination is a source of environmental methylene chloride seems in error. The kinetics of reaction between chlorine and organic material in water is such that only chloroform is released to any appreciable extent.

Response: We agree that the kinetics of reaction between chlorine and organic material in water is not likely to favor the formation of appreciable amounts of methylene chloride. However, limited data suggests that a minor source of methylene chloride emissions may result from the chlorination of water.

k) Comment: The draft report should integrate the pharmacokinetic information addressed in Part B with the exposure information in Part A.

Response: The Overview, which will be released with the final draft of the methylene chloride report, integrates the information in the risk assessment (Part B report) with the information presented in the exposure assessment (Part A Report).

l) Comment: Health effects information suggests that the effects of methylene chloride at high exposure levels are unlike the effects after chronic low exposure.

Response: All comments concerning the health effects of methylene chloride will be responded to by the Department of Health Services.

m) Comment: Page III-5 - Methylene chloride use has been declining at approximately 10 percent per year since 1984. 1987 production estimates are approximately 500 million pounds. In 1984 the market was over 600 million pounds. Aerosols and certain formulated products have shown the biggest reduction in methylene chloride use.

Response: Refer to our response to comment (a).

n) Comment: Page III-6, line 2 - We question whether 7,500 tons of methylene chloride were used in paint removers in California.

Response: The basis for estimating emissions of methylene chloride from paint removers in California is provided in the report. If you have information which indicates that this estimate is not accurate, we would be interested in reviewing it.

o) Comment: Page III-8, 2nd and 3rd lines - The percentage of methylene chloride in the total aerosol formulation depends on what the product is. Aerosol paint strippers contain 85% methylene chloride.

Response: The report was revised to clarify that the amount of methylene chloride in product formulations depends upon what the product is.

p) Comment: Page III-19, last line of second paragraph - Voluntary labeling will begin in 1988, not 1986.

Response: In 1986, some manufacturers of consumer products containing methylene chloride voluntarily labeled their products with additional health warnings and use recommendations. However, enforcement of these labeling requirements becomes effective for products whose labels are printed after March 14, 1988 and after September 14, 1988 for products that are packaged.

q) Comment: Page III-20, last few lines - Air stripping would be adequate for this volatile compound. Steam stripping would not be necessary.

Response: Should methylene chloride be identified as a toxic air contaminant, appropriate levels of control for methylene chloride emissions sources will be evaluated in the subsequent risk management phase.

r) Comment: Page III-9, last paragraph - Some methylene chloride is typically retained in the foam. Methylene chloride is also used as a flush for urethane foam nozzles and for cleanup purposes in polyester molding operations.

Response: We acknowledge that a small fraction of the methylene chloride used in the manufacture of urethane foam may be retained in the foam after curing. Staff also acknowledge that there may be other uses for methylene chloride in the foam manufacturing industry that are not discussed in the report. However, if methylene chloride is identified as a toxic air contaminant, its uses by the foam manufacturing industry will be evaluated in greater detail during the subsequent risk management phase.

s) Comment: Page III-10 - Few degreasers use methylene chloride. The estimates given in this paragraph appear to be for stripping and circuit board stripping.

Response: The EPA estimated methylene chloride emissions from degreasing operations in a variety of industries, primarily within five distinct Standard Industrial Classifications (SICs). Based on information provided by HSIA, the EPA estimated U.S. emissions of methylene chloride from degreasing operations at 23,700 tons in 1983 (U.S. EPA, 1985). In addition, ARB's emissions data system identifies a number of sources in California that use substantial amounts of methylene chloride for degreasing.

t) Comment: Page III-11 - to our knowledge, film cleaning is done instead with 1,1,1-trichloroethane.

Response: Based on comments from the National Association of Photographic Manufacturers, Inc., the discussion on photographic film processing has been deleted from the text.

u) Comment: Page III-11, 3rd line - 800 tons seems to high.

Response: This use estimate is based on information provided by the EPA for one pesticide manufacturing facility in California for 1983 (see Table III-1). This estimate is only for process use and does not include any methylene chloride consumed as an ingredient in pesticide formulations.

v) Comment: Page III-11 and 1st paragraph of III-12 - This section is confusing. Is there one facility that used 800 tons in 1983 as a solvent for process use plus 90 more tons for extraction, phase separation, purification, crystallization, and as a general transport solvent? The first paragraph indicates that there is one facility.

Response: The text has been revised to clarify that the source which used 800 tons of methylene chloride is the same source that is estimated to have emitted 90 tons.

2) The National Association of Photographic Manufacturers, Inc., January 7, 1988

The NAPM states that the draft report is incorrect in identifying "photographic film processing" as a use category. The NAPM's comments were accompanied with well documented letters describing the uses and emissions of methylene chloride by the photographic industry. The NAPM concluded their comments by recommending the following changes to the report.

a) Comment: Page III-2, Figure III-2 - Remove the category and data for "photo film processing" from the chart.

Response: The category photographic film processing has been removed from Figure III-2 and replaced with photographic film manufacturing.

b) Comment: Page III-3, Section B - Remove "photographic film processing" from the list of uses.

Response: The category photographic film processing has been removed from the list.

c) Comment: Page III-5, Table III-1 - Change "photographic film processing" to "photographic film manufacturing" and change the estimated California use to zero (0).

Response: The report has been revised to reflect your comments.

d) Comment: Page III-7, Table III-2 - Remove "photographic film processing" and associated data from this table.

Response: The report has been revised to reflect your comments.

e) Comment: Page III-11 - Remove section on "photographic film processing" entirely.

Response: The report has been revised to reflect your comment.

f) Comment: Page III-15 - Under "Miscellaneous Uses," insert another example as follows: "a constituent in photographic film cement."

Response: The use of methylene chloride as a constituent in film cement is now listed under miscellaneous uses.

g) Comment: Appendix II, Page 1 - Remove "photographic film processing" from entry "paint removers, aerosols, photographic film processing and miscellaneous."

Response: The report was revised to reflect your comment.

h) Comment: Appendix II, Page IV - Add section on photographic film manufacturing as follows:

Photographic Film Manufacturing

Methylene chloride is used as a solvent in the manufacture of cellulose triacetate photographic film base. It is also used in the manufacture of specialized chemicals for photographic emulsions and for certain coating operations. Methylene chloride is not used in the above manner in California.

Response: The purpose of Appendix II is to describe the methods used to estimate California methylene chloride use. Since methylene chloride is not used for this category in California, Appendix II was not revised to reflect your comment.

3) Bendix Environmental Research, Inc., November 30, 1987

The comments concerned the use of methylene chloride to degrease sampling tubes used for soil and sediment samples at contaminated or potentially contaminated sites.

a) Comment: "I believe that regulatory agencies should encourage the use of safer solvents in procedures designed to evaluate and abate existing environmental problems."

Response: If methylene chloride is identified as a toxic air contaminant, the subsequent risk management phase will evaluate its uses in more detail. Part of the evaluation will be to consider strategies intended to reduce exposure, one of which is to encourage the use substitute compounds.

4) University of California, Riverside, December 22, 1987

a) Comment: Despite the uncertainties in the ambient atmospheric OH concentrations, the annually and diurnally averaged OH radical concentration is, based upon the measured lifetimes of various tracers and computer modeling studies, approximately 5×10^5 molecules cm^{-3}.

Response: The report relies on a range of average OH radical concentrations (5×10^5 to 1×10^6 molecules cm^{-3}) which includes the concentration that you recommend.

b) Comment: As stated on Page IV-5, the initial reaction with OH radical does form the $CHCl_2$ radical (reaction 2). The subsequent reactions of this radical are as follows. The $CHCl_2$ radical will rapidly react with O_2 to form a peroxy radical.

$$CHCl_2 + O_2 = OOCHCl_2$$

This peroxy radical will then react with NO (if present), HO_2 radicals or other peroxy (RO_2) radicals.

$$OOCHCl_2 + NO = OCHCl_2 + NO_2$$

$$OOCHCl_2 + HO_2 = HOOCHCl_2 + O_2$$
(forming dichloromethylhydroperoxide)

$$OOCHCl_2 + RO_2 = OCHCl_2 + \text{other products}$$

These reactions have not been experimentally studied; rather they are expected to occur based on our knowledge of the atmospheric reactions of other simple alkyl peroxy and haloalkyl peroxy radials. The $CHCl_2O$ radical is known to eliminate a chlorine atom to form formyl chloride:

$$OCHCl_2 = HC(O)Cl + Cl$$

The Cl atom will react with whichever organics are present (in the cleaner troposphere this will be mainly with methane) to yield HCl.

The above paragraph should replace the last four lines of page IV-5 and all of page IV-6.

<u>Response</u>: The report was revised to reflect your comments with your letter cited as the source.

c) <u>Comment</u>: It would be helpful to the reader to reference the five groups cited at the bottom of page IV-2, in which case the sentence stating the years in which they published their data could be eliminated.

<u>Response</u>: The report was revised to reflect your comment.

d) <u>Comment</u>: In the first paragraph on page IV-3, it is not clear what the phrase "included to illustrate the variation among the different studies" refers to. Also, in this paragraph it should be "authors'" not "author's".

Response: The sentence has been deleted and the typographical error corrected.

e) Comment: At the bottom of page IV-3, you may want to state explicitly for the uninitiated reader the OH radicals are only present during daylight.

Response: The report has been revised to state this fact.

f) Comment: In the last sentence of the first paragraph on page IV-5, it is not clear which OH radical concentration "this" refers to.

Response: The range of OH radical concentration being discussed in the paragraph was used to estimate a range of atmospheric lifetime estimates. Therefore, the word "this" was replaced with "these".

III - Department of Health Services Responses to Part B - Related Comments

DEPARTMENT OF HEALTH SERVICES
STAFF RESPONSES TO PUBLIC COMMENTS
ON THE PRELIMINARY DRAFT REPORT TO THE AIR RESOURCES BOARD
ON METHYLENE CHLORIDE:
PART B -- HEALTH EFFECTS OF METHYLENE CHLORIDE

TOPIC: Genotoxicity

Comment: Methylene chloride does not appear to act by a direct genotoxic mechanism. In light of the overall negative results in genetic toxicity studies in higher order animals, including several important DNA-binding studies, genotoxicity would not appear to be a significant factor in inducing tumorigenic effects. (Halogenated Solvents Industry Alliance [HSIA]).

Response: Methylene chloride is consistently positive, in a dose-related manner, in *Salmonella* strains TA1535, TA98, and TA100, in both the presence and absence of metabolic activation systems. In the case of methylene chloride, the chemical tests positive in the Ames assay and has been shown to produce tumors in two species. As indicated on page 6-2 of the health effects document, methylene chloride has also exhibited mutagenic activity in *E. coli*. Three of the arguments listed by HSIA (page 14) against applying the Ames test results to higher animals are generic for testing any chemical in a bacterial system. However, since tests in *Salmonella* and *E. coli* have been shown to be useful in evaluating mutagenicity of chemicals and in indicating possible mechanisms of carcinogenicity, the generic criticisms of bacterial test systems (e.g., bacterial cells are different from mammalian cells) will not be addressed in this response. The one HSIA comment on bacterial test systems specific to methylene chloride is

that the bacteria may "metabolize methylene chloride to a transient reactive intermediate(s)." However, HSIA comments also indicate that methylene chloride's metabolism to an active intermediate in animals may be a determining factor of its tumorigenicity and HSIA's pharmacokinetic model is based on this assumption. Thus, the fact that bacteria may metabolize methylene chloride to a reactive intermediate is a reason to regard the bacterial mutagenicity data as relevant. There is no convincing evidence that bacteria produce a reactive intermediate that is not produced in animals. Furthermore, a reactive intermediate, solely responsible for the positive response in Salmonella and E. coli., has not been identified.

The results in yeast and Drosophilla, as discussed in the DHS report (page 6-3), are listed as equivocal. However, the most recent review by IARC (IARC Monographs, 1987, Supplement 7:194-195) states: "...It induced sex-linked recessive lethal mutations in Drosophila. It was mutagenic to plants and induced mutation, mitotic recombination and gene conversion in Saccharomyces cerevisiae under conditions in which endogenous levels of cytochrome P450 were enhanced..." Thus, although the genotoxic results in yeast and Drosophilla are not strong, they do not support the HSIA argument that positive genetic toxicity test results were confined to bacteria.

As indicated in the DHS report, methylene chloride has induced transformation of virus-infected Fischer rat cells and has produced equivocal indications of cell transformation in Syrian hamster embryo cells. The compound has induced chromosomal aberrations in rodent cells in vitro and has induced positive results of sister chromatid exchange in Chinese hamster ovary cells. Thus, as concluded in the DHS report, methylene

chloride appears to exhibit clastogenic activity. These results refute the assertion that genetic toxicity studies in higher order animals are negative overall.

Reactive intermediates, which may be genotoxic, appear to be formed in mammalian cells during methylene chloride metabolism. Mechanism studies of Clara cell toxicity indicate that metabolism of methylene chloride in the mouse lung by P450 results in P450 damage (supplemental information submitted by HSIA, Green et al., Report No. CTL/R/935). This implies that the Clara cell P450 is metabolizing methylene chloride to a reactive intermediate. Also, in support of its physiologically-based pharmacokinetic models, HSIA explicitly accepts the assumption that methylene chloride may produce tumors by production of a reactive intermediate via the glutathione-S-transferase route. Consequently, mammalian cells may metabolize methylene chloride to a reactive intermediate via both the P450 route and the glutathione-S-transferase route. Since reactive intermediates are formed in mammalian cells, methylene chloride is potentially genotoxic to mammalian cells.

In conclusion, the comment hypothesizes that methylene chloride is not genotoxic and that the positive results in bacteria do not reflect the negative genetic toxicity results in higher order animals. However, the consistent positive results in Salmonella, along with the positive and borderline results in higher order test systems, cannot be ignored. A negative or equivocal response in another genotoxic test system may simply reflect the test's evaluation of a different mechanism of genotoxic action. Two potential genotoxic mechanisms of carcinogenesis, that is, mutagenesis

and clastogenesis, have been discussed in the DHS health effects document (page 8-8 to 8-12). Furthermore, reactive intermediates may be formed via two metabolic routes in mammalian cells. Currently, there is no direct evidence to provide support for only one of the hypothesized mechanisms of methylene chloride carcinogenicity. Because the data indicate that methylene chloride is weakly genotoxic, and because reactive intermediates have been identified, DHS staff do not agree that methylene chloride produces its tumorigenic effects by a nongenotoxic mechanism.

TOPIC: Teratogenicity

Comment: The draft report incorrectly concludes that experimental data are inadequate to make inferences about the teratogenic potential of methylene chloride in man. No medical reports suggest a need for more exhaustive testing. The available data merit a more straightforward conclusion than that human reproductive and developmental effects are not expected at ambient concentrations of methylene chloride in California (HSIA).

Response: As indicated in the conclusion of Section 5 of the DHS report: "Experiments performed to date have been limited in that either they employed only one dose level, utilized relatively few test animals, or demonstrated signs of maternal toxicity possibly as a result of carboxyhemoglobin formation." Additionally, rats were used in all studies except one, which tested mice at one dose level that was maternally toxic. For these reasons, DHS staff conclude that methylene chloride has not been sufficiently tested for developmental toxicity. Positive human medical reports linking methylene chloride to reproductive or birth defects in

humans are not necessary to indicate a need for further testing. Considering the large volume of methylene chloride used in industry and by consumers, more information is needed on the potential developmental toxicity of methylene chloride. However, the assessment by DHS staff is that no birth defects or other reproductive effects are expected at ambient levels of methylene chloride.

TOPIC: Carcinogenicity

Comment: The International Agency for Research on Cancer (IARC) considers methylene chloride to be "possibly carcinogenic to humans," rather than a probable human carcinogen. Classifications based strictly on scoring bioassay evidence of carcinogenicity cannot adequately characterize the overall likelihood of a substance to cause cancer in humans. The draft report does not adequately elucidate the staff reasoning regarding hazard identification (HSIA).

Response: Generally, DHS follows the procedures for carcinogen identification that are utilized by IARC and have been described in numerous IARC monographs. A specific discussion on DHS's reasoning regarding hazard identification is presented in the report "Guidelines for Chemical Carcinogen Risk Assessments and their Scientific Rationale," published by the Health and Welfare Agency in 1985. Since methylene chloride exhibits sufficient evidence of carcinogenicity in animals (Group 2), IARC could have classified it as a probable (2A) or possible (2B) human carcinogen. The distinction reflects the quantity and quality of human epidemiologic data available for consideration. There is no clear reason to regard the line

between these two groups as signaling action/no action or concern/no concern. Methylene chloride was classified by IARC as a "possible" human carcinogen as a result of inadequate epidemiologic data indicating carcinogenicity. As described in the DHS report, two epidemiologic studies are available. Although the results reported were negative, the studies had limited power to detect excess risk.

Comment: The weight of evidence suggests that levels of methylene chloride in the California environment do not pose a human cancer risk (HSIA). No carcinogenic effects would be expected to occur at current ambient levels and there is no compelling scientific evidence to refute this conclusion (Allied Corporation [Allied]).

Response: DHS staff do not agree that the weight of evidence indicates that ambient levels of methylene chloride do not pose a human cancer risk. Methylene chloride exhibits genotoxic potential and has been shown to be an animal carcinogen in two species. There is no "proof" that it is carcinogenic at ambient levels because an experiment to test this hypothesis cannot be conducted. High doses are used in animal studies to ensure a sensitive test, given a relatively small number of animals. DHS staff conducted a quantitative risk assessment using the best available data and standard methods of extrapolation to estimate the risk at ambient levels. After reviewing the health effects literature for methylene chloride, including the evidence referred to by HSIA and Allied, DHS staff conclude that methylene chloride is a potential human carcinogen and that a threshold for its carcinogenicity has not been established. Thus, DHS staff conclude

that methylene chloride poses a carcinogenic risk to humans at ambient levels.

Comment: Lung and liver tumors in mice were observed only at toxic doses. Cytotoxic damage in mouse lung may have played a significant role in the enhanced lung tumor response observed in the National Toxicology Program (NTP) study. Cytotoxic effects were not observed in rats, a finding consistent with known metabolic differences between lung tissue of mice, rats, and humans. This may indicate that levels of methylene chloride in the California environment do not pose a human cancer risk (HSIA). Clinically observable hepatic damage is considered to be a prerequisite to liver tumor formation (Allied). The 1982 National Coffee Association drinking water study in rats indicated that 5 ppm is a no observable adverse effect level for a carcinogenic effect (Allied). Even at higher doses, a repair mechanism may operate to prevent liver tumors following insult from methylene chloride (Allied).

Response: The relationship between cytotoxic damage and enhanced lung or hepatic tumor production is unclear. The NTP inhalation study in mice did not report any increase in nonneoplastic changes in the lungs. There was no increase in epithelial hyperplasia, inflammation, or edema. However, the NTP study did report that "alveolar/bronchiolar adenomas, alveolar bronchiolar carcinomas, and alveolar/bronchiolar adenomas or carcinomas (combined) in male and female mice occurred with significant positive trends..." Thus, an association between cytologic damage in the lung and tumor production is not evident in the NTP study. However, HSIA states (page 30) that an unpublished 10-day inhalation study (Reference 28 in HSIA

comments) showed histopathologic damage to Clara cells. While this report might suggest cytotoxicity in mice at the NTP exposure level, it is by no means conclusive. Note that while there is some evidence that the Clara cell is the origin of some chemically induced bronchial tumors (e.g., Kaufmann et al., 1979. Lab Invest 40:708), there is also immunocytochemical evidence suggesting that the vast majority of naturally occurring and experimentally induced pulmonary neoplasms of mice are alveolar Type II cell adenomas and carcinomas (Ward et al., 1985. Am J Pathol 118:493). Furthermore, cell proliferation could be occurring simultaneously with genotoxicity, and still exhibit a linear response at low concentrations.

HSIA also indicates that in the 10-day mouse inhalation study mentioned above there were statistically significant changes in liver weight. Increased liver size does not invariably lead to toxicity, as stated on page 30 of the HSIA comments. Thus, the 10-day study described by HSIA is not considered conclusive evidence that hepatic cytotoxicity preceded tumor formation in the mouse NTP inhalation study. The NTP inhalation study with mice does indicate "cytologic degeneration" was observed in three of the four dose groups. However, an increase in hepatic tumors was observed in all four groups (male and female, low and high doses). Other hepatic tissue changes such as congestion, inflammation, or necrosis were not observed in any of the dose groups. Clinical signs of hepatoxicity (such as SGOT or SGPT changes) were not reported in the NTP study. Thus, clinically observable hepatic damage was not observed in the treated animals.

Thus, the preliminary information presented by HSIA may suggest the possibility of hepatic cytotoxicity at the doses used in the NTP mouse

study, but the relationship of cytotoxicity to tumorigenicity has not been established in this case. The NTP mouse inhalation study does not in itself lend strong support to the hypothesis that methylene chloride cytotoxicity results in tumorigenicity. Furthermore, cytotoxicity was not observed in the NTP rat inhalation study even though rats exhibited a dose-related increase in mammary gland tumors in both sexes. Methylene chloride may cause tumors by mechanisms other than cytotoxicity, even at high doses. Thus, the available data do not indicate that any single mechanism is operating exclusive of other hypothesized mechanisms.

Comment: The final report might include estimates from a Moolgavkar-Knudson model for methylene chloride, which would offer many advantages (HSIA).

Response: As indicated in the report, DHS staff fit several low-dose risk assessment models to the data, including the multistage, time-dependent multistage, probit, logit, Weibull, gamma multihit, and two-stage models. DHS also applied a physiologically-based pharmacokinetic model to the data. The Moolgavkar-Knudson model allows utilization of mechanistic information of carcinogenesis. Risk estimates from this type of model are provided on pages 8-9 and 8-23 of the DHS report.

Comment: The draft report fails to make use of available data on metabolism, pharmacokinetics and mechanism, including those discussed in a recent update to the U.S. Environmental Protection Agency (US EPA) hazard assessment document (HSIA).

Response: Estimates of risk using pharmacokinetic models were calculated by DHS staff and are presented in the DHS report, particularly in Appendix E. DHS staff conducted an extensive examination of data on metabolism, pharmacokinetics and mechanism, which included discussions with US EPAstaff and representatives from industry. The DHS report does not mention the US EPA document specifically because the US EPA document is a review draft labeled "Do not cite or quote." In the DHS report, Section 2 considers metabolism and pharmacokinetics. Sections 6 and 8.4 discuss the mechanism of carcinogenic action. DHS staff applied a physiologically-based pharmacokinetic model to estimate low-dose risks. The model suggested lower human risks than predicted by the other methods. However, as indicated in the Executive Summary "...DHS staff concluded that the relative absence of detailed information on human metabolism of [methylene chloride], the failure of the NTP bioassay to demonstrate saturation of the carcinogenic effect, and other factors indicate that application of a pharmacokinetic approach to risk assessment is premature." This physiologically-based pharmacokinetic model has not been validated by independent experimental data. The model has not been shown to predict any toxicologic effect with greater precision than direct use of the exposure concentration. Current validation of the physiologically-based pharmacokinetic model rests on theoretical considerations. DHS staff is willing to use this model approach when: 1) additional human and animal parameters have been measured, 2) when it is independently validated experimentally, and 3) when the approach, for the compound under consideration, is shown to exhibit a better correlation with response than the applied dose. Consequently, DHS staff considered the factors indicated by the comment.

Comment: Interspecies differences in metabolism may be substantial. Available data explain the observed sensitivity of the mouse. B6C3F1 mice normally exhibit high spontaneous lung and liver tumor rates. This suggests that the mouse is a less appropriate model than the rat or hamster for predicting human response to methylene chloride, and may indicate that levels of methylene chloride in the California environment do not pose a human cancer risk (HSIA).

Response: This argument does not distinguish between published results, unpublished results and inference. DHS staff agrees that interspecies differences in enzymatic rates exist. Nevertheless, the connection between a specific metabolic intermediate and carcinogenicity has not been shown. The available data mentioned in the comment are unpublished results submitted by HSIA. Several key problems with these data are: 1) the human metabolism of methylene chloride by glutathione-S-transferase was reported to be zero in one of the reports. Another unpublished report indicates that the human metabolism is in the range of the rat and hamster. Knowledge of the interindividual variability among humans of this enzyme activity is very incomplete. The current pool of subjects tested is very small. 2) the available data do not have the power to associate only the glutathione (GSH) pathway with tumors. Other pathways may have a greater effect proportionately at low doses. As indicated above, the Clara cell has been shown in mice to produce reactive intermediates in the lung via the P450 pathway. Since these reactive intermediates have the ability to alkylate protein they may also alkylate DNA. This assumption would be consistent with tumor production exhibited in the mouse lung.

Comment: The draft report's presentation of epidemiological studies is confusing. This is due in part to intermixing data from five articles, rather than presenting them in chronological order (Eastman Kodak Company [Kodak]).

Response: The final draft to the Scientific Review Panel will be revised to make the presentation less confusing and to clarify statements that may be misinterpreted by those unfamiliar with the studies.

Comment: The findings from the latest epidemiological studies should have been emphasized, because these studies provided the most complete follow-up and exposure information (Kodak).

Response: DHS staff felt that the latest findings were emphasized in the document, but it is hoped that in the revised version this will be more readily apparent.

Comment: Epidemiological studies show no evidence of an increased cancer risk for all sites or for the primary sites associated with lesions found in animal studies. The finding among Eastman Kodak employees of 8 observed cases of pancreatic cancer versus 3.2 expected does not imply a causal association with methylene chloride; it may have been due to chance (Allied, Kodak). No dose-related effect was observed in the mortality study; the results may have been confounded by smoking, alcohol consumption, diabetes, other chemical exposures, and other risk factors (Kodak). This may indicate that levels of methylene chloride in the California environment do not pose

a human cancer risk. The report should give greater weight to the negative findings at the high exposure levels in the studies of workers (HSIA).

Response: DHS staff does not believe that the epidemiological studies are definitive, due to their limited power. As indicated on pages 7-2, 7-3, 7-4, and 7-7 of the draft report, the available epidemiological data do not indicate a significantly increased cancer rate from methylene chloride exposure, where the test was done with an α-level (two-sided) of 0.01. The actual p-value associated with the observed pancreatic cancer deaths is 0.017 using New York State Controls, and 0.014 using Kodak controls. To conclude that a compound is likely to be carcinogenic in humans does not require positive human evidence; strong animal evidence may be sufficient. To rule out carcinogenicity on the basis of human data, for any compound, would require multiple, negative, well-designed studies. Such negative studies would need to have historical exposure information (i.e., industrial hygiene samples) for all relevant job sites, and individual employee records relating work periods to job sites. These studies would also need to have sufficient power, a long follow-up period, and substantial data on confounding. In light of the clear carcinogenicity of methylene chloride in two animal species, additional negative epidemiologic data would be required to separate the animal and human responses. For these reasons, DHS staff do not give great weight to the negative findings in the epidemiological studies as is suggested by the comment.

Comment: There is no apparent evidence of biologic plausibility for pancreatic cancer in man (Kodak). Pancreatic cancer has been found in association with certain chemical exposures in the rat and hamster, (e.g.,

bis(chloromethyl)ether, 4-hydroxyamino-quinoline-1-oxide, the diazoketone azaserine, the methylnitrosourea-containing amino acid N-(N-methyl-N-nitroso carbamoyl)-L-ornithine, nafenopin and clofibrate) but following exposure to methylene chloride it was not observed in rats dosed orally or in rats or hamsters dosed by inhalation (Kodak). It is unlikely that methylene chloride is a human pancreatic carcinogen (Kodak).

Response: A substantial amount of information on the potential carcinogenicity of these six compounds in animals is not available, but the IARC monographs contained the following information. Methylnitrosourea produced tumors in the pancreas of rats under certain protocols, but it produced pancreatic tumors consistently in guinea pigs. In contrast, methylnitrosourea did not produce pancreatic tumors in mice, hamsters, pigs, dogs, rabbits, or gerbils; in these species other tumors were produced following methylnitrosourea exposure. These other tumors include those in the central and peripheral nervous tissue, stomach, esophagus, respiratory tract, intestine, lymphoreticular tissues, skin and kidney. The tumor site(s) for methylnitrosourea depends on the species tested and the route of exposure. The production of pancreatic tumors in rats or guinea pigs following exposure to methylnitrosourea did not predict the presence of pancreatic tumors in the other species tested. Nafenopin produced pancreatic and hepatocellular carcinomas in the rat but produced only hepatocellular carcinomas in mice. Thus, the production of pancreatic tumors in rats was not predictive of pancreatic tumors in mice. Clofibrate has been tested only in rats and produced pancreatic carcinomas (3/36), hepatocellular carcinomas (14/36), and numerous types of tumors in various other tissues. Thus, these studies indicate that the production of a

specific tumor type cannot necessarily be extrapolated across species. This lack of concordance for specific tumor sites is not restricted to pancreatic tumors, but is characteristic of many chemically induced tumor types. In addition, although pancreatic tumors have been produced by some chemicals in some studies with rats and hamsters, these two species have not been shown to be specifically sensitive to the development of pancreatic cancer. A negative result in the pancreas from any specific chemical would therefore not provide assurance that the chemical would not produce pancreatic cancer in humans.

With this information, one can focus on whether pancreatic tumors in man are inconsistent with tumors produced by methylene chloride in animals. Methylene chloride produces tumors at different sites across species. As a result of methylene chloride inhalation, rats have developed salivary gland tumors, mammary tumors and liver tumors, while mice have experienced increased incidences of liver tumors and lung tumors. Thus, although pancreatic tumors were not produced in rats or mice by inhalation, the specific tumor types that might occur in man following methylene chloride exposure cannot be accurately predicted. The Kodak comment states that pancreatic cancer in rats has not been asociated with oral or inhalation exposure to methylene chloride. The studies cited were the NTP inhalation studies and several industry-sponsored studies. However, a draft NTP gavage study reports that 19 of 50 male rats given 500 mg/kg-day methylene chloride developed pancreatic adenomas (Draft NTP Technical Report on the Carcinogenesis Bioassay of Dichloromethane in F344/N Rats and B6C3F1 mice, 1982). For male rats given 1000 mg/kg-day, 15 of 47 developed pancreatic tumors. In contrast, 2 of 96 control male animals developed pancreatic

tumors. Thus, methylene chloride produces tumors in several tissues and there is some suggestive evidence that methylene chloride can induce pancreatic tumors in rats.

In summary, strong concordance does not exist between tumor sites in animals and in man. Compounds that produce tumors in the liver, lung or other sites in one species may produce pancreatic tumors in another species. Some limited evidence indicates that methylene chloride may produce pancreatic tumors in the rat. Evidence for the biological plausibility for methylene chloride's production of pancreatic tumors in man is available in the literature. Consequently, although methylene chloride has not been causally associated with pancreatic cancer in humans, the suggestive human evidence is not inconsistent with the location of tumors produced by methylene chloride in experimental animals.

Comment: To evaluate the validity of the risk estimates from animal data, the staff of DHS derived risk estimates from epidemiological data. To do so, the staff used a modeling procedure that allowed only linear changes with dose and forced the (linear) response function through the origin. The upper bound risk estimate generated from the model used in the draft report is highly insensitive to differences in the number of observed deaths and the dose-response relationship within the cohort. It should therefore have been expected that the resulting estimates would be consistent with those derived from animal data and the linearized multistage model (Kodak). Using mortality findings from a tumor site of questionable significance and ignoring information contained in the lower bound estimates contributed to the lack of contradiction between the estimates derived from the animal and

epidemiological studies. Thus, the human and animal data are not consistent with one another (Kodak).

Response: DHS staff did not ignore the information in the human lower bound estimates; the human lower bound estimates are consistent with no excess cancer risk. The purpose of examining the upper bound estimates was to determine if the human finding was consistent with the carcinogenic potency of methylene chloride estimated in animals. The lack of sensitivity of the multiplicative model does not diminish the consistency between animal and human data. It may simply indicate the lack of power of the epidemiological study to clearly distinguish a negative human result from risks predicted by a positive animal result.

A second approach taken by DHS staff to assess the consistency of the animal-based risk assessment with the results of epidemiologic studies was not mentioned in the comment. This approach has been used to evaluate other compounds, and in two cases, ethylene dibromide and cadmium, detected a discrepancy of over ten-fold. This approach is sensitive enough to detect major discrepancies between the observed epidemiologic data and predictions based on extrapolation from animal bioassay. As stated in the draft document (pages 8-14), this approach showed predictions to fall within a factor of two of the observed. Thus, two approaches indicated that the human epidemiologic data was consistent with that predicted from the animal bioassays.

Comment: The probability of observing eight or fewer pancreatic cancer deaths, given an animal-based expectation of 12.5, is relatively low

(0.125). Similarly, although the observed-versus-expected lung and liver cancer deaths were within a factor of 3, the human findings at these sites are highly unlikely, given the animal model predictions (probability less than 0.0001) (Kodak).

Response: A probability of 0.125 associated with the observed data, under a null hypothesis of 12.5 or greater, is not particularly low and does not seem to be a basis to reject the hypothesis. Although the animal-based model predicts higher lung and liver cancer rates than observed in humans, DHS staff believes that considering the enormous uncertainties in extrapolating from high to low doses and from animals to man, a factor of three should be viewed as consistent.

Comment: The draft report should have evaluated data for all primary cancers rather than only those from the site (pancreas) for which there was a suggestive increase (Kodak).

Response: In the DHS staff analysis, the most sensitive animal site was compared to the human site exhibiting the greatest response in the epidemiologic study. The suggestion by Kodak that the analysis should now be redone looking at all cancers in humans and all cancers in animals would not resolve the issue. Use of all primary cancer sites is a poor way to evaluate a specific carcinogen, since individual chemical compounds tend to affect certain sites more than others. In light of the high background cancer rates in humans and other animals, combining chemically-induced and background sites would substantially dilute the sensitivity of the analysis.

IV. Air Resources Board
Letters to Comment Originators

STATE OF CALIFORNIA

GEORGE DEUKMEJIAN, Governor

AIR RESOURCES BOARD
1102 Q STREET
P.O. BOX 2815
SACRAMENTO, CA 95812

April 11, 1988

Paul A. Cammer, President
Halogenated Solvents Industry Alliance
1225 19th Street, N.W., Suite 300
Washington, D.C. 20036

Dear Mr. Cammer:

Thank you for your comments on Parts A and B of the Preliminary Draft Report on Methylene Chloride. My staff has prepared responses concerning comments on the Part A report while the California Department of Health Services is responding to comments on Part B. Both sets of responses will be incorporated into Part C of the final draft report, which is expected to be released to the public within a month.

If you have any questions concerning the report, please contact Richard Corey of my staff at (916) 323-8511.

Sincerely,

Joan E. Denton

Joan Denton, Ph.D., Manager
Substance Evaluation Section
Stationary Source Division

cc: George Alexeeff
Department of Health Services

STATE OF CALIFORNIA

GEORGE DEUKMEJIAN, Governor

AIR RESOURCES BOARD
1102 Q STREET
P.O. BOX 2815
SACRAMENTO, CA 95812

April 11, 1988

Thomas J. Dufficy
National Association of Photographic Manufacturers, Inc.
600 Mamaroneck Ave.
Harrison, New York 10528

Dear Mr. Dufficy:

Thank you for your comments on Part A of the Preliminary Draft Report on Methylene Chloride. My staff has prepared responses to your comments which will be incorporated into Part C of the final draft report, which is expected to be released to the public within a month.

If you have any questions concerning the report, please contact Richard Corey of my staff at (916) 323-8511.

Sincerely,

Joan E. Denton

Joan Denton, Ph.D., Manager
Substance Evaluation Section
Stationary Source Division

STATE OF CALIFORNIA GEORGE DEUKMEJIAN, Governor

AIR RESOURCES BOARD
1102 Q STREET
P.O. BOX 2815
SACRAMENTO, CA 95812

April 11, 1988

Roger W. Reinhold, Manager
Department of Toxicology
Allied Corporation
P.O. Box 1021 R
Morristown, New Jersey 07960

Dear Mr. Reinhold:

Thank you for your comments on Part B of the Preliminary Draft Report on Methylene Chloride. Your comments have been forwarded to the California Department of Health Services. Their staff is preparing responses which will be incorporated into Part C of the Final Draft Report, which is expected to be released to the public within a month.

If you have any questions concerning the report, please contact Richard Corey of my staff at (916) 323-8511.

Sincerely,

Joan E. Denton

Joan Denton, Ph.D., Manager
Substance Evaluation Section
Stationary Source Division

cc: George Alexeeff
Department of Health Services

STATE OF CALIFORNIA GEORGE DEUKMEJIAN, Governor

AIR RESOURCES BOARD
1102 Q STREET
P.O. BOX 2815
SACRAMENTO, CA 95812

April 11, 1988

Selina Bendix, President
Bendix Environmental Research, Inc.
Fox Plaza, Suite 902
1390 Market St.
San Francisco, CA 94102

Dear Ms. Bendix:

Thank you for your comments on Part A of the Preliminary Draft Report on Methylene Chloride. My staff has prepared responses to your comments which will be incorporated into Part C of the final draft report, which is expected to be released to the public within a month.

If you have any questions concerning the report, please contact Richard Corey of my staff at (916) 232-8511.

Sincerely,

Joan E. Denton

Joan Denton, Ph.D., Manager
Substance Evaluation Section
Stationary Source Division

STATE OF CALIFORNIA

GEORGE DEUKMEJIAN, Governor

AIR RESOURCES BOARD
1102 Q STREET
P.O. BOX 2815
S AMENTO, CA 95812

April 11, 1988

Dr. Roger Atkinson
University of California, Riverside
Statewide Air Pollution Research Center
Riverside, CA 92521

Dear Dr. Atkinson:

Thank you for your comments on Part A of the Preliminary Draft Report on Methylene Chloride. My staff has prepared responses to your comments which will be incorporated into Part C of the final draft report, which is expected to be released to the public within a month.

If you have any questions concerning the report, please contact Richard Corey of my staff at (916) 323-8511.

Sincerely,

Joan E. Denton

Joan Denton, Ph.D., Manager
Substance Evaluation Section
Stationary Source Division

STATE OF CALIFORNIA

GEORGE DEUKMEJIAN, Governor

AIR RESOURCES BOARD
1102 Q STREET
P.O. BOX 2815
SACRAMENTO, CA 95812

April 11, 1988

Dr. Arthur Winer
University of California, Riverside
Statewide Air Pollution Research Center
Riverside, CA 92521

Dear Dr. Winer:

Thank you for your comments on Part A of the Preliminary Draft Report on Methylene Chloride. My staff has prepared responses to your comments which will be incorporated into Part C of the final draft report, which is expected to be released to the public within a month.

If you have any questions concerning the report, please contact Richard Corey of my staff at (916) 323-8511.

Sincerely,

Joan E. Denton

Joan Denton, Ph.D., Manager
Substance Evaluation Section
Stationary Source Division

PART C ADDENDUM

PUBLIC COMMENTS AND RESPONSES TO THE FINAL DRAFT METHYLENE CHLORIDE REPORT

Prepared by the staff of the Air Resources Board

March 1989

(Part C Addendum reflects the comments received from the public during May 3, 1988 to may 24, 1988 public review period for the Final Draft Report. The responses of the air Resources Board staff to those comments are contained in Part C Addendum)

ADDENDUM TO PART C - CONTENTS

I. Comments Received from the public

II. Air Resources Board Responses to Comments

III. Department of Health Services Responses to Comments

IV. Air Resources Board Letters to Comment Originators

I. Comments Received from the Public

A. The Halogenated Solvents Industry Alliance

Heron, Burchette, Ruckert & Rothwell

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May 24, 1988

BY COURIER

Mr. Robert Barham
Chief
Toxic Air Contaminant Identification Branch
Stationary Source Division
P.O. Box 2815
Sacramento, California 95812

Re: Methylene Chloride

Dear Mr. Barham:

Enclosed are the comments of the Halogenated Solvents Industry Alliance (HSIA) on the final draft report on methylene chloride. In light of the significant scientific and science policy questions raised by the recommendations and the health effects assessment in the draft report, HSIA requests an opportunity to appear before the Scientific Review Panel when it addresses methylene chloride. HSIA representatives will be in touch with you concerning this request.

Sincerely,

Caffey Norman

W. Caffey Norman, III

Enclosure

UNITED STATES ENVIRONMENTAL PROTECTION AGENCY
WASHINGTON D.C. 20460

March 9, 1988

SAB-EHC-88-013

OFFICE OF
THE ADMINISTRATOR

Hon. Lee M. Thomas
Administrator
U.S. Environmental Protection
Agency
401 M Street SW
Washington, D.C. 20460

Dear Mr. Thomas:

On August 13-14 1987 the Halogenated Organics Subcommittee of the Science Advisory Board's Environmental Health Committee met in Washington, D.C. to review two documents prepared by EPA's Office of Research and Development that assess health effects associated with dichloromethane (methylene chloride). These documents included:

- a June 1987 Draft Technical Analysis of New Methods and Data Regarding Dichloromethane Hazard Assessments, and

- a July 1987 Draft Addendum to the Health Assessment Document for Dichloromethane: Pharmacokinetics, Mechanism of Action and Epidemiology.

The Subcommittee's evaluation of these documents is presented in two parts: 1) a discussion of scientific issues related to pharmacokinetics and metabolism, and 2) review of specific issues pertinent to the addendum. The Subcommittee focused less attention on the former document because much of its scientific content overlapped with the Addendum.

The Subcommittee concludes that the Addendum was one of the best documents it has reviewed in terms of its clarity, coverage of the data and analysis of scientific issues. This document clearly demonstrates the potential utility of pharmacokinetic data in risk assessment. EPA should continue to use this approach in future risk assessments, whenever scientifically possible.

For reasons discussed in the attached report, the Subcommittee concludes that the level of uncertainty is greater and the hazard posed by dichloromethane may be less than that expressed by the categories of EPA's cancer risk assessment guidelines.

The Subcommittee appreciates the opportunity to conduct this scientific review. In behalf of the Subcommittee we request that the Agency formally respond to the scientific advice provided in its attached report.

Sincerely,

Norton Nelson, Chair
Executive Committee

Richard A. Griesemer, Chair
Environmental Health Committee

John Doull, Chair
Halogenated Organics Subcommittee

Halogenated Organics Subcommittee
Review of the June 1987 Draft Technical Analysis of New Methods and Data Regarding Dichloromethane Hazard Assessments: and July 1987 Draft Addendum to the Health Assessment Document for Dichloromethane: Pharmacokinetics, Mechanism of Action and Epidemiology

Pharmacokinetics and Metabolism

The application of a physiologically based pharmacokinetics (PBPK) model presented in the document is generally well conceptualized and organized. This application represents a novel approach that can sharpen EPA's ability to refine human risk estimates in the future. The Subcommittee commends EPA for incorporating such information into the weight of evidence determination of the carcinogenic potential of dichloromethane. Adoption of the Reitz-Anderson model, with certain modifications, is a positive step forward for the Agency's risk assessment process. The critical analysis of the constraints of the model are thoroughly discussed and scientifically balanced. EPA, for example, is justified in adjusting the estimates of Reitz-Anderson for breathing rates traditionally used in EPA models. The rationale for using surface area factor adjustment, and contrary arguments, are clearly described.

The PBPK model has appeared in the published peer reviewed literature. The novel applications of the latest data concerning the model that were presented at the meeting are new and received enthusiastic support from the Subcommittee, which also recommends publication of this approach. The Subcommittee recognizes that validation will be required for this and other compounds before using this approach generally for human risk calculations.

One possible problem is that the metabolic conversion of dichloromethane by different animal species by either the cytochrome P-450 oxidase system (mixed function oxidase or MFO) or the glutathione-S-transferase system (GST) was not supported by data indicating that measurements in each species were conducted at conditions optimal for pH, ionic strength or temperature for that substrate in that tissue for each specific species. Unless such conditions are utilized, interspecies quantitative data may be meaningless, and the basis for the use of this approach in human risk estimation could be flawed.

Comparative in vitro studies with rat, mouse, hamster and human cytosol showed that the dichloromethane-GST conjugation rates in humans were at least 50 times lower than in mice. The Subcommittee points out that: 1) mice have the highest activity and liver tumor induction that correlates with GSH-metabolite production above saturation of the MFO system; 2) hamsters have much lower activity and no liver tumors; and 3) humans have even lower activity indicating very low, if any, liver tumor inducing potential for dichloromethane. There was a good correlation between the relative rates of dichloromethane-GSH conjugation and susceptibility of liver tumors. The conclusion that, at low exposure levels, the carcinogenic hazard to humans

from dichloromethane appears very low needs to be clearly stated in the document.

The document could be simplified by eliminating the Chapter 7 discussion of a "rationale" for surface area scaling and replacing it with the statement on page 107 that, "The fact that there is no clear basis for choosing the use of surface area correction or not...is a weakness of the current state-of-the-art of quantitative risk assessment."

Discussion of Specific Scientific Issues Related to the Addendum

1. In considering an overall weight of evidence approach to risk assessment, other factors, such as the nature of the animal tumor response, mechanistic data (such as binding of the chemical to DNA), genotoxic activity and epidemiological data should also be discussed.

2. In evaluating the tumor data, the Subcommittee urges caution in extrapolating the existing animal bioassay to humans. Although dichloromethane induced both lung and liver tumors in the mouse models, these observations occurred only at high doses which likely influenced the compound's overall metabolism. Other bioassays in other species, or at lower doses in mice, induced negative results. The fact that the Reitz-Anderson model is able to predict these responses suggests that an interspecies correction factor based on surface area may not be necessary for extrapolating the tumor data to humans. This is particularly true when hamster and rat data (GSH transferase) are considered using the PBPK analysis. The observation of benign mammary tumors and salivary gland tumors in rats should not be used as strong evidence for human carcinogenic potential given the uncertain significance of these lesions. The benign mammary tumors have very low potential for predicting malignancy even in the rat, and salivary gland tumors were reported in only one of the studies.

3. EPA should discuss the findings of several investigators (Shumann et. al., Dow Chemical; Green et. al., ICI, U.K.) that indicate that dichloromethane or its metabolites do not exhibit any potential to alkylate liver or lung DNA following in vivo exposure. Such findings raise the clear possibility that dichloromethane may have produced its carcinogenic responses in mice by non-genotoxic mechanisms, and may include an important contribution of cytotoxicity in the overall tumorigenic process. Such data become particularly relevant as carcinogenicity was observed only at extremely high exposures and was absent at lower, potentially noncytotoxic doses.

4. Critical uncertainties remain regarding the relationship between dose to target tissues and tumor incidence, since little information on the mechanism of action is available for dichloromethane. The Subcommittee accepts EPA's use of a surface area scaling factor for delivered dose as appropriate for calculating an upper bound estimate, but it views this usage as more conservative than the usual "default" assumption from the Agency cancer guidelines, scaling administered dose by surface area from animals to humans. Further research may indicate that, at least for some substances, scaling delivered dose on the basis of body weight is more appropriate than scaling by surface area.

5. The degree of nonlinearity in the dose reponse relationship for delivered dose is an important source of uncertainty. As noted on page 110 of the Addendum, EPA uses the linearized multi-stage model to calculate an upper bound estimate. The true dose response curve may fall off more rapidly than a linear relationship at low doses. Biological information supporting a non-linear or threshold type of dose response relationship is potentially important for risk management decision making because it becomes less likely that the default plausible upper bound linear estimate will be an accurate estimate of human risk, especially at low exposure levels in the ambient environment.

6. The Subcommittee was presented with a brief report on the current status of the Kodak epidemiological study of dichloromethane. A slight excess of pancreatic cancer deaths has been separately reported. However, the study is based only on death certificate data and has not included a histopathologic review of biopsies or surgical specimens from such patients. The incidence of pancreatic cancers tended to cluster, and only with further surveillance of the population can a more definitive statement be made on human health risk. The clinical diagnosis of pancreatic cancer is difficult and may be easily confused with other abdominal malignancies. Thus, without pathologic confirmation, the Subcommittee cannot necessarily conclude that an excess of pancreatic cancer deaths has occurred. However, neither can it be concluded that dichloromethane is safe for humans at the occupational exposure levels seen in the study. The Agency should determine the criteria of the Kodak epidemiological study necessary to substituting the animal derived risk estimate with a human derived risk estimate. Finally, the Subcommittee recommends the continuation of this important study.

7. Although there is an impressive weight of evidence implicating metabolites of dichloromethane in tumors, the possibility should not be discounted that the actual tumor inducing agent is the parent compound(s). In order to present a more balanced document, this possibility should be discussed at greater length, perhaps in Chapter 8.

8. Both the scaling factor and the shape of the dose response relationship are important areas for further work in order to aid development of risk assessment methods that incorporate available scientific data and judgement on biological mechanisms. As better information is developed on pharmacokinetics, pharmacodynamics and mechanisms for chemical carcinogenesis, it should be possible to further reduce uncertainties in human risk estimates.

9. For all of the above reasons, therefore, the Subcommittee concludes that the level of uncertainty is greater and that the hazard for dichloromethane may be less than that expressed by the Agency's classification system in its cancer risk assessment guidelines.

More detailed discussion of these and other issues by individual Subcommittee members has been forwarded to the Office of Research and Development.

U.S. Environmental Protection Agency
Science Advisory Board
Environmental Health Committee
Halogenated Organics Subcommittee
Roster for August 13-14, 1987 Review of the Draft
Assessment Documents for Dichloromethane

Dr. John Doull, Chairman, Professor of Pharmacology and Toxicology, University of Kansas Medical Center, Kansas City, Kansas 66103

Dr. Seymour Abrahamson, Vice-Chairman, Professor of Zoology and Genetics, Department of Zoology, University of Wisconsin, Madison, Wisconsin 53706

Subcommittee Members and Consultants

Dr. Linda Birnbaum, National Institute of Environmental Health Sciences, P.O. Box 12233, Research Triangle Park, North Carolina 27709

Dr. George T. Bryan, Department of Human Oncology, University of Wisconsin, K-4, Room 528, 608 Clinical Science Center, 600 Highland Ave., Madison, Wisconsin 53792

Dr. James Bus, Pathology and Toxicology Research, Upjohn Company, Kalamazoo, Michigan 49001

Dr. Robert Dedrick, Chief, Chemical Engineering Section, National Institutes Health, Building 13, Room 3W13, Bethesda, Maryland 20892

Dr. David Gaylor, National Center for Toxicological Research, Jefferson, Arkansas 72079

Dr. Ronald D. Hood, Professor and Coordinator, Cell and Developmental Biology Section, Department of Biology, University of Alabama, and Principal Associate, R.D. Hood and Associates, Consulting Toxicologists, P.O. 1927, University, Alabama, 35486

Dr. K. Roger Hornbrook, Department of Pharmacology, P.O. Box 26901, University of Oklahoma, Oklahoma City, Oklahoma 73190

Dr. Curtis Klaassen, Professor of Pharmacology and Toxicology, University of Kansas Medical Center, 39th and Rainbow Blvd., Kansas City, Kansas 66103

Dr. Karl K. Rozman, Department of Pharmacology, Toxicology and Therapeutics, University of Kansas, Kansas City, Kansas 66103

Dr. Stephen Safe, Department of Veterinary Medicine, Physiology and Pharmacology, College of Veterinary Medicine, Texas A&M University, College Station, Texas 77843-4

Dr. Robert Squire, 1515 Labelle Ave., Ruxton, Maryland 21204

Dr. Thomas Starr, CIIT, P.O. Box 12137, Research Triangle Park, North Carolina 27709

Participating Members of the Environmental Health Committee

Dr. Richard A. Griesemer, Biology Division, Oak Ridge National Laboratory, Martin Marietta Energy Systems, Inc., P.O. Box Y, Oak Ridge, Tennessee 37831

Dr. D. Warner North, Principal, Decision Focus Inc., Los Altos Office Center, Suite 200, 4984 El Camino Real, Los Altos, California 94022

Executive Secretary

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BEFORE THE CALIFORNIA AIR RESOURCES BOARD

PROPOSED IDENTIFICATION OF METHYLENE CHLORIDE AS A TOXIC AIR CONTAMINANT

Comments of the
Halogenated Solvents Industry Alliance
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Paul A. Cammer, Ph.D.
President

Of Counsel:

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May 24, 1988

PROPOSED IDENTIFICATION OF METHYLENE
CHLORIDE AS A TOXIC AIR CONTAMINANT

By letter dated April 28, 1988, the final draft Report to the Air Resources Board on Methylene Chloride (the draft Report), prepared by the staffs of the Air Resources Board (the Board) and the Department of Health Services (DHS), was made available for review and comment before submission to the Scientific Review Panel (the Panel). The draft Report consists of Parts A (use and exposure), B (health effects), and C (public comments on Parts A and B and the DHS staff response), and also contains an overview and recommendation that methylene chloride be identified as a toxic air contaminant under Cal. Health and Safety Code § 39660.

The Halogenated Solvents Industry Alliance (HSIA) commented extensively in January 1988 on the preliminary draft report on methylene chloride, released in late November 1987. HSIA's 60-page comment focused primarily on Part B (health effects). Accordingly, we were very surprised and disappointed to find that Part B has not been revised at all by DHS staff, and is the same document (November 1987 draft) that was sent out during the first comment period. HSIA expended significant effort and resources to respond within 45 days to the DHS staff assessment. No purpose would be served by repeating the earlier HSIA comments, which are reprinted in Part C of the draft Report.

The letter soliciting comment on the final draft Report states that any revisions to the DHS staff health effects assessment (Part B) will be incorporated into the Report after the comment period and before review by the Panel. This highly unusual procedure for peer reviewing a health effects assessment would seem to be inconsistent with Health and Safety Code § 39660, which requires that "the report, together with the scientific data on which the report is based, shall . . . be made available to the public and shall be formally reviewed by the scientific review panel" (emphasis added). The law does not provide for one draft of the report to be made available for public review and a later, different version to be reviewed by the Panel. We submit that the Board could not sustain a decision to identify methylene chloride as a toxic air contaminant based on a health effects assessment that the public was not allowed to review prior to Panel consideration.

HSIA's expression of concern is not an effort to rely on a technicality to delay or subvert the statutory process for identifying toxic air contaminants. The staff recommendation to the Board is premised on a risk assessment that rejects the use of scientific information that has been acknowledged by academic and government scientists alike as a milestone in efforts to predict more accurately the health risk, if any,

from atmospheric concentrations of pollutants.[1] Indeed, the DHS staff assessment will not be helpful to the Board in addressing a critical regulatory issue -- whether there is a threshold exposure level below which no significant adverse health effects can realistically be anticipated. See Health and Safety Code § 39662(c).

None of the key points in the January 1988 HSIA comments has been adequately addressed by DHS staff. The DHS response is confusing in several respects, e.g., its use of the International Agency for Research on Carcinogens (IARC) classification of methylene chloride as a "possible," but not a "probable," human carcinogen to support identification of methylene chloride as a toxic air contaminant. The DHS response fails to note as well that EPA's Science Advisory Board, an independent peer review panel established by federal statute, has formally stated that "the level of uncertainty is greater and the hazard posed by dichloromethane [methylene chloride] may be less than that expressed by the categories of EPA's cancer risk assessment guidelines."[2]

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[1] DHS staff is required by law to consider all available scientific data on health effects, including potency, mode of action, and other relevant biological factors. Health and Safety Code § 39660(b),(c).

[2] Letter to Lee M. Thomas, EPA Administrator, from Drs. Nelson, Griesemer, and Doull, EPA Science Advisory Board (Mar. 9, 1988) (attached).

Rather than repeating our January 1988 comments, two key issues will be discussed below to show the importance of a thorough review by the Panel.[3] On both points, the DHS staff health effects assessment is inconsistent with (i) EPA's evaluation of methylene chloride, culminating an 8-year effort involving independent scientific peer review on a number of separate occasions; (ii) the recommendations made by EPA's Science Advisory Board; and/or (iii) the overwhelming weight of current scientific consensus.

A. Available Human Evidence Indicates No Increased Cancer Risk from Methylene Chloride

The DHS health effects assessment transforms a state-of-the-art epidemiology study, showing no indication of increased overall cancer risk, into evidence for the staff's position that methylene chloride causes cancer in humans. Since the study has been reported in the scientific literature as showing no evidence of increased overall cancer risk,[4] and

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[3] The DHS staff response, contained in Part C of the draft Report, rejects virtually every public comment on its health effects assessment. HSIA disagrees with DHS staff on each of these points and reserves the right to raise before the Panel each of the points that warrant a determination by the Panel that the DHS health effects assessment is seriously deficient.

[4] Hearne, et al., Methylene Chloride Mortality Study: Dose-Response Characterization and Animal Model Comparison, J. Occ. Med. 29:217-228 (1987).

accepted as a negative study by EPA, an unusual interpretation of the results was necessary in order for DHS staff to consider the study to be consistent with "human risk estimates based on animal studies." The only way the results could be so interpreted would be to ignore the overall mortality results (i.e., total cancer and hypothesized tumor sites such as lung and liver), and to restrict the analysis to the only site (pancreas) showing an increased number of cancer deaths (8 observed vs. 3.2 expected). This is the approach taken in the DHS health effects assessment.

Without repeating the criticisms of the DHS staff interpretation that have been submitted by Eastman Kodak Company, we note that there were several sites (colon-rectum, genito-urinary) where a result of the same magnitude was seen, but in the opposite direction (2 observed vs. 7 expected, 3 observed vs. 8 expected, respectively). Observed-expected differences of this type are likely, due to chance alone. Perhaps in recognition of the weakness of its interpretation, the DHS staff response (p. 15-16) dredges up animal bioassay results and relies on these results as "[e]vidence for the biological plausibility for methylene chloride's production of pancreatic tumors in man." The source for this purported evidence is a draft NTP technical report.[6] Based on "significant discrepancies in experimental data," however, NTP

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[6] Draft NTP Technical Report on the Carcinogenesis Bioassay of Dichloromethane (Methylene Chloride) (T.R. No. 254) (Sept. 22, 1982).

announced the cancellation of this report in 1983.[5] We submit that a strange interpretation of human evidence, supported by admittedly flawed animal data, has no place in a scientific risk assessment.

B. Failure to Use Available Pharmacokinetic Data Is Inconsistent with Current Scientific Practice

The DHS staff health effects assessment recognizes that extensive scientific data on the metabolism, pharmacokinetics, and mechanism of methylene chloride are currently available, but declines to make use of this information either in its assessment of whether atmospheric concentrations of methylene chloride are likely to pose a cancer risk to humans or in its estimate of the magnitude of the theoretical cancer risk. The failure to incorporate this information, after a request from a Panel member that "future documents include a

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[5] 48 Fed. Reg. 35508 (Aug. 4, 1983). The deficiencies in the study on which the DHS staff response relies triggered a Congressional investigation of the NTP bioassay program and its quality-assurance procedures, and the termination of the contractor that had performed the defective study and some fifty others. See Hearings before the Subcommittee on Oversight and Investigations of the House Committee on Energy and Commerce, 98th Cong., 1st Sess. (Nov. 7, 1983), Serial No. 98-97; General Accounting Office, Report to the Chairman, Subcommittee on Oversight and Investigations, House Committee on Energy and Commerce, National Toxicology Program: Efforts to Improve Oversight of Contractors Testing Chemicals (June 25, 1985).

section on pharmacokinetics,"[6] has not been adequately explained.

In its response to public comments, DHS staff asserts that its refusal to make use of these relevant data is appropriate because the data are unpublished and EPA's risk assessment, which does make use of these data, is in draft form. Extensive research on the metabolism and pharmacokinetics of methylene chloride has, however, been published in the open scientific literature and provided to DHS.[7] Published articles include Andersen et al., Physiologically Based Pharmacokinetics and the Risk Assessment Process for Methylene Chloride, Tox. Appl. Pharm. 87:185-205 (1987); Reitz et al., Development of Multispecies, Multiroute Pharmacokinetic Models for Methylene Chloride and 1,1,1-Trichloroethane (Methyl Chloroform), in Pharmacokinetics and Risk Assessment 391-409 (National Academy Press 1987); and Green et al., Macromolecular Interactions of Inhaled Methylene Chloride in Rats and Mice, Tox. Appl. Pharm. 93:1:10 (1988). Numerous other articles are mentioned in our January 1988 comments.

The Addendum to the EPA Health Assessment Document on Methylene Chloride is, as the DHS response correctly states, a

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[6] Meeting Summary, Scientific Review Panel on Toxic Air Contaminants (July 7, 1987), at 3.

[7] HSIA sponsored a seminar on this research for the benefit of DHS staff on June 4, 1986, at the DHS offices in Berkeley.

review draft. It has, however, now been formally approved by EPA's Science Advisory Board, which stated:

> The Subcommittee [Halogenated Organics] concludes that the Addendum was one of the best documents it has reviewed in terms of its clarity, coverage of the data and analysis of scientific issues. This document clearly demonstrates the potential utility of pharmacokinetic data in risk assessment. EPA should continue to use this approach in future risk assessments, whenever scientifically possible.[8]

Moreover, as indicated in the January 1988 HSIA comments, it has been clear since well before release of the DHS draft Report in November 1987 that there is strong scientific support for the use of metabolic and pharmacokinetic information on methylene chloride in risk assessment.[9]

In reviewing an earlier draft Addendum to the EPA Health Assessment Document on Methylene Chloride, Dr. John Doull had stated:

> [W]e criticize modeling because we say that it ignores the relevant biology. Here we have, if anyplace, we have relevant biology, and gee whiz, if we can't make it work with methylene chloride, we're in deep trouble.[10]

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[8] Letter to Lee M. Thomas, EPA Administrator, from Drs. Nelson, Griesemer, and Doull, EPA Science Advisory Board (March 19, 1988).

[9] See, e.g., New Data Indicate Methylene Chloride Not Carcinogenic, Some SAB Members Say, Chem. Reg. Rep. (BNA) 11:862 (Aug. 14, 1987).

[10] Transcript, Science Advisory Board Review of Draft Addendum to the Health Assessment Document for Methylene Chloride (May 23, 1985), at 86.

In response to similar statements from scientists and regulators, a major research program was undertaken in late 1985 by the European Council of Chemical Manufacturer's Federation (CEFIC) and by Dow Chemical U.S.A. in an attempt to investigate the strikingly different responses seen in various long-term bioassays of methylene chloride. This research program has cost several million dollars and taken over two years of effort. The results have been published as rapidly as possible, and use of the information in risk assessment has been recommended by a National Academy of Sciences Workshop, praised by the FDA official responsible for methylene chloride risk assessment as an "interesting and useful . . . approach," and recognized by a broad scientific consensus as an important step forward.[11] In light of the widespread review of this scientific research, the willingness of EPA to make use of the data, and the fact that HSIA and CEFIC have made every effort to ensure that DHS staff receive all relevant information as soon as it is available, the failure to make use of the data in the DHS health effects assessment seems inexplicable.[12]

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[11] See, e.g., Pharmacokinetics Seen Reducing Risk Assessment Uncertainties, Food Chemical News at 8-10 (Mar. 14, 1988) (report of Toxicology Forum meeting); Physiologically-Based Pharmacokinetic Risk Assessment Model Hailed, Food Chemical News at 29-31 (Feb. 24, 1986) (same).

[12] We note also that DHS staff apparently had no reservations about using data in a draft NTP report to support its position on pancreatic tumors, discussed above, even though the report was formally withdrawn after peer review. In light of this, it is difficult to understand an unwillingness to use the analysis in a draft EPA report that has been approved after peer review and will shortly be published.

Conclusion

The foregoing discussion addresses only two of the most serious shortcomings in the DHS health effects assessment. In addition, the Board and the Panel should give careful consideration to the regulatory and science policy consequences of approving a health effects evaluation that disregards available data and calculates risk estimates at significant variance from estimates that have been scientifically peer reviewed and will form the basis for federal regulatory evaluations of methylene chloride. Continued reliance on an archaic approach to risk assessment will set DHS staff apart from the scientific community and could hinder the Board's ability to make rational regulatory decisions. The Panel should conclude that the draft health effects assessment is "seriously deficient" for purposes of Health and Safety Code § 39661(c).

Attachment

II. Air Resources Board Responses to Comments

1) The Halogenated Solvents Industry Alliance

a) Comment: The letter soliciting comment on the final draft report states that any revisions to the DHS staff health effects assessment (Part B) will be incorporated into the report after the comment period and before review by the Panel. This highly unusual procedure for peer reviewing a health effects assessment would seem to be inconsistent with Health and Safety Code Section 39660, which requires that "the report, together with the scientific data on which the report is based, shall ... be made available to the public and shall be formally reviewed by the Scientific Review Panel" (emphasis added). The law does not provide for one draft of the report to be made available for public review and a later, different version to be reviewed by the Panel. We submit that the Board could not sustain a decision to identify methylene chloride as a toxic air contaminant based on a health effects assessment that the public was not allowed to review prior to Panel consideration.

Response: See section IV for ARB's July 1, 1988 letter to HSIA regarding their comments.

III. Department of Health Services Responses to Comments

DEPARTMENT OF HEALTH SERVICES
STAFF RESPONSES TO PUBLIC COMMENTS
ON THE DRAFT REPORT TO THE AIR RESOURCES BOARD
ON METHYLENE CHLORIDE:

SECOND COMMENT PERIOD

General Comment

Comment: Several comments were made by the Halogenated Solvents Industry Alliance (HSIA) with regard to the procedures in submitting the draft report for public comment. These procedural questions are under the jurisdiction of the Air Resources Board. However, one of HSIA's comments expressed concerns that Part B had not been revised after the first public review, which requires a response from Department of Health Services' (DHS) staff.

Response: As indicated in the previous DHS staff responses to comments, the presentation of the epidemiologic studies would be revised to provide better clarity as requested by Kodak. Only minor changes (such as the numerical error noted in the Kodak comment) were to be made in response to the first comment period. Since no substantial changes were to be made in response to the first public review period, a revised draft was not submitted.

TOPIC: Risk Assessment

Comment: The DHS response during the first comment period reported that a draft National Toxicology Program (NTP, 1982) study found pancreatic tumors in rats and that this was evidence for the biological plausibility of pancreatic tumor induction in humans by methylene chloride. The report in which these findings were published was subsequently canceled by its sponsoring agency (the National Toxicology Program), based on discrepancies in experimental data. In light of this, it is difficult to understand the unwillingness of DHS to use information in draft U.S. Environmental Protection Agency (EPA) reports (the June 1987 Draft Technical Analysis of New Methods and Data Regarding Dichloromethane Hazard Assessments, and the July 1987 Draft Addendum to the Health Assessment Document for Dichloromethane: Pharmacokinetics, Mechanism of Action and Epidemiology). The NTP report was withdrawn after it was peer reviewed, and the EPA reports have been approved by peer reviewers. (HSIA)

Response: The draft results of the NTP gavage study are not central to DHS' conclusions regarding biological plausibility. Methylene chloride has been shown to be carcinogenic in two animal species at several sites. These tumors include salivary gland sarcomas in rats (Burek et al. 1984), mammary gland tumors in rats (Burek et al. 1984, NTP 1986), combined liver tumors in rats (Serota et al. 1986a), hepatocellular adenomas and carcinomas in mice (Serota et al. 1986b, NTP 1986), and alveolar/bronchiolar adenomas and carcinomas in mice (NTP 1986). DHS staff concluded that the induction of pancreatic tumors by methylene chloride in humans is biologically plausible based on the lack site specificity in rodents. The Kodak comment (page 13) in the first public response period indicated that since rodents tumors had never been found in the pancreas resulting from methylene chloride exposure, there was no biological plausibility for pancreatic tumor induction. The

DHS staff comment merely pointed out that such a position is very weak due to the lack of site concordance and since a draft NTP study had indicated the pancreas as a potential tumor site. However, the fact that the NTP report has been withdrawn underscores the weakness in using any draft report (including the 1987 EPA reports, "Draft Technical Analysis of New Methods and Data Regarding Dichloromethane Hazard Assessments," and "Draft Addendum to the Health Assessment Document for Dichloromethane: Pharmacokinetics, Mechanism of Action and Epidemiology") to support a scientific argument. This would appear to be particularly important when the document has the words "Do not cite or quote" imprinted on it, as do the above-mentioned EPA documents; furthermore EPA staff had specifically asked DHS staff not to cite the reports since they were not final. However, subsequent to the first comment period EPA staff indicated that since much of the information in the document had been presented at scientific meetings and is present in a Consumer Product Safety Commission report (Cohn 1987), the document could be cited as a draft. DHS staff have evaluated the information in the EPA draft reports on its own merits. As a result, DHS staff have incorporated much of the EPA draft analysis into the current document. Consequently, a risk estimate based on the EPA draft report is included in the DHS document and reference is made to the EPA documents to acknowledge their source.

Comment: The DHS health effects assessment transformed an epidemiology study showing no indication of increased overall cancer risk "into evidence for the staff's position that methylene chloride causes cancer in humans." The DHS analysis is based on an observed excess of pancreatic cancer. Note, however, that there were several sites (colon-rectum, genito-urinary) where a result of the same magnitude was seen, but in the opposite direction. Observed-expected differences of this type can occur by chance alone. On this point, the DHS staff health effects assessment is inconsistent with EPA's evaluation of methylene chloride and the overwhelming current scientific consensus. (HSIA)

Response: The DHS health effects assessment document concurs with the EPA in concluding that human data for carcinogenicity are inadequate. The Hearne et al. (1987) study reported an excess in pancreatic malignancy deaths and this result is reported in the DHS health effects assessment document. As discussed in the health efffects document, and in the following comment, DHS staff used the reported excess of pancreatic cancer in its comparison of risk estimates from animal and human exposure. Such comparisons can be made even when there is no excess cancer risk observed by taking into account the statistical power of the epidemiological study.

Comment: The DHS health effects assessment employs an unusual interpretation of an epidemiological study in order to find it to be consistent with human risk estimates based on animal data. The interpretation is unusual in that it ignores the overall mortality results and restricts the analysis to the only site (pancreas) showing an increased number of cancer deaths. (HSIA)

Response: Hearne et al. (1987) and DHS staff both conducted comparisons of human and animal risk estimates. The differences in the procedures for comparing human and animal risks are spelled out in the DHS health effects document on methylene chloride. In the DHS staff analysis, the most sensitive animal site was compared to the human site exhibiting the greatest

response in the epidemiologic study. Use of all primary cancer sites to evaluate a specific carcinogen will dilute any effect that might observed, since individual chemical compounds affect certain sites more than others. Furthermore, in light of the high background rates of some cancers in humans and other animals, combining chemically induced and background sites would substantially decrease the sensitivity of the analysis. A similar type of analysis was conducted by EPA and Consumer Products Safety Commission (EPA 1987a) where they concluded (page 102): "Even if the pancreatic cancer deaths are discounted, the 95% upper-limit estimates based on lung tumors from the epidemiologic analysis ... are comparable to the upper-limit estimates derived from the mouse metabolized dose data."

Comment: The DHS staff declines to make use of extensive data on the metabolism, pharmacokinetics, and mechanism of methylene chloride that are currently available and has been provided to DHS staff. As a result, the DHS staff health effects assessment is inconsistent with EPA's draft evaluation of methylene chloride which makes use of the published and unpublished data provided to them. HSIA sponsored a seminar on this research for the benefit of DHS staff. The European Council of Chemical Manufacturers Federation (CEFIC) has undertaken an intense, costly investigation of the striking differences among the results of various long-term bioassays of methylene chloride. Use of information from this investigation has been recommended by a National Academy of Sciences Workshop and recognized by a broad scientific consensus as an important step forward. This scientific research has been widely reviewed, the EPA is willing to make use of the data, and HSIA and CEFIC have made every effort to ensure that DHS staff receive all relevant information as soon as it is available. In light of the above, the failure to make use of the data in the DHS health effects assessment seems inexplicable. Furthermore, a Scientific Review Panel member has requested that future documents include a section on pharmacokinetics. (HSIA)

Response: As indicated in the health effects document and in the response to a similar question in the first comment period (page 9), risk estimates based on pharmacokinetic data were calculated in the document. However, the DHS staff did not incorporate the information into the final risk assessment due to the numerous assumptions required and the extensive use of unpublished data in the model. Since the first comment period, much of the data has been published or extensively reviewed by independent scientific groups and DHS staff have reassessed the use of the model. After critical review of the pharmacokinetic information submitted by HSIA, DHS staff concluded that application of pharmacokinetics to calculate the internal dose of the sustance represented a plausible scientific approach for evaluating the cancer risks of methylene chloride. As discussed extensively in the document, DHS staff still have many reservations regarding the use of the Andersen et al. (1987) model, particularly since the pharmacokinetic adjustment may add uncertainty to the final potency values due to the additional assumptions necessary to estimate the dose. These assumptions include the following: the identification of the methylene chloride-glutathione conjugate as the sole metabolite responsible for carcinogenicity in laboratory animals and humans; the relative tissue distribution of glutathione and metabolic activities in laboratory animals versus humans; the relative tissue susceptibility to carcinogenic methylene chloride metabolites; the use of *in vitro* constants and acute metabolic values for

the prediction of chronic responses; and the use of estimates of physiologic parameters, partition coefficients, and metabolic constants that may not accurately reflect the actual human values. Substantial additional research is needed to externally validate the pharmacokinetic model and to determine the extent of human variability in the pharmacokinetics and pharmacodynamics of methylene chloride and its metabolites. However, DHS staff believe that pharmacokinetic modeling represents a scientifically plausible approach to evaluating the internal dose. Consequently, pharmacokinetic information submitted by HSIA was used in the final risk assessment range presented by DHS staff in Part B.

REFERENCES

ANDERSEN, M.E., CLEWELL, M.J., III, GARGAS, M.L., SMITH, F.A., and REITZ, R.H. 1987. Physiologically-based Pharmacokinetics and the Risk Assessment Process for Methylene Chloride. Toxicol. Appl. Pharmacol. 87:185-205

BUREK, J.D., NITSCHKE, K.D., BELL, T.J., WACKERLE, D.L., CHILDS, R.C., BEYER, J.E., DITTENBER, D.A., RAMPY, L.W., and McKENNA, M.J. 1984. Methylene chloride: A two year inhalation toxicity and oncogenicity study in rats and hamsters. Fundam. Appl. Toxicol. 4:30-47

COHN, M.S. 1987. Updated risk assessment for methylene chloride (dichloromethane). In: Methylene chloride briefing package for U. S. Consumer Products Safety Commission, Washington, D. C.

ENVIRONMENTAL PROTECTION AGENCY. 1987a. External Review Draft, Technical analysis of new methods and data regarding dichloromethane. EPA 600-8-8-7/029A.

ENVIRONMENTAL PROTECTION AGENCY (EPA). 1987b. External Review Draft, Update to the Health Assessment Document and Addendum for Dichloromethane (Methylene Chloride): Pharmacokinetics, Mechanism of Action, and Epidemiology. EPA 600-8-87/030A.

HEARNE, F.T., GROSE, R., PIFER, J.W., FRIEDLANDER, B.R., and RALEIGH, R.L. 1987. Methylene Chloride Mortality Study: Dose response to characterization and animal model comparison. J. Occup. Med. 29:217-228

NATIONAL TOXICOLOGY PROGRAM (NTP). 1982. Draft technical report on the carcinogenesis bioassay of dichloromethane (methylene chloride), gavage study. Research Triangle Park, NC, and Bethesda, Maryland

NATIONAL TOXICOLOGY PROGRAM (NTP). 1986. Toxicology and Carcinogenesis Studies of Dichloromethane (Methylene Chloride) (CAS No. 75-09-2) in F344/N Rats and B6C3F1 Mice (Inhalation Studies). TR No. 306, NIH (USDHHS, PHS, NIH) Publication No. 86-2562

SEROTA, D.G., THAKAR, A.K., ULLAND, B.M., KIRSCHMAN, J.C., BROWN, N.M., COOTS, R.H. and MORGANEIDGE, K. 1986a. A two year drinking water study of dichloromethane on rodents: I. Rats Food Chem. Toxicol. 24:951-958

SEROTA, D.G., THAKAR, A.K., ULLAND, B.M., KIRSCHMAN, J.C., BROWN, N.W.M, COOTS, R.H. and MORGAREIGE, K. 1986b. A two year drinking water study of dichloromethane on rodents: II Mice. Food Chem. Toxicol. 24:959-963

IV. Air Resources Board Letters to Comment Originators

TE OF CALIFORNIA

GEORGE DEUKMEJIAN, Governor

R RESOURCES BOARD
2 Q STREET
. BOX 2815
D··ENTO, CA 95812

July 1, 1988

W. Caffey Norman, III
Heron, Burchette, Ruckert & Rothwell
Suite 700
1025 Thomas Jefferson Street, N.W.
Washington, D.C. 20007

Dear Mr. Norman:

Thank you for providing HSIA's comments on the final draft report on methylene chloride. HSIA's comments on Part B have been forwarded to the Department of Health Services (DHS). DHS staff are preparing responses which will be incorporated as an addendum to Part C of the report submitted to the Scientific Review Panel (SRP).

In addition to commenting on Part B, HSIA requested the opportunity to appear before the SRP when it reviews Parts A and B of the methylene chloride report. We have discussed your request with Dr. James Kendrick, Chair of the Scientific Review Panel. Dr. Kendrick supports the SRP's long-standing policy of not accepting written or oral testimony at SRP meetings. The SRP decided that by requiring public comment and agency responses in advance of the submission of the report to the SRP, the SRP's review would be both comprehensive and timely. The ARB Legal Office has advised the SRP that its process meets the statutory requirements set forth in Health and Safety Code Section 39661. The SRP receives the information submitted by the public, as all comments received regarding the substance under review and the report are included in Part C. Additionally, the report which is reviewed by the SRP is available to the public.

Before making a recommendation as to whether methylene chloride should be identified as a TAC, the SRP will carefully consider all comments that have been received. They will also evaluate the adequacy of ARB and DHS responses to comments as well as revisions to the report. The Panel may request further clarification if it believes this is necessary. There will also be an opportunity to testify before the Board when it considers the Panel's findings.

If you have any questions regarding this letter, please call me at (916) 322-7072.

Sincerely,

Robert D. Barham, Chief
Toxic Air Contaminant
Identification Branch

cc: Dr. Kendrick, Chairman
Scientific Review Panel
David Nawi
Bill Lockett
Peter Venturini

Appendix D.3 Chronic RELs and toxicity summaries using the previous version of the Hot Spots Risk Assessment guidelines (OEHHA 1999)

Acrylonitrile 11
Ammonia 19
Benzene 24
Beryllium and Beryllium Compounds 35
1,3-Butadiene 48
Cadmium and Cadmium Compounds 59
Carbon Disulfide 66
Carbon Tetrachloride 81
Chlorinated Dibenzo-*p*-Dioxins and Chlorinated Dibenzofurans 90
Chlorine 106
Chlorine Dioxide 114
Chloroform 124
Chromium, Hexavalent (Soluble Compounds) 138
Cresol Mixtures 157
1,4-Dichlorobenzene 165
1,1-Dichloroethylene 170
Diethanolamine 177
N,N-Dimethylformamide 184
1,4-Dioxane 189
Epichlorohydrin 196
1,2-Epoxybutane 200
Ethyl Chloride 204
Ethylbenzene 208
Ethylene Dibromide 214
Ethylene Dichloride 226
Ethylene Glycol 234
Ethylene Glycol Monoethyl Ether 240
Ethylene Glycol Monoethyl Ether Acetate 248
Ethylene Glycol Monomethyl Ether 253
Ethylene Glycol Monomethyl Ether Acetate 260
Ethylene Oxide 264
Fluorides *including* Hydrogen Fluoride 270
Glutaraldehyde 286
n-Hexane 292
Hydrazine 303
Hydrogen Chloride 309
Hydrogen cyanide 313
Hydrogen Sulfide 321
Isophorone 329
Isopropanol 337
Maleic Anhydride 348
Methanol 354
METHYL BROMIDE 360
Methyl Chloroform 366
Methyl Isocyanate 372
Methyl *t*-Butyl Ether 383

Methylene Chloride 389
4,4′-Methylene Dianiline 399
Methylene Diphenyl Isocyanate 407
Naphthalene 413
Nickel and Nickel Compounds: Nickel Oxide 420
Phenol 429
Phosphine 437
Phosphoric Acid 446
Phthalic Anhydride 453
Propylene 460
Propylene Glycol Monomethyl Ether 466
Propylene oxide 471
Selenium and Selenium Compounds 476
SILICA (CRYSTALLINE, RESPIRABLE) 486
Styrene 534
Sulfuric Acid 551
Toluene 560
2,4- and 2,6-Toluene Diisocyanate 574
Trichloroethylene 581
Triethylamine 590
Vinyl Acetate 596
Xylenes 602

Table of Chronic RELs determined using the previous Hot Spots Risk Assessment Guidelines (OEHHA 1999)

Substance (CAS #)	Listed in CAPCOA (1993)	Chronic Inhalation REL ($\mu g/m^3$)	Oral REL (mg/kg Body Weight)	Hazard Index Target Organs	Human Data
Acrylonitrile (107-13-1)	☑	5		Respiratory system	
Ammonia (7664-41-7)		200		Respiratory system	
Benzene (71-43-2)		60		Hematopoietic system; development; nervous system	
Beryllium (7440-41-7) and beryllium compounds	☑	0.007	0.002	Respiratory system; immune system	☑
Butadiene (106-99-0)		20		Reproductive system	
Cadmium (7440-43-9) & cadmium compounds	☑	0.02	0.0005	Kidney; respiratory system	☑
Carbon tetrachloride (56-23-5)	☑	40		Alimentary system; development; nervous system	
Carbon disulfide (75-15-0)		800		Nervous system; reproductive system	☑
Chlorinated dioxins (1746-01-6) & dibenzofurans (5120-73-19)		0.00004	1×10^{-8}	Alimentary system (liver); reproductive system; development; endocrine system; respiratory system; hematopoietic system	
Chlorine (7782-50-5)		0.2		Respiratory system	

Table of Chronic RELs determined using the previous Hot Spots Risk Assessment Guidelines (OEHHA 1999)

Substance (CAS #)	Listed in CAPCOA (1993)	Chronic Inhalation REL ($\mu g/m^3$)	Oral REL (mg/kg Body Weight)	Hazard Index Target Organs	Human Data
Chlorine dioxide (10049-04-4)		0.6		Respiratory system	
Chlorobenzene (108-90-7)	☑	1000		Alimentary system; kidney; reproductive system	
Chloroform (67-66-3)	☑	300		Alimentary system; kidney; development	
Chloropicrin (76-06-2)	☑	0.4		Respiratory system	
Chromium hexavalent: soluble except chromic trioxide	☑	0.2	0.02	Respiratory system	
Chromic trioxide (as chromic acid mist)	☑	0.002		Respiratory system	☑
Cresol mixtures (1319-77-3)	☑	600		Nervous system	
Dichlorobenzene (1,4-) (106-46-7)	☑	800		Nervous system; respiratory system; alimentary system; kidney	
Dichloroethylene (1,1) (75-35-4)	☑	70		Alimentary system	
Diesel Exhaust*		5		Respiratory system	
Diethanolamine (111-42-2)		3		Cardiovascular system; nervous system	
Dimethylformamide (N,N-) (68-12-2)		80		Alimentary system ; respiratory system	☑

Table of Chronic RELs determined using the previous Hot Spots Risk Assessment Guidelines (OEHHA 1999)

Substance (CAS #)	Listed in CAPCOA (1993)	Chronic Inhalation REL ($\mu g/m^3$)	Oral REL (mg/kg Body Weight)	Hazard Index Target Organs	Human Data
Dioxane (1,4-) (123-91-1)	. .	3,000		Alimentary system; kidney; cardiovascular system	
Epichlorohydrin (106-89-8)	☑	3		Respiratory system; eyes	
Epoxybutane (1,2-) (106-88-7)		20		Respiratory system; cardiovascular system	
Ethylbenzene (100-41-4)		2,000		Development; alimentary system (liver); kidney; endocrine system	
Ethyl chloride (75-00-3)	. .	30,000		Development; alimentary system	
Ethylene dibromide (106-93-4)	☑	0.8		Reproductive system	☑
Ethylene dichloride (107-06-2)	☑	400		Alimentary system (liver)	
Ethylene glycol (107-21-1)		400		Respiratory system; kidney; development	☑
Ethylene glycol monoethyl ether (110-80-5)	. .	70		Reproductive system; hematopoietic system	
Ethylene glycol monoethyl ether acetate (111-15-9)	. .	300		Development	
Ethylene glycol monomethyl ether (109-86-4)	. .	60		Reproductive system	
Ethylene glycol monomethyl ether acetate (110-49-6)	. .	90		Reproductive system	

Table of Chronic RELs determined using the previous Hot Spots Risk Assessment Guidelines (OEHHA 1999)

Substance (CAS #)	Listed in CAPCOA (1993)	Chronic Inhalation REL ($\mu g/m^3$)	Oral REL (mg/kg Body Weight)	Hazard Index Target Organs	Human Data
Ethylene oxide (75-21-8)	☑	30		Nervous system	
Fluoride including Hydrogen Fluoride		13 F 14 HF	0.04	Bone and teeth; respiratory system	☑
Glutaraldehyde (111-30-8)	☑	0.08		Respiratory system	
Hexane (n-) (110-54-3)		7000		Nervous system	
Hydrazine (302-01-2)	☑	0.2		Alimentary system; endocrine system	
Hydrogen chloride (7647-01-0)	. .	9		Respiratory system	
Hydrogen cyanide (74-90-8)	. .	9		Nervous system; endocrine system; cardiovascular system	☑
Hydrogen sulfide (7783-06-4)	. .	10		Respiratory system	
Isophorone (78-59-1)		2000		Development; liver	
Isopropanol (67-63-0)		7,000		Kidney; development	
Maleic anhydride (108-31-6)	☑	0.7		Respiratory system	
Methanol (67-56-1)	. .	4,000		Development	
Methyl bromide (74-83-9)	. .	5		Respiratory system; nervous system; development	
Methyl chloroform (71-55-6)	. .	1,000		Nervous system	

Table of Chronic RELs determined using the previous Hot Spots Risk Assessment Guidelines (OEHHA 1999)

Substance (CAS #)	Listed in CAPCOA (1993)	Chronic Inhalation REL ($\mu g/m^3$)	Oral REL (mg/kg Body Weight)	Hazard Index Target Organs	Human Data
Methyl isocyanate (624-83-9)		1		Respiratory system; reproductive system	
Methyl t-butyl ether (1634-04-4)		8,000		Kidney; eyes; alimentary system (liver)	
Methylene chloride (75-09-2)	. .	400		Cardiovascular system; nervous system	. .
Methylene dianiline (4,4'-) (101-77-9)	☑	20		Eyes; alimentary system (hepatotoxicity)	
Methylene Diphenyl Isocyanate (101-68-8)		0.7		Respiratory system	
Naphthalene (91-20-3)	. .	9		Respiratory system	
Nickel & compounds (except nickel oxide)	. .	0.05	0.05	Respiratory system; hematopoietic system	
Nickel oxide (1313-99-1)		0.1	?	Respiratory system; hematopoietic system	
Phenol (108-95-2)	. .	200		Alimentary system; cardiovascular system; kidney; nervous system	
Phosphine (7803-51-2)	☑	0.8		Respiratory system; alimentary system; nervous system; kidney; hematopoietic system	

Table of Chronic RELs determined using the previous Hot Spots Risk Assessment Guidelines (OEHHA 1999)

Substance (CAS #)	Listed in CAPCOA (1993)	Chronic Inhalation REL ($\mu g/m^3$)	Oral REL (mg/kg Body Weight)	Hazard Index Target Organs	Human Data
Phosphoric acid (7664-38-2)		7		Respiratory system	
Phthalic anhydride (85-44-9)	☑	20		Respiratory system	☑
Propylene (115-07-1)		3,000		Respiratory system	
Propylene glycol monomethyl ether (107-98-2)		7,000		Alimentary system (liver)	
Propylene oxide (75-56-9)	☑	30		Respiratory system	
Selenium and selenium compounds (other than hydrogen selenide)	☑	20	0.005	Alimentary system; cardiovascular system; nervous system	☑
Silica (crystalline, respirable)		3		Respiratory system	☑
Styrene (100-42-5)	☑	900		Nervous system	☑
Sulfuric acid (7664-93-9)	▪	1		Respiratory system	
Tetrachloroethylene* (perchloroethylene) (127-18-4)	☑	35		Kidney; alimentary system (liver)	
Toluene (108-88-3)	☑	300		Nervous system; respiratory system; development	
Toluene diisocyanates (2,4- & 2,6-)	☑	0.07		Respiratory system	☑
Trichloroethylene (79-01-6)	☑	600		Nervous system; eyes	☑
Triethylamine (121-44-8)		200		Eyes	
Vinyl acetate (108-05-4)		200		Respiratory system	

Table of Chronic RELs determined using the previous Hot Spots Risk Assessment Guidelines (OEHHA 1999)

Substance (CAS #)	Listed in CAPCOA (1993)	Chronic Inhalation REL ($\mu g/m^3$)	Oral REL (mg/kg Body Weight)	Hazard Index Target Organs	Human Data
Xylenes (m-, o-, p-)	☑	700		Nervous system; respiratory system	☑

*These peer-reviewed values were developed under the Toxic Air Contaminant (TAC) Program mandated by AB1807.

CHRONIC TOXICITY SUMMARY

ACRYLONITRILE

(Acrylonitrile monomer, cyanoethylene, propenenitrile, 2-propenenitrile, VCN, vinyl cyanide.)

CAS Number: 107-13-1

I. Chronic Toxicity Summary

Inhalation reference exposure level	**5 µg/m^3** (2 ppb)
Critical effect(s)	Degeneration and inflammation of nasal epithelium in rats
Hazard index target(s)	Respiratory system

II. Chemical Property Summary (HSDB, 1994)

Description	Clear, colorless to pale yellow liquid (technical grades)
Molecular formula	C_3H_3N
Molecular weight	53.1 g/mol
Density	0.81 g/cm^3 @ 25°C
Boiling point	77.3^{o}C
Melting point	–82°C
Vapor pressure	100 torr @ 23°C
Solubility	Soluble in isopropanol, ethanol, ether, acetone, and benzene
Conversion factor	1 ppm = 2.17 mg/m^3 @ 25 ^{o}C

III. Major Uses or Sources

Acrylonitrile is produced commercially by propylene ammoxidation, in which propylene, ammonia, and air are reacted by catalyst in a fluidized bed. Acrylonitrile is used primarily as a co-monomer in the production of acrylic and modacrylic fibers. Uses include the production of plastics, surface coatings, nitrile elastomers, barrier resins, and adhesives. It is also a chemical intermediate in the synthesis of various antioxidants, pharmaceuticals, dyes, and surface-active agents. Formerly, acrylonitrile was used as a fumigant for food commodities, flour milling, and bakery food processing equipment (HSDB, 1994). The annual statewide industrial emissions from facilities reporting under the Air Toxics Hot Spots Act in California based on the most recent inventory were estimated to be 3948 pounds of acrylonitrile (CARB, 2000). US EPA (1993) reported a mean ambient air concentration of acrylonitrile at four urban locations in the U.S.of 0.66 µg/m^3.

IV. Effects of Human Exposure

Many occupational epidemiology studies have investigated retrospectively the morbidity and mortality of acrylonitrile exposed workers. An increased incidence of lung cancer was associated with acrylonitrile exposure. No significant excess mortality has been observed for any noncarcinogenic endpoint. One early cross-sectional study (Wilson *et al.*, 1948) observed multiple deleterious effects in synthetic rubber manufacturing workers acutely exposed (20 to 45 minutes) to various concentrations of acrylonitrile (16 to 100 ppm, 34.7 to 217 mg/m^3). Mucous membrane irritation, headaches, feelings of apprehension, and nervous irritability were observed in the majority of workers. Other less common symptoms observed included low-grade anemia, leukocytosis, and mild jaundice. These effects were reported to subside with cessation of exposure. Human volunteers exposed for a single 8 hour period to acrylonitrile vapors exhibited no deleterious CNS effects at concentrations ranging from 5.4 to 10.9 mg/m^3 (2.4 to 5.0 ppm) (Jakubowski *et al.*, 1987).

A cross-sectional study (Sakurai *et al.*, 1978) found no statistically significant increases in adverse health effects in chronically exposed workers (minimum 5 years) employed at 6 acrylic fiber factories (n = 102 exposed, n = 62 matched controls). Mean acrylonitrile levels ranged from 0.1 to 4.2 ppm (0.2 to 9.1 mg/m^3) as determined by personal sampling. Although not statistically significant, slight increases in reddening of the conjunctiva and pharynx were seen in workers from the plant with the highest mean levels (4.2 ppm arithmetic mean). However, this study has limitations, including small sample size and examiner bias, since the medical examiner was not blind to exposure status. The time-weighted average exposure of the group occupationally exposed to 4.2 ppm (9.1 mg/m^3) acrylonitrile can be calculated as: TWA = 9.1 mg/m^3 x (10/20) m^3/day x 5 days/7 days = 3 mg/m^3. This level is comparable to the LOAEL (HEC) of 2 mg/m^3 derived by the U.S. EPA from the animal study of Quast *et al*. (1980).

Czeizel *et al*. (1999) studied congenital abnormalities in 46,326 infants born between 1980 and 1996 to mothers living within a 25 km radius of an acrylonitrile factory in Nyergesujfalu, Hungary. Ascertainment of cases with congenital abnormalities was based on the Hungarian Congenital Abnormality Registry plus review of pediatric, pathology and cytogenetic records. Particular attention was paid to indicators of germinal mutations (sentinel anomalies, Down's syndrome, and unidentified multiple congenital abnormalities) and to indicators of teratogens (specific pattern of multiple congenital abnormalities). Three congenital abnormalities: pectus excavatum in Tata, 1990-1992 (OR = 78.5, 95%CI = 8.4-729.6), undescended testis in Nyergesujfalu between 1980 and 1983 (8.6, 1.4-54.3) and in Esztergom, 1981-1982 (4. 2, 1.3-13.5) and clubfoot in Tata, 1980-1981 (5.5, 1.5-20.3) showed significant time-space clusters in the study area. The risk of undescended testis decreased with increasing distance from the factory. An unusual increase for the combination of oral cleft and cardiac septal defects was seen in multimalformed babies in Tatabanya in 1990. Unfortunately there were no data on levels of acrylonitrile or any other exposure.

V. Effects of Animal Exposure

Quast *et al.* (1980) exposed Sprague-Dawley rats (100/sex/ concentration) 6 hours/day, 5 days/week for 2 years to concentrations of 0, 20, or 80 ppm acrylonitrile vapors (0, 43, or 174 mg/m^3). A statistically significant increase in mortality was observed in the first year among 80 ppm exposed rats (male and female). Additionally, the 80 ppm exposed group had a significant decrease in mean body weight. Two tissues, the nasal respiratory epithelium and the brain, exhibited treatment-related adverse effects due to acrylonitrile exposure. Proliferative changes in the brain glial cells (i.e., tumors and early proliferation suggestive of tumors) were significantly increased in the 20 ppm (8/100) and 80 ppm (20/100) females versus female controls (0/100), and in the 80 ppm males (22/99) versus male controls (0/100). Noncarcinogenic, extrarespiratory effects were observed in the nasal turbinate epithelium at both exposure concentrations, 20 and 80 ppm (see table below). Thus the LOAEL was 20 ppm. No treatment-related effects in the olfactory epithelium, trachea, or lower respiratory epithelium were observed at either concentration.

Effects of acrylonitrile reported by Quast *et al*. (1980)

Effect	*Sex*	0 ppm	20 ppm	80 ppm
Respiratory epithelium hyperplasia in the nasal turbinates	Male	0/11	4/12	10/10*
Hyperplasia of the mucous secreting cells	Male	0/11	7/12*	8/10*
Focal inflammation in the nasal turbinates	Female	2/11	6/10	7/10*
Flattening of the respiratory epithelium of the nasal turbinates	Female	1/11	7/10*	8/10*
Lung: pneumonia, consolidation, atelectasis, or edema	Male	14/100	27/100*	30/100*
Lung: pneumonia, consolidation, atelectasis, or edema	Female	7/100	2/100	7/100

* statistically significant difference from controls ($p<.05$)

Maltoni and associates exposed Sprague-Dawley rats (30/sex/concentration) to 0, 5, 10, 20, or 40 ppm acrylonitrile vapor for 5 days/week over 52 weeks, and at 60 ppm for 4 to 7 days, 5 days/week for 104 weeks (Maltoni *et al.*, 1977; Maltoni *et al.*, 1988). Histopathologic examinations were performed, including on lungs, brain, kidney, and liver. No noncarcinogenic effects were reported.

Gagnaire et al. (1998) studied motor and sensory conduction velocities (MCV and SCV, respectively) and amplitudes of the sensory and motor action potentials (ASAP and AMAP) of the tail nerve in male Sprague-Dawley rats during chronic treatment with acrylonitrile. (Four other unsaturated aliphatic nitriles were also given orally to other rats.) Rats were given doses of 12.5, 25, and 50 mg/kg of acrylonitrile once a day, 5 days per week for 12 weeks. Rats were also exposed by inhalation to 25, 50, and 100 ppm of acrylonitrile vapors for 6 h/day, 5 days per week, for 24 weeks and neurophysiological examinations were carried out. After oral acrylonitrile, animals developed behavioral sensitization characterized by salivation, locomotor hyperactivity, and moderately intense stereotypies. Rats dosed with 50 mg/kg developed hindlimb weakness associated with decreases in sensory conduction velocity (SCV) and in the amplitude of the sensory action potential (ASAP). Rats exposed to acrylonitrile by inhalation exhibited time- and concentration-dependent decreases in motor conduction velocity (MCV), SCV, and ASAP, which were partially reversible after 8 weeks of recovery. The authors concluded that the nervous system of the rat appears to be a target following either oral or inhalation exposures of acrylonitrile. The NOAEL by inhalation for 24 weeks was 25 ppm.

Changes in electrophysiological parameters after 24 wks of exposure (Gagnaire et al., 1998)

Acrylonitrile	MCV (m/sec)	SCV (m/sec)	AMAP (mvolts)	ASAP (μvolts)
0 ppm	42.9 ± 0.9[a]	53.3 ± 1.0	17.8 ± 1.2	186 ± 8
25 ppm	41.6 ± 0.8	50.5 ± 0.8*	16.1 ± 0.8	164 ± 11
50 ppm	38.1 ± 0.9**	49.1 ± 0.5***	15.7 ± 1.0	159 ± 5*
100 ppm	38.5 ± 1.2**	48.4 ± 1.0***	17.4 ± 0.9	133 ± 11***

[a] Mean ± SEM; * $p<0.05$; ** $p<0.01$; ***$p<0.001$

In a developmental study, Murray *et al.* (1978) exposed rats to acrylonitrile vapors at 0, 40 ppm (87 mg/m^3), or 80 ppm (174 mg/m^3) for 6 hours/day during gestational days 6 to 15. In the 80 ppm exposed group, significant increases in fetal malformations were observed including short tail, missing vertebrae, short trunk, omphalocoele, and hemivertebra (Murray *et al.*, 1978). No differences in implantations, live fetuses, or resorptions were seen in the exposed (40 and 80 ppm) versus the control group. Maternal toxicity was observed as decreased body weight at both exposure levels. After adjustment to continuous exposure, this study identified a developmental NOAEL of 10 ppm and a LOAEL of 20 ppm (with maternal toxicity).

Saillenfait *et al*. (1993) studied the developmental toxicity of eight aliphatic mononitriles in Sprague-Dawley rats after inhalation exposure for 6 hr/day during days 6 to 20 of gestation. The range of exposure levels for acrylonitrile was 12, 25, 50, and 100 ppm; group sizes were 20-23 females. Embryolethality was observed after exposure to 25 ppm (54 mg/m^3) acrylonitrile in the presence of overt signs of maternal toxicity. Fetal weights were significantly lower at 25 ppm. Thus 12 ppm (26 mg/m^3) is a NOAEL for developmental toxicity using this study design.

VI. Derivation of Chronic Reference Exposure Level

Study	Quast *et al.*, 1980
Study population	Sprague-Dawley rats (100/sex/concentration)
Exposure method	Discontinuous whole-body inhalation exposures (0, 20, or 80 ppm)
Critical effects	Degeneration and inflammation of nasal respiratory epithelium; hyperplasia of mucous secreting cells
LOAEL	20 ppm
NOAEL	Not observed
BMC_{05}	1.5 ppm
Exposure continuity	6 hours/day, 5 days/week
Average experimental exposure	0.27 ppm for BMC_{05} (1.5 x 6/24 x 5/7)
Human equivalent concentration	0.067 ppm (gas with extrathoracic respiratory effects; RGDR = 0.25 based on MV = 0.33 m^3/day, SA(ET) = 11.6 cm^2)
Exposure duration	2 years
LOAEL uncertainty factor	Not needed in the BMC approach
Subchronic uncertainty factor	1
Interspecies uncertainty factor	3
Intraspecies uncertainty factor	10
Cumulative uncertainty factor	30
Inhalation reference exposure level	0.002 ppm (2 ppb; 0.005 mg/m^3; 5 µg/m^3)

Sprague-Dawley rats (100/sex/concentration) were exposed 6 hours/day, 5 days/week for 2 years to 0, 20, or 80 ppm acrylonitrile (0, 43, and 174 mg/m^3, respectively). Significant degenerative and inflammatory changes were observed in the respiratory epithelium of the nasal turbinates at both exposure concentrations (20 and 80 ppm). This treatment-related irritation of the nasal mucosa appeared in the 20 ppm exposed male rats as either epithelial hyperplasia of the nasal turbinates, or as hyperplasia of the mucous secreting cells. In the 20 ppm exposed females it appeared as either focal inflammation in the nasal turbinates or flattening of the respiratory epithelium of the nasal turbinates. In 80 ppm exposed rats the effects were more severe, including suppurative rhinitis, hyperplasia, focal erosions, and squamous metaplasia of the respiratory epithelium. No treatment-related effects in the olfactory epithelium, trachea, or lower respiratory system were observed at either concentration. This study identified a LOAEL for pathological alterations in the respiratory epithelium of the extrathoracic region of the respiratory tract of 20 ppm (43 mg/m^3). The U.S. EPA (1994) based its RfC of 2 µg/m^3 on the same study but included a Modifying Factor (MF) of 10 for database deficiencies. The criteria for use of modifying factors are not well specified by U.S. EPA. Such modifying factors were not used by OEHHA.

OEHHA used a benchmark dose approach to determine the chronic REL for acrylonitrile. The cumulative gamma distribution model in the U.S. EPA's BMDS sotware was individually fit to the data on respiratory epithelium hyperplasia in the nasal turbinates in males, hyperplasia of the

mucous secreting cells in males, focal inflammation in the nasal turbinates in females, and flattening of the respiratory epithelium of the nasal turbinates in females. The resulting BMC_{05} values (1.27, 1.33, 2.18, 1.35) were averaged to yield a value of 1.5 ppm. The RGDR adjustment and appropriate uncertainty factors were applied as indicated in the above table and resulted in a chronic REL of 5 $\mu g/m^3$.

For comparison, Gagnaire *et al*. (1998) found a NOAEL for nervous system effects at 24 weeks of 25 ppm, which is equivalent to a continuous exposure of 4.5 ppm. Use of the default RGDR of 1 for systemic effects, a subchronic UF of 3, an interspecies UF of 3, and an intraspecies UF of 10 results in an estimated REL of 45 ppb (100 $\mu g/m^3$). We were unable to derive a BMC from the neurotoxicity data due partly to the tendency of the animals in the 100 ppm group to yield values for two of the four endpoints measured closer to the controls than those in the 50 ppm group.

As another comparison, Saillenfait *et al*. (1983) found a 12 ppm (26 mg/m^3) NOAEL for fetal weight reduction (6 h/d exposure). This is equivalent to a continuous exposure of 3 ppm (on days 6 to 20 of gestation). Use of the default RGDR of 1 for systemic effects, an interspecies UF of 3, and an intraspecies UF of 10 results in an estimated REL of 100 ppb (200 $\mu g/m^3$).

Finally, after adjustment to continuous exposure, Murray *et al*. (1978) identified a developmental NOAEL, adjusted to continuous exposure, of 10 ppm and a LOAEL of 20 ppm (with maternal toxicity at both levels). Use of the default RGDR of 1 for systemic effects, an interspecies UF of 3, and an intraspecies UF of 10 results in an estimated REL of 30 ppb (70 $\mu g/m^3$).

VII. Data Strengths and Limitations for Development of the REL

Significant strengths in the chronic REL for acrylonitrile include (1) the availability of chronic inhalation exposure data from a well-conducted study with histopathological analysis and (2) the demonstration of a dose-response relationship. Major uncertainties are (1) the lack of adequate human exposure data, (2) the lack of a NOAEL in the 2 year study, (3) lack of inhalation bioassay in a second species, and (4) lack of reproductive data for inhalation exposures when an oral study showed adverse reproductive effects

When assessing the health effects of acrylonitrile, its carcinogenicity must also be assessed.

VIII. Potential for Differential Impacts on Children's Health

The chronic REL is considerably lower than the comparison estimate based on developmental effects. Although neurotoxicity, an endpoint which is often associated with increased sensitivity of younger animals or humans, was evaluated as one of the alternative endpoints, the comparison reference level for this end point in adults was more than an order of magnitude higher that the REL based on histological changes in the upper respiratory tract. It is therefore considered that the REL is likely to be adequately protective of infants and children.

IX. References

CARB. 2000. California Air Resources Board. California Emissions Inventory Development and Reporting System. (CEIDARS). Data from Data Base Year 1998. February 12, 2000.

Czeizel AE, Hegedus S and Timar L. 1999. Congenital abnormalities and indicators of germinal mutations in the vicinity of an acrylonitrile producing factory. Mutat. Res. 427(2):105-123

Gagnaire F, Marignac B, and Bonnet P. 1998. Relative neurotoxicological properties of five unsaturated aliphatic nitriles in rats. J. Appl. Toxicol. 18(1):25-31.

HSDB. 1994. Hazardous Substances Data Bank. TOMES® Vol. 20. Denver, CO: Micromedex, Inc.

Jakubowski M, Linhart I, Pielas G, and Kopecky J. 1987. 2-Cyanoethylmercapturic acid (CEMA) in the urine as a possible indicator of exposure to acrylonitrile. Br. J. Ind. Med. 44:834-840.

Maltoni C, Ciliberti A, and Di Maio V. 1977. Carcinogenicity bioassays on rats of acrylonitrile administered by inhalation and by ingestion. Med. Lav. 68(6):401-411.

Maltoni C, Ciliberti A, Cotti G, and Perino G. 1988. Long-term carcinogenicity bioassays on acrylonitrile administered by inhalation and by ingestion to Sprague-Dawley rats. Ann. NY Acad. Sci. 534:179-202.

Murray FJ, Schwetz BA, Nitschke KD, John JA, Norris JM, and Gehring PJ. 1978. Teratogenicity of acrylonitrile given to rats by gavage or by inhalation. Food Cosmet. Toxicol. 16(6):547-552.

Quast JF, Schwetz DJ, Balmer MF, Gunshow TS, Park CN, and McKenna MJ. 1980. A two-year toxicity and oncogenicity study with acrylonitrile following inhalation exposure of rats. Toxicology Research Laboratory. Midland, MI: Dow Chemical Co.

Saillenfait AM, Bonnet P, Guenier JP and de Ceaurriz J. 1993. Relative developmental toxicities of inhaled aliphatic mononitriles in rats. Fundam. Appl. Toxicol. 20(3):365-375.

Sakurai H, Onodera T, Utsunomiya T, Minakuchi H, Iwai H, and Matsumura H. 1978. Health effects of acrylonitrile in acrylic fibre factories. Br. J. Ind. Med. 35:219-225.

United States Environmental Protection Agency (U.S. EPA) (1993). Kelly TJ RMPASCCL. Ambient Concentration Summaries for Clean Air Act Title III Hazardous Air Pollutants. U.S. EPA Contract No. 68-D80082.

U.S. EPA 1994. U.S. Environmental Protection Agency. Integrated Risk Information System (IRIS) Database. Reference concentration (RfC) for acrylonitrile. Available online at http://www.epa.gov/ngispgm3/iris

Wilson RH, Hough GV and McCormick WE. 1948. Medical problems encountered in the manufacture of American-made rubber. Ind. Med. 17:199-207.

CHRONIC TOXICITY SUMMARY

AMMONIA

(Anhydrous ammonia; aqueous ammonia)

CAS Registry Number: 7664-41-7

I. Chronic Toxicity Summary

Inhalation reference exposure level	**200 μg/m^3 (300 ppb)**
Critical effect(s)	Pulmonary function tests or subjective symptomatology in workers
Hazard index target(s)	Respiratory system

II. Physical and Chemical Properties (From HSDB, 1994; 1999)

Description	Colorless gas
Molecular formula	NH_3
Molecular weight	17.03 g/mol
Density	0.7710 g/L @ 0°C
Boiling point	-33.35° C
Vapor pressure	7510 torr @ 25°C
Solubility	Soluble in water, alcohol, and ether
Conversion factor	1 ppm = 0.71 mg/m^3

III. Major Uses or Sources

This strongly alkaline chemical is widely used in industry as a feed stock for nitrogen-based chemicals such as fertilizers, plastics and explosives (ATSDR, 1990). Ammonia is also used as a refrigerant. The general public is exposed by off-gasing from cleaning solutions containing aqueous ammonia. Household ammonia solutions contain 5-10% ammonia in water while industrial strength can be up to 28%. The annual statewide industrial emissions from facilities reporting under the Air Toxics Hot Spots Act in California based on the most recent inventory were estimated to be 21,832,909 pounds of ammonia (CARB, 1999).

IV. Effects of Human Exposures

Comparisons were made between 52 workers and 31 control subjects in a soda ash plant for pulmonary function and eye, skin and respiratory symptomatology (Holness *et al.*, 1989). The pulmonary function tests included FVC (forced vital capacity – the total amount of air the subject can expel during a forced expiration), FEV_1 (forced expiratory volume in one second), FEF_{50} (forced expiratory flow rate at 50% of the FVC) and FEF_{75} (forced expiratory flow rate at

75% of the FVC). Age, height, and pack-years smoked were treated as covariates for the comparisons. The workers were exposed on average for 12.2 years to mean (time-weighted average) ammonia concentrations of 9.2 ppm (6.4 mg/m^3) ± 1.4 ppm, while controls were exposed to 0.3 ppm (0.21 mg/m^3) ± 0.1 ppm. No differences in any endpoints (respiratory or cutaneous symptoms, sense of smell, baseline lung function, or change in lung function over a work shift at the beginning and end of a workweek) were reported between the exposed and control groups.

Groups of human volunteers were exposed to 25, 50, or 100 ppm (0, 17.8, 35.5, or 71 mg/m^3) ammonia 5 days/week for 2, 4, or 6 hours/day, respectively, for 6 weeks (Ferguson *et al.*, 1977). Another group of 2 volunteers was exposed to 50 ppm ammonia for 6 hours/day for 6 weeks.

Group	Exposure	Week 1	Week 2	Week 3	Week 4	Week 5	Week 6
A	ppm NH_3 hours	25 2	50 4	100 6	25 2	50 4	100 6
B	ppm NH_3 hours	50 6	50 6	50 6	50 6	50 6	50 6
C	ppm NH_3 hours	100 6	50 4	25 2	25 6	50 4	100 2

Pulmonary function tests (respiration rate, FVC and FEV_1) were measured in addition to subjective complaints of irritation of the eyes and respiratory tract. The difficulty experienced in performing simple cognitive tasks was also measured, as was pulse rate. There were reports of transient irritation of the nose and throat at 50 or 100 ppm. Acclimation to eye, nose, and throat irritation was seen after two to three weeks (in addition to the short-term subjective adaption). No significant differences between subjects or controls on common biological indicators, in physical exams, or in performance of normal job duties were found. After acclimation, continuous exposure to 100 ppm, with occasional excursions to 200 ppm, was easily tolerated and had no observed effect on general health.

V. Effects of Animal Exposures

Rats were continuously exposed to ammonia at 0, 25, 50, 150, or 250 ppm (0, 18, 36, 107, or 179 mg/m^3) ammonia for 7 days prior to intratracheal inoculation with *Mycoplasma pulmonis*, and from 28 to 42 days following *M. pulmonis* exposure (Broderson *et al.*, 1976). All exposures to ammonia resulted in significantly increased severity of rhinitis, otitis media, tracheitis, and pneumonia characteristic of *M. pulmonis* infection, therefore 25 ppm was a LOAEL in this subchronic study. Exposure to 250 ppm ammonia alone resulted in nasal lesions (epithelial thickening and hyperplasia) which were not like those seen in *M. pulmonis*-infected rats.

The growth of bacteria in the lungs and nasal passages, and the concentration of serum immunoglobulin were significantly increased in rats exposed to 100 ppm (71 mg/m^3) ammonia over that seen in control rats (Schoeb *et al.*, 1982).

Guinea pigs (10/group) and mice (20/group) were continuously exposed to 20 ppm (14.2 mg/m^3) ammonia for up to 6 weeks (Anderson *et al.*, 1964). Separate groups of 6 guinea pigs and 21 chickens were exposed to 50 ppm and 20 ppm ammonia for up to 6 and 12 weeks, respectively. All species displayed pulmonary edema, congestion, and hemorrhage after 6 weeks exposure, whereas no effects were seen after only 2 weeks. Guinea pigs exposed to 50 ppm ammonia for 6 weeks exhibited enlarged and congested spleens, congested livers and lungs, and pulmonary edema. Chickens exposed to 200 ppm for 17-21 days showed liver congestion and slight clouding of the cornea. Anderson and associates also showed that a 72-hour exposure to 20 ppm ammonia significantly increased the infection rate of chickens exposed to Newcastle disease virus, while the same effect was observed in chickens exposed to 50 ppm for just 48 hours.

Coon *et al*. (1970) exposed groups of rats (as well as guinea pigs, rabbits, dogs, and monkeys) continuously to ammonia concentrations ranging from 40 to 470 mg/m^3. There were no signs of toxicity in 15 rats exposed continuously to 40 mg/m^3 for 114 days or in 48 rats exposed continuously to 127 mg/m^3 for 90 days. Among 49 rats exposed continuously to 262 mg/m^3 for 90 days, 25% had mild nasal discharge. At 455 mg/m^3 50 of 51 rats died. Thus 127 mg/m^3 (179 ppm) is a subchronic NOAEL for upper respiratory effects in rats. Coon *et al*. (1970) also found no lung effects in 15 guinea pigs exposed continuously to 40 mg/m^3 (28 ppm) ammonia for 114 days.

VI. Derivation of Chronic Reference Exposure Level

Study	Holness *et al.*, 1989 (supported by Broderson *et al.*, 1976)
Study population	52 workers; 31 controls
Exposure method	Occupational inhalation
Critical effects	Pulmonary function, eye, skin, and respiratory symptoms of irritation
LOAEL	25 ppm (Broderson *et al.*, 1976) (rats)
NOAEL	9.2 ppm (Holness *et al.*, 1989)
Exposure continuity	8 hours/day (10 m^3/day occupational inhalation rate), 5 days/week
Exposure duration	12.2 years
Average occupational exposure	3 ppm for NOAEL group (9.2 x 10/20 x 5/7)
Human equivalent concentration	3 ppm for NOAEL group
LOAEL uncertainty factor	1
Subchronic uncertainty factor	1
Interspecies uncertainty factor	1
Intraspecies uncertainty factor	10
Cumulative uncertainty factor	10
Inhalation reference exposure level	0.3 ppm (300 ppb; 0.2 mg/m^3; 200 µg/m^3)

The Holness *et al*. (1989) study was selected because it was a chronic human study and was published in a respected, peer-reviewed journal. It is also the only chronic study available. The USEPA (1995) based its RfC of 100 µg/m^3 on the same study but included a Modifying Factor

(MF) of 3 for database deficiencies. The criteria for use of modifying factors are not well specified by U.S. EPA. Such modifying factors were not used by OEHHA.

For comparison with the proposed REL of 200 $\mu g/m^3$ based on human data, we estimated RELs from 2 animal studies. (1) Anderson *et al*. (1964) exposed guinea pigs continuously to 50 ppm (35 mg/m^3) ammonia for 6 weeks and observed pulmonary edema. Use of an RGDR of 0.86 and a cumulative uncertainty factor of 3000 (10 for use of a LOAEL, 10 for subchronic, 3 for interspecies, and 10 for intraspecies) resulted in a REL of 10 $\mu g/m^3$. Staff note that the nearly maximal total uncertainty factor of 3000 was used in this estimation. (2) Coon *et al*. (1970) exposed rats continuously to 127 mg/m^3 ammonia for 90 days and saw no signs of toxicity. Use of an RGDR(ET) of 0.16 for nasal effects (observed in rats exposed to higher levels of ammonia in Broderson *et al*. (1976)) and a cumulative uncertainty factor of 100 (3 for subchronic, 3 for interspecies, and 10 for intraspecies) resulted in a REL of 200 $\mu g/m^3$.

VII. Data Strengths and Limitations for Development of the REL

Significant strengths in the ammonia REL include (1) the availability of long-term human inhalation exposure data (Holness *et al*., 1989), (2) the demonstration of consistent effects in experimentally exposed human volunteers following short-term exposures (Ferguson *et al.*, 1977), and (3) reasonable consistency with animal data (Coon *et al*., 1970).

Major areas of uncertainty are (1) the lack of a NOAEL and LOAEL in a single study, (2) a lack of animal data with chronic exposure and histopathological analyses, and (3) difficulties in estimated human occupational exposures. The overall database for this common chemical is limited.

VIII. References

ATSDR. 1990. Agency for Toxic Substances and Disease Registry. Toxicological Profile for Ammonia. Atlanta, GA: ATSDR, U.S. Public Health Service.

Anderson DP, Beard CW, and Hanson RP. 1964. The adverse effects of ammonia on chickens including resistance to infection with Newcastle disease virus. Avian. Dis. 8:369379.

Broderson JR, Lindsey JR, and Crawford JE. 1976. The role of environmental ammonia in respiratory mycoplasmosis of rats. Am. J. Pathol. 85(1):115-130.

CARB. 1999. Air toxics emissions data collected in the Air Toxics Hot Spots Program CEIDARS Database as of January 29, 1999.

Coon RA, Jones RA, Jenkins LJ Jr, and Siegel J. 1970. Animal inhalation studies on ammonia, ethylene glycol, formaldehyde, dimethylamine, and ethanol. Toxicol. Appl. Pharmacol. 16(3):646-655.

Ferguson WS, Koch WC, Webster LB, and Gould JR. 1977. Human physiological response and adaptation to ammonia. J. Occup. Med. 19(5):319-326.

HSDB. 1994. Hazardous Substances Data Bank. TOMES®. Vol .20. Denver, CO: Micromedex, Inc.

HSDB. 1999. Hazardous Substances Data Bank. Available online at http://sis.nlm.nih.gov

Holness DL, Purdham JT, and Nethercott JR. 1989. Acute and chronic respiratory effects of occupational exposure to ammonia. Am. Ind. Hyg. Assoc. J. 50(12):646-650.

Schoeb TR, Davidson MK, and Lindsey JR. 1982. Intracage ammonia promotes growth of Mycoplasma pulmonis in the respiratory tract of rats. Infect. Immun. 38:212-217.

U.S. EPA. 1995. U. S. Environmental Protection Agency. Integrated Risk Information System (IRIS) database. Reference concentration (RfC) for ammonia.

CHRONIC TOXICITY SUMMARY

BENZENE

(Benzol; Benzole; Cyclohexatriene)

CAS Registry Number: 71-43-2

I. Chronic Toxicity Summary

Inhalation reference exposure level	**60 μg/m^3 (20 ppb)**
Critical effect(s)	Lowered red and white blood cell counts in occupationally exposed humans
Hazard index target(s)	Hematopoietic system; development; nervous system

II. Physical and Chemical Properties (HSDB, 1994; 1999)

Description	Colorless liquid
Molecular formula	C_6H_6
Molecular weight	78.1 g/mol
Density	0.879 g/cm^3 @ 25° C
Boiling point	80.1°C
Vapor pressure	100 torr @ 26.1°C
Solubility	Soluble in ethanol, chloroform, ether, carbon disulfide, acetone, oils, and glacial acetic acid; slightly soluble in water
Conversion factor	1 ppm = 3.2 mg/m^3 @ 25° C

III. Major Uses or Sources

Benzene has been widely used as a multipurpose organic solvent. This use is now discouraged due to its high toxicity, including carcinogenicity. Present uses include use as a raw material in the synthesis of styrene, phenol, cyclohexane, aniline, and alkyl benzenes in the manufacture of various plastics, resins, and detergents. Syntheses of many pesticides and pharmaceuticals also involve benzene as a chemical intermediate (HSDB, 1994). The tire industry and shoe factories use benzene extensively in their manufacturing processes. Annual demand in the U.S. was estimated to be 6 million tons in 1990 (HSDB, 1994). Benzene exposure also occurs as a result of gasoline and diesel fuel use and combustion (Holmberg and Lundberg, 1985). In 1996, the latest year tabulated, the statewide mean outdoor monitored concentration of benzene was approximately 0.7 ppb (CARB, 1999a). Annual statewide industrial emissions from facilities reporting under the Air Toxics Hot Spots Act in California based on the most recent inventory were estimated to be 750,364 pounds of benzene (CARB, 1999b). (This does not include the large amount of benzene emitted by mobile sources.)

IV. Effects of Human Exposure

The primary toxicological effects of chronic benzene exposure are on the hematopoeitic system. Neurological and reproductive/developmental toxic effects are also of concern at slightly higher concentrations. Impairment of immune function and/or various anemias may result from the hematotoxicity. The hematologic lesions in the bone marrow can lead to peripheral lymphocytopenia and/or pancytopenia following chronic exposure. Severe benzene exposures can also lead to life-threatening aplastic anemia. These lesions may lead to the development of leukemia years after apparent recovery from the hematologic damage (DeGowin, 1963).

Kipen *et al.* (1988) performed a retrospective longitudinal study on a cohort of 459 rubber workers, examining the correlation of average benzene exposure with total white blood cell counts taken from the workers. These researchers found a significant ($p < 0.016$) negative correlation between average benzene concentrations in the workplace and white blood cell counts in workers from the years 1940-1948. A reanalysis of these data by Cody *et al.* (1993) showed significant decreases in RBC and WBC counts among a group of 161 workers during the 1946-1949 period compared with their pre-exposure blood cell counts. The decline in blood counts was measured over the course of 12 months following start of exposure. During the course of employment, workers who had low monthly blood cell counts were transferred to other areas with lower benzene exposures, thus potentially creating a bias towards non-significance or removing sensitive subjects from the study population. Since there was a reported 75% rate of job change within the first year of employment, this bias could be highly significant. In addition, there was some indication of blood transfusions used to treat some “anemic” workers, which would cause serious problems in interpreting the RBC data, since RBCs have a long lifespan in the bloodstream. The exposure analysis in this study was performed by Crump and Allen (1984). The range of monthly median exposures was 30-54 ppm throughout the 12-month segment examined. Despite the above-mentioned potential biases, workers exposed above the median concentrations displayed significantly decreased WBC and RBC counts compared with workers exposed to the lower concentrations using a repeated measures analysis of variance.

Tsai *et al.* (1983) examined the mortality from all cancers and leukemia, in addition to hematologic parameters in male workers exposed to benzene for 1-21 years in a refinery from 1952-1978. The cohort of 454 included maintenance workers and utility men and laborers assigned to benzene units on a “regular basis”. Exposures to benzene were determined using personal monitors; the median air concentration was 0.53 ppm in the work areas of greatest exposure to benzene. The average length of employment in the cohort was 7.4 years. The analysis of overall mortality in this population revealed no significant excesses. Mortality from all causes and from diseases of the circulatory system was significantly below expected values based on comparable groups of U.S. males. The authors concluded the presence of a healthy worker effect. An internal comparison group of 823 people, including 10% of the workers who were employed in the same plant in operations not related to benzene, showed relative risks for 0.90 and 1.31 for all causes and cancer at all sites, respectively ($p < 0.28$ and 0.23). A subset of 303 workers was followed for medical surveillance. Up to four hematological tests per year were conducted on these workers. Total and differential white blood cell counts, hemoglobin,

hematocrit, red blood cells, platelets and clotting times were found to be within normal (between 5% and 95% percentile) limits in this group.

Collins *et al*. (1997) used routine data from Monsanto's medical/industrial hygiene system to study 387 workers with daily 8-hour time-weighted exposures (TWA) averaging 0.55 ppm benzene (range = 0.01 – 87.69 ppm; based on 4213 personal monitoring samples, less than 5% of which exceeded 2 ppm). Controls were 553 unexposed workers. There was no increase in the prevalence of lymphopenia, an early, sensitive indicator of benzene toxicity, among exposed workers (odds ratio = 0.6; 95% confidence interval = 0.2 to 1.8), taking into account smoking, age, and sex. There also was no increase in risk among workers exposed 5 or more years (odds ratio = 0.6; 95% confidence interval = 0.2 to 1.9). There were no differences between exposed and unexposed workers for other measures of hematotoxicity, including mean corpuscular volume and counts of total white blood cells, red blood cells, hemoglobin, and platelets.

Rothman *et al*. (1996) compared hematologic outcomes in a cross-sectional study of 44 male and female workers heavily exposed to benzene (median = 31 ppm as an 8-hr TWA) and 44 age and gender-matched unexposed controls from China. Hematologic parameters (total WBC, absolute lymphocyte count, platelets, red blood cells, and hematocrit) were decreased among exposed workers compared to controls; an exception was the red blood cell mean corpuscular volume (MCV), which was higher among exposed subjects. In a subgroup of 11 workers with a median 8 hr TWA of 7.6 ppm (range = 1-20 ppm) and not exposed to more than 31 ppm on any of 5 sampling days, only the absolute lymphocyte count was significantly different between exposed workers and controls ($p = 0.03$). Among exposed subjects, a dose response relationship with various measures of current benzene exposure (i.e., personal air monitoring, benzene metabolites in urine) was present only for the total WBC count, the absolute lymphocyte count, and the MCV. Their results support the use of the absolute lymphocyte count as the most sensitive indicator of benzene-induced hematotoxicity.

An examination of 32 patients, who were chronically exposed to benzene vapors ranging from 150 to 650 ppm for 4 months to 15 years, showed that pancytopenia occurred in 28 cases. Bone marrow punctures revealed variable hematopoeitic lesions, ranging from acellularity to hypercellularity (Aksoy *et al.*, 1972).

Central nervous system disorders have been reported in individuals with pancytopenia following chronic occupational benzene exposure to unknown concentrations for an average length of time of 6 years (Baslo and Aksoy, 1982).

Runion and Scott (1985) estimated a composite geometric mean benzene concentration in various workplaces containing benzene to be 0.1 ppm (0.32 mg/m^3) (geometric standard deviation = 7.2 ppm, 23.3 mg/m^3). This estimate was based on samples collected by industrial hygienists between the years 1978 and 1983.

V. Effects of Animal Exposure

A number of animal studies have demonstrated that benzene exposure can induce bone marrow damage, changes in circulating blood cells, developmental and reproductive effects, alterations of the immune response, and cancer. With respect to chronic toxicity, hematological changes appear to be the most sensitive indicator.

Wolf *et al*. (1956) studied the effects of repeated exposure to benzene in rabbits (80 ppm, 175 total exposures), rats (88 ppm, 136 total exposures) and guinea pigs (88 ppm, 193 total exposures). The observed effects included leukopenia, increased spleen weight, and histological changes to the bone marrow. Hematologic effects, including leukopenia, were observed in rats exposed to mean concentrations of 44 ppm (143 mg/m^3) or greater for 5-8 weeks (Deichmann *et al.*, 1963). Exposure to 31 ppm (100 mg/m^3) benzene or less did not result in leukopenia after 3-4 months of exposure. Snyder *et al*. (1978) exposed Sprague-Dawley rats and AKR/J mice to 300 ppm benzene, 6 hours/day, 5 days/week for life. Lymphocytopenia, anemia and decreased survival time were observed in both species. Cronkite *et al*. (1982) exposed male mice to 400 ppm benzene, 6 hours/day, 5 days/week for 9.5 weeks and observed depressed bone marrow cellularity, decreased stem cell count, and altered morphology in spleen colony-forming cells.

Mice have been shown to be more sensitive than rats or rabbits to the hematologic and leukemic effects of benzene (Sabourin *et al.*, 1989; IARC, 1982). Sabourin *et al.* (1988) showed that metabolism of benzene to the toxic hydroquinone, muconic acid, and hydroquinone glucuronide was much more prevalent in the mouse than in rats, whereas the detoxification pathways were approximately equivalent between the two species.

A study on the chronic hematological effects of benzene exposure in C57 Bl/6 male mice (5-6 per group) showed that peripheral lymphocytes, red blood cells and colony-forming units (CFUs) in the bone marrow and spleen were significantly decreased in number after treatment with 10 ppm (32.4 mg/m^3) benzene for 6 hours/day, 5 days/week for 178 days (Baarson *et al.*, 1984).

Inhalation of 0, 10, 31, 100, or 301 ppm (0, 32.4, 100.4, 324, or 975 mg/m^3) benzene for 6 hours/day for 6 days resulted in a dose-dependent reduction in peripheral lymphocytes, and a reduced proliferative response of B- and T-lymphocytes to mitogenic agents in mice (Rozen *et al.*, 1984). In this study, total peripheral lymphocyte numbers and B-lymphocyte proliferation to lipopolysaccharide were significantly reduced at a concentration of 10 ppm (32.4 mg/m^3). The proliferation of T-lymphocytes was significantly reduced at a concentration of 31 ppm (100.4 mg/m^3).

Male and female mice (9-10 per group) exposed to 100 ppm (324 mg/m^3) benzene or greater for 6 hours/day, 5 days/week for 2 weeks showed decreased bone marrow cellularity and a reduction of pluripotent stem cells in the bone marrow (Cronkite *et al.*, 1985). The decrease in marrow cellularity continued for up to 25 weeks following a 16-week exposure to 300 ppm (972 mg/m^3) benzene. Peripheral blood lymphocytes were dose-dependently decreased with benzene exposures of greater than 25 ppm (81 mg/m^3) for 16 weeks, but recovered to normal levels following a 16-week recovery period.

Ward *et al*. (1985) exposed 50 Sprague-Dawley rats and 150 CD-1 mice of both sexes to 0, 1, 10, 30, or 300 ppm benzene, 6 hours/day, 5 days/week for 13 weeks. Serial sacrifices were

conducted at 7, 14, 28, 56, and 91 days. No hematological changes were found for mice and rats at 1, 10, or 30 ppm in this study. Significant increases in mean cell volume and mean cell hemoglobin values and decreases in hematocrit, hemoglobin, lymphocyte percentages, and decreases in red cell, leukocyte and platelet counts were observed in male and female mice at 300 ppm. The changes were first observed after 14 days of exposure. Histological changes in mice included myeloid hypoplasia of the bone marrow, lymphoid depletion in the mesenteric lymph node, increased extramedullary hematopoiesis in the spleen, and periarteriolar lymphoid sheath depletion. Effects were less severe in the rats.

Aoyama (1986) showed that a 14-day exposure of mice to 50 ppm (162 mg/m^3) benzene resulted in a significantly reduced blood leukocyte count.

The NTP (1986) conducted a bioassay in F344 rats and B6C3F1 mice of benzene by corn oil gavage. Doses were 0, 25, 50, and 100 mg/kg-day for females and 0, 50, 100, and 200 mg/kg-day for males. Dose-related lymphocytopenia and leukocytopenia were observed in both species in all dosed groups but not controls. Mice exhibited lymphoid depletion of the thymus and spleen and hyperplasia of the bone marrow.
Cronkite *et al*. (1989) exposed CBA/Ca mice to 10, 25, 100, 300, 400 and 3000 ppm benzene 6 hours/day, 5 days/week for up to 16 weeks. No effects were observed at the 10 ppm level. Lymphopenia was observed in the 25 ppm exposure group. Higher concentrations of benzene produced dose-dependent decreases in blood lymphocytes, bone marrow cellularity, spleen colony-forming units, and an increased percentage of CFU-S in S-phase synthesis.
Farris *et al*. (1997) exposed B6C3F$_1$ mice to 1, 5, 10, 100, and 200 ppm benzene for 6 hr/day, 5 days/week, for 1, 2, 4, or 8 weeks. In addition some animals were allowed to recover from the exposure. There were no significant effects on hematopoietic parameters from exposure to 10 ppm benzene or less. Exposure to higher levels reduced the number of total bone marrow cells, progenitor cells, differentiating hematopoietic cells, and most blood parameters. The replication of primitive progenitor cells was increased. The authors suggested that this last effect, in concert with the genotoxicity of benzene, could account for the carcinogenicity of benzene at high concentrations.

Reproductive and developmental effects have been reported following benzene exposure. Coate *et al.* (1984) exposed groups of 40 female rats to 0, 1, 10, 40, and 100 ppm (0, 3.24, 32.4, 129.6, or 324 mg/m^3) benzene for 6 hours/day during days 6-15 of gestation. In this study, teratologic evaluations and fetotoxic measurements were done on the fetuses. A significant decrease was noted in the body weights of fetuses from dams exposed to 100 ppm (324 mg/m^3). No effects were observed at a concentration of 40 ppm (129.6 mg/m^3).

Keller and Snyder (1986) reported that exposure of pregnant mice to concentrations as low as 5 ppm (16 mg/m^3) benzene on days 6-15 of gestation (6 hr/day) resulted in bone-marrow hematopoietic changes in the offspring that persisted into adulthood. However, the hematopoietic effects (e.g. bimodal changes in erythroid colony-forming cells) in the above study were of uncertain biological significance. In a similar later study, Keller and Snyder (1988) found that exposure of mice *in utero* to 20 ppm (64 mg/m^3) benzene on days 6-15 of gestation resulted in neonatal suppression of erythropoietic precursor cells and persistent,

enhanced granulopoiesis. This effect was considered significant bone-marrow toxicity by the authors. No hematotoxicity was seen in this study at 10 ppm (32 mg/m^3).

An exposure of 500 ppm (1,600 mg/m^3) benzene through days 6-15 gestation was teratogenic in rats while 50 ppm (160 mg/m^3) resulted in reduced fetal weights on day 20 of gestation. No fetal effects were noted at an exposure of 10 ppm (Kuna and Kapp, 1981). An earlier study by Murray *et al.* (1979) showed that inhalation of 500 ppm benzene for 7 hours/day on days 6-15 and days 6-18 of gestation in mice and rabbits, respectively, induced minor skeletal variations in the absence of maternal toxicity. Red and white blood cell counts in the adults of either species were measured by Murray *et al.* (1979) but were not significantly different from control animals. However, fetal mouse hematological effects were not measured.

Tatrai *et al.* (1980) demonstrated decreased fetal body weights and elevated liver weights in rats exposed throughout gestation to 150 mg/m^3 (47 ppm).

VI. Derivation of Chronic Reference Exposure Level (REL)

Study	Tsai *et al.* (1983)
Study population	303 Male refinery workers
Exposure method	Occupational exposures for 1-21 years
Critical effects	Hematological effects
LOAEL	Not observed
NOAEL	0.53 ppm
Exposure continuity	8 hr/day (10 m^3 per 20 m^3 day), 5 days/week
Exposure duration	7.4 years average (for the full cohort of 454); 32% of the workers were exposed for more than 10 years
Average occupational exposure	0.19 ppm
Human equivalent concentration	0.19 ppm
LOAEL uncertainty factor	1
Subchronic uncertainty factor	1
Interspecies uncertainty factor	1
Intraspecies uncertainty factor	10
Cumulative uncertainty factor	10
Inhalation reference exposure level	0.02 ppm (20 ppb; 0.06 mg/m^3; 60 μg/m^3)

Staff identified Tsai *et al.* (1983) as the most appropriate study for a chronic REL derivation. The authors examined hematologic parameters in 303 male workers exposed to benzene for 1-21 years in a refinery from 1952-1978. Follow-up success was 99.3% in the entire cohort of 359. A total of approximately 1400 samples for hematological tests and 900 for blood chemistry tests were taken between 1959 and 1979. Exposures to benzene were determined using personal monitors. Data consisting of 1394 personal samples indicated that 84% of all benzene samples were less than 1 ppm; the median air concentration of benzene was 0.53 ppm in the work areas of greatest exposure to benzene ("benzene related areas", for example, production of benzene

and cyclohexane and also of cumene). The average length of employment in the cohort was 7.4 years. Mortality from all causes and from diseases of the circulatory system was significantly below expected values based on comparable groups of U.S. males. The authors concluded the presence of a healthy worker effect. An analysis using an internal comparison group of 823 people, including 10% of the workers who were employed in the same plant in operations not related to benzene, showed relative risks for 0.90 and 1.31 for all causes and cancer at all sites, respectively ($p < 0.28$ and 0.23). Total and differential white blood cell counts, hemoglobin, hematocrit, red blood cells, platelets and clotting times were found to be within normal (between 5% and 95% percentile) limits in this group. Although the exposure duration averaged only 7.4 years, the study was considered to be chronic since 32% of the workers had been exposed for more than 10 years.

VII. Data Strengths and Limitations for Development of the REL

Both the animal and human databases for benzene are excellent. Although the study by Tsai *et al.* (1983) is a free-standing NOAEL, the endpoint examined is a known sensitive measure of benzene toxicity in humans. In addition, the LOAEL for the same endpoint in workers reported by Cody *et al.* (1993) help form a dose-response relationship and also yield an REL which is consistent with that derived from Tsai *et al.* (1983). The study by Cody *et al.* (1993), since it failed to identify a NOAEL and was only for a period of 1 year, contained a greater degree of uncertainty in extrapolation to a chronic community Reference Exposure Level. The recent results of Collins *et al*. (1997) that included a NOAEL of 0.55 ppm and of Rothman *et al*. (1996) that included a LOAEL of 7.6 ppm are consistent with those of Tsai *et al*. Therefore the study by Tsai *et al.* (1983) was used as the basis for the chronic REL for benzene.

In the Cody *et al.* (1993) study, significant hematological effects, including reduced RBC and WBC counts, were observed in 161 male rubber workers exposed to median peak concentrations (i.e., only the peak concentrations for any given exposure time were reported) of 30-54 ppm or more for a 12-month period during 1948. The 30 ppm value was considered a 1-year LOAEL for hematological effects. In this rubber plant, workers who had blood dyscrasias were excluded from working in the high benzene units. Furthermore, individual workers having more than a 25% decrease in WBC counts from their pre-employment background count were removed from the high benzene units and placed in other units with lower benzene concentrations. Sensitive individuals therefore could have been excluded from the analysis. The 30 ppm value is the low end of the range of median values (30-54 ppm) reported by Crump and used in the Kipen *et al.* (1988) and Cody *et al.* (1993) studies. An equivalent continuous exposure of 10.7 ppm can be calculated by assuming that workers inhaled 10 m^3 of their total 20 m^3 of air per day during their work-shift, and by adjusting for a normal 5 day work week. Application of uncertainty factors for subchronic exposures, estimation of a NOAEL, and for protection of sensitive subpopulations (10 for each) results in an REL of 0.01 ppm (10 ppb; 30 $\mu g/m^3$). This is comparable to the REL based on Tsai *et al*. (1983).

Ward *et al*. (1996) determined a relationship between occupational exposures to benzene and decreased red and white cell counts. A modeled dose-response relationship indicated a possibility for hematologic effects at concentrations below 5 ppm. However, no specific measures of the actual effects at concentrations below 2 ppm were taken, and the Tsai *et al*. (1983) data were not considered in their analysis. The purpose of this study was to characterize

the trend for effects at low concentrations of benzene. A NOAEL or LOAEL was not identified in the study. The selection of a NOAEL of 0.53 ppm is therefore not inconsistent with the results of the Ward *et al*. (1996) study.

The human data presented by Tsai and associates were selected over animal studies because the collective human data were considered adequate in terms of sample size, exposure duration, and health effects evaluation.

For comparison with the REL of 20 ppb based on human data, we estimated a REL based on the chronic inhalation study in mice by Baarson *et al.* (1984), which showed that bone-marrow progenitor cells were markedly suppressed after intermittent exposures (6 hr/day, 5 days/week) to 10 ppm benzene for 6 months. An extrapolation of this value to an equivalent continuous exposure resulted in a concentration of 1.8 ppm. Application of an RGDR of 1 for a systemic effect and uncertainty factors of 3 and 10 for inter- and intraspecies variability, and 10 for estimation of a NOAEL from the LOAEL would result in an REL of 6 ppb (20 $\mu g/m^3$). The Farris *et al*. (1997) 8 week study indicated a LOAEL of 100 ppm and a NOAEL of 10 ppm for hematological effects. Application of an RGDR of 1 and UFs of 10 for subchronic, 3 for interspecies and 10 for intraspecies extrapolation (total UF = 300) also resulted in an estimated REL of 6 ppb, in reasonable agreement with the proposed REL of 20 ppb. One could also crudely approximate an inhalation REL from the oral NTP bioassay where a dose of 25 mg/kg-day was associated with hematological effects. The concentration approximately equivalent to a 25 mg/kg dose for a 70 kg human breathing 20 cubic meters per day is 27 ppm. Assuming this is a LOAEL and applying an RGDR of 1 for systemic effects, a 3 fold UF for extrapolation to humans, a 10-fold UF for LOAEL to NOAEL extrapolation and a 10-fold UF for intraspecies extrapolation yields a REL of 90 ppb. There are a number of uncertainties to this approach, yet it comes within a factor of 5 of the proposed REL based on human studies.

VIII. References

Aksoy M, Dincol K, Erdem S, Akgun T, and Dincol G. 1972. Details of blood changes in 32 patients with pancytopenia associated with long-term exposure to benzene. Br. J. Ind. Med. 29:56-64.

Aoyama K. 1986. Effects of benzene inhalation on lymphocyte subpopulations and immune response in mice. Toxicol. Appl. Pharmacol. 85:92-101.

Baarson KA, Snyder CA, and Albert RE. 1984. Repeated exposure of C57Bl/6 mice to inhaled benzene at 10 ppm markedly depressed erythrocyte colony formation. Toxicol. Lett. 20:337-342.

Baslo A, and Aksoy M. 1982. Neurological abnormalities in chronic benzene poisoning. A study of six patients with aplastic anemia and two with preleukemia. Environ. Res. 27:457-465.

CARB. 1999a. California Air Resources Board. Toxics Air Quality Data. Substance Chooser. Benzene. Available online at http:/www.arb.ca.gov/aqd/toxics.htm

CARB. 1999b. Air toxics emissions data collected in the Air Toxics Hot Spots Program CEIDARS Database as of January 29, 1999.

Coate WB, Hoberman AM, and Durloo RS. 1984. Inhalation teratology study of benzene in rats. In: Advances in Modern Environmental Toxicology, Vol. VI. Applied Toxicology of Petroleum Hydrocarbons. MacFarland HN, ed. Princeton, NJ: Princeton Scientific Publishers, Inc.

Cody RR, Strawderman WW, and Kipen HM. 1993. Hematologic effects of benzene. J. Occup. Med. 35(8):776-782.

Collins JJ, Ireland BK, Easterday PA, Nair RS, Braun J. 1997. Evaluation of lymphopenia among workers with low-level benzene exposure and the utility of routine data collection. J. Occup. Environ. Med. 39(3):232-237.

Cronkite EP, Inoue T, Carten AL, Miller ME, Bullis JE, and Drew RT. 1982. Effects of benzene inhalation on murine pluripotent stem cells. J. Toxicol. Environ. Health 9(3):411-421.

Cronkite EP, Drew RT, Inoue T, and Bullis JE. 1985. Benzene hematotoxicity and leukemogenesis. Am. J. Ind. Med. 7:447-456.

Cronkite EP, Drew RT, Inoue T, Hirabayashi Y and Bullis JE. 1989. Hematotoxicity and carcinogenicity of inhaled benzene. Environ. Health Perspect. 82:97-108.

Crump K, and Allen B. 1984. Quantitative estimates of risk of leukemia from occupational exposure to benzene. Occupational Safety and Health Administration; Docket H-059B. [As cited in: Cody RR, Strawderman WW, and Kipen HM. 1993. Hematologic effects of benzene. J. Occup. Med. 35(8):776-782.]

DeGowin RL. 1963. Benzene exposure and aplastic anemia followed by leukemia 15 years later. J. Am. Med. Assoc. 185(10):748-751.

Deichmann WB, MacDonald WE, and Bernal E. 1963. The hematopoietic tissue toxicity of benzene vapors. Toxicol. Appl. Pharmacol. 5:201-224.

Farris GM, Robinson SN, Gaido, KW, Wong BA, Wong VA, Hahn WP, and Shah RS. 1997. Benzene-induced hematoxicity and bone marrow compensation in B6C3F1 mice. Fundam. Appl. Toxicol. 36:119-129.

HSDB. 1994. Hazardous Substances Data Bank. National Library of Medicine, Bethesda, MD (CD-ROM version). Micromedex, Inc., Denver, CO (edition expires 11/31/94).

HSDB. 1999. Hazardous Substances Data Bank. Available online at http://sis.nlm.nih.gov

Holmberg B, and Lundberg P. 1985. Benzene: Standards, occurrence, and exposure. Am. J. Ind. Med. 7:375-383.

IARC (International Agency for Research on Cancer). 1982. IARC Monographs on the Evaluation of the Carcinogenic Risk of Chemicals to Humans: Some Industrial Chemicals and Dyestuffs. Volume 29. Lyon: IARC. pp. 95-148.

Keller KA, and Snyder CA. 1986. Mice exposed in utero to low concentrations of benzene exhibit enduring changes in their colony forming hematopoietic cells. Toxicology 42:171-181.

Keller KA, and Snyder CA. 1988. Mice exposed in utero to 20 ppm benzene exhibit altered numbers of recognizable hematopoietic cells up to seven weeks after exposure. Fundam. Appl. Toxicol. 10:224-232.

Kipen HM, Cody RP, Crump KS, Allen BC, and Goldstein BD. 1988. Hematologic effects of benzene: A thirty-five year longitudinal study of rubber workers. Toxicol. Ind. Health. 4(4):411-430.

Kuna RA, and Kapp RW. 1981. The embryotoxic/teratogenic potential of benzene vapor in rats. Toxicol. Appl. Pharmacol. 57:1-7.

Murray FJ, John JA, Rampy LW, Kuna RA, and Schwetz BA. 1979. Embryotoxicity of inhaled benzene in mice and rabbits. Am. Ind. Hyg. Assoc. J. 40:993-998.

NTP, National Toxicology Program 1986. NTP Technical Report on the Toxicology and Carcinogenesis Studies of Benzene (CAS No. 71-43-2) in F344/N Rats and B6C3F1 Mice (Gavage Studies). U.S. Department of Health and Human Servcies, Public Health Service, National Institutes of Health NIH Publication No. 86-2545.

Rothman N, Li GL, Dosemeci M, Bechtold WE, Marti GE, Wang YZ, Linet M, Xi LQ, Lu W, Smith MT, Titenko-Holland N, Zhang LP, Blot W, Yin SN, Hayes RB. 1996. Hematotoxocity among Chinese workers heavily exposed to benzene. Am. J. Ind. Med. 29(3):236-246.

Rozen MG, Snyder CA, and Albert RE. 1984. Depressions in B- and T-lymphocyte mitogen-induced blastogenesis in mice exposed to low concentrations of benzene. Toxicol. Lett. 20:343-349.

Runion HE, and Scott LM. 1985. Benzene exposure in the United States 1978-1983: An overview. Am. J. Ind. Med. 7:385-393.

Sabourin PJ, Bechtold WE, Birnbaum LS, Lucier G, and Henderson RF. 1988. Differences in the metabolism and disposition of inhaled [3H]benzene by F344/N rats and B6C3F1 mice. Toxicol. Appl. Pharmacol. 94:128-140.

Sabourin PJ, Bechtold WE, Griffith WC, Birnbaum LS, Lucier G, and Henderson RF. 1989. Effect of exposure concentration, exposure rate, and route of administration on metabolism of benzene by F344 rats and B6C3F1 mice. Toxicol. Appl. Pharmacol. 99:421-444.

Snyder CA, Goldstein BD, Sellakamur A, Wolman S, Bromberg I, Erlichman MN, and Laskin S. 1978. Hematotoxicity of inhaled benzene to Sprague-Dawley rats and AKR mice at 300 ppm. J. Toxicol. Environ. Health 4:605-618.

Tatrai E, Ungvary GY, Hudak A, Rodics K, Lorincz M, and Barcza GY. 1980. Concentration dependence of the embryotoxic effects of benzene inhalation in CFY rats. J. Hyg. Epidem. Micro. Immunol. 24(3):363-371.

Tsai SP, Wen CP, Weiss NS, Wong O, McClellan WA, and Gibson RL. 1983. Retrospective mortality and medical surveillance studies of workers in benzene areas of refineries. J. Occup. Med. 25(9):685-692.

Ward CO, Kuna RA, Snyder NK, Alsaker RD, Coate WB, and Craig PH. 1985. Subchronic inhalation toxicity of benzene in rats and mice. Am. J. Ind. Med. 7:457-473.

Ward E, Hornburg R, Morris J, Rinsky R, Wild D, Halperin W, and Guthrie W. 1996. Risk of low red or white blood cell count related to estimated benzene exposure in a rubberworker cohort (1940-1975). Am. J. Ind. Med. 29:247-257.

Wolf MA, Rowe VK, McCollister DD *et al*. 1956. Toxicological studies od certain alkylated benzenes and benzene. Arch. Ind. Health 14:387-398.

CHRONIC TOXICITY SUMMARY

BERYLLIUM AND BERYLLIUM COMPOUNDS

(beryllium-9; glucinium; glucinum; beryllium metallic)
CAS Registry Number: 7440-41-7

(beryllium oxide; beryllia; beryllium monoxide)
CAS Registry Number: 1304-56-9

(*beryllium hydroxide; beryllium hydrate; beryllium dihydroxide)*
CAS Registry Number: 13327-32-7

(beryllium sulfate; sulfuric acid; beryllium salt)
CAS Registry Number: 13510-49-1

I. Chronic Toxicity Summary

Inhalation reference exposure level	**0.007 μg Be/m^3**
Critical effect(s)	Beryllium sensitization and chronic beryllium disease in occupationally exposed humans
Hazard index target(s)	Respiratory system; immune system
Oral reference exposure level	0.002 mg/kg-day
Critical efect	Small intestinal lesions in dogs
Hazard index target(s)	Gastrointestinal tract/liver

II. Physical and Chemical Properties Summary (ATSDR, 1993)

	Metallic beryllium	*Beryllium oxide*	*Beryllium hydroxide*	*Beryllium sulfate*
Description	Solid gray, hexagonal structure	White light, amorphous powder	White amorphous powder or crystalline	Colorless tetragonal crystals
Molecular formula	Be	BeO	$Be(OH)_2$	$BeSO_4$
Molecular weight	9.012 g/mol	25.01 g/mol	43.03 g/mol	105.07 g/mol
Solubility	Insoluble in water			Soluble
Conversion factor	Not applicable			

III. Major Uses and Sources

Beryllium is a metallic element mined as bertrandite and beryl mineral ores. As the lightest structural metal, beryllium is used in the space, aircraft, and nuclear industries in a variety of components including aircraft disc brakes, x-ray transmission windows, vehicle optics, nuclear reactor neutron reflectors, fuel containers, precision instruments, rocket propellants, navigational systems, heat shields, and mirrors. In addition to the four species listed, there are many other beryllium-containing compounds, including other salts, ores, and alloys (see, e.g., CRC, 1994). The annual statewide industrial emissions from facilities reporting under the Air Toxics Hot Spots Act in California based on the most recent inventory were estimated to be 2279 pounds of beryllium (CARB, 2000).

Beryllium alloys, especially the hardest alloy beryllium copper, are used in electrical equipment, precision instruments, springs, valves, non-sparking tools, and in molds for injection-molded plastics for automotive, industrial, and consumer applications. Beryllium oxide is used in high-technology ceramics, electronic heat sinks, electrical insulators, crucibles, thermocouple tubing, and laser structural components. Other beryllium compounds, including the chloride, nitrate, fluoride, and sulfate, are utilized as chemical reagents or generated from the refining of beryllium-containing ores.

Beryllium is naturally emitted into the atmosphere by windblown dusts and volcanic particles. However, the major emission source is the combustion of coal and fuel oil, which releases beryllium-containing particulates and ash. Other beryllium-releasing industrial processes include ore processing, metal fabrication, beryllium oxide production, and municipal waste incineration (ATSDR, 1993). Beryllium also occurs in tobacco smoke (0-0.0005 μg/cigarette) (Smith *et al*., 1997).

IV. Effects of Human Exposure

The respiratory tract is the major target organ system in humans following the inhalation of beryllium. The common symptoms of chronic beryllium disease (CBD) include shortness of breath upon exertion, weight loss, cough, fatigue, chest pain, anorexia, and overall weakness. Most studies reporting adverse respiratory effects in humans involve occupational exposure to beryllium. Exposure to soluble beryllium compounds is associated with acute beryllium pneumonitis (Eisenbud *et al.*, 1948). Exposure to either soluble or insoluble beryllium compounds may result in obstructive and restrictive diseases of the lung, called chronic beryllium disease (berylliosis) (Cotes *et al.*, 1983; Johnson, 1983; Infante *et al.*, 1980; Kriebel *et al.*, 1988a; Metzner and Lieben, 1961). The total number of beryllium-related disease cases has declined since the adoption of industrial standards (Eisenbud and Lisson, 1983; ATSDR, 1993).

Historically, beryllium pneumonitis has been associated with occupational concentrations over 0.1 mg Be/m^3, primarily as beryllium sulfate or beryllium fluoride (Eisenbud *et al.*, 1948). The atmospheric concentrations related to chronic beryllium disease have been more difficult to define, in part due to the lack of individual exposure estimates, especially in the studies derived

from the berylliosis case registries (Infante *et al.*, 1980; Lieben and Metzner, 1959). However, Infante and associates (1980) reported significantly increased mortality due to non-neoplastic respiratory disease in beryllium-exposed workers, and noted one case of chronic berylliosis in a worker following seven years exposure to $\leq$ 2 µg Be/m^3. In a 30-year follow-up study of 146 beryllium-exposed workers, Cotes *et al.* (1983) identified seven cases of chronic beryllium related disease. All the cases were exposed to beryllium oxide or hydroxide, but in a wide range of retrospectively estimated doses (over 3000 samples from 1952 to 1960). The estimated average daily exposure did not exceed 2 µg/m^3 for the ten site/process classifications, but 318 samples did exceed 2 µg Be/m^3 (and 20 samples were greater than 25 µg Be/m^3). No atmospheric samples were available after 1963, even though the exposure occurred through 1973. The LOAEL for occupationally induced berylliosis observed in this study was estimated from uncertain exposure data to be less than 2 µg Be/m^3.

One cross-sectional study (Kriebel *et al.*, 1988a; Kriebel *et al.*, 1988b) estimated beryllium exposure levels for 309 workers originally surveyed in 1977, with a median duration of exposure of 17 years (range 2 to 39 years). Historic plant levels were estimated to be as high as 100 µg Be/m^3, and, even as late as 1975, some job classifications exceeded 10 µg Be/m^3. The workers' median cumulative exposure was 65 µg Be/m^3-year (range 0.1 to 4400 µg Be/m^3-years); the median lifetime exposure estimate was 4.3 µg/m^3 (range 0.01 to 150 µg/m^3). Spirometric measurement of pulmonary function, chest x-rays, and arterial blood gas measurements were collected. Decrements in lung function, as defined by forced vital capacity (FVC) and forced expiratory volume in one second (FEV_1), were associated with cumulative exposure up to 20 years prior to the health survey, even in workers with no radiographic abnormalities. Differences in alveolar-arterial oxygen gradient were associated with cumulative exposure in the 10 years prior to the study. These endpoints give a LOAEL of 39 µg/m^3-years (geometric mean cumulative exposure) for decrements in pulmonary function and changes in arterial blood gases.

Non-occupational beryllium-related chronic disease has been reported in individuals residing in the vicinity of beryllium manufacturing industries (Eisenbud *et al.*, 1949; Metzner and Lieben, 1961). An early cross-sectional study (Eisenbud *et al.*, 1949) described 11 cases of non-occupational berylliosis after x-ray and clinical examination of approximately 10,000 residents near a beryllium fabrication facility in Lorain, Ohio. Ten of the cases resided within 3/4 mile of the plant (up to 7 years duration), and five cases resided within 1/4 mile. The authors estimated a 1% disease incidence within 1/4 mile (500 individuals). Atmospheric sampling in 1947 identified an average level of 0.2 µg Be/m^3 at 1/4 mile decreasing to 0 µg Be/m^3 at 10 miles, but samples varied up to 100 fold over the 10 week sampling period. Utilizing current and historical exposure estimates based on discharge, process, inventory, and building design changes, this study estimated a chronic LOAEL in the range of 0.01 to 0.1 µg Be/m^3 for continuous exposure to beryllium compounds, based on the development of chronic berylliosis.

Metzner and Lieben (1961) also reported 26 cases of chronic berylliosis in a population of approximately 100,000, living within 7 miles of a refining and alloy fabrication plant (duration 6 to 19 years). Neighborhood exposure assessment conducted over 14 months during 1958 and 1959 identified a mean level of 0.0155 µg Be/m^3, with 10% of the samples registering over 0.03 µg Be/m^3. Limited measurements conducted earlier at the site were higher (1.0 to 1.8 µg Be/m^3 in 1953 and 0.91 to 1.4 µg Be/m^3 in 1954).

Chronic beryllium disease appears to involve a cell-mediated immune response, especially granulomatous reactions found in the lungs of sensitive individuals. Humans exposed to beryllium compounds have demonstrated increased T-cell activity (*in vitro*) and histological abnormalities of the lymph nodes (Cullen *et al.*, 1987; Johnson, 1983). Johnson (1983) described granuloma of lymph nodes and chronic interstitial pneumonitis in a small number of beryllium metal handling machinists (LOAEL = 4.6 μg Be/m^3). A second study identified granulomatous lung lesions, scarred lung tissue, and breathing difficulties in workers from a precious metal refining facility exposed to a mixture of beryllium and other metals (Cullen *et al.*, 1987). Also, altered proliferative responses of lymphocytes obtained by bronchoalveolar lavage indicated increased T-cell activity *in vitro*. Cullen *et al.* (1987) reported a mean exposure level of 1.2 μg Be/m^3 (range = 0.22 – 43 μg/m^3). USEPA (1998) and ATSDR (2000) considered 0.52 μg Be/m^3 to be the LOAEL for CBD from this study since this was the average concentration in the furnace area where 4 of the 5 CBD cases worked.

Sensitization to beryllium, as measured by the beryllium lymphocyte proliferation test (BeLPT), can occur in the absence of chronic beryllium disease (Kreiss *et al*., 1989). The authors hoped that the identification of sensitized individuals without disease might prevent clinical disease, presumably by removing the individuals from exposure to beryllium. Some beryllium-sensitized individuals progress to having clinical disease (Newman *et al*., 1992). Data obtained from a four-year survey conducted at beryllium-copper alloy manufacturing factories in Japan (Yoshida *et al*., 1997) indicated that the T cells of workers continuously exposed to more than 0.01 μg Be/m^3 were activated and that the cell-mediated immune (CMI) response was promoted. The BeLPT in workers exposed to less than 0.01 μg Be/m^3 was unaffected.

Genetic influences on development of CBD have been identified. CBD is associated with the allelic substitution of glutamic acid for lysine at position 69 in the HLA-DPB1 protein (Richieldi *et al*., 1993). Up to 97% of CBD patients may have the Glu69 marker, but only 30-45% of beryllium-exposed, unaffected individuals carry the same marker. Because CBD occurs in only 1-6% of exposed workers, Glu69 is not likely to be the only genetic factor influencing the development of CBD. Changes in other sequences of the HLA-DPB1 gene and in the copy number of Glu69 are also involved (Wang *et al*., 1999).

The Rocky Flats Environmental Technology Site in Colorado is part of the U.S. Department of Energy nuclear weapons complex. Operations using Be began in 1953, Be production operations began in 1957, and the first case of CBD was diagnosed in a machinist in 1984. Exposures could have occurred during foundry operations, casting, shearing, rolling, cutting, welding, machining, sanding, polishing, assembly, and chemical analysis operations. Since 1991, 29 cases of CBD and 76-78 cases of beryllium sensitization have been identified (Stange *et al*., 1996). Several cases appear to have had only minimal Be exposure, since the employees were in administrative functions, not primary beryllium operations. Personal air monitoring devices used over a period of 4 years showed a breathing zone level of 1.04 μg Be/m^3. ATSDR (2000) considered 1.04 μg Be/m^3 to be the LOAEL for this study. A recent case-control study of workers at Rocky Flats (Viet *et al*., 2000) suggested that exposures of workers to lower Be levels might lower the future incidence of CBD, but not necessarily the incidence of sensitivity to Be.

Kreiss *et al*. (1996) investigated the prevalence of beryllium sensitization in relation to work process and beryllium exposure measurements in a beryllia ceramics plant that had operated since 1980. In 1992 they interviewed 136 employees (97.8% of the workforce), ascertained beryllium sensitization with the beryllium lymphocyte proliferation blood test (BeLPT), and reviewed industrial hygiene measurements. Eight employees were beryllium-sensitized (5.9%); six of the eight had granulomatous disease based on transbronchial lung biopsy. Machinists had a Be sensitization rate of 14.3% compared to 1.2% among other employees. Machining operations (drilling, dicing, centerless grinding, and/or surface grinding) had significantly higher general area and breathing zone measurements than other work processes during the time in which most beryllium-sensitized cases had started machining. Daily weighted average estimates of exposure for matching processes also exceeded estimates for other work processes in that time period (median daily weighted average = 0.9 $\mu g/m^3$). Daily weighted averages for the machining process accounted for the majority of exceedances of the 2.0 $\mu g/m^3$ OSHA Permissible Exposure Limit (PEL); 8.1% of machining daily weighted averages were above the PEL. The LOAEL from this study was 0.55 $\mu g/m^3$, the median exposure of the sensitized workers.

The facility was again surveyed in 1998 after some attempts were made to lower exposure to beryllium (Henneberger *et al*., 2001). The investigators separated the workers into 77 long-term workers hired before the 1992 screening and 74 short-term workers hired after 1992. Among 20 short-term workers exposed to the lowest mean Be level (0.05 to 0.19 $\mu g/m^3$), two showed Be sensitivity by the BeLPT test. Thus a fraction of workers appears to be exquisitely sensitive to beryllium.

Based on a review of this and other occupational studies Wambach and Tuggle (2000) have suggested that the workplace standard of 2 $\mu g/m^3$ be lowered to 0.1 $\mu g/m^3$. Some workers might still be sensitized to beryllium at this level (Yoshida *et al*., 1997).

V. Effects of Animal Exposure

Three chronic studies, two in rats (Vorwald and Reeves, 1959; Reeves *et al.*, 1967) and one in guinea pigs (Reeves *et al.*, 1970), observed adverse inflammatory and proliferative respiratory changes following inhalation exposure to beryllium compounds. Vorwald and Reeves (1959) observed inflamed lungs and fibrosis in rats exposed to 0.006 mg Be/m^3 (as BeO) for an unspecified duration. A later study exposed Sprague-Dawley CD rats for 72 weeks (7 hr/d, 5 d/wk) to 34.25 µg Be/m^3 from $BeSO_4$ (Reeves *et al.*, 1967). Gross and histological changes observed in exposed versus unexposed rats included increased lung weight, inflamed lungs, emphysema, arteriolar wall thickening, granulomas, fibrosis, and proliferative responses within the alveoli (LOAEL = 34.25 µg Be/m^3). Guinea pigs were exposed to 0, 3.7, 15.4, or 29.3 µg Be/m^3 (from the sulfate) for 6 hours/day, 5 days/week for up to 1 year (Reeves *et al.*, 1970). Respiratory alterations observed in the beryllium-exposed groups included increased tracheobronchial lymph node and lung wet weights, interstitial pneumonitis, and granulomatous lesions. These adverse respiratory effects were observed in all the beryllium dosed groups and indicated a chronic inhalation LOAEL of 3.7 µg Be/m^3.

Wagner *et al.* (1969) exposed monkeys, rats, and hamsters to 0.21 and 0.62 mg Be/m^3 as fumes from bertrandite or beryl ore, respectively, for 6 hours/day, 5 days/week for up to 17 months. Exposed animals displayed severe effects, including (1) bronchial lymphocytic infiltrates, abscesses, consolidated lobes, and granulomatous lesions after exposure to 0.21 mg Be/m^3 from bertrandite ore, and (2) inflamed lungs, fibrosis, and granuloma after exposure to 0.62 mg Be/m^3 from beryl ore. Lung inflammation was observed in the exposed monkeys, and a few granulomatous lung lesions were observed in the hamsters after similar exposure conditions (up to 23 months).

Immunological effects have been observed in a few subchronic studies (Schepers, 1964; Schepers *et al.*, 1957; Stiefel *et al.*, 1980). Schepers (1964) exposed monkeys (*Macacus mullata*) to three soluble forms of beryllium (BeF_2, $BeSO_4$, $BeHPO_4$) daily for 6 hours/day over 7 to 30 days. Increased lung weight, inflammation, emphysema, and fibrosis of the lung were observed after 17 days at 0.198 mg Be/m^3 (as $BeSO_4$). Histological examination found pleuritis, congestion, emphysema, consolidation, and edema of the lung. Immunological effects were seen as hyperplasia of the lymph nodes typical of immune activation after 7 to 18 days exposure to either 0.198 or 0.184 mg Be/m^3 as the sulfate or fluoride. A subchronic inhalation study reported immunological effects as increased, beryllium-specific stimulation of T-lymphocytes *in vitro* from Wistar rats and guinea pigs exposed daily (6 hours/day) over 10 weeks (LOAEL = 0.5 mg/m^3) (Stiefel *et al.*, 1980). However, a subchronic inhalation study in Wistar and Sherman rats (Schepers *et al.*, 1957) observed multiple lung alterations including granulomas (LOAEL = 35 µg Be/m^3) but did not find any accompanying immunological effects after 30 days discontinuous exposure (5-6 d/wk, 4-8 hr/d) to beryllium fumes from $BeSO_4$.

VI. Derivation of Chronic Reference Exposure Levels

Derivation of Inhalation Reference Exposure Level

Key study	Kreiss *et al*., 1996
Study population	8 beryllium-sensitized workers among 136 employees in a beryllia ceramics plant
Exposure method	Workplace
Critical effects	Beryllium sensitization (chronic beryllium disease)
LOAEL	0.55 µg/m^3 (median exposure of sensitized workers)
NOAEL	Not observed
Exposure continuity	Workplace
Average experimental exposure	0.2 µg/m^3 for LOAEL group (0.55 x 10/20 x 5/7)
Human equivalent concentration	0.2 µg/m^3
Exposure duration	6.1 years (5 mo – 10 yr)
LOAEL uncertainty factor	10 (low incidence but serious, irreversible chronic disease)
Subchronic uncertainty factor	1
Interspecies uncertainty factor	1
Intraspecies uncertainty factor	3 (sensitized may not be only sensitive subpopulation) (see below)
Cumulative uncertainty factor	30
Inhalation chronic REL	0.007 µg/m^3

Supportive study	Eisenbud *et al.* (1949)
Study population	Approximately 10,000 individuals within 2 miles of a beryllium manufacturing plant
Exposure method	Environmental exposure
Critical effects	Pulmonary berylliosis in 11 residents
LOAEL	0.03 µg/m^3 (geometric mean of range of measured exposures associated with berylliosis of 0.01 to 0.1 µg/m^3)
NOAEL	Not observed
Exposure continuity	Continuous
Average exposure	Estimated to be approximately 0.3 µg/m^3 (historical exposures estimated to be 10-fold higher than measured values) for LOAEL group
Human equivalent concentration	0.3 µg/m^3 for LOAEL group
Exposure duration	Up to 7 years
LOAEL uncertainty factor	10
Subchronic uncertainty factor	3
Interspecies uncertainty factor	1
Intraspecies uncertainty factor	3
Cumulative uncertainty factor	100
Inhalation chronic REL	0.003 µg/m^3

U.S. EPA (1998) developed an RfC of 0.02 µg/m^3 based on beryllium sensitization and progression to chronic beryllium disease (CBD) identified by Kreiss *et al*. (1996). The Kreiss *et al*. (1996) occupational exposure study identified a LOAEL for beryllium sensitization in workers of 0.55 µg/m^3 (median of average exposure concentrations of the 8 Be sensitized workers). The Eisenbud *et al*. (1949) study, which U.S. EPA used as a co-principal study and which in U.S. EPA's opinion used relatively insensitive screening methods, suggested a NOAEL of 0.01-0.1 µg/m^3 in community residents living near a beryllium plant. U.S. EPA used the LOAEL from the Kreiss *et al*. (1996) study for the operational derivation of the RfC, because the screening method used in the Eisenbud *et al*. (1949) study was considered to be less sensitive than the method used in the Kreiss *et al*. (1996) study. The LOAEL was time adjusted to 0.2 µg/m^3, then a total UF of 10 was used to obtain the RfC of 0.02 µg/m^3. The UF of 10 was comprised of a UF of 3 to account for the sensitive nature of the subclinical endpoint (beryllium sensitization) and a database UF of 3 to account for the poor quality of exposure monitoring in the Kreiss *et al*. and Eisenbud *et al*. studies. Poor exposure monitoring was also a problem in other epidemiology studies that assessed the incidence of beryllium sensitization. The U.S. EPA did not explicitly apply a LOAEL to NOAEL uncertainty factor. Thus implicitly the factor is 1.

OEHHA prefers to use the methodology for assignment of UFs, which is described in OEHHA (2000) and used in our derivation of the REL for beryllium, including use of a LOAEL to NOAEL Uncertainty Factor of 10. Since chronic beryllium disease (CBD) is serious, chronic, disabling, usually irreversible, and often fatal (Newman *et al*., 1997), it is difficult to justify use of a LOAEL to NOAEL factor of only 3. OEHHA has not used database deficiency UFs since the criteria for use of such factors are not well specified by U.S. EPA. The people who get CBD are likely that part of the population who are by nature more sensitive to beryllium, for example those with the human leukocyte antigen (HLA) class II marker HLA-DP Glu69 (Richeldi *et al*., 1993; Saltini *et al*., 1998). Although it is likely that the effects are seen in a "sensitive subpopulation," OEHHA applied an intraspecies uncertainty factor (UF_H). OEHHA used an intermediate UF_H of 3, since 1) there may be other population factors involved in being sensitive, such as immature lungs, and 2) all the diseased were initially healthy adult workers.

For comparison the LOAEL from guinea pigs of 3.7 µg Be/m^3 (Reeves *et al*., 1970) is equivalent to a continuous exposure of 0.66 µg/m^3. Division by UFs of 10 for intraspecies, 10 for interspecies (since HEC adjustments are not available yet for guinea pigs), and 10 for use of a LOAEL results in a REL of 0.0007 µg/m^3

VII. Data Strengths and Limitations for Development of the REL

The major strength of the inhalation chronic REL for beryllium is the use of human data from persons occupationally exposed. The major uncertainties are the lack of a NOAEL observation in the key study, the lack of long-term exposure data, the difficulty of estimating exposures, and the lack of chronic exposure data.

VIII. Potential for Differential Impacts on Children's Health

No evidence to support a differential effect of beryllium on infants or children was found in the literature. However, children have developed beryllium disease from metal brought home on the parents' work clothes and by living near a facility using beryllium. Unfortunately the number of children and their ages were not published (Eisenbud *et al*., 1948).

Derivation of Chronic Oral Reference Exposure Level

In addition to being inhaled, airborne beryllium can settle onto crops and soil and enter the body by ingestion. Thus an oral chronic reference exposure level for beryllium is also required for conducting Air Toxics Hot Spots risk assessments.

Study	Morgareidge *et al*., 1976
Study population	Male and female dogs (5/sex/group)
Exposure method	Diet containing 0, 1, 5, 50 or 500 ppm Be as beryllium sulfate tetrahydrate
Critical effects	Small intestinal lesions
LOAEL	500 ppm
NOAEL	50 ppm (1.2 mg/kg bw-day)
Exposure continuity	Continuous
Exposure duration	Up to 3 years, 4 months
Average experimental exposure	1.2 mg/kg bw-day (males, 1.1; females, 1.3)
BMD_{05}	0.244 mg/kg-day
LOAEL uncertainty factor	Not needed in BMD approach
Subchronic uncertainty factor	1
Interspecies uncertainty factor	10
Intraspecies factor	10
Cumulative uncertainty factor	100
Oral reference exposure level	0.002 mg/kg-day

Morgareidge *et al*. (1976) conducted a long-term feeding study in which beagle dogs (aged 8 to 12 mo) were fed diets (for 1 h per day) containing 0, 5, 50, or 500 ppm Be for 172 weeks. The 500 ppm group was terminated at 33 weeks because of overt signs of toxicity, and an additional group was added to the study and fed a diet containing 1 ppm Be (for 143 weeks). The 1, 5, 50, and 500 ppm concentrations corresponded to doses of 0.023, 0.12, 1.1, and 12.2 mg/kg-day for males and 0.029, 0.15, 1.3, and 17.4 mg/kg-day for females. All animals in the 500 ppm group showed fairly extensive erosive (ulcerative) and inflammatory lesions in the gastrointestinal tract. These occurred predominantly in the small intestine and to a lesser extent in the stomach and large intestine, and were considered treatment related. All animals with stomach or large intestinal lesions also had lesions in the small intestine, except for one animal (whose stomach lesions were very localized and not very severe). Lesions in the small intestine (4/5 males and 5/5 females) were considered to be treatment-related and included desquamation of the epithelium, edema, fibrin thrombi, acute inflammation, subacute/chronic inflammation, necrosis and thinning/atrophy of the epithelium, and ulceration. High-dose animals also showed

moderate to marked erythroid hypoplasia of the bone marrow, which the authors also considered treatment related. (Bile stasis and vasculitis in the liver, acute inflammation in the lymph nodes, and kidney occurring in these animals was attributed to a likely systemic bacterial invasion through the damaged intestinal mucosa.) In the 50 ppm group, one female dog, which died after 70 weeks of treatment, showed gastrointestinal lesions, which were less severe, but occurred in the same locations and appeared to be the same types of lesions as those in dogs administered 500 ppm. The observation that beryllium is poorly absorbed by the gastrointestinal tract (Owen, 1990; ATSDR, 2000) probably explains why lesions were not seen outside the gastrointestinal tract. In addition the predominance of lesions in the small intestine may have been partly due to precipitation of beryllium phosphate there due to the slightly alkaline pH (Reeves, 1965). Thus 500 ppm was a LOAEL and 50 ppm was a NOAEL (statistically) for gastrointestinal lesions.

USEPA used the same study to derive its RfD of 0.002 mg/kg-day. The U.S. EPA stated its confidence in the RfD as: study - medium; database – low to medium, and RfD - low to medium. USEPA used a BD_{10} approach and included a database UF of 3. OEHHA used a BD_{05} approach (specifically a Weibull model in the USEPA's BMDS software) and did not include a database UF since the criteria for use of modifying factors such as this are not well specified by U.S. EPA. However, the final value for the oral chronic REL was the same as the USEPA's RfD.

This RfD and the oral REL are limited to soluble beryllium salts. Data on the teratogenicity or reproductive effects of beryllium are limited. Beryllium has been reported to produce terata and increased mortality in chick embryos.

When assessing the health effects of beryllium, its carcinogenicity must also be assessed.

IX. References

ATSDR. 1993. Agency for Toxic Substances and Disease Registry. U.S. Department of Health and Human Services, Public Health Service, Agency for Toxic Substances and Disease Registry. Toxicological Profile for Beryllium. TP-92/04. Atlanta, GA: ATSDR. April 1993.

ATSDR. 2000. Agency for Toxic Substances and Disease Registry. U.S. Department of Health and Human Services, Public Health Service, Agency for Toxic Substances and Disease Registry. Toxicological Profile for Beryllium (update). Dreaft for Public Comment. Atlanta, GA: ATSDR. September 2000.

CARB. 2000. California Air Resources Board. California Emissions Inventory Development and Reporting System. (CEIDARS). Data from Data Base Year 1998. February 12, 2000.

Cotes JE, Gilson JC, McKerrow CB, and Oldham P. 1983. A long-term follow-up of workers exposed to beryllium. Br. J. Ind. Med. 40:13-21.

CRC. 1994. CRC Handbook of Chemistry and Physics, 75th edition. Lide DR, ed. Boca Raton, FL: CRC Press Inc.

Cullen M, Kominsky J, Rossman M, Cherniack M, Rankin J, Balmes J, Kern J, Daniele R, Palmer L, Naegel G, McManus K, and Cruz R. 1987. Chronic beryllium disease in a precious metal refinery. Clinical epidemiologic and immunologic evidence for continuing risk from exposure to low level beryllium fume. Am. Rev. Respir. Dis. 135:201-208.

Eisenbud M, and Lisson J. 1983. Epidemiological aspects of beryllium-induced nonmalignant lung disease: A 30 year update. J. Occup. Med. 25:196-202.

Eisenbud M, Berghout CF, and Steadman L. 1948. Environmental studies in plants and laboratories using beryllium: the acute disease. J. Ind. Hyg. Toxicol. 30:282-285.

Eisenbud M, Wanta R, Dustan C, Steadman L, Harris W, and Wolf B. 1949. Nonoccupational berylliosis. J. Ind. Hyg. Toxicol. 31:282-294.

Henneberger PK, Cumro D, Deubner DD, Kent MS, McCawley M and Kreiss K. 2001. Beryllium sensitization and disease among long-term and short-term workers in a beryllium ceramics plant. Int. Arch. Occup. Environ. Health. 74(3):167-176.

Infante PF, Wagoner JK, and Sprince NL. 1980. Mortality patterns from lung cancer and nonneoplastic respiratory disease among white males in the beryllium case registry. Environ. Res. 21:35-43.

Johnson NR. 1983. Beryllium disease among workers in a spacecraft-manufacturing plant - California. MMWR. 32:419-425.

Kreiss K, Newman LS, Mroz MM, and Campbell PA. 1989. Screening blood test identifies subclinical beryllium disease. J. Occup. Med. 31(7):603-608.

Kreiss K, Mroz MM, Newman LS, Martyny J, and Zhen B. 1996. Machining risk of beryllium disease and sensitization with median exposures below 2 micrograms/m^3. Am. J. Ind. Med. 30(1):16-25.

Kriebel D, Sprince NL, Eisen EA, Greaves I, Feldman H and Greene R. 1988a. Beryllium exposure and pulmonary function: A cross sectional study of beryllium workers. Br. J. Ind. Med. 45:167-173.

Kriebel D, Sprince NL, Eisen EA, and Greaves I. 1988b. Pulmonary function in beryllium workers: Assessment of exposure. Br. J. Ind. Med. 45:83-92.

Lieben J, and Metzner F. 1959. Epidemiological findings associated with beryllium extraction. Am. Ind. Hyg. Assoc. 20:494-499.

Metzner F, and Lieben J. 1961. Respiratory disease associated with beryllium disease - A case study. J. Occup. Med. 3:341-345.

Morgareidge K, Cox GE and Gallo MA. (1976) Chronic feeding studies with beryllium in dogs. Food and Drug Research Laboratories, Inc. Submitted to the Aluminum Company of America, Alcan Research & Development, Ltd., Kawecki-Berylco Industries, Inc., and Brush-Wellman, Inc.

Newman LS, Mroz MM, Schumacher B, Daniloff E, and Kreiss K. 1992. Beryllium sensitization precedes chronic beryllium disease. Am. Rev. Resp. Dis. (Suppl) 145:A324.

Newman LS, Lloyd J, and Daniloff E. 1996. The natural history of beryllium sensitization and chronic beryllium disease. Environ. Health Perspect. 104S(5):937-943.

OEHHA. 2000. Office of Environmental Health Hazard Assessment. Air Toxics Hot Spots Program Risk Assessment Guidelines. Part III. Technical Support Document for the Determination of Noncancer Chronic Reference Exposure Levels. Available on-line at http://www.oehha.ca.gov

Owen BA. 1990. Literature-derived absorption coefficients for 39 chemicals via oral and inhalation routes of exposure. Regul. Toxicol. Pharmacol. 11(3):237-252.

Reeves AL. 1965. The absorption of beryllium from the gastrointestinal tract. Arch. Environ. Health. 11(2):209-214.

Reeves AL, Deitch D, and Vorwald AJ. 1967. Beryllium carcinogenesis. I. Inhalation exposure of rats to beryllium sulfate aerosol. Cancer Res. 27:439-443.

Reeves AL, Swanborg RH, Busby EK, and Krivanek ND. 1970. In: Target Organ Toxicology Series: Immunotoxicology and Immunopharmacology. Dean J, Luster M, Munson A, and Amos H, eds. New York, NY: Raven Press, Inc. pp. 441-456.

Richeldi L, Sorrentino R, and Saltini C. 1993. HLA-DPB1 glutamate 69: a genetic marker of beryllium disease. Science 262(5131):242-244.

Saltini C, Amicosante M, Franchi A, Lombardi G and Richeldi L. 1998. Immunogenetic basis of environmental lung disease: lessons from the berylliosis model. Eur Respir J. 12(6):1463-75.

Schepers GW. 1964. The biological action of beryllium: Reaction of the monkey to inhaled aerosols. Ind. Med. Surg. 33:1-16.

Schepers GW, Durkhan TM, Delahunt AB, and Creedon FT. 1957. The biological action of inhaled beryllium sulfate. A preliminary chronic toxicity study in rats. AMA Arch. Ind. Health 15:32-58.

Schroeder HA, and Mitchner M. 1975. Life-term studies in rats: Effects of aluminum, barium, beryllium and tungsten. J. Nutr. 105:421-427.

Smith CJ, Livingston SD and Doolittle DJ. 1997. An international literature survey of "IARC Group I carcinogens" reported in mainstream cigarette smoke. Food Chem. Toxicol. 35(10-11):1107-1130.

Stange AW, Hilmas DE, and Furman FJ. 1996. Possible health risks from low level exposure to beryllium. Toxicology 111(1-3):213-224.

Stiefel T, Schulze K, Zorn H, and Toelg G. 1980. Toxicokinetic and toxicodynamic studies of beryllium. Arch. Toxicol. 45:81-92.

U.S. Environmental Protection Agency. 1998. Integrated Risk Information System (IRIS) Database. Available online at http://www.epa.gov/iris

Viet SM, Torma-Krajewski J, and Rogers J. 2000. Chronic beryllium disease and beryllium sensitization at Rocky Flats: a case-control study. AIHAJ. 61(2):244-254.

Vorwald A, and Reeves A. 1959. Pathologic changes induced by beryllium compounds. Arch. Indust. Health 19:190-199.

Wagner W, Groth D, Holtz J, Madden G, and Stokinger H. 1969. Comparative chronic inhalation toxicity of beryllium ores bertrandite and beryl, with production of pulmonary tumors by beryl. Toxicol. Appl. Pharmacol. 15:10-29.

Wambach PF, and Tuggle RM. 2000. Development of an eight-hour occupational exposure limit for beryllium. Appl. Occup. Environ. Hyg. 15(7):581-587

Wang Z, White PS, Petrovic M, Tatum OL, Newman LS, Maier LA, and Marrone BL. 1999. Differential susceptibilities to chronic beryllium disease contributed by different Glu69 HLA-DPB1 and -DPA1 alleles. J. Immunol. 163(3):1647-1653.

Yoshida T, Shima S, Nagaoka K, Taniwaki H, Wada A, Kurita H, and Morita K. 1997. A study on the beryllium lymphocyte transformation test and the beryllium levels in working environment. Ind. Health 35(3):374-379.

CHRONIC TOXICITY SUMMARY

1,3-BUTADIENE

(butadiene; buta-1,3-diene; biethylene; bivinyl; divinyl; vinylethylene)

CAS Registry Number: 106-99-0

I. Chronic Toxicity Summary

Inhalation reference exposure level	**20 µg/m^3** (8 ppb)
Critical effect(s)	Increased incidence of ovarian atrophy in mice
Hazard index target(s)	Female reproductive system

II. Physical and Chemical Properties Summary (HSDB, 2000; CRC, 1995)

Description	Colorless gas
Molecular formula	C_4H_6
Molecular weight	54.09 g/mol
Boiling point	–4.4^{o}C
Melting point	–108.9^{o}C
Vapor pressure	910 torr at 20°C
Solubility	Very slightly soluble in water (735 mg/L); soluble in ethanol, ether, acetone, benzene and organic solvents
Conversion factor	1 ppm = 2.21 mg/m^3 at 25°C

III. Major Uses and Sources

1,3-Butadiene is a major commodity product of the petrochemical industry, usually produced as a by-product of ethylene. The majority of 1,3-butadiene is used in the production of styrene-butadiene rubber copolymers (SBR). Other applications include use as a polymer component for polybutadiene, hexamethylene diamine, styrene-butadiene latex, acrylonitrile-butadiene-styrene (ABS) resins, chloroprene and nitrile rubbers. A variety of industrial syntheses use 1,3-butadiene resins (AB as a chemical intermediate, such as in the production of adiponitrile (a nylon precursor), captan and captofol fungicides, ethylidene norbornene and sulfolane, boron alkyls, and hexachlorobutadiene. Additionally, 1,3-butadiene is found in automobile exhaust, gasoline vapor, fossil fuel incineration products, and cigarette smoke (HSDB, 2000). In 1996, the latest year tabulated, the statewide mean outdoor monitored concentration of 1,3-butadiene was approximately 0.2 ppb (CARB, 1999). The South Coast Air Quality Management District (SCAQMD, 2000) detected ambient levels of 1,3-butadiene ranging from 0.1 to 0.8 ppb at 10 stationary monitors placed throughout the South Coast Air Basin. The annual statewide industrial emissions from facilities reporting under the Air Toxics Hot Spots Act in California

based on the most recent inventory were estimated to be 20,846 pounds of 1,3-butadiene (CARB, 2000).

IV. Effects of Human Exposure

An early occupational study reported complaints of irritation of eyes, nasal passages, throat, and lungs in rubber manufacturing workers following acute exposure to unknown levels of 1,3-butadiene (Wilson, 1944). Additional symptoms reported included coughing, fatigue, and drowsiness; however, all symptoms ceased on removal from the exposure.

Studies on the chronic effects of 1,3-butadiene have been centered in the styrene-butadiene rubber manufacturing industry, which uses large quantities of 1,3-butadiene, and in the 1,3-butadiene monomer industry. One retrospective epidemiological study reported an increase in overall mortality, emphysema, and cardiovascular diseases (chronic rheumatic and arteriosclerotic heart disease) among rubber workers (McMichael *et al.*, 1976). Two other occupational studies (Divine and Hartman, 1996; Matanoski *et al.*, 1990) indicated that the standardized mortality ratio for deaths from arteriosclerotic heart disease was elevated (~1.4-1.8) among black workers in the 1,3-butadiene rubber industry. Other occupational studies have described the potential for adverse hematological effects due to butadiene exposure (Checkoway and Williams, 1982; McMichael *et al.*, 1975). A survey of workers at a styrene-butadiene rubber plant revealed slightly lower levels (but within normal range) of red blood cells, hemoglobin, platelets, and neutrophils in exposed (mean = 20 ppm) versus unexposed workers (Checkoway and Williams, 1982). And 1,3-butadiene has been implicated in hematopoietic malignancies among styrene-butadiene rubber workers at levels lower than 20 ppm (McMichael *et al.*, 1975). Since the workers in these studies were exposed to mixtures of chemicals, the specific contribution of butadiene to the adverse respiratory and hematopoietic effects remains unclear.

V. Effects of Animal Exposure

The few available chronic animal inhalation studies have focused on the potential carcinogenicity of 1,3-butadiene. The National Toxicology Program (NTP) has sponsored two chronic inhalation studies in $B6C3F_1$ mice (NTP, 1984; Melnick *et al.*, 1990; NTP, 1993), while Hazelton Laboratories Europe (HLE) Ltd. conducted a chronic inhalation study in Sprague-Dawley rats (HLE, 1981; Owen *et al.*, 1987; Owen and Glaister, 1990).

The two $B6C3F_1$ mice inhalation studies sponsored by NTP (Huff *et al.*, 1985; Melnick *et al.*, 1990; NTP, 1984; NTP, 1993), although focused on carcinogenicity, identified other adverse chronic effects. The earlier NTP (1984) study in mice administered 0, 625 or 1250 ppm 1,3-butadiene for 6 hours/day, 5 days/week for up to 61 weeks. Nonneoplastic changes observed were elevated testicular and ovarian atrophy at both doses (625 and 1250 ppm); liver necrosis in male mice at both doses and in female mice at 1250 ppm; and nonneoplastic lesions in the nasal cavity at 1250 ppm. At the highest dose, adverse changes in the nasal cavity included chronic inflammation, fibrosis, cartilaginous metaplasia, osseous metaplasia, and atrophy of the sensory epithelium. No nasal or respiratory lesions were seen in the controls. This study identified a chronic LOAEL of 625 ppm for gonadal atrophy in both sexes.

The later NTP study (Melnick *et al.*, 1990; NTP, 1993) used lower exposure concentrations of 1,3-butadiene (0, 6.25, 20, 62.5, 200 or 625 ppm) administered 6 hours/day, 5 days/week for up to 2 years. Two-year survival was significantly decreased in mice exposed to 20 ppm and greater, primarily due to chemical-related malignant neoplasms. Increased incidences of non-neoplastic lesions in exposed mice included bone marrow atrophy, gonadal atrophy (testicular, ovarian and uterine), angiectasis, alveolar epithelial hyperplasia, forestomach epithelial hyperplasia, and cardiac endothelial hyperplasia. Gonadal atrophy was observed at 200 ppm and 625 ppm for males and at 6.25 ppm and higher for females. Bone marrow toxicity (regenerative anemia) was seen at 62.5 ppm and higher. This study identified a chronic LOAEL of 6.25 ppm for reproductive toxicity, and a NOAEL of 200 ppm and a LOAEL of 625 for non-neoplastic hematotoxic effects.

Table 1. Reproductive system atrophy and 2 year survival (NTP, 1993)

	Butadiene (ppm)	*Female survival*	*Atrophy of ovary*	*Atrophy of uterus*	*Male survival*	*Atrophy of testicle*
	0	37/50	4/49	1/50	35/50	1/50
	6.25	33/50	19/49	0/49	39/50	3/50
	20	24/50	32/48	1/50	24/50	4/50
	62.5	11/50	42/50	1/49	22/50	2/48
	200	0/50	43/50	8/50	4/50	6/49
	625	0/80	69/79	41/78	0/70	53/72

The U.S. EPA (1985) reviewed data from a 2-year chronic inhalation toxicity study sponsored by the International Institute of Synthetic Rubber Producers (IISRP) at Hazelton Laboratories Europe, Ltd (1981) on Sprague-Dawley rats exposed to 0, 1000 or 8000 ppm 1,3-butadiene. Results from the study were also reported later by Owen *et al.* (1987; 1990). Minor clinical effects, including excessive eye and nose secretions plus slight ataxia, were observed between 2 and 5 months in rats exposed to 8000 ppm 1,3-butadiene. Alterations in organ weight were also observed in this high exposure group. A dose-related increase in liver weights was observed at both the 52-week interim kill and at study termination. Absolute and relative kidney weight was also significantly increased and associated with nephrosis. No reproductive organ atrophy was reported in this rat study; however, tumors were found in reproductive tissues (Owen *et al.*, 1987).

Penn and Snyder (1996a,b) exposed cockerels (young male chickens) to 0 or 20 ppm 1,3-butadiene 6 hr/day, 5 days/week for 16 weeks to study arteriosclerotic plaque development. The cockerel is a sensitive animal model for studying the effects of environmental arteriosclerotic plaque-promoting agents. Plaque frequency and location were not affected. However, plaque sizes were significantly larger in 1,3-butadiene-treated cockerels than in controls.

The U.S. EPA (1985) described another secondary report, that of Miller (1978), which reviewed a group of Russian studies of subchronic 1,3-butadiene exposure in rats. One study (reported by Ripp in 1967) continuously exposed rats to relatively lower concentrations of 0.45, 1.4 or 13.5 ppm. At 13.5 ppm, blood cholinesterase was elevated, blood pressure was lowered, and motor

activity was decreased. Histopathological changes reported at 0.45 ppm were congestion in the spleen and hyperemia and leukocyte infiltration of cardiac tissue. Alterations in lung tissue noted at 1.4 and 13.5 ppm included atelectasis, interstitial pneumonia, and emphysema. No other studies used such low exposure levels or measured such endpoints. Unfortunately, the specific research methods and results for this study are unavailable for direct review and comparison.

A series of reproductive and developmental toxicity studies undertaken by U.S. EPA was summarized by Morrissey *et al*. (1990). In developmental toxicity studies, pregnant female rats and mice were exposed to 0, 40, 200, or 1000 ppm 1,3-butadiene for 6 hrs/day on days 6-15 of gestation. In rats, maternal body weight gain and extra-gestational body weight gain was reduced at the highest exposure. However, no evidence of developmental toxicity was observed. In mice, maternal body weight gain and extra-gestational body weight gain were reduced at 200 and 1000 ppm. Gravid uterine weight was reduced at 1000 ppm. Fetal and placental weights were reduced in an exposure-dependent manner with reduced male fetal body weight reaching statistical significance at 40 ppm and above. In the sperm head morphology assay and the dominant lethality study, groups of male mice were exposed to 200, 1000, and 5000 ppm 1,3-butadiene for 5 consecutive days. Concentration-related small increases in the percentages of abnormal sperm heads were observed, but were statistically significant only at the two highest exposures. Dominant lethal effects were observed only in the first two weeks following exposure. At week 1, the percentage of dead implants/total implants was increased only at 1000 ppm, and the percentage of females with $\geq$ 2 dead implants was increased at 200 and 1000 ppm. The number of dead implants/pregnancy was increased beginning at 1000 ppm at week 1, and 200 and 1000 ppm at week 2. While not strongly concentration dependent, the dominant lethality results are consistent with an adverse effect of 1,3-butadiene on more mature cells (spermatozoa and spermatids).

An acute and subchronic (10 week) study identified male-mediated F_1 effects in mice exposed to 12.5 or 1250 ppm 1,3-butadiene for 6 hours/day, 5 days/week (Anderson *et al.*, 1996). An additional group of mice were also exposed to 6250 ppm 1,3-butadiene in the acute study. Meaningful toxic effects were not observed in the acute study and no reproductive parameters were affected in either study. In the 10-week study, 1250 ppm (2762.4 mg/m^3) resulted in a statistically significant reduction in the number of implantations, an induction of dominant lethal mutations, an increased incidence of early and late deaths, and an increase in abnormalities. The lower level of 12.5 ppm (27.63 mg/m^3) resulted in an increase of late deaths and fetal abnormalities.

A follow-up of the Anderson *et al.* (1996) dominant lethality study exposed male mice to 12.5 or 125 ppm 1,3-butadiene under the same subchronic exposure conditions (Brinkworth *et al*., 1998). A statistically significant increase in early deaths was observed at 125 ppm. The incidences of late deaths, dead fetuses, and abnormalities were elevated at 125 ppm but were not statistically significant. Testicular DNA damage, as detected by the Comet assay, was observed at 125 ppm.

Further dominant lethality studies in rodents by the same research group exposed male mice to 12.5, 65, and 130 ppm 1,3-butadiene 6 hr/day, 5 days/week for four weeks (Anderson *et al*., 1998). Groups of male rats were also exposed to 65, 400, and 1250 ppm 1,3-butadiene 6 hr/day, 5 days/week for 10 weeks. In mice, a statistically significant increase in early deaths was

observed at 65 and 130 ppm but was not dose-related. Male-mediated effects in rats were not observed at any exposure level.

Pacchierotti *et al*. (1998) investigated 1,3-butadiene-induced toxic effects on spermatogenic cell stages and first-cleavage embryos. Exposure of male mice to 130, 500, and 1300 ppm 1,3-butadiene 6 hr/day for 5 days did not result in an increase of unfertilized oocytes after pairing with untreated females. However, statistically significant increases of cytogenetic aberrations in first-cleavage embryos were observed in the first mating week in mice exposed to 500 and 1300 ppm, and in the second mating week in mice treated with 1300 ppm. Treatment-related effects on differentiating spematogonia were shown by a concentration-dependent decrease of round spermatids occurring 21 days after exposure, and confirmed 7 days later by a similar decrease of elongated spermatids. Testis weight was significantly reduced at all doses tested, 21 days after the end of exposure. A dose-dependent increase of variant sperm with single-stranded DNA content was observed 28 days after exposure, and attained statistical significance at 1300 ppm.

VI. Derivation of Chronic Reference Exposure Level (REL)

Study	NTP (1993)
Study population	$B6C3F_1$ mice (70/sex/group)
Exposure method	Discontinuous inhalation (0, 6.25, 20, 62.5, 200, 625 ppm) over 2 years
Critical effects	Increased incidence of ovarian atrophy
LOAEL	6.25 ppm
NOAEL	Not observed
BMC_{05}	1.40 ppm
Exposure continuity	6 hr/d, 5 d/wk
Exposure duration	103 weeks
Average experimental exposure	0.25 ppm for BMC_{05} (1.40 ppm x 6/24 hr/day x 5/7 days/week)
Human equivalent concentration	0.25 ppm (gas with systemic effects, based on RGDR = 1.0 using default assumption that lambda (a) = lambda (h))
LOAEL uncertainty factor	Not needed in the BMC approach
Subchronic uncertainty factor	1
Interspecies uncertainty factor	3
Intraspecies uncertainty factor	10
Cumulative uncertainty factor	30
Inhalation reference exposure level	8 ppb (0.008 ppm; 0.02 mg/m^3; 20 $\mu g/m^3$)

The chronic REL for butadiene is based on an increased incidence of ovarian atrophy in mice. Characteristically, affected females had no evidence of oocytes, follicles, or corpora lutea. Significant reproductive toxicity was observed in both sexes of mice at the interim 9-month, interim 15-month, and 2-year study termination as gonadal atrophy (NTP, 1993). Testicular atrophy was induced in male B6C3F1 mice at 625 ppm or above in this principal study and in a previous study (NTP, 1984). In female mice exposed for 9-months, ovarian atrophy was

observed at 200 and 625 ppm (442 or 1381 mg/m^3, respectively). After 15 months, ovarian atrophy was observed at exposure levels of 20 ppm (44.2 mg/m^3) and above. In mice exposed for up to 2 years (103 weeks), the incidence of ovarian atrophy increased at all exposure concentrations relative to controls, which establishes a chronic LOAEL of 6.25 ppm (13.81 mg/m^3) for reproductive toxicity.

Presentation of the ovarian atrophy data in quantal form (see Table 1) allows the use of the benchmark concentration (BMC) approach to determine the REL. A log-normal probit analysis (U.S. EPA, National Center for Environmental Assessment, benchmark dose software, version 1.20) using only the control group and the log-dose of the three lowest butadiene exposure groups provided the lowest chi-square value (i.e., the best line fit to the data points). The proportion of mice developing ovarian atrophy in the two highest exposure groups did not increase appreciably with increasing exposure concentration, and therefore, deviated from the log-normal probit plot. The significantly shortened survival rate in these two groups may be one reason for this deviation. Another possible cause is that a relatively resistant subgroup of mice (to ovarian atrophy) is revealed at the two highest doses following 2-year exposure to 1,3-butadiene. Thus, it may be biologically plausible to remove these resistant subgroups when using a BMC approach. The maximum likelihood estimate (MLE) for a 5% response was 1.53 ppm. The resulting 95% lower confidence limit at the MLE provided a BMC_{05} of 1.40 ppm. A BMC_{05} is considered to be similar to a NOAEL in estimating a concentration associated with a low level of risk.

The mouse ovary is more sensitive to butadiene's epoxide metabolites than the rat ovary. Doerr *et al*. (1996) administered butadiene monoepoxide (BMO) or butadiene diepoxide (BDE) intraperitoneally to female B6C3F1 mice and Sprague-Dawley rats for 30 days and found that BMO and BDE exhibited a greater ovotoxic potential in the mice compared to the rats. Dahl *et al*. (1991) reported that, for equivalent inhalation exposures, the concentrations of total butadiene metabolites in blood were 5-50 times lower in the monkeys than in the mice and 4-14 times lower than in the rats. People may be more like the monkey than the mouse or the rat in their formation of epoxides from butadiene. In vitro metabolism studies with human liver tissue present conflicting results regarding whether humans would be more like rats or mice in forming epoxide metabolites (Bond *et al*., 1996; Duescher and Elfarra, 1994). The considerable degree of interindividual variability in human samples was a reason given for the inconsistencies. Several pharmacokinetic models (Sweeney *et al*., 1997; reviewed by Himmelstein *et al*., 1997) have been developed to adjust for species differences in pharmacokinetics. However, an interspecies pharmacodynamic adjustment for this ovarian atrophy endpoint with butadiene is still needed. Therefore OEHHA staff use an interspecies uncertainty factor of 3 to account for pharmacodynamic differences between mice and women.

Christian (1996) has postulated that it may be inappropriate to develop health-protective values for 1,3-butadiene based on 2-year ovarian atrophy in mice because the mice are beyond their normal reproductive age. It was suggested that the 15-month evaluation of ovarian atrophy conducted by the NTP (1993) would be a better indicator of reproductive risk. However, OEHHA staff believes that butadiene-induced ovarian atrophy represents a toxic manifestation in an organ system. The fact that it occurs in a reproductive organ is immaterial for the development of a chronic REL. Nonetheless, a comparison REL based on the 15-month interim

evaluation for ovarian atrophy can be estimated. Quantal data at the 15-month interim evaluation shows that no mice developed ovarian atrophy (0/10) in the control group or at the lowest exposure. Ovarian atrophy was observed in 1/10, 9/10, 7/10, and 2/2 mice at the 20, 62.5, 200, and 625 ppm exposure groups, respectively. A log-normal probit analysis (U.S. EPA, National Center for Environmental Assessment, benchmark dose software draft, beta version 1.1b) based on the 15-month ovarian atrophy data provided an MLE of 8.12 ppm and a BMC_{05} of 3.08 ppm. Following adjustment for exposure continuity (6/24 hr/day, 5/7 days/wk) to 0.55 ppm and dividing by a total UF of 30 (3 for interspecies variability and 10 for intraspecies variability), a REL of 20 ppb (40 $\mu g/m^3$) was attained.

Another comparison to the proposed REL can be made using the dominant lethality study of Anderson *et al*. (1998). Early fetal deaths were observed at 65 and 125 ppm, but not 12.5 ppm. An earlier dominant lethality study (Anderson *et al*., 1996) indicated that early deaths may occur at 12.5 ppm but the toxicological effect could not be repeated at this concentration in subsequent studies. The average exposure duration at the NOAEL is 3.125 ppm (12.5 ppm x 6 hr/24 hr). Use of an RGDR of 1 and a cumulative uncertainty factor of 30 (3 for interspecies and 10 for intraspecies) resulted in a REL of 0.1 ppm (0.2 mg/m^3). Since the endpoint is a function of exposure during sperm maturation, no subchronic UF was used. The U.S. EPA had observed developmental toxicity in fetal rats (reduced male fetal body weight) at 40 ppm (Morrissey *et al*., 1990). However, unlike the Anderson *et al*. (1998) study, a NOAEL was not determined.

Recent studies have implicated 1,3-butadiene in accelerating arteriosclerotic plaque development in cockerels (Penn and Snyder, 1996a,b), although no animal studies in mammals have implicated 1,3-butadiene in this disease. The worker study by McMichael *et al*. (1976) observed a slight increase in mortality from arteriosclerosis among all rubber workers. But more recent mortality studies in the rubber industry found no association or found an actual mortality decrement from arteriosclerosis and other circulatory diseases when compared to a reference population, suggesting a 'healthy worker' effect (Divine and Hartman, 1996; Matanoski *et al*., 1990; Sathiakumar *et al*., 1998).

When mortality among rubber workers was adjusted for race, two studies found that black rubber workers had a small, although statistically significant, increased mortality from arteriosclerosis compared to the black male U.S. population (Divine and Hartman, 1996; Matanoski *et al*., 1990). But a larger study of black workers in the rubber industry found no association between circulatory diseases, which includes arteriosclerosis, and mortality (Sathiakumar *et al*., 1998). Weaknesses in these worker analyses include relatively small cohort sizes, the bias of having racial information on all deaths and not on all living workers, the lack of racial data on some workers (up to 15% of cohort), and the lack of complete or specific work histories of the subjects. Also, black men of certain age groups are known to have an increased standardized mortality ratio for arteriosclerotic (ischemic) heart disease compared to white men (CDC, 2000). Limited data, conflicting worker mortality results, and lack of underlying mechanisms of action prevent the use of these findings in 1,3-butadiene REL development. However, there clearly is a need for further animal and epidemiological studies to determine if there is a true association between 1,3-butadiene exposure and arteriosclerotic diseases.

VII. Data Strengths and Limitations for Development of the REL

The major strength of the 1,3-butadiene REL is the observation of a dose-response effect in a well-conducted lifetime inhalation exposure study. The major weaknesses are the lack of adequate human health effects and metabolism data and the lack of a NOAEL observation in the key study.

VIII. References

Anderson D, Edwards AJ, Brinkworth MH, and Hughes JA. 1996. Male-mediated F_1 effects in mice exposed to 1,3-butadiene. Toxicology 113:120-127.

Anderson D, Hughes JA, Edwards AJ, and Brinkworth MH. 1998. A comparison of male-mediated effects in rats and mice exposed to 1,3-butadiene. Mutat. Res. 397:77-84.

Bond JA, Himmelstein MW, Seaton M, Boogaard P, and Medinsky MA. 1996. Metabolism of butadiene by mice, rats, and humans: a comparison of physiologically based toxicokinetic model predictions and experimental data. Toxicology 113:48-54.

Brinkworth MH, Anderson D, Hughes JA, Jackson LI, Yu T-W, and Nieschlag E. 1998. Genetic effects of 1,3-butadiene on the mouse testis. Mutat. Res. 397:67-75.

CARB. 1999. California Air Resources Board. Toxics Air Quality Data. Substance Chooser. 1,3-Butadiene. Available online at http:/www.arb.ca.gov/aqd/toxics.htm

CARB. 2000. California Air Resources Board. California Emissions Inventory Development and Reporting System. (CEIDARS). Data from Data Base Year 1998. February 12, 2000.

CDC. 2000. Centers for Disease Control and Prevention. National Center for Health Statistics. Available online at http://www.cdc.gov/nchs/datawh/statab/unpubd.htm

Checkoway H, and Williams TM. 1982. A hematology survey of workers at a styrene-butadiene synthetic rubber manufacturing plant. Am. Ind. Hyg. Assoc. J. 43(3):164-169.

CRC. 1995. CRC Handbook of Chemistry and Physics, 76th edition. Lide DR, ed. Boca Raton, FL: CRC Press Inc.

Christian MS. 1996. Review of reproductive and developmental toxicity of 1,3-butadiene. Toxicology 113:137-143.

Dahl AR, Sun JD, Birnbaum LS, Bond JA, Griffith WC Jr, Mauderly JL, Muggenburg BA, Sabourin PJ, Henderson RF. 1991. Toxicokinetics of inhaled 1,3-butadiene in monkeys: comparison to toxicokinetics in rats and mice. Toxicol. Appl. Pharmacol. 110(1):9-19. (Published erratum appears in Toxicol. Appl. Pharmacol. 1992. 116(1):152.)

Divine BJ and Hartman CM. 1996. Mortality update of butadiene production workers. Toxicology 113:169-181.

Doerr JK, Hollis EA, and Sipes IG. 1996. Species difference in the ovarian toxicity of 1,3-butadiene epoxides in B6C3F1 mice and Sprague-Dawley rats. Toxicology 113(1-3):128-136.

Duescher RJ and Elfarra AA. 1994. Human liver microsomes are efficient catalysts of 1,3-butadiene oxidation: evidence for major roles by cytochromes P450 2A6 and 2E1. Arch. Biochem. Biophys. 311(2):342-349.

Himmelstein MW, Acquavella JF, Recio L, Medinsky MA, and Bond JA. 1997. Toxicology and epidemiology of 1,3-butadiene. Crit. Rev. Toxicol. 27(1):1-108.

HLE. 1981. Hazelton Laboratories Europe, Ltd. 1,3-Butadiene: The toxicity and carcinogenicity of butadiene gas administered to rats by inhalation for approximately 24 months. Prepared for the International Institute of Synthetic Rubber Producers, New York, NY. Unpublished. [as reported in U.S. EPA, 1985].

HSDB. 2000. Hazardous Substances Data Bank. National Library of Medicine, Bethesda, MD. Available online through Toxicology Data Network at http://toxnet.nlm.nih.gov

Huff JE, Melnick RL, Solleveld HA, Haseman JK, Powers M, and Miller RA. 1985. Multiple organ carcinogenicity of 1,3-butadiene in B6C3F1 mice after 60 weeks of inhalation exposure. Science 227(4686):548-549.

Matanoski GM, Santos-Burgoa C, and Schwartz L. 1990. Mortality of a cohort of workers in the styrene-butadiene polymer manufacturing industry (1943-1982). Environ. Health Perspect. 86:107-117.

McMichael AJ, Spirtas R, and Kupper KK. 1975. Solvent exposure and leukemia among rubber workers: An epidemiologic study. J. Occup. Med. 17:234-239.

McMichael AJ, Spirtas, R, Gamble JF, and Tousey PM. 1976. Mortality among rubber workers. Relationship to specific jobs. J. Occup. Med. 18(3):178-185.

Melnick RL, Huff J, Chou BJ, and Miller R. 1990. Carcinogenicity of 1,3-butadiene in C57BL/6xC3H F1 mice at low exposure concentrations. Cancer Res. 50(20):6592-6599.

Miller LM. 1978. Investigation of selected potential environmental contaminants: Butadiene and its oligomers. Philadelphia, PA: Franklin Research Center. [as reported in U.S. EPA, 1985].

Morrissey RE, Schwetz BA, Hackett PL, Sikov MR, Hardin BD, McClanahan BJ, Decker JR, and Mast TJ. 1990. Overview of reproductive and developmental toxicity studies of 1,3-butadiene in rodents. Environ. Health Perspect. 86:79-84.

NTP. 1984. U.S. National Toxicology Program. Toxicology and Carcinogenesis Studies of 1,3-Butadiene (CAS No.106-99-0) in B6C3F1 Mice (Inhalation Studies) (TR No. 288) Research Triangle Park, NC: National Institute of Environmental Health Sciences.

NTP. 1993. U.S. National Toxicology Program. Toxicology and Carcinogenesis Studies of 1,3-Butadiene (CAS No.106-99-0) in B6C3F1 Mice (Inhalation Studies) (TR No. 434). Research Triangle Park, NC: National Institute of Environmental Health Sciences.

OEHHA. 2000. Office of Environmental Health Hazard Assessment. Air Toxics Hot Spots Program Risk Assessment Guidelines. Part III. Technical Support Document for the Determination of Noncancer Chronic Reference Exposure Levels. Available on-line at http://www.oehha.ca.gov

Owen PE, Glaister JR, Gaunt IF, and Pullinger DH. 1987. Inhalation toxicity studies with 1,3-butadiene. 3. Two-year toxicity/carcinogenicity studies in rats. Am. Ind. Hyg. Assoc. J. 48:407-413.

Owen PE, and Glaister JR. 1990. Inhalation toxicity and carcinogenicity of 1,3-butadiene in Sprague-Dawley rats. Environ. Health Perspect. 86:18-25.

Pacchierotti F, Tiveron C, Ranaldi R, Bassini B, Cordelli E, Leter G, and Spanò M. 1998. Reproductive toxicity of 1,3-butadiene in the mouse: cytogenetic analysis of chromosome aberrations in first-cleavage embryos and flow cytometric evaluation of spermatogonial cell killing. Mutat. Res. 397:55-66.

Penn A and Snyder CA. 1996. Butadiene inhalation accelerates arteriosclerotic plaque development in cockerels. Toxicology 113:351-354.

Penn A and Snyder CA. 1996. 1,3 Butadiene, a vapor phase component of environmental tobacco smoke, accelerates arteriosclerotic plaque development. Circulation 93:552-557.

Sathiakumar N, Delzell E, Hovinga M, Macaluso M, Julian JA, Larson R, Cole P, Muir DCF. 1998. Mortality from cancer and other causes of death among synthetic rubber workers. Occup. Environ. Med. 55:230-235.

SCAQMD. 2000. South Coast Air Quality Management District. MATES-II. Multiple Air Toxics Exposure Study in the South Coast Air Basin. Final Report. Diamond Bar, CA: SCAQMD. March. p. 3-10.

Sweeney LM, Schlosser PM, Medinsky MA, and Bond JA. 1997. Physiologically based pharmacokinetic madeling of 1,3-butadiene, 1,2-epoxy-3-butene, and 1,2:3,4-diepoxybutane toxicokinetics in mice and rats. Carcinogenesis 18:611-625.

U.S. EPA. 1985. U.S. Environmental Protection Agency. Mutagenicity and Carcinogenicity Assessment of 1,3-Butadiene. Publication No. EPA/600/8-85/004F. Washington, DC: U.S. Environmental Protection Agency, Office of Health and Environmental Assessment.

Wilson RH. 1944. Health hazards encountered in the manufacture of synthetic rubber. JAMA 124(11):701-703.

CHRONIC TOXICITY SUMMARY

CADMIUM AND CADMIUM COMPOUNDS

CAS Registry Number: 7440-43-9

I. Chronic Toxicity Summary

Inhalation reference exposure level	**0.02 μg/m^3** (respirable)
Critical effect(s)	Kidney effects (proteinuria) and respiratory effects (reduction in forced vital capacity and reduction in peak expiratory flow rate) in occupationally exposed humans
Hazard index target(s)	Kidney; respiratory system

II. Physical and Chemical Properties (ATSDR, 1993)

Description	Blue-white solid
Molecular formula	Cd
Molecular weight	112.41 g/mol
Density	8.642 g/cm^3 @ 20ºC
Boiling point	765ºC (CRC, 1994)
Melting point	320.9ºC
Vapor pressure	1 torr @ 394ºC
Conversion factor	Not applicable

III. Major Uses or Sources

The production of nickel-cadmium batteries is currently the primary use of cadmium (ATSDR, 1993). Cadmium, a by-product of zinc- and sulfide-ore processing, is also used for metal plating and in pigments and plastics. The annual statewide industrial emissions from facilities reporting under the Air Toxics Hot Spots Act in California based on the most recent inventory were estimated to be 3672 pounds of cadmium (CARB, 2000).

IV. Effects of Human Exposure

Pulmonary and renal function were examined in three worker groups: women with less than 20 years of exposure [group E1]; men with less than 20 years of exposure [group E2], and men with more than 20 years of exposure [group E3] (Lauwerys *et al.*, 1974). Exposed groups were matched to control groups in terms of age, body size, cigarettes smoked per day, duration of smoking, and duration of employment. Although urine cadmium concentrations were significantly elevated, the subjects in E1 did not exhibit pulmonary function changes or

proteinuria indicative of renal impairment. The workers in E1 had been exposed for a mean of 4.08 years to 31 μg/m^3 total cadmium (1.4 μg/m^3 respirable cadmium). The 27 workers in E2 had been exposed for a mean of 8.6 years to 134 μg/m^3 total cadmium (88 μg/m^3 respirable cadmium). The blood and urinary cadmium levels of these workers were also significantly elevated compared to matched controls. Glomerular proteinuria was observed in 15% of the workers in E2 and in 68% of workers in E3. The 22 workers of E3 had been exposed for a mean of 27.8 years to 66 μg/m^3 total cadmium (21 μg/m^3 respirable cadmium). Significantly increased levels of cadmium were observed in the blood and urine, and workers in E3 also exhibited significant decreases in some measures of pulmonary function (forced vital capacity, forced expiratory volume in one second, and peak expiratory flow rate). This study identifies the kidney as the key target organ of chronic cadmium exposure. For respirable cadmium, this study indicates a LOAEL of 21 μg/m^3 for workers exposed for 28 years and a NOAEL of 1.4 μg/m^3 for workers exposed for 4 years.

A study of 82 cadmium exposed workers reports the time-weighted cumulative exposure index (TWE) and cadmium body burden determined in vivo (Ellis *et al.*, 1985). Evidence of renal dysfunction (usually elevated urinary β_2-microglobulin) was consistently observed when the worker's liver cadmium burden exceeded 40 ppm and the time-weighted cumulative exposure index exceeded 400-500 μg years/m^3.

A detailed investigation of renal function in 75 male cadmium-exposed workers identified significant increases in urinary excretion of several low- and high molecular weight proteins, including β_2-microglobulin, and significant decreases in renal reabsorption of calcium, urate, and phosphate compared to controls (Mason *et al.*, 1988). Exposures, which ranged from 36 to 600 μg/m^3, were determined from background or personal exposure measurements made between 1964 and 1983, or were estimated. A time-weighted cumulative exposure index (TWE) was determined for each subject. A two phase linear regression model was applied to the data to identify inflection points for each biochemical parameter. The biochemical indicators most highly correlated to exposure were urinary retinol binding protein and urinary β_2-microglobulin. Of these, the most sensitive parameter, urinary β_2-microglobulin, demonstrated an inflection point at 1108 μg years/m^3 with a 95% lower confidence limit of 509 μg years/m^3. The endpoint selected is indicative of defects in tubular reabsorption of proteins.

Diminished sensitivity of smell has also been observed in cadmium exposed workers (Rose *et al.*, 1992). Cadmium body burden, β_2-microglobulin levels, and olfactory function were measured in a group of 55 male workers exposed to cadmium fumes in a brazing operation. A group of 15 control workers was also tested. Exposed workers exhibited high urinary cadmium levels, tubular proteinuria, and a significant, selective defect in odor detection threshold.

V. Effects of Animal Exposure

Interstitial infiltration of lymphocytes and leukocytes and hyaline casts were observed in the kidneys of rabbits following exposure to 6.5 mg/m^3 cadmium-iron dust for 3 hours per day, 21 days per month for 9 months (Friberg, 1950). Proteinuria was observed in the majority of exposed rabbits by the fourth month of exposure. Increased lung weights and emphysema were

also observed. The trachea and nasal mucous membranes exhibited chronic inflammatory changes (not specified) and lymphocyte infiltration. The kidney contained the greatest concentration of cadmium. This study also exposed a group of rabbits to 9.1 mg/m^3 cadmium-iron dust for 3 hours per day, 23 days per month, for 7 months. Two rabbits in this group died from acute pneumonia at one month, and one rabbit was terminated at 3 months of exposure. Findings at necropsy were similar, although more severe than those observed in rabbits exposed to 6.5 mg/m^3. Chronic bronchitis and hyperplasia of the bronchiolar epithelium were observed in the higher dose group in addition to the findings previously noted.

Male and female rats were exposed to 0, 0.3, 1.0, or 2.0 mg Cd/m^3 (as $CdCl_2$) 6 hours per day, 5 days per week for a total of 62 exposures (Kutzman *et al.*, 1986). Rapid, shallow breathing and marked weight loss were observed in the highest dose group; all animals in this group died within the first 45 days of exposure. A dose-dependent increase in lung weight was observed in the remaining dose groups and a statistically significant increase in lung collagen and elastin was observed in rats exposed to 1.0 mg/m^3. Pathological changes noted in the terminal bronchioles include flattening and hyperplasia of type II cells, and infiltration of macrophages, mononuclear cells, and polymorphonuclear leukocytes. Proliferation of fibroblasts with deposition of collagen was also noted.

Male rats were exposed continuously to 0, 30, or 90 µg Cd/m^3 cadmium oxide (CdO) dust for up to 18 months (Takenaka *et al.*, 1990). Animals exposed to 30 $\mu g/m^3$ were sacrificed at 6 and 18 months of exposure. Although some rats in the high dose group were terminated after 6 months of exposure, the remaining rats were terminated after 7 months due to increased mortality and were not included in the study. Inflammation and hyperplasia of the alveolar epithelium occurred in animals of both groups after 6 months of exposure with more marked changes observed in the high dose group. Abnormal proliferation of the epithelium was observed in the low dose group following 18 months of exposure. Lung tumors observed in both dose groups were characterized as being duration dependent.

VI. Derivation of Chronic Reference Exposure Levels (REL)

Derivation of Chronic Inhalation Reference Exposure Level

Study	Lauwerys *et al.*, 1974
Study population	Humans (22 exposed men and 22 unexposed men in LOAEL group; 31 exposed women and 31 non-exposed women in NOAEL group)
Exposure method	Occupational exposures
Critical effects	Kidney effects - proteinuria in 68% of LOAEL group Respiratory effects – reduction in forced vital capacity (FVC), forced expiratory flow in 1 second (FEV_1); reduction in peak expiratory flow rate
LOAEL	21 $\mu g/m^3$ respirable cadmium
NOAEL	1.4 $\mu g/m^3$ respirable cadmium
Exposure continuity	Assumed to be 5 days/week for 8 hours/day during which 10 m^3 air is breathed
Average occupational exposure	0.5 $\mu g/m^3$ for NOAEL group (1.4 x 10/20 x 5/7)
Human equivalent concentration	0.5 $\mu g/m^3$ for NOAEL group
Exposure duration	Average of 4.1 years (1 to 12 years) for NOAEL group
LOAEL uncertainty factor	1
Subchronic uncertainty factor	3
Interspecies uncertainty factor	1
Intraspecies uncertainty factor	10
Cumulative uncertainty factor	30
Inhalation reference exposure level	0.02 $\mu g/m^3$

VII. Data Strengths and Limitations for Development of the REL

This evaluation of a chronic REL for cadmium is strengthened by being based on a human exposure study of workers exposed to cadmium for periods of 1 to over 20 years. The exposed group was matched to a control group in terms of age, body size, cigarettes smoked per day, duration of smoking, and duration of employment. The factory process was unchanged over the study period suggesting that exposures may have remained relatively constant over time. Significant areas of uncertainty include an incomplete knowledge of the past exposures over the full study interval and the relatively small number of subjects in the study.

A similar evaluation of the LOAEL group led to an alternate estimate for an inhalation reference exposure level of 0.05 $\mu g/m^3$. The LOAEL group had an average occupational exposure of 5.0 $\mu g/m^3$ and an average exposure duration of 27.8 years (21 to 40 years). Default uncertainty

factors included a 10-fold LOAEL uncertainty factor and a 10-fold intraspecies uncertainty factor (UF).

For comparison, using data presented by Ellis and associates (1985) and Mason and associates (1993) correlating human cumulative exposures (in terms of µg-years/m^3) and renal tubular protein reabsorption, a LOAEL of 500 µg-years/m^3 was predicted. This correlates to 7 µg/m^3 over 70 years. A time-weighted exposure to account for continuous exposure rather than 40 hour per week occupational exposure is 1.7 µg/m^3. Applying a 10-fold LOAEL uncertainty factor and a 10-fold intraspecies uncertainty factor results in a REL value of 0.02 µg/m^3, the same value obtained using the Lauwerys *et al*. data. U.S. EPA has not published an RfC for cadmium.

In addition to being inhaled, airborne cadmium can settle onto crops and soil and enter the body by ingestion. Thus an oral chronic reference exposure level for cadmium is also required. We propose adopting the U.S. EPA RfD as the chronic oral REL.

***Derivation of Chronic Oral Reference Exposure Level* (U.S. EPA RfD)**

Study	U.S. EPA, 1985
Study population	Humans
Exposure method	Food and drinking water
Critical effects	Significant proteinuria
LOAEL	Not observed
NOAEL	0.005 mg/kg bw-day
Exposure continuity	Chronic
Exposure duration	Up to lifetime
Average exposure	0.005 mg/kg bw-day
LOAEL uncertainty factor	1
Subchronic uncertainty factor	1
Interspecies uncertainty factor	1
Intraspecies factor	10
Cumulative uncertainty factor	10
Oral reference exposure level	0.0005 mg/kg bw-day

The oral REL is the U.S. EPA's Reference Dose (RfD) (U.S. EPA, 1996). A concentration of 200 µg cadmium (Cd)/gm wet human renal cortex is the highest renal level not associated with significant proteinuria (U.S. EPA, 1985). A toxicokinetic model is available to determine the level of chronic human oral exposure (NOAEL) which results in 200 µg Cd/gm wet weight human renal cortex. The model assumes that 0.01% of the Cd body burden is eliminated per day (U.S. EPA, 1985). Assuming 2.5% absorption of Cd from food or 5% from water, the toxicokinetic model predicts that the NOAEL for chronic Cd exposure is 0.005 and 0.01 mg Cd/kg/day from water and food, respectively (i.e., levels which would result in 200 µg Cd/gm wet weight human renal cortex). Thus, based on an estimated NOAEL of 0.005 mg Cd/kg/day for Cd in drinking water and an UF of 10, an RfD of 0.0005 mg Cd/kg/day (water) was calculated; an equivalent RfD for Cd in food is 0.001 mg Cd/kg/day.

Cd is unusual in relation to most, if not all, of the substances for which an oral RfD has been determined in that a vast quantity of both human and animal toxicity data are available. The RfD is based on the highest level of Cd in the human renal cortex (i.e., the critical level) not associated with significant proteinuria (i.e., the critical effect). A toxicokinetic model has been used to determine the highest level of exposure associated with the lack of a critical effect. Since the fraction of ingested Cd that is absorbed appears to vary with the source (e.g., food vs. drinking water), it is necessary to allow for this difference in absorption when using the toxicokinetic model to determine an RfD.

The uncertainty factor of 10 is used to account for intrahuman variability to the toxicity of this chemical in the absence of specific data on sensitive individuals. No modifying factor was used.

U.S. EPA stated its confidence in the RfD as: Study - Not applicable; Data Base - High; and RfD – High. The choice of NOAEL does not reflect the information from any single study. Rather, it reflects the data obtained from many studies on the toxicity of cadmium in both humans and animals. These data also permit calculation of pharmacokinetic parameters of cadmium including absorption, distribution, metabolism, and elimination. All this information considered together gives high confidence in the data base. High confidence in the RfD follows as well.

VIII. References

ATSDR. 1993. Agency for Toxic Substances and Disease Registry. Toxicological Profile for Cadmium. Prepared under contract No. 205-88-0606 for: US Department of Health and Human Services, Public Health Service. Atlanta, GA: ATSDR.

CARB. 1999. Air toxics emissions data collected in the Air Toxics Hot Spots Program CEIDARS Database as of January 29, 1999.

CRC. 1994. CRC Handbook of Chemistry and Physics, 75th edition. Lide DR, ed. Boca Raton, FL: CRC Press Inc.

Ellis KJ, Cohn SH, and Smith TJ. 1985. Cadmium inhalation exposure estimates: Their significance with respect to kidney and liver cadmium burden. J. Toxicol. Environ. Health 15:173-187.

Friberg L. 1950. Health hazards in the manufacture of alkaline accumulators with special reference to chronic cadmium poisoning. Acta Med. Scand. (Suppl. 240) 138:1-124.

Lauwerys RR, Buchet JP, Roels HA, Brouwers J, and Stanescu D. 1974. Epidemiological survey of workers exposed to cadmium. Arch. Environ. Health 28:145-148.

Kutzman RS, Drew RT, Shiotsuka RN, and Cockrell BY. 1986. Pulmonary changes resulting from subchronic exposure to cadmium chloride aerosol. J. Toxicol. Environ. Health 17:175-189.

Mason HJ, Davison AG, Wright AL, Guthrie CJG, Fayers PM, Venables KM, *et al.* 1988. Relations between liver cadmium, cumulative exposure, and renal function in cadmium alloy workers. Br. J. Ind. Med. 45:793-802.

Rose CS, Heywood PG, and Costanzo RM. 1992. Olfactory impairment after chronic occupational cadmium exposure. J. Occup. Med. 34(6):600-605.

Takenaka S, Glaser U, Oldiges H, and Mohr U. 1990. Morphological effects of Cd-aerosols on the rat lung. Toxicol. Environ. Chem. 27:163-172.

U.S.EPA. 1985. United States Environmental Protection Agency. Drinking Water Criteria Document on Cadmium. Final draft. Office of Drinking Water. Washington, DC: U.S. EPA.

U.S. Environmental Protection Agency. 1996. Integrated Risk Information System (IRIS) Database. Cadmium.

CHRONIC TOXICITY SUMMARY

CARBON DISULFIDE

(carbon bisulfide; carbon sulfide; dithiocarbonic anhydride)

CAS Registry Number: 75-15-0

I. Chronic Toxicity Summary

Inhalation reference exposure level	**800 µg/m^3** (300 ppb)
Critical effect(s)	CNS/PNS (reduction in motor nerve conduction velocities in occupationally-exposed humans)
Hazard index target(s)	Nervous system; reproductive system

II. Physical and Chemical Properties Summary (HSDB, 1995; CRC, 1994)

Description	Clear, colorless or faintly yellow liquid
Molecular formula	CS_2
Molecular weight	76.14 g/mol
Boiling point	46.5^{o}C
Melting point	–111.5 ^{o}C
Vapor pressure	297 torr @ 20°C
Solubility	Slightly soluble in water (2.94 g/L); miscible in anhydrous methanol, ethanol, ether, benzene, chloroform, and carbon tetrachloride
Conversion factor	3.1 mg/m^3 per ppm at 25°C

III. Major Uses and Sources

The most prominent industrial use of carbon disulfide is in the production of viscose rayon fibers. Carbon disulfide is also used in the production of carbon tetrachloride and cellophane, and, as a solvent for rubber, sulfur, oils, resins, and waxes. In the past, carbon disulfide was used in soil fumigation and insect control in stored grain. Industrial processes that produce carbon disulfide as a by-product include coal blast furnaces and oil refining (HSDB, 1995). Carbon disulfide is also a breakdown product of metam sodium. The annual statewide industrial emissions from facilities reporting under the Air Toxics Hot Spots Act in California based on the most recent inventory were estimated to be 1562 pounds of carbon disulfide (CARB, 2000).

IV. Effects of Human Exposure

A primary target of carbon disulfide (CS_2) toxicity is the nervous system. The major neurotoxic action of carbon disulfide is the development of mental disturbances. These include change of personality, irritability, and forgetfulness, often with accompanying neurophysiological and neuropathological changes after prolonged exposure. Such changes include decreased peripheral nerve impulse conduction, motor and/or sensory neuropathies, cerebral or cerebellar atrophy, and neuropsychological organic changes (Aaserud *et al.* 1988, 1990, 1992; Foa *et al.*, 1976; Hirata *et al.* 1992; Ruijten *et al.* 1990, 1993). Alterations in behavioral indices have historically been associated with high levels of CS_2, often in excess of 20 ppm (Foa *et al.* 1976; Hanninen *et al.*, 1978).

Studies have identified alterations in the nerve conduction of workers chronically exposed to lower CS_2 levels (Hirata *et al.*, 1992a; Johnson *et al.*, 1983; Ruijten *et al.*, 1990; Ruijten *et al.*, 1993). A cross-sectional study of Japanese spinning workers identified alterations in the central nervous system as measured by brain stem auditory evoked potential (BAEP) (Hirata *et al.*, 1992). The latencies of the three main BAEP components increased significantly in workersexposed to CS_2 for more than 20 years when compared to controls. CS_2 exposures ranged from 3.3 to 8.2 ppm (mean = 4.76 ppm). Ruijten *et al.* (1993) identified mild presymptomatic nerve impairment (decreased conduction velocities and response amplitudes) in 44 CS_2-exposed workers with an average cumulative exposure range from 192 to 213 ppm-year (mean duration = 26.1 years).

A NIOSH occupational study evaluated the effects of CS_2 on the peripheral nervous system. Johnson *et al.* (1983) identified a significant dose related reduction in the maximum motor nerve conduction velocities (MCV) in the calves and ankles of male viscose rayon workers exposed to high (median = 7.6 ppm) CS_2 levels versus a comparison group exposed to low concentrations (median = 0.2 ppm). The workers were all employed in artificial fiber production in the same plant. Since these reduced MCVs were still within the normal range, the authors considered the measured difference an indication of minimal neurotoxicity. The mean exposure concentration for all exposed workers (n = 145) ranged from 0.6 to 16 ppm (mean = 7.6 ppm) with a mean duration of 12.1 years. This study identified a chronic LOAEL of 7.6 ppm for minor neurological effects (decreased peroneal nerve MCV and sural nerve conduction velocity).

Another epidemiological study evaluated a group of 111 Belgian viscose rayon factory workers exposed to 4 to 112 mg/m^3 CS_2 (time-weighted average 1 to 40 mg/m^3) (Vanhoorne et al., 1995). Among four categories of cumulative exposure (0, 1 to 300, 301 to 600, and greater than 600 mg/m^3-years), a clear dose-response effect was observed for reduced mean peroneal motor nerve conduction velocities in both fast and slow fibers. Unfortunately, the data are incompletely reported, and the mean duration of exposure is not given. Subgroups of workers whose exposures ever exceeded 10 ppm (n=64) and never exceeded 10 ppm (n=30) each showed significantly reduced fibular nerve motor conduction velocities compared with non-exposed workers.

Vascular atherosclerotic changes are also considered a major effect of chronic carbon disulfide exposure. Several occupational studies have demonstrated an increase in the mortality due to

ischemic heart disease in CS_2 exposed workers (Hernberg *et al.*, 1970; MacMahon and Monson, 1988; Tiller *et al.*, 1968; Tolonen *et al.*, 1979). A 2.5-fold excess in mortality from coronary heart disease in workers exposed to CS_2 was first reported by Tiller *et al.* (1968). A subsequent prospective study by Hernberg *et al.* (1970) found a 5.6-fold increased risk in coronary heart disease mortality and a 3-fold increased risk of a first nonfatal myocardial infarction in CS_2 exposed workers.

Male workers (n=177) in a Polish fiber plant were exposed to CS_2 for an average of 14 years (range of 5 to 38 years). Controls were 93 healthy male workers from other factories that did not use carbon disulfide. Carbon disulfide exposed workers had higher rates (42%) of 24-hour electrocardiographic abnormalities than non-exposed workers (24%, p=0.006) (Bortkiewicz et al., 2001). The most common abnormalities were ventricular extrasystoles and repolarization disturbances, the latter occurring most often in workers with the longest CS_2 exposures. Long-term blood pressure monitoring did not reveal any differences between exposed and control groups.

Male workers in a Belgian viscose rayon factory (n=85) were estimated by personal active sampling to have exposures of 2 to 32 mg/m^3 CS_2. Controls were 37 non-exposed workers from factories that did not use CS_2. Exposed workers had reduced common carotid artery distensibility as measured with ultrasound sonography, while the carotid artery compliance coefficient was not significantly affected. Also, blood pressures and cholesterol levels were not significantly different than observed among control workers (Kotseva et al., 2001a). Differences in carotid artery distensibility remained significant after adjustment for age, smoking, alcohol consumption, ethnicity, body mass index, heart rate, and systolic blood pressure.

Egeland *et al.* (1992) and Vanhoorne *et al.* (1992) have reported that human exposure to CS_2 for more than one year causes increases in biochemical changes often associated with cardiovascular disease - diastolic blood pressure, low density lipoprotein cholesterol, and apolipoproteins A1 and B. Egeland *et al.* (1992) used cross sectional data on 165 CS_2-exposed workers (245 controls) collected in 1979 by Fajen *et al.* (1981). The affected workers were exposed for at least 1 year in a viscose rayon factory to an estimated median TWA (8-hour) of 7.6 ppm. The Egeland *et al.* (1992) study indicated that modest CS_2 exposure (range = 3.4 to 5.1 ppm, median = 4.1 ppm) was associated with increased low density lipoprotein cholesterol (LDLc), the type of increase associated with atherosclerotic heart disease. No significant differences were seen between controls and the low CS_2 exposed group (range = 0.04 to 1.02 ppm, median = 1.00 ppm). Study NOAEL and LOAEL for increased LDLc and diastolic blood pressure were thus 1.0 ppm and 4.1 ppm , respectively. Vanhoorne *et al.* (1992) identified increased LDL-cholesterol, apolipoprotein B, systolic and diastolic blood pressure as indicative of an increased coronary risk in workers from a Belgian viscose rayon factory (115 exposed and 76 controls). CS_2 concentrations ranged from 1 to 36 ppm. Duration of exposure was not indicated. Even though these biochemical changes were observed, no significant increases in cardiovascular disease, such as angina, myocardial infarction, or ischemia, were determined by ECG changes.

Workers (n=141) with a minimum of 1 year employment in viscose rayons factories were compared with 141 age and gender-matched plastic industry workers. Current exposures were estimated as 1 to 30 mg/m^3 (03 to 10 ppm). Exposed workers were categorized as group 1 or

group 2, with cumulative exposures of less than or greater than 100 mg/m^3-years, respectively. Group 2 ($p<0.001$) but not group 1 workers had increased mean total cholesterol (5.3 and 4.5 mmol/l) compared with controls (4.6 mmol/l) (Kotseva, 2001b).

CS_2 causes reproductive toxicity in both males and females. Lancranjan *et al.* (1969), Lancranjan (1972), Cirla *et al.* (1978), and Wagar *et al.* (1983) studied male reproductive effects of occupational exposure to CS_2 and showed significant adverse effects on spermatogenesis, levels of serum FSH and LH, and libido; these effects persisted in 66% of the workers subject to follow-up. Zhou *et al.* (1988) investigated pregnancy outcomes and menstrual disturbances in 265 women occupationally exposed to CS_2 in five facilities and 291 controls. The CS_2-exposed women had a significantly higher incidence of menstrual disturbances versus the control group (overall 34.9% vs. 18.2%). CS_2 levels varied between the five facilities (exposure category means of low = 3.1 mg/m^3, intermediate = 6.5 mg/m^3, and high = 14.8 mg/m^3), but all workers from these CS_2 facilities had significantly higher incidences of menstrual disturbance. Irregularity of menstruation was the most common disturbance, followed by abnormal bleeding. No evidence was observed to indicate an adverse effect on the term and outcome of pregnancy.

An abstract of an epidemiological study of birth defects among female workers occupationally exposed to CS_2, was reported by Bao et al. (1991). Exposures were at rayon factories in four Chinese provinces and began at least 6 months prior to pregnancy and continued during pregnancy. An increased rate of birth defects (2.6% vs. 1.3%) among 682 exposed women was noted compared to 745 women in the control group. The most common defects were congenital heart defects, inguinal hernia, and CNS defects. However, there was no significant difference in birth defects between those with estimated exposures greater than 10 mg/m^3 compared to those with lower exposures. There were no differences in rates of stillbirth, low birth weight, or neonatal or perinatal deaths among any of the groups.

The possibility of determining LOAEL and/or NOAEL values for the major CS_2-related adverse effects from epidemiology studies, which predominately use workers from the viscose rayon industry, is limited. The limitations include incomplete historical exposure measurements, concurrent exposure to other chemicals (including hydrogen sulfide or methylene chloride), lack of personal exposure determinations, and a high variability of individual exposures due to decreases of plant CS_2 concentrations over time.

V. Effects of Animal Exposure

Studies investigating the potential for CS_2 toxicity in animals have usually been limited by intermediate or subchronic duration (less than 1 year) and a lack of multiple dose or exposure groups. The neuropathologic changes consistently observed in rodents following CS_2 exposure include axonal swelling, demyelination, swelling at neuromuscular junctions, muscle atrophy and degeneration, damage to terminal axons, and nerve fiber breakdown (Clerici and Fechter, 1991; Colombi *et al.* 1981; Eskin *et al.*, 1988; Jirmanova and Lukas, 1984; Maroni *et al.*, 1979; Szendzikowski *et al.*, 1973). These adverse effects have been observed over a range of

exposures (250 to 800 ppm), but few studies have attempted to establish a dose response for this CS_2-induced neurotoxicity.

In a 90 day subchronic inhalation study, Sprague-Dawley and Fischer 344 rats exposed discontinuously (6 hours/day, 5 days/week) to CS_2 developed morphological alterations in nerves including axonal swelling and myelin degradation (Gottfried *et al.*, 1985). This study established a subchronic NOAEL of 50 ppm and a LOAEL of 300 ppm for morphological changes in nerves. A longer inhalation study in Wistar rats observed impairment in the conduction velocity of the sciatic and tibial nerves after 6 and 12 months of intermittent exposure to 289 ppm CS_2 (LOAEL of 289 ppm) (Knobloch *et al.*, 1979).

In a 13-week subchronic study, male and female F344 rats inhaled 0, 50, 500, or 800 ppm CS_2 discontinuously (6 h/day, 5 days per week) (Sills *et al.*,1998). Development of distal axonopathy in the muscular branch of the posterior tibial nerve (MBPTN) and spinal cord was examined. After 13 weeks, giant swollen axons were observed with thin myelin sheaths as well as some degenerated and regenerated axons. Axonal swelling was noted in the spinal cords of rats exposed to 500 or 800 ppm CS_2. In the 800 ppm group, additional axonal swelling was observed in the muscular branch of the posterior tibial nerve, . Neurofilament deposits were found in swollen axons in the spinal cord and MBPTN. The NOAEL for axonal swelling was 50 ppm.

Wronska-Nofer (1973) showed a positive relationship between the level of triglycerides, the rate of cholesterol synthesis, and CS_2 exposure in Wistar rats exposed to 0, 73.8, 160, 321, or 546 ppm CS_2 for 5 hours/day, 6 days/week over 8 months. This study found a subchronic LOAEL of 73.8 ppm for disturbances in lipid metabolism (increase in serum cholesterol and serum triglycerides).

Lewis et al. (1999) investigated the capacity of CS2 to induce arterial fatty deposits by itself, and its ability to enhance the rate of fatty deposit formation induced by a high fat diet. Groups of 20 female C57BL/6 mice were exposed to 0, 50, 500, or 800 ppm CS2 by inhalation. Half the animals in each group were placed on an atherogenic high fat diet and half on a control diet. Mice were necropsied after 1, 4, 8, 12, 16, or 20 weeks of exposure, and the rates of fatty deposit formation under the aortic valve leaflets were evaluated. Exposure of mice on the control diet to 500 and 800 ppm CS2 induced a small but significant increase in the rate of fatty deposit formation over non-exposed controls. In the animals on the high fat diet there was marked enhancement of the rate of fatty deposit formation in mice exposed to 500 and 800 ppm over the animals on the high fat diet alone. In addition, there was a small but significant enhancement in mice exposed to 50 ppm over the rate of fatty deposit formation induced by the high fat diet alone. Thus CS2 is atherogenic at high concentrations and in conjunction with other risk factors, CS2 at relatively low concentrations can enhance atherogenesis in mice. Fifty ppm is thus the study LOAEL.

Hepatic toxicity has also been induced in rats exposed to relatively high doses of CS_2, usually following pretreatment with liver inducers such as phenobarbital. Bond *et al.* (1969) showed that high doses of CS_2 to rats produced an increase in periportal liver fat, and decreases in hepatic cytochrome P450 content and in microsomal mixed function oxidase (MFO) activity. After phenobarbital induction, exposed rats exhibited more severe hepatoxicity characterized by hydropic degeneration and necrosis. Other hepatotoxic effects seen after CS_2 exposures greater

than 400 ppm include increases in relative liver weight (Sokal, 1973), stimulation of liver microsomal lipid peroxidation (Wronska-Nofer *et al.*, 1986), and decreases in hepatic cholesterol synthesis (Simmons *et al.*, 1988).

The 24-hr lethal ip LD_{50} values for CS_2 were estimated in 1-, 5-, 10-, 20-, 30- and 40-day-old rats (sample size not specified) (Green and Hunter, 1985). 1-day-old rats (LD_{50} 583 mg/kg, ip) were about 3-times more susceptible than 20-day-old rats (LD_{50} 1545 mg/kg, ip).

^{14}C- and ^{35}S-labelled CS_2 was given ip to 1-, 5-, 10-, 20-, 30-, and 40-day-old rats (Snyderwine and Hunter, 1987). Thirty- and forty-day-old rats (sample size not reported) metabolized significantly more CS_2 to CO_2 and expired significantly less CS_2 than 1- to 20-day-old rats. Twenty-four hr after administration, up to 13 times more ^{35}S -label (radioactivity per g of tissue) were present in organs from 1-day-old rats than in similar organs from 40-day-old rats. The study does not specifically address the toxicological implications of the metabolic differences, and did not include fully mature animals. However, inability to detoxify CS_2 would lead to higher tissue concentrations and thus, potentially, increased toxicity.

The metabolite responsible for CS_2 hepatotoxicity is believed to be reactive sulfur atoms that covalently bind to cellular macromolecules (Dalvi, 1988). Similarly, the correlation between increased lethality (Green and Hunter, 1985) and increasing binding of ^{35}S –label (Snyderwine and Hunter, 1987) in younger CS_2 -exposed animals is consistent with a role for reactive sulfur. Neurotoxicity of CS_2 results from the formation of thiourea lysine cross-links between neurofilament proteins (DeCaprio et al., 1992; Valentine et al., 1997; Erve et al., 1998).

New Zealand white rabbits (24 per group) inhaled 0, 60, 100, 300, 600 or 1200 ppm CS_2 for 6 h/d on gestation days 6 to 18 (Pathology Associates, 1991). Developmental toxicity (NOAEL = 300 ppm; 930 mg/m^3) was noted at concentrations lower than those associated with significant maternal toxicity (NOAEL = 600 ppm; 1860 mg/ m^3) (Pathology Associates, 1991). The adults did have some slight hematological changes at the 600 ppm level, but the authors questioned the biological significance of these marginal findings. Reduced fetal body weights were noted at 600 and 1200 ppm. Cumulative malformations were increased in the 1200 (3720 mg/m^3) but not 600 ppm group, though there were no significant increases in any specific malformation in any group. Maternal effects at 1200 ppm included decreased body weight, ataxia, wheezing, and tremors. In an initial range-finding study, exposure to 3000 ppm was associated with significant lethality.

Rats were exposed to 100 mg/m^3 (32 ppm) for 4 hr/d on gestation days 7 and 8, and the embryos explanted to culture medium at day 9.5. Growth of explants of 10 treated and 17 control embryos was monitored for 44 hours. CS_2 at this concentration induced growth retardation in treated embryos relative to controls (Zhao et al., 1997).

In a two-generation study, Tabacova et al. (1983) exposed pregnant Albino rats (30-32 pregnant females per group) to 0.03, 10, 100, or 200 mg/m^3 (0.01, 3, 32, or 64 ppm) CS_2. The two highest dose levels were both teratogenic and maternally neurotoxic. There were no significant adverse effects in the F1 generation at the 2 low dose levels. However, significant increases in teratogenicity were found in the F2 generation at 10 mg/m^3, as well as increased postnatal

neurological effects including hypoactivity, mild ataxia and gait disturbances, hind-limb weakness, spinning and tremor (Tabacova et al., 1983). While the overall rate of malformations (club foot, hydrocephalus, microcephalus, generalized edema) exhibited a dose-response trend, with increased effects in the F2 generation, the specific malformations exhibited a less-consistent pattern. For example, while club foot was the predominant malformation in the F1 fetuses (occurring at 100 and 200 mg/m^3); much lower rates of club foot were noted in the F2 generation (including none in the 200 mg/m^3 group). Limitations of the study include a lack of information on chemical purity and exposure methods, lack of concurrent controls, lack of clear dose-response trend, and incomplete reporting on the statistical significance of reported behavioral effects.

Wistar albino rats (32 animals per group) were exposed to 50, 100, or 200 mg/m^3 (16, 32, or 64 ppm) CS_2 for 8 hours per day throughout gestation. There were no statistically significant results in the 50 mg/m^3 group. In the 100 and 200 mg/m^3 groups, there were statistically significant increases in reduced fetal body weights, and reduced post natal body weights for 21 days, which subsequently disappeared. There was an increase in external malformations (hydrocephalus, club foot, and tail deformations) at the two higher doses (Tabacova et al., 1978).

Behavioral effects were examined in the offspring of Lati:CFY rats (8 per group) exposed to 0, 10, 700, or 2000 mg/m^3 CS_2 (3, 230, or 640 ppm) for 6 hours per days over days 7 to 15 of gestation. The two high doses caused significant perinatal mortality. Avoidance conditioning was tested using a bell as a conditional stimulus prior to an electric shock. The animals learned to avoid the shock by jumping onto a pole at the sound of the bell. The latency to jump onto the pole and errors were measured as a means to evaluate avoidance conditioning in the treated versus control animals. The authors reported that there was a dose-related change in avoidance conditioning among male pups over the first 15 days (Lehotsky et al., 1985). While the magnitude of the effect on avoidance conditioning was greater at all doses relative to controls, and at 2000 mg/m^3 compared with 700 mg/m^3, the effect was virtually identical between the 10 and 700 mg/m^3. This lack of dose-response effect raises some question about the significance of this finding.

Effects of low (0.03 and 10 mg/m^3; 0.01 and 3 ppm) prenatal exposures (8 hours per day throughout gestation) of CS_2 were studied in Wistar albino rats. No congenital malformations or significant prenatal effects were found in the 9-11 litters evaluated at each dose. Mortality during postnatal days 10 through 21 was increased in the 10 mg/m^3 group. Delays in the development of visual and auditory function were reported in the higher dose group (Tabacova and Balabaeva, 1980). There was no mention of maternal toxicity in this study.

Several other studies yielded either no teratogenic effects or effects only at maternally toxic exposures. Saillenfait et al. (1989) exposed rats via inhalation to 0, 100, 200, 400, or 800 ppm CS_2 for 6h/d during days 6-20 of gestation. Lower exposures (100 or 200 ppm; 310 or 620 mg/m^3) were not associated with maternal toxicity or adverse effects on the developing embryo or fetus. Higher concentrations (400 or 800 ppm; 1240 or 2480 mg/m^3) yielded a significant reduction of maternal weight gain as well as reductions of fetal body weight and a low incidence of club foot. Significant increases in unossified sternebrae were reported following 800 ppm (2480 mg/m^3) exposures. Nemec et al. (1993) reported no teratogenicity or maternal,

developmental, or reproductive toxicity among pregnant CD rats and their offspring following exposure to 125 or 250 ppm (388 or 775 mg/m^3) from 2 weeks prior to mating through gestation day 19. At 500 ppm, dams had decreased body weight gain and food consumption; decreased litter viability but no teratogenic effects were noted. CS_2 was not found to be teratogenic or embryotoxic following intraperitoneal administration to rats on days 1-15 of gestation (Beliles et al., 1980; Hardin et al., 1981). No significant effects were noted in animal inhalation exposures (20 to 40 ppm; 62 to 125 mg/m^3 CS_2) with either rats on days 1-19 of gestation or rabbits on days 1-24 of gestation.

VI. Derivation of Chronic Reference Exposure Level

Study	Johnson *et al.* (1983)
Study population	145 occupationally exposed workers and 212 comparison workers
Exposure method	Discontinuous occupational inhalation exposures (mean of 7.6 ppm and range of 0.6 to 16 ppm)
Critical effects	Reduction in motor nerve conduction velocities (decreased peroneal nerve MCV and sural nerve SVC)
LOAEL	7.6 ppm
NOAEL	Not observed
Exposure continuity	8 hr/day, 5 days/week
Average occupational exposure	2.7 ppm for LOAEL group (7.6 x 10/20 x 5/7)
Benchmark concentration (BMC_{05})	6.86 ppm (continuity-weighted exposure of 2.54 ppm)
Human equivalent concentration	2.54 ppm for BMC_{05} (6.86 x 10/20 x 5/7)
Exposure duration	Mean of 12.1 years (SD 6.9 years)
Subchronic uncertainty factor	1
LOAEL uncertainty factor	Not needed in BMC approach
Interspecies uncertainty factor	1
Intraspecies uncertainty factor	10
Cumulative uncertainty factor	10
Inhalation reference exposure level	0.3 ppm (300 ppb; 0.8 mg/m^3; 800 µg/m^3)

A benchmark dose analysis was performed on the peroneal MCV data. The NIOSH exposure data were regrouped into 8 geometrically spaced dose groups (Table 1).

Table 1. Peroneal MCV data used for benchmark dose modeling

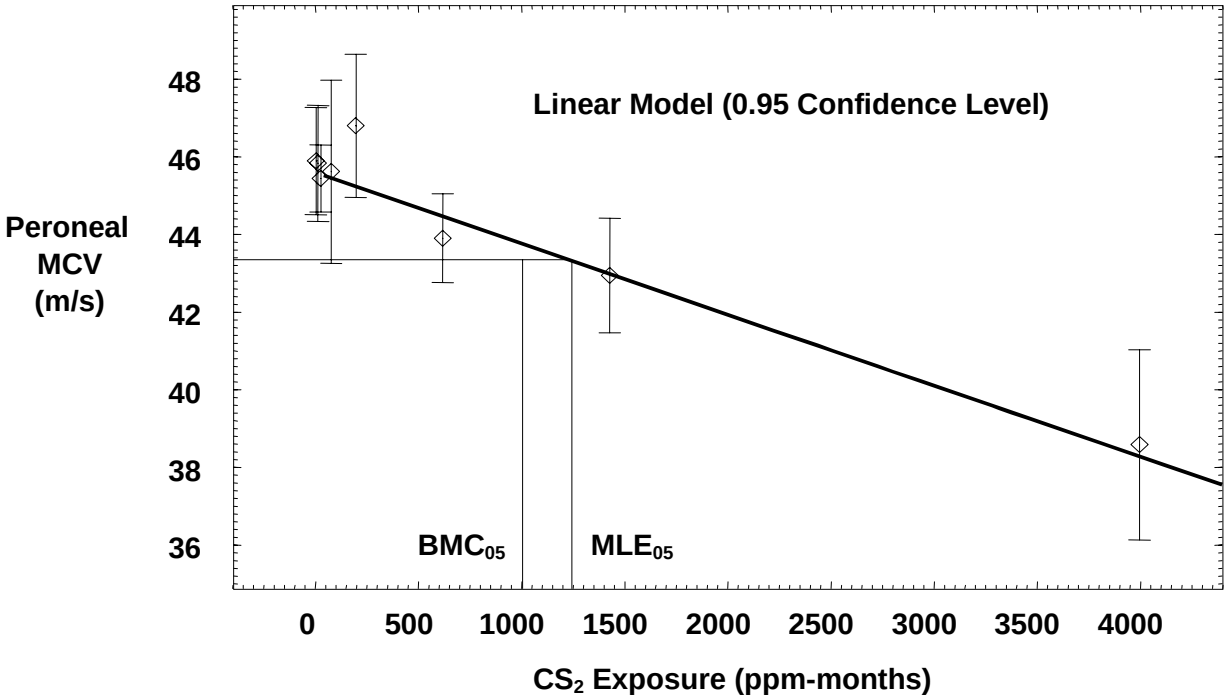
Exposure (ppm-months)	***Subjects***	***Peroneal MCV (m/s)***	
		Mean	***Std. Dev.***
3.8 (2 - 6)	32	45.9	3.8
13.1 (6 - 16)	61	45.8	5.8
26.5 (16-44)	140	45.4	5.2
77.3 (44-122)	17	45.6	4.6
197 (122 – 336)	17	46.8	3.6
619 (336 – 929)	54	43.9	4.2
1428 (929-2563)	61	42.9	5.8
3997 (2563 – 7075)	19	38.6	5.1

Model fitting was conducted with U.S. Environmental Protection Agency BMDS Benchmark Dose Software, Version 1.3. Four continuous data models were compared: linear, polynomial (v. 2.1), power (v. 2.1) and hill (v. 2.1) models. All four models adequately fit the data set (Table 2).

Table 2. Benchmark dose modeling results

Model	***MLE_{05} (ppm-mo)***	***BMC_{05} (ppm-mo)***	***p value***
Linear	1245	1005	0.84
Polynomial	1100	736	0.78
Hill	1092	670	0.65
Power	1245	1005	0.58

The BMC_{05} from the best-fitting linear model was used. An occupational BMC_{05} of 6.9 ppm was derived by dividing the 1005 ppm-month value by the average exposure duration of 145 months (12.1 years). The time-weighted average value was thus 2.5 ppm (6.9 ppm x 10/20 x 5/7).



The U.S. EPA (1995) based its RfC of 700 $\mu g/m^3$ on the same study but used a BMC_{10} and included a Modifying Factor (MF) of 3 for database deficiencies. The criteria for use of modifying factors are not well specified by U.S. EPA. Such modifying factors are not used by OEHHA. In addition OEHHA prefers use of a BMC_{05} since in practice it tends to be closer to the NOAEL while the BMC_{10} is often closer to the LOAEL (OEHHA, 2000).

For comparison, 50 ppm was a 13 week NOAEL in rats for axonal swelling (Sills *et al*., 1998). The equivalent continuous exposure is 8.9 ppm. Use of an RGDR of 1, an interspecies UF of 3, a subchronic UF of 3, and an intraspecies UF of 10 results in a REL of 90 ppb.

VII. Data Strengths and Limitations for Development of the REL

The major strengths of the REL for carbon disulfide are the use of human data, the observation of a dose-response effect, and the duration of exposures. The major uncertainties are the poor quantitation of actual exposure magnitude over time and the limited nature of the health effects studies which have been conducted.

VIII. Potential for Differential Impacts on Children's Health

The data available on the developmental toxicity of carbon disulfide are equivocal. Several studies reported that adverse developmental effects are only noted with exposures exceeding 100 ppm, while Tabacova and Balabaeva (1980) and Lehotsky et al. (1985) reported transient effects

at levels as low as 10 mg/m3 (3 ppm). The results of these two studies are not consistent with the database as a whole. While further research into behavioral effects of low concentrations of CS2 would better clarify the risks associated with such exposures, no adverse effects have been reported at concentrations below the REL of 800 µg/m3 (300 ppb).

IX. References

Aaserud O, Gjerstad L, Nakstad P, Nyberg-Hansen R, Hommeren OJ, Tvelt B, Russell D, and Rootwelt K. 1988. Neurological examination, computerized tomography, cerebral blood flow and neuropsychological examination in workers with long-term exposure to carbon disulfide. Toxicology 49(2-3):277-282.

Aaserud O, Hommeren OJ, Tvedt B, Nakstad P, Mowe G, Efskind J, Russell D, Jorgensen EB, Nyberg-Hansen R, and Rootwelt K. 1990. Carbon disulfide exposure and neurotoxic sequelae among viscose rayon workers. Am. J. Ind. Med. 18(1):25-37.

Aaserud O, Russell D, Nyberg-Hansen R, Rootwelt K, Jorgensen EB, Nakstad P, Hommeren OJ, Tvedt B, and Gjerstad L. 1992. Regional cerebral blood flow after long-term exposure to carbon disulfide. Acta Neurol. Scand. 85(4):266-271.

Bao YS, Cai S, Zhao SF, Xhang XC, Huang MY, Zheng O, Jiang Y. 1991. Birth defects in the offspring of female workers occupationally exposed to carbon disulfide in China. Teratology 43:451-452.

Beliles RP, Brusick DJ, Mecler FJ. 1980. Teratogenic-mutagenic risk of workplace contaminants. Litton Bionetics, Kensington, MD. NIOSH 210-77-0047.

Bortkiewicz A, Gadzicka E, Szymczak W. 2001. Cardiovascular disturbances in workers exposed to carbon disulfide. Appl Occup Environ Hyg 16(4):455-63.

Bond EJ, Butler WH, De Matteis F, and Barnes JM. 1969. Effects of carbon disulphide on the liver of rats. Br. J. Ind. Med. 26(4):335-337.

CARB. 2000. California Air Resources Board. California Emissions Inventory Development and Reporting System. (CEIDARS). Data from Data Base Year 1998. February 12, 2000.

CRC. 1994. CRC Handbook of Chemistry and Physics, 75th edition. Lide DR, ed. Boca Raton, FL: CRC Press Inc.

Cirla AM, Bertazzi PA, Tomasini M, Villa A, Graziano C, Invernizzi R, Gilioli R. 1978. Study of endocrinological functions and sexual behaviour in carbon disulphide workers. Med. Lav. 69(2):118-129.

Clerici WJ, and Fechter LD. 1991. Effects of chronic carbon disulfide inhalation on sensory and motor function in the rat. Neurotoxicol. Teratol. 13(3):249-255.

Colombi A, Maroni M, Picchi O, Rota E, Castano P, and Foa V. 1981. Carbon disulfide neuropathy in rats. A morphological and ultrastructural study of degeneration and regeneration. Clin. Toxicol. 18(12):1463-1474.

Dalvi RR. 1988. Mechanism of the neurotoxic and hepatotoxic effects of carbon disulfide. Drug Metabol Drug Interact 6(3-4):275-84.

DeCaprio AP, Spink DC, Chen X, Fowke JH, Zhu M, Bank S. 1992. Characterization of isothiocyanates, thioureas, and other lysine adduction products in carbon disulfide-treated peptides and protein. Chem Res Toxicol. 5(4):496-504.

Egeland GM, Burkhart GA, Schnorr TM, Hornung RW, Fajen JM, and Lee ST. 1992. Effects of exposure to carbon disulphide on low density lipoprotein cholesterol concentration and diastolic blood pressure. Br. J. Ind. Med. 49(4):287-293.

Erve JC, Amarnath V, Graham DG, Sills RC, Morgan AL, Valentine WM. 1998. Carbon disulfide and N,N-diethyldithiocarbamate generate thiourea cross-links on erythrocyte spectrin in vivo. Chem Res Toxicol. 11(5):544-9.

Eskin TA, Merigan WH, and Wood RW. 1988. Carbon disulfide effects on the visual system. II. Retinogeniculate degeneration. Invest. Ophthalmol. Vis. Sci. 29(4):519-527.

Fajen J, Albright B, and Leffingwell SS. 1981. A cross-sectional medical and industrial hygiene survey of workers exposed to carbon disulfide. Scand. J. Work Environ. Health. 7(Suppl 4):20-27.

Foa V, Cassitto MG, and Forzi M. 1976. Mental performance and personality disorders among workers exposed to carbon disulphide: Comparison between two different rayon plants. In: Adverse Effects of Environmental Chemicals and Psychotrophic Drugs, Neurophysiological and Behavioural Tests. Vol. 2. pp. 173-182.

Gottfried MR, Graham DG, Morgan M, Casey HW, and Bus JS. 1985. The morphology of carbon disulfide neurotoxicity. Neurotoxicology. 6(4):89-96.

Green EC, Hunter A (1985). Toxicity of carbon disulfide in developing rats: LD50 values and effects on the hepatic mixed-function oxidase enzyme system. Toxicol Appl Pharmacol 78(1):130-138.

Hanninen H, Nurminen M, and Tolonen M. 1978. Psychological tests as indicators of excessive exposure to carbon disulfide. Scand. J. Psychol. 19:163-174.

Hernberg S, Partanen T, Nordman CH, and Sumari P. 1970. Coronary heart disease among workers exposed to carbon disulphide. Br. J. Ind. Med. 27:313-325.

Hirata M, Ogawa Y, Okayama A, and Goto S. 1992. A cross-sectional study on the brainstem auditory evoked potential among workers exposed to carbon disulfide. Int. Arch. Occup. Environ. Health 64(5)321-324.

HSDB. 1995. Hazardous Substances Data Bank. (Edition of January 1995). National Library of Medicine, Bethesda, MD (TOMES® CD-Rom Version). Denver, CO: Micromedex, Inc.

Jirmanova I, and Lukas E. 1984. Ultrastructure of carbon disulphide neuropathy. Acta Neuropathologica 63(3):255-263.

Johnson BL, Boyd J, Burg JR, Lee ST, Xintaras C, and Albright BE. 1983. Effects on the peripheral nervous system of workers' exposure to carbon disulfide. Neurotoxicology 4(1)53-65.

Knobloch K, Stetkiewicz J, and Wronska-Nofer T. 1979. Conduction velocity in the peripheral nerves of rats with chronic carbon disulphide neuropathy. Br. J. Ind. Med. 36(2):148-152.

Kotseva K, Braeckman L, Duprez D, De Bacquer D, De Buyzere M, Van De Veire N, Vanhoorne M. 2001a. Decreased carotid artery distensibility as a sign of early atherosclerosis in viscose rayon workers. Occup Med (Lond) 51(4):223-9.

Kotseva K. 2001b. Occupational exposure to low concentrations of carbon disulfide as a risk factor for hypercholesterolaemia. Int Arch Occup Environ Health 74(1):38-42.

Lancranjan I, Popescu HI, Klepsch I. 1969. Changes of the gonadic function in chronic carbon disulphide poisoning. Med. Lav. 60(10):566-571.

Lancranjan I. 1972. Alterations of spermatic liquid in patients chronically poisoned by carbon disulphide. Med. Lav. 63(1):29-33.

Lehotsky K, Szeberenyi JM, Ungvary G, Kiss A (1985). Behavioural effects of prenatal exposure to carbon disulfide and to aromatol in rats. Arch Toxicol Suppl 8:442-446.

Lewis JG, Graham DG, Valentine WM, Morris RW, Morgan DL, Sills RC. 1999. Exposure of C57BL/6 mice to carbon disulfide induces early lesions of atherosclerosis and enhances arterial fatty deposits induced by a high fat diet. Toxicol. Sci. 49(1):124-132.

MacMahon B, and Monson RR. 1988. Mortality in the US rayon industry. J. Occup. Med. 30(9):698-705.

Maroni M, Colombi A, Rota E, Antonini C, Picchi O, Foa V, Caimi L, Tettamanti G, and Castano P. 1979. Biochemical and morphological investigations on nervous tissue of rats inhaling carbon disulphide. Med. Lav. 70(6):443-451.

OEHHA. 2000. Office of Environmental Health Hazard Assessment. Air Toxics Hot Spots Program Risk Assessment Guidelines. Part III. Technical Support Document for the Determination of Noncancer Chronic Reference Exposure Levels. Available on-line at http://www.oehha.ca.gov

Nemec MD, Holson JF, Naas DJ, Shour MH, Gerhart JM (1993). An assessment of reproduction in female rates exposed to carbon disulfide vapor. Teratol Soc Abstracts 47:430.

Pathology Associates (1991). Developmental inhalation toxicity study of carbon disulfide in the New Zealand white rabbit. January 31, 1991. Pathology Associates, Frederick, MD. Project Number 2100-202.

Price B, Berner T, Henrich RT, Stewart JM, and Moran EJ. 1996. A Benchmark Concentration for carbon disulfide: Analysis of the NIOSH carbon disulfide exposure database. Regul. Toxicol. Pharmacol. 24:171-176.

Ruijten MW, Salle JH, Verberk MM, and Muijser H. 1990. Special nerve functions and colour discrimination in workers with long term low level exposure to carbon disulphide. Br. J. Ind. Med. 47(9):589-595.

Ruijten MW, Salle HJ, and Verberk MM. 1993. Verification of effects on the nervous system of low level occupational exposure to CS_2. B. J. Ind. Med. 50(4):301-307.

Saillenfait AM, Bonnet P, De Caurriz J (1989). Effects of inhalation exposure to carbon disulfide and its combination with hydrogen sulfide on embryonic and fetal development in rats. Toxicol Lett 1989 Jul;48(1):57-66.

Sills RC, Harry GJ, Morgan DL, Valentine WM, Graham DG. 1998. Carbon disulfide neurotoxicity in rats: V. Morphology of axonal swelling in the muscular branch of the posterior tibial nerve and spinal cord. Neurotoxicology 19(1):117-127.

Simmons JE, Sloane RA, and Van Stee EW. 1988. Hepatic cholesterol metabolism as a function of carbon disulfide concentration and treatment with phenobarbital. Am. Ind. Hyg. Assoc. J. 49(9):427-433.

Snyderwine EG, Hunter A (1987). Metabolism and distribution of 14C- and 35S-labeled carbon disulfide in immature rats of different ages. Drug Metab Dispos 15(3):289-294

Sokal JA. 1973. Effect of chronic exposure to carbon disulphide upon some components of the electron transport system in rat liver microsomes. Biochem. Pharmacol. 22(1):129-132.

Szendzikowski S, Karasek M, Stetkiewicz J, and Wronska-Nofer T. 1973. Ultrastructure of the peripheral nerve in rats chronically exposed to carbon disulphide, preliminary report. Folia Histochemica et Cytochemica 11(3):353-354.

Tabacova S, Balabaeva L (1980). Subtle consequences of prenatal exposure to low carbon disulphide levels. Arch Toxicol Suppl. 4:252-4.

Tabacova S, Hinkova L, Balabaeva L (1978). Carbon disulfide teratogenicity and post natal effects in rat. Toxicol Lett 2:129-133.

Tabacova S, Nikiforov B, Balabaeva L (1983). Carbon disulphide intrauterine sensitization. J Appl Toxicol 3(5):223-229.

Tiller JR, Schilling RS, and Morris JM. 1968. Occupational toxic factors in mortality from coronary heart disease. Br. Med. J. 4:407-411.

Tolonen M, Nurminen M, and Hernberg S. 1979. Ten-year coronary mortality of workers exposed to carbon disulfide. Scand. J. Work Environ. Health 5(2):109-114.

USDHHS. 1994. U.S. Department of Health and Human Services, Public Health Service, Agency for Toxic Substances and Disease Registry. Toxicological Profile for Carbon Disulfide. Draft.

U.S. Environmental Protection Agency. 1995. Carbon Disulfide. CASRN 75-15-0. Integrated Risk Information System, National Center for Environmental Assessment, Office of Research and Development, U.S. Environmental Protection Agency.

Valentine WM, Amarnath V, Graham DG, Morgan DL, Sills RC. 1997. CS_2-mediated cross-linking of erythrocyte spectrin and neurofilament protein: dose response and temporal relationship to the formation of axonal swellings. Toxicol Appl Pharmacol. 142(1):95-105.

Vanhoorne M, De Bacquer D, and De Backer G. 1992. Epidemiological study of the cardiovascular effects of carbon disulphide. Int. J. Epidemiol. 21(4):745-752.

Vanhoorne MH, Ceulemans L, De Bacquer DA, De Smet FP (1995). An epidemiologic study of the effects of carbon disulfide on the peripheral nerves. Int J Occup Environ Health 1(4):295-302.

Wagar G, Tolonen M, Tanner P, and Helpio E. 1983. Serum gonadotropins and testosterone in men occupationally exposed to carbon disulfide. J. Toxicol. Environ. Health 11(4-6):691-701.

Wronska-Nofer T. 1973. Disturbances of lipids metabolism in rats in dependence upon carbon disulphide concentrations in the air. Med. Lav. 64(1):8-12.

Wronska-Nofer T, Klimczak J, Wisniewska-Knypl JM, Jajte J, and Opalska B. 1986. Combined effect of ethanol and carbon disulphide on cytochrome P-450 mono-oxygenase, lipid peroxidation and ultrastructure of the liver in chronically exposed rats. J. Appl. Toxicol. 6(4):297-302.

Zhao SF, Zhang XC, Zhang LF, Zhou SS, Zhang F, Wang QF, Wang YL, Bao YS (1997). The evaluation of developmental toxicity of chemicals exposed occupationally using whole embryo culture. Int J Dev Biol 41(2):275-82.

Zhou SY, Liang YX, Chen ZQ, and Wang YL. 1988. Effects of occupational exposure to low-level carbon disulfide (CS_2) on menstruation and pregnancy. Ind. Health 26(4):203-214.

CHRONIC TOXICITY SUMMARY

CARBON TETRACHLORIDE

(carbon chloride; carbon tet; freon 10; halon-104; methane tetrachloride; necatrine; tetrachlorocarbon; tetrachloromethane; tetraform; tetrasol; univerm)

CAS Registry Number: 56-23-5

I. Chronic Toxicity Summary

Inhalation reference exposure level	**40 μg/m^3** (6 ppb)
Critical effect(s)	Increased liver weight and hepatic fatty infiltration in guinea pigs
Hazard index target(s)	Alimentary system; development (teratogenicity); nervous system

II. Physical and Chemical Properties (HSDB, 1995; CRC, 1994)

Description	Colorless liquid
Molecular formula	CCl_4
Molecular weight	153.8 g/mol
Density	1.59 g/cm^3 @ 20°C
Boiling point	76.7°C
Melting point	–23°C
Vapor pressure	91.3 torr @ 20°C
Solubility	Soluble in acetone, ethanol, benzene, carbon disulfide, slightly soluble in water
Conversion factor	1 ppm = 6.3 mg/m^3 @ 25°C

III. Major Uses or Sources

Carbon tetrachloride was formerly used for metal degreasing and as a dry-cleaning fluid, fabric-spotting fluid, fire-extinguisher fluid, grain fumigant and reaction medium (DeShon, 1979). Carbon tetrachloride is used as a solvent for the recovery of tin in tin-plating waste and in the manufacture of semiconductors. It is used in petrol additives, refrigerants, metal degreasing, and as a catalyst in the production of polymers. Carbon tetrachloride is also used as a chemical intermediate in the production of fluorocarbons and some pesticides (HSDB, 1995). In 1996, the latest year tabulated, the statewide mean outdoor monitored concentration of carbon tetrachloride was approximately 0.08 ppb (CARB, 1999a). The annual statewide industrial emissions from facilities reporting under the Air Toxics Hot Spots Act in California based on the most recent inventory were estimated to be 8781 pounds of carbon tetrachloride (CARB, 2000).

IV. Effects of Human Exposure

Kazantzis *et al.* (1960) evaluated 17 employees of a quartz processing factory who were occupationally exposed to 45-100 ppm (284-630 mg/m^3) carbon tetrachloride (CCl_4) vapor. Fifteen of the 17 workers complained of symptoms including nausea, anorexia, vomiting, flatulence, epigastric discomfort or distention, depressive symptoms, headache or giddiness for up to 4 months prior to the evaluation. A week after CCl_4 concentrations were reduced to 0-9 ppm with control measures, workers were symptom-free.

V. Effects of Animal Exposure

Adams *et al.* (1952) chronically exposed albino Wistar rats, guinea pigs, albino rabbits and rhesus monkeys to 0, 5, 10, 25, 50, 100, 200 and 400 ppm CCl_4 for varying duration. For each exposure group, two control groups were devised (unexposed and air-exposed controls) consisting of animals similar in age, sex, weight and number. The 2 control groups responded similarly to the experimental protocol.

In the 100, 200 and 400 ppm exposure groups (Adams *et al*., 1952), mortality was excessive with moderate to severe liver cirrhosis and other various pathological changes in all the species tested. Fifteen male and 15 female rats were exposed to 50 ppm CCl_4 134 times for 187 days. They experienced decreased body weight gain and liver weight increase as well as moderate fatty degeneration and slight to moderate liver cirrhosis. Females showed kidney weight increase and four rats showed slight to moderate swelling of the kidney tubular epithelium. Guinea pigs (8 males and 8 females; 143 exposures in 200 days) showed depressed growth in the first two weeks, enlarged livers, moderate fatty degeneration and liver cirrhosis, and increased levels of liver total lipids, neutral fat, esterified cholesterol and plasma prothrombin clotting time.

The rabbit group of 2 males and 2 females, which underwent 155 exposures to 50 ppm in 216 days, showed slightly depressed growth and increased kidney weights, prolonged plasma prothrombin clotting time, and moderate fatty degeneration and cirrhosis of the liver.

No change was seen in the group of 2 male monkeys exposed 198 times to 50 ppm in 277 days (Adams *et al*., 1952). One monkey experienced depressed weight gain compared to the other monkey and the controls, but no other adverse effects were seen with respect to organ weights, tissue examination, total liver lipid, blood urea nitrogen, blood non-protein nitrogen, serum phosphatase, plasma prothrombin clotting time, phospholipid, neutral fat, and free esterified cholesterol.

At 25 ppm CCl_4, 15 male and 15 female rats were exposed 137 times for 191 days. Early growth depression in males was observed, although final body weights did not significantly differ from the controls. Significant liver weight increase and slight to moderate fatty degeneration occurred. Liver lipid content was nearly twice the level of the controls and esterified cholesterol was five times that of the controls. For this exposure, phospholipid and neutral fat were not measured. Five male guinea pigs were exposed 133 times over 185 days and 5 female guinea pigs were exposed 93 times over 126 days. Symptoms included growth depression, liver weight

increase, increased plasma prothrombin clotting time, slight to moderate fatty degeneration, twice the level of the control total liver lipid, and five times the control level of esterified cholesterol. After 178 exposures to 25 ppm over 248 days, rabbits (2 per sex) showed increased liver weights and slight to moderate liver cirrhosis and fatty degeneration.

Twenty male and 20 female rats were exposed 136 times over a period of 192 days to 10 ppm CCl_4. These rats exhibited increase in liver weight, slight to moderate fatty degeneration and total lipid, neutral fat and esterified cholesterol levels that were twice the control levels. Guinea pigs (8 male and 8 female), which were exposed 139 times over 197 days, experienced liver weight increase, slight to moderate fatty degeneration without cirrhosis, and increased levels of total lipid, neutral fat, and esterified cholesterol. In an additional group of 18 male rats exposed 13 times to 10 ppm, slight fatty degeneration was seen as early as 17 days. Two male and two female rabbits tolerated the same regimen as the guinea pigs and showed no symptoms as a result of the exposure. Sixteen additional guinea pigs developed hepatic changes after 12 exposures in 16 days.

Twenty-five male and 23 female rats, exposed 145 times over 205 days to 5 ppm CCl_4, had no adverse effects. Nine male and nine female guinea pigs exposed 143 times over 203 days showed a statistically significant increase in the liver weights (females only), but only slightly higher liver lipid content. No additional histopathological effects were seen at this level of exposure.

In a more recent study, Prendergast *et al.* (1967) exposed 15 Long-Evans or Sprague-Dawley rats, 15 guinea pigs, 3 rabbits, 2 dogs, and 3 monkeys 30 times to a concentration of 515 $\pm$39 mg/m^3 (81.7 ppm) carbon tetrachloride (CCl_4) 8 hours a day, 5 days a week, for 6 weeks. (This intermittent exposure is equivalent to a continuous exposure to 123 mg/m^3.) Additionally, two 90 day continuous exposure studies were conducted. One study exposed 15 rats, 15 guinea pigs, 2 rabbits, 2 dogs and 3 monkeys to 61$\pm$5.2 mg/m^3 CCl_4 and the other exposed 15 rats, 3 rabbits, 2 dogs and 3 monkeys continuously to 6.1$\pm$0.3 mg/m^3 CCl_4 in inhalation chambers. Control groups consisted of 304 rats, 314 guinea pigs, 34 dogs, 48 rabbits and 57 monkeys. All the animals' weights were recorded prior to the study, at monthly intervals throughout the study, and at the conclusion of the study.

During the 6 week study, one monkey died following the 7th exposure, and 3 guinea pigs died following the 20th, 22nd, and 30th exposures, respectively. Monkeys, guinea pigs, dogs and rabbits all exhibited weight loss. A high percentage of mottled livers was seen in all species except dogs. Histopathologic examination of the lungs and livers showed morphological changes in all the animals exposed to CCl_4 (most prominently the guinea pigs). The guinea pigs were the most sensitive species displaying discolored lungs, fatty livers, bile duct proliferation, fibrosis, focal inflammatory cell infiltration, hepatic cell degeneration and regeneration, early portal cirrhosis, and alteration of lobular structure. Hepatic lipid content in the guinea pigs was 35.4$\pm$10.7% compared to the control value of 11.0$\pm$3.6%. Alterations of liver lipid content were also observed, to a lesser extent, in the other four species; the most severe alteration occurred in the rats, less severe alteration in rabbits and dogs, and the least severe in the monkeys.

During the 61 mg/m^3 (9.7 ppm) CCl_4 continuous exposure study, 3 guinea pigs died (one each after 47, 63, and 71 days). All the monkeys were emaciated and experienced hair loss. Depressed body weight increases were seen in all exposed animals compared to the controls. Autopsies showed enlarged and/or discolored livers in a high percentage (not given) of monkeys, guinea pigs, rabbits, and rats. Rats and guinea pigs showed hepatic fatty acid changes, and a moderate reduction in succinic dehydrogenase activity was also evident in guinea pigs. Varying but lesser degrees of these changes were also seen in the other species tested.

The low concentration of 6.1 mg/m^3 (1 ppm) CCl_4 was attained by diluting the CCl_4 to 10% of the above concentration with *n*-octane, resulting in a solution of 6.1 mg/m^3 CCl_4 in 61 mg/m^3 of *n*-octane (Prendergast *et al.,* 1967)). The level of *n*-octane used was shown to be nontoxic by an *n*-octane control, which yielded no effects. (The current TLV for n-octane is 1400 mg/m^3 (300 ppm) (ACGIH, 1992).) No animals died during this study, and no signs of toxicity were noted. All exposed animals except the rats showed reduced weight gain when compared to the controls, and all species exhibited nonspecific inflammatory lung changes. Guinea pig liver lipid contents and serum urea nitrogen concentrations were similar to the control values. In several animals there were some nonspecific inflammatory changes in the liver, kidney and heart, but the authors did not attribute these to the chemical exposure. There was no other observed hematologic or histopathologic toxicity at this level.

Shimizu *et al.* (1973) exposed groups of 4 female Sprague-Dawley rats to 10, 50 and 100 ppm of CCl_4 vapor for 3 hours a day, 6 days a week for up to 6-8 weeks. The rats were terminated two days after the last inhalation. Accumulation of CCl_4 occurred in the adipose tissue and was measured after 1 and 3 weeks of exposure. For the 10 ppm group, accumulation was gradual, reaching a level of 1/3 the amount found in the 50 ppm group after 6 weeks. A slight increase of triglycerides in the liver (6.2-6.4 mg/g) was observed in the 10 ppm group, but no control group was used for comparison.

The intermittent exposure caused a more pronounced and higher number of change indices to occur (34 as opposed to the 17 change indices of the monotonous regimen), indicating a greater intensity of liver damage. Changes included a significant decrease in hippuric acid synthesis, presence of mitochondrial enzymes (glutamate dehydrogenase and ornithine carbonyl transferase) in the blood (indicating severe damage to hepatocytes), significant increase in cytoplasmic enzyme activity, and a decrease in the level of cytochrome P-450 in liver tissue. The effects seen in the monotonous group were the same variety as those in the intermittent group, but were less intense. The content of CCl_4 in the blood was similar for both the intermittent and monotonous exposure groups. Another test was performed over a period of 27 days varying the regimen, and therefore the concentration, of intermittent exposure while keeping the TWA level of CCl_4 stable. Increasing the concentration threefold or fivefold with five 10 minute peaks did not potentiate the toxic effects. Varying the regimen tenfold to five 5-minute peaks (peak exposure 402 mg/m^3 (63.8 ppm)) with a time weighted average exposure of 6.5 ppm (41$\pm$1 mg/m^3) did, however, result in more severe liver damage.

Sakata *et al.* (1987) exposed 10-15 male Sprague-Dawley rats to <10 ppm CCl_4 vapor for 15 minutes a day, twice a week for 8 weeks. All the rats had chronic liver damage involving

nodular liver surfaces and extensive fibrosis. Researchers also found similar results in rats after 8 weeks of subcutaneous injections of 0.1 mL of 50% CCl_4 solution in olive oil twice a week.

Ideura *et al.* (1993) exposed male Wistar rats to CCl_4 vapor for 7 minutes, 3 times a week for 6-10 weeks (concentration unspecified). Six experimental groups of 4-5 rats were used, two of which were exposed for 10 weeks, another two for 6 weeks, and two unexposed control groups. Following the last exposures, rats were injected with varying amounts of endotoxin (1.0 mL lipopolysaccharide (LPS)). The rats were sacrificed 24 hours after the injection and processed for histological examination. Examination of the rats' left kidneys and livers revealed liver cirrhosis with destruction of normal structure and massive ascites retention after 10 weeks of exposure as compared to the controls. Those exposed for 6 weeks exhibited an increase in fibrous tissue. The control groups displayed normal liver structure. Researchers found that rats previously resistant to endotoxin became susceptible following CCl_4 exposure, which was manifested as induced acute renal tubular necrosis in cirrhotic rats.

Yoshimura *et al.* (1993) performed a similar experiment to that of Ideura *et al.* (1992) by exposing male Wistar rats for 6 (5 rats) and 10 weeks (5 rats) to 99% CCl_4 vapor for 3 minutes a day. A control group of 5 rats was given phenobarbitone for 10 weeks. After 24 hours following the final exposure, rats were injected with endotoxin. Six weeks of CCl_4 exposure caused liver fibrosis with bridging fibrosis, while 10 weeks of exposure to CCl_4 caused liver cirrhosis and destruction of the normal liver architecture.

Pregnant rats were exposed to 0, 300, or 1000 ppm (0, 1938, or 6460 mg/m^3) carbon tetrachloride for 7 hours/day on days 6-15 of gestation (Schwetz *et al*., 1974). Significant fetal growth retardation, measured by decreased crown-rump length and body weight, was observed in the offspring of the exposed groups (n = 22 litters) compared with controls (n = 43 litters). Subcutaneous edema was observed in the 300 ppm group but not in the 1000 ppm group. Sternebral anomalies were observed in the 1000 ppm group.

Effects of Chronic CCl_4 Exposure (Adams *et al.*, 1952)

Species	*Concentration (ppm)*	*Group size*	*Endpoint*	*Exposure scenario (days exposed/ experiment length)*
Rats (male)	50 ppm	15	liver damage: fatty degeneration and cirrhosis; growth depression	134/187
Rats (female)	50 ppm	15	same effects as males with the addition of increased kidney weight	134/187
Guinea pigs	50 ppm	16	liver damage: fatty degeneration and cirrhosis; growth depression	143/200
Rabbits	50 ppm	4	enlarged kidney; liver damage: fatty degeneration and cirrhosis; growth depression	155/216
Monkeys	50 ppm	2	one experienced growth depression	198/277
Rats	25 ppm	30	liver damage; early growth depression	137/191
Guinea pigs (male)	25 ppm	5	liver damage: fatty degeneration; growth depression	133/185
Guinea pigs (female)	25 ppm	5	liver damage: fatty degeneration; growth depression	93/126
Rabbits	25 ppm	4	liver damage: fatty degeneration; and cirrhosis	178/248
Rats	10 ppm	40	liver damage: fatty degeneration	136/192
Guinea pigs	10 ppm	16	liver damage: fatty degeneration	139/197
Rats	5 ppm	48	no adverse effects	145/205
Guinea pigs (male)	5 ppm	9	no adverse effects	143/203
Guinea pigs (female)	5 ppm	9	liver damage	143/203

Data from Guinea Pigs and Rats Exposed to 5 ppm CCl_4 for 7 Months (Adams *et al*., 1952)

			←----- g organ weight/g body weight---→			
Group	*n*	*BW (g)*	*Lung*	*Heart*	*Liver*	*Kidneys*
Rats, male						
Unexposed controls	11	336	0.65	0.32	2.38	0.65
Air-exposed controls	16	322	0.62	0.31	2.25	0.66
5 ppm CCl_4	13	336	0.62	0.31	2.23	0.65
Rats, female						
Unexposed controls	14	204	0.86	0.38	2.41	0.73
Air-exposed controls	17	209	0.76	0.37	2.76	0.76
5 ppm CCl_4	18	214	0.81	0.38	2.58	0.73
Guinea pigs, male						
Air-exposed controls	7	695	0.79	0.27	3.07	0.63
5 ppm CCl_4	8	669	0.82	0.27	3.14	0.65
Guinea pigs, female						
Air-exposed controls	9	611	0.81	0.27	2.58	0.59
5 ppm CCl_4	6	636	0.78	0.26	2.82*	0.57

* p =0.004

VI. Derivation of Chronic Reference Exposure Level (REL)

Study	Adams *et al.* (1952)
Study population	9 male and 9 female guinea pigs
Exposure method	Discontinuous whole-body inhalation
Critical effects	Increase in liver weight and liver lipid content in females
LOAEL	5 ppm
NOAEL	Not observed
Exposure continuity	7 hours/day, 5 days/week
Average experimental exposure	1.0 ppm
Human equivalent concentration	1.7 ppm (gas with systemic effects, based on RGDR = 1.7 for lambda (a) : lambda (h) (Gargas *et al.* 1989))
Exposure duration	143 exposures over 203 days (7.3 months)
LOAEL uncertainty factor	3 (mild effect; only in one sex of one species)
Subchronic uncertainty factor	3 (7.3 mo/6 yr guinea pig life-span = 10.1%)
Interspecies uncertainty factor	3
Intraspecies uncertainty factor	10
Cumulative uncertainty factor	300
Inhalation reference exposure level	0.006 ppm (6 ppb; 40 $\mu g/m^3$; 0.04 mg/m^3)

Of the 2 adequate chronic inhalation studies available on CCl_4, the Adams *et al.* (1952) study was chosen over the Prendergast *et al.* (1967) study as the key reference for the carbon tetrachloride chronic REL. The Adams *et al.* (1952) experiment was conducted over a longer

duration. In addition, the Adams study contained more specific endpoints of liver damage that were consistent with the mechanism of carbon tetrachloride toxicity. Both studies resulted in hepatic effects with exposed rats appearing less sensitive than the affected monkeys or guinea pigs.

For comparison, conversion of the oral U.S. EPA RfD value of 0.7 μg/kg/day to an equivalent inhalation value by route-to-route extrapolation yields an inhalation REL estimate of 2.5 $\mu g/m^3$. As another comparison, if the 6.1 mg/m^3 continuous exposure in Prendergast *et al*. (1967) is a NOAEL (for rats), the resulting REL estimate would be 60 $\mu g/m^3$. If the 6.1 mg/m^3 continuous exposure is a mild LOAEL, the resulting REL estimate would be 20 $\mu g/m^3$.

VII. Data Strengths and Limitations for Development of the REL

The major strengths of the REL for carbon tetrachloride are the chronic exposure study used and the target tissue affected. The major uncertainties are the lack of human data, the lack of a NOAEL observation, the small sample sizes used, and the lack of comprehensive multiple dose studies.

VIII. References

Adams E, Spencer H, Rowe V, McCollister D, and Irish D. 1952. Vapor toxicity of carbon tetrachloride determined by experiments on laboratory animals. Arch. Indust. Hyg. Occup. Med. 6(1):50-66.

ACGIH. 1992. American Conference of Governmental and Industrial Hygienists. Documentation of the Threshold Limit Values and Biological Exposure Indices. 6th ed. Vol. I. Cincinnati, OH: ACGIH.

CARB. 1999. California Air Resources Board. Toxics Air Quality Data. Substance Chooser. Carbon tetrachloride. Available online at http://www.arb.ca.gov/aqd/toxics.htm

CARB. 2000. California Air Resources Board. California Emissions Inventory Development and Reporting System. (CEIDARS). Data from Data Base Year 1998. February 12, 2000.

CRC. 1994. CRC Handbook of Chemistry and Physics, 75th edition. Lide DR, ed. Boca Raton, FL: CRC Press Inc.

DeShon ND. 1979. Carbon Tetrachloride. Kirk-Othmer Encyclopedia of Chemical Technology, 3rd ed. Grayson M, and Eckroth D, eds. New York: John Wiley and Sons, Inc. 5:704-714. [as cited by Santodonato J. 1985. Monograph on human exposure to chemicals in the workplace: Carbon tetrachloride; PB86-143377; SRC-TR-84-1123.]

Gargas ML, Burgess RJ, Voisard DE, Cason GH, and Andersen ME. 1989. Partition coefficients of low-molecular-weight volatile chemicals in various liquids and tissues. Toxicol. Appl. Pharmacol. 98(1):87-99.

HSDB. 1995. Hazardous Substances Data Bank. National Library of Medicine, Bethesda, MD (CD-ROM version). Denver, CO: Micromedex, Inc. (edition expires 7/31/95).

Ideura T, Yoshimura A, Shirai M, Taira T, and Koshikawa S. 1993. Endotoxin induced acute tubular necrosis in cirrhotic rats. Scand. J. Urol. Nephrol. 27:433-439.

Kazantzis G, Bomford RR, and Oxon DM. 1960. Dyspepsia due to inhalation of carbon tetrachloride vapour. Lancet 360-362.

Prendergrast J, Jones R, Jenkins L, and Siegel J. 1967. Effects on experimental animals of long-term inhalation of trichloroethylene, carbon tetrachloride, 1,1,1-trichloroethane, dichlorodifluoromethane, and 1,1-dichloroethylene. Toxicol. Appl Pharmacol 10:270-289.

Sakata T, Watanabe N, Hobara N, and Nagashima H. 1987. Chronic liver injury in rats by carbon tetrachloride inhalation. Bull. Environ. Contam. Toxicol. 38:959-961.

Santodonato J. 1985. Monograph on human exposure to chemicals in the workplace: Carbon tetrachloride; PB86-143377; SRC-TR-84-1123. NTIS.

Schwetz BA, Leong BK, and Gehring PJ. 1974. Embryo - and fetotoxicity of inhaled carbon tetrachloride, 1,1-dichloroethane and methyl ethyl ketone in rats. Toxicol. Appl. Pharmacol. 28:452-464.

Shimizu Y, Nagase C, and Kawai K. 1973. Accumulation and toxicity of carbon tetrachloride after repeated inhalation in rats. Ind. Health 11:48-54.

Yoshimura A, Ideura T, Shirai M, Tiara T, Iwasaki S, Kitaoka T, and Koshikawa S. 1993. The distribution of ^{3}H-labeled endotoxin in the kidney of liver cirrhotic rats. Scand. J. Urol. Nephrol. 27:295-299.

CHRONIC TOXICITY SUMMARY

CHLORINATED DIBENZO-*p*-DIOXINS and CHLORINATED DIBENZOFURANS

(INCLUDING 2,3,7,8-TETRACHLORODIBENZO-P-DIOXIN)

(Polychlorinated dibenzo-p-dioxins (PCDDs) and polychlorinated dibenzofurans (PCDFs) including 2,3,7,8-Tetrachlorodibenzo-p-dioxin (2,3,7,8-TCDD) which is the principal congener of concern based on toxicity)

CAS Registry Number: 1746-01-6 (TCDD); 5120-73-19 (TCDF)

I. Chronic Toxicity Summary

Inhalation reference exposure level	**0.00004 µg/m^3 (40 pg/m^3)**
Oral reference exposure level	**1 x 10^{-8} mg/kg/day (10 pg/kg/day)**
Critical effect(s)	Increased mortality, decreased weight gain, depression of erythroid parameters, increased urinary excretion of porphyrins and delta-aminolevulinic acid, increased serum activities of alkaline phosphatase, gamma-glutamyl transferase and glutamic-pyruvic transaminase, gross and histopathological changes in the liver, lymphoid tissue, lung and vascular tissues in rats.
Hazard index target(s)	Alimentary system (liver); reproductive system; development; endocrine system; respiratory system; hematopoietic system

II. Physical and Chemical Properties (HSDB, 1995; 1999)

Description	All are white crystalline powders at 25° C.
Molecular Formula	$C_{12}H_4C_{l4}O_2$ (TCDD)
Molecular Weight	321.97 g/mol (TCDD)
Density	1.827 g/ml (estimated for TCDD)
Boiling Point	412.2°C (estimated for TCDD)
Melting Point	305-306°C (TCDD)
Vapor Pressure	1.52 x 10^{-9} torr at 25°C (TCDD)
Solubility	In water: 19.3 ng/L at 22°C (TCDD)
Log K_{ow} *(octanol/water partition coefficient)*	6.15-7.28 (6.8 for TCDD)
Log K_{oc} *(organic-carbon distribution coefficient)*	6.0-7.39
Henry's Law Constant	8.1 x 10^{-5} ATM-m^3/mol

III. Major Uses and Sources

The chlorinated dioxins and furans are generated as by-products from various combustion and chemical processes. PCDDs are produced during incomplete combustion of chlorine containing wastes like municipal solid waste, sewage sludge, and hospital and hazardous wastes. Various metallurgical processes involving heat, and burning of coal, wood, petroleum products and used tires for energy generation also generate PCDDs. Chemical manufacturing of chlorinated phenols (e.g., pentachlorophenol), polychlorinated biphenyls (PCBs), the phenoxy herbicides (e.g., 2,4,5 T), chlorinated benzenes, chlorinated aliphatic compounds, chlorinated catalysts and halogenated diphenyl ethers are known to generate PCDDs as a by-product under certain conditions. While manufacture of many of these compounds and formulations has been discontinued in the United States, continued manufacture elsewhere in the world combined with use and disposal of products containing PCDD by-products results in the inadvertent release of PCDDs into the environment. Industrial and municipal processes in which naturally occurring phenolic compounds are chlorinated can produce PCDDs; the best example is chlorine bleaching of wood pulp in the manufacture of paper products. Additionally, municipal sewage sludge has been documented to occasionally contain PCDDs and PCDFs. Annual statewide industrial emissions from facilities reporting under the Air Toxics Hot Spots Act in California based on the most recent inventory were estimated to be 0.123 pounds of 2,3,7,8-TCDD, 0.244 pounds of 1,2,3,4,7,8-hexachlorodibenzodioxin and lesser amounts of other polychlorinated dibenzodioxins and dibenzofurans (CARB, 1999).

IIIa. 2,3,7,8 Tetrachlorodibenzo-p-dioxin Toxic Equivalents

2,3,7,8-Tetrachlorodibenzo-p-dioxin is considered the most potent congener of the polychlorinated dibenzo-p-dioxins (PCDDs) and polychlorinated dibenzofurans (PCDFs) families of compounds. Potency of PCDD and PCDF congeners correlates with the binding affinity to the cytosolic Ah receptor. Structure activity studies have demonstrated that optimal biological activity and Ah-receptor binding requires congeners with a planar conformation and chlorines at the corners of the molecule at the 2,3,7,8 positions (Poland and Knutson, 1982; Safe, 1986). Chlorines at both ortho positions in these molecules (i.e., positions 1 and 9) sterically hinder a planar conformation that lessens the congeners' biological activity. Thus only 15 of 210 different PCDDs and PCDFs congeners possess significant biological activity based on chlorines in the 2,3,7,8 positions and some degree of planar conformation (Safe, 1986; U.S. EPA 1989). These include two tetrachloro-congeners: 2,3,7,8-tetrachlorodibenzo-p-dioxin and 2,3,7,8-tetrachlorodibenzofuran; three pentachloro congeners: 1,2,3,7,8-pentachlorodibenzo-p-dioxin, 1,2,3,7,8-pentachlorodibenzofuran, and 2,3,4,7,8-pentachlorodibenzofuran; seven hexachloro congeners: 1,2,3,4,7,8 or 1,2,3,6,7,8 or 1,2,3,7,8,9-hexachlorodibenzo-p-dioxins and hexachlorodibenzofurans and 2,3,4,6,7,8-hexachlorodibenzofuran; and three heptachloro congeners: 1,2,3,4,6,7,8-heptachlorodibenzo-p-dioxin, 1,2,3,4,6,7,8-heptachlorodibenzofuran and 1,2,3,4,7,8,9-heptachlorodibenzofuran (U.S. EPA, 1989). The structures of the dibenzo-p-dioxins and dibenzofurans along with their numbering schemes are shown in Figure 1. Toxic equivalents are calculated relative to the most potent congener, 2,3,7,8-tetrachlorodibenzo-p-dioxin, and are determined based on structure activity studies examining relative affinity for the

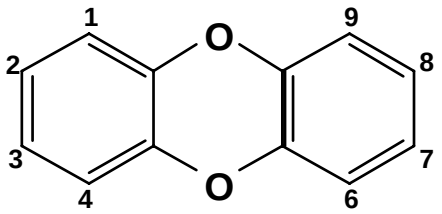
Ah receptor as well as on relative toxicity of different congeners. Values for the international system of toxic equivalents are provided in Table 1 (U.S. EPA, 1989).

Table 1. International Toxic Equivalency Factors (I-TEFs) for PCDDs and PCDFs Chlorinated in the 2,3,7, and 8 Positions. (U.S. EPA 1989.)

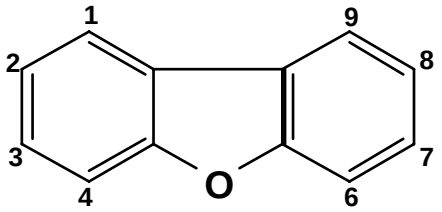
Compound[1,2]	**I-TEF**
Mono-, Di-, and Tri-CDDs and CDFs	0
TetraCDD	
2,3,7,8-substituted	1.0
Others	0
PentaCDD	
2,3,7,8-substituted	0.5
Others	0
HexaCDD	
2,3,7,8-substituted	0.1
Others	0
HeptaCDD	
2,3,7,8-substituted	0.01
Others	0
OctaCDD	0.001
TetraCDF	
2,3,7,8	0.1
Others	0
PentaCDF	
1,2,3,7,8-PentaCDF	0.05
2,3,4,7,8-PentaCDF	0.5
others	0
HexaCDF	
2,3,7,8-substituted	0.1
Others	0
HeptaCDF	
2,3,7,8-substituted	0.01
Others	0
OctaCDF	0.001

[1] CDD designates chlorinated dibenzo-p-dioxin
[2] CDF designates chlorinated dibenzofuran

Figure 1. Structures of the Dibenzo-p-dioxins and Dibenzofurans



Dibenzo-p-dioxin



Dibenzofuran

IV. Effects of Human Exposure

The information available on possible chronic toxic effects in humans is complicated by the relative insensitivity of epidemiological studies, the limited ability of case studies of exposed individuals to establish cause and effect relationships, the heterogeneous nature of human populations, the broad spectrum of exposures to other toxic agents in the human environment, and the episodic exposure of many of the exposed human populations which have been studied (e.g., Seveso, Italy). As a result, a limited number of effects have been associated with exposure to dioxins in humans. The meaning of these effects in terms of toxicity in most cases remains to be clarified. The majority of information comes from cross-sectional medical studies. Chloracne is the most widely recognized effect of exposure to 2,3,7,8-TCDD and TCDD-like PCDDs and PCDFs. Chloracne is a persistent condition, which is characterized by comedones, keratin cysts and inflamed papules and is seen after acute and chronic exposure to various chlorinated aromatic compounds (Moses and Prioleau, 1985). Other dermal effects include hyperpigmentation and hirsutism or hypertrichosis (Jirasek *et al.*, 1974; Goldman, 1972; Suskind *et al.*, 1953; Ashe and Suskind, 1950); both appear to resolve themselves more quickly over time than chloracne, making them more of an acute response rather than a chronic response (U.S. EPA, 1994a). Epidemiological data available for 2,3,7,8-TCDD have not allowed a determination of the threshold dose required for production of chloracne (U.S. EPA, 1994b). Case studies suggest that there may be a relationship between 2,3,7,8-TCDD exposure and hepatomegaly (Reggiani, 1980; Jirasek *et al.*, 1974; Suskind *et al.*, 1953; Ashe and Suskind, 1950) and hepatic enzyme changes (Mocarelli *et al.*, 1986; May, 1982; Martin 1984; Moses *et al.*, 1984). Nevertheless, cross sectional epidemiological studies of trichlorophenol (TCP) production workers (Suskind and Hertzberg., 1984; Bond *et al.*, 1983; Moses *et al.*, 1984; Calvert *et al.* 1992), Vietnam veterans (Centers for Disease Control Vietnam Experience Study, 1988; Roegner *et al.*, 1991) and Missouri residents (Webb *et al.*, 1989; Hoffman *et al.*, 1986)

found little evidence for an association between exposure and hepatomegaly suggesting that this is not a chronic response. There is a consistent pattern of increased levels of serum gamma glutamyl transferase in populations exposed to 2,3,7,8-TCDD which is presumably of hepatic origin (Mocarelli, 1986; Caramaschi *et al.*, 1981, May, 1982; Martin, 1984; Moses *et al.*, 1984; Calvert *et al.*, 1992; Centers For Disease Control Vietnam Experience Study, 1988). Two cross sectional studies have associated diabetes and elevated fasting serum glucose levels with relatively high serum 2,3,7,8-TCDD levels (Sweeney *et al.*, 1992; Roegner *et al.*, 1991). However other studies provided mixed results (Moses *et al.*, 1984; Centers for Disease Control Vietnam Experience Study, 1988; Ott *et al.*, 1993). TCDD has been associated with effects on reproductive hormonal status in males. The likelihood of abnormally low testosterone levels was 2 to 4 times greater in individuals with serum 2,3,7,8-TCDD levels above 20 pg/ml (Egeland *et al.* 1994) and increased serum levels of luteinizing hormone and follicle stimulating hormone have been documented (Egeland *et al.*, 1994). A number of other effects have been reported that were either not seen as chronic effects or effects seen long term in only one population of exposed persons. These include elevated liver enzymes (aspartate aminotransferase and alanine aminotransferase), pulmonary disorders, neurologic disorders, and changes in porphyrin metabolism and kidney disorders (U.S. EPA, 1994c). Areas in which there is presently insufficient information to draw solid conclusions include effects on the circulatory system, reproductive effects, immunological effects, effects on metabolism and handling of lipids, and on thyroid function (U.S. EPA, 1994c). Recent findings in Rhesus monkeys have shown 2,3,7,8-TCDD to cause endometriosis (Reier *et al.*, 1993) and epidemiological studies are currently underway to determine if there is an association between TCDD exposure and endometriosis in human populations exposed by the Seveso accident.

Potential effects of a toxicant on normal fetal development include fetal death, growth retardation, structural malformations and organ system dysfunction. Evidence for all four of these responses has been seen in human populations exposed to dioxin-like compounds. In these poisoning episodes populations were exposed to a complex mixture of halogenated aromatic hydrocarbons contained within PCBs, PCDFs and PCDDs mixtures thus limiting the conclusions that could be drawn from the data. In the Yusho and Yu-Cheng poisoning episodes, human populations consumed rice oil contaminated with PCBs, PCDFs and PCDDs. Yu-Cheng women experienced high perinatal mortality in hyperpigmented infants born to affected mothers (Hsu *et al.* 1985). This occurred in women with overt signs of toxicity (chloracne) (Rogan, 1982) and Rogan notes that, when there is no sign of toxicity in the mother, the likelihood of fetotoxicity appears to lessen considerably in the infants. Signs of toxicity from dioxin like compounds were absent in infants born to mothers apparently not affected in the Seveso, Italy and Times Beach, Missouri, incidents (Reggiani, 1989; Hoffman and Stehr-Green, 1989), which supports Rogan's conclusion. There was an increased incidence of decreased birth weight in infants born to affected mothers in the Yusho and Yu-Cheng incidents suggesting fetal growth retardation (Wong and Huang, 1981; Law *et al.*, 1981; Lan *et al.*, 1989; Rogan *et al.*, 1988). The structural malformation, rocker bottom heel, was observed in Yusho infants (Yamashita and Hayashi, 1985) making this malformation a possible result of exposure to dioxin-like compounds. Nevertheless, it is unknown if these compounds produce malformations in humans. Evidence for possible organ system dysfunction in humans comes from a study of Yu-Cheng children which found that children exposed in utero experienced delays in attaining developmental milestones, and exhibited neurobehavioral abnormalities (Rogan *et al.*, 1988)

suggesting involvement of CNS function. Dysfunction of dermal tissues is noted in exposed infants of the Yusho and Yu-Cheng incidents and is characterized by hyperpigmentation of the skin, fingernails, and toenails, hypersecretion of the meibomian glands, and premature tooth eruption (Taki *et al.*, 1969; Yamaguchi *et al.*, 1971; Funatsu *et al.*, 1971; Wong and Huang, 1981; Hsu *et al.*, 1985; Yamashita and Hayashi, 1985; Rogan *et al.*, 1988; Rogan, 1989; Lan *et al.*, 1989).

V. Effects of Animal Exposure

The toxicity to laboratory animals encompasses a number of areas including changes in energy metabolism manifested as wasting syndrome, hepatotoxicity, effects on tissue of epithelial origin, various endocrine effects, effects on vitamin A storage and use, immune system effects and reproductive and developmental toxicity. The limited number of chronic studies available do not examine all these endpoints. Therefore subchronic exposures are included here in order to provide a more complete coverage of potential chronic toxic effects of these compounds.

Wasting syndrome is one of the most broadly occurring toxic effects. The wasting syndrome is characterized by loss of adipose tissue and lean muscle mass and is produced in all species and strains tested, but there are difference in sensitivity (U.S. EPA 1994d; Peterson *et al.*, 1984; Max and Silbergeld, 1987). Numerous studies have not yet established the mechanism of wasting syndrome (U.S. EPA, 1994e). Hepatotoxicity is also seen in all species tested, but there is considerable variation in species sensitivity (U.S. EPA, 1994d). TCDD induces hyperplasia and hypertrophy of liver parenchymal cells. Morphological and biochemical changes in the liver include increased SGOT and SGPT, induction of microsomal monooxygenases and proliferation of the smooth endoplasmic reticulum, porphyria, increased regenerative DNA synthesis, hyperlipidemia, hyperbilirubinemia, hyperchloesterolemia, hyperproteinemia, degenerative and necrotic changes, mononuclear cell infiltration, multinucleated giant hepatocytes, increased numbers of mitotic figures, and parenchymal cell necrosis (U.S. EPA, 1994d; WHO/IPCS, 1989). Epithelial effects seen include chloracne (rabbit ear and the hairless mouse) (Jones and Krizek, 1962; Schwetz *et al.*, 1973) and hyperplasia and/or metaplasia of gastric mucosa, intestinal mucosa, the urinary tract, the bile duct and the gall bladder (U.S. EPA 1994f). TCDD exposure results in endocrine like effects including epidermal growth factor like effects such as early eye opening and incisor eruption in the mouse neonate (Madhukar *et al.*, 1984), glucocorticoid like effects such as involution of lymphoid tissues (U.S. EPA, 1994g; Sunahara *et al.*, 1989), alteration in thyroid hormone levels and in some cases thyroid hormone like effects (WHO/IPCS, 1989; Rozman *et al.*, 1984), decreases in serum testosterone and dihydrotestosterone (Mittler *et al.*, 1984; Keys *et al.*, 1985; Moore and Peterson, 1985), and changes in arachidonic acid metabolism and prostaglandin synthesis (Quilley and Rifkind, 1986; Rifkind *et al.*, 1990). TCDD is known to decrease hepatic vitamin A storage (Thunberg *et al.*, 1979). TCDD and other dioxin like PCDDs and PCDFs are potent suppressors of both cellular and humoral immune system function, characteristically producing thymic involution at low doses and involution of other lymphoid tissues at higher doses (U.S. EPA 1994h).

In animal studies there is a large body of information available documenting both developmental and reproductive toxicity of 2,3,7,8-TCDD and other PCDDs and PCDFs. These compounds are

acutely toxic to early life stages of fish and birds with fish being most sensitive (LD_{50} of 0.4 µg/kg for rainbow trout sac fry eggs and LD_{50} of 34 ng/kg for lake trout eggs); some species of birds are also relatively sensitive (LD_{50} of 0.25 µg/kg for chicken eggs) (Peterson *et al.*, 1993). 2,3,7,8-TCDD has been documented to increase the incidence of prenatal mortality in a number of species of laboratory animals including the Rhesus monkey, Guinea pig, rabbit, rat, hamster, and mouse (Peterson *et al.*, 1993). Exposure to 2,3,7,8-TCDD during gestation produces a characteristic set of fetotoxic responses in most laboratory animals which includes: thymic hypoplasia, subcutaneous edema, and decreased growth (Peterson *et al.*, 1993). More species specific responses include cleft palate formation in the mouse at doses below maternal toxicity (Moore *et al.*, 1973; Smith *et al.*, 1976; Couture *et al.*, 1990), intestinal hemorrhage in the rat (Sparschu *et al.*, 1971), hydronephrosis in the mouse and hamster (Moore *et al.*, 1973; Smith *et al.*, 1976; Couture *et al.*, 1990; Birnbaum *et al.*, 1989; Olson *et al.*, 1990), and extra ribs in the rabbit (Giavini *et al.*, 1982). Female rats have also been found to be affected by perinatal exposure to 2,3,7,8-TCDD with clefting of the clitoris, incomplete or absent vaginal opening and a smaller vaginal orifice after a dose of 1 µg/kg to the mother on day 15 of gestation (Gray *et al.*, 1993).

A number of effects on adult reproductive function are seen in male animals exposed in utero to 2,3,7,8-TCDD. TCDD reduces plasma androgen levels in the adult male rat and perinatal exposure decreases spermatogenesis, spermatogenic function and reproductive capability, feminizes male sexual behavior, and feminizes male gonadotrophic function (LH secretion) (Mably *et al.*, 1991; Mably *et al.*, 1992a,b,c). Evidence suggests that these effects are the result of impaired sexual differentiation of the CNS, which in male rats is dependent on exposure of the developing brain to testosterone.

There are numerous studies detailing the effects of the PCDDs, PCDFs and other dioxin like compounds, however a large number of these studies were conducted as either acute or subchronic exposures, studies in which it is unlikely that body burdens had reached steady state levels. Detailed below are three chronic studies that were considered in the setting of a chronic toxicity exposure level.

The most definitive study of chronic toxicity in rats is that of Kociba *et al.* (1978). This study involved the administration of 2,3,7,8-TCDD in the diet at doses of 1 ng/kg/day, 10 ng/kg/day, and 100 ng/kg/day to groups of 50 male and 50 female Sprague Dawley rats for two years. A group of 86 male and 86 female rats received diet with solvent vehicle alone and served as controls. The following observations (excluding carcinogenic effects) were seen at the 100 ng/kg/day dose: increased mortality, decreased weight gain, depressed erythroid values, increased urinary excretion of porphyrins and delta-aminolevulinic acid, and increased serum activities of alkaline phosphatase, gamma-glutamyl transferase, and glutamic-pyruvic transaminase. Histopathologic changes were noted in the liver, lymphoid tissue, respiratory and vascular tissues. The primary ultrastructural change in the liver was proliferation of the rough endoplasmic reticulum. At the 10 ng/kg/day dose the severity of toxic symptoms was less than that of the 100 ng/kg/day dose and included increased urinary excretion of porphyrins in females as well as liver and lung lesions. The 1 ng/kg/day dose produced no discernible significant toxic effects. Interpretation of this study by the authors was that the 1 ng/kg/day dose was a NOAEL.

Two chronic toxicity studies are available in the mouse. The first is a one year study conducted by Toth *et al.* (1979) using male Swiss mice administered weekly oral doses of 7, 700, and 7000 ng/kg/day. In this study 2,3,7,8-TCDD administration resulted in amyloidosis and dermatitis in 0 of 38 control animals, 5 of 44 animals receiving 7 ng/kg/day, 10 of 44 animals receiving 700 ng/kg/day and 17 of 43 animals receiving 7,000 ng/kg/day. The other study was from the NTP 1982 gavage study (NTP, 1982) in B6C3F1 mice. This study employed groups of 50 male and 50 female mice. The males received doses of 0, 10, 50, and 500 ng/kg/week by gavage for two years while female mice received doses of 0, 40, 200, and 2000 ng/kg/week by gavage for two years. No adverse effects were seen at the lowest doses tested in each sex, which correspond to NOAELs of approximately 1.4 and 6 ng/kg/day for males and females, respectively. Neither chronic toxicity study in mice reported data on enzyme activity.

VI. Derivation of Chronic Reference Exposure Level (REL)

Study	Kociba *et al.* (1978)
Study population	Sprague-Dawley rats of both sexes (50/treatment group/sex)
Exposure method	Continuous dietary exposure starting at seven weeks of age for 2 years
Critical effects	Increased mortality, decreased weight gain, depression of hematologic measures, increased urinary excretion of porphyrins and delta-aminolevulinic acid, increased serum activities of alkaline phosphatase, gamma-glutamyl transferase and glutamic-pyruvic transaminase, gross and histopathological changes in the liver, lymphoid tissue, lung and vascular tissues
Observed LOAEL	210 ppt in diet (0.01 µg/kg/day)
Observed NOAEL	22 ppt in diet (0.001 µg/kg/day)
Exposure continuity	Continuous exposure via the diet
Exposure duration	2 years
Subchronic uncertainty factor	1
LOAEL uncertainty factor	1
Interspecies uncertainty factor	10
Intraspecies uncertainty factor	10
Cumulative uncertainty factor	100
Oral reference exposure level	10 pg/kg/day
Route-to-route extrapolation	3,500 $\mu g/m^3$ per mg/kg/day
Inhalation reference exposure level	40 pg/m^3 (0.00004 $\mu g/m^3$)

The data available for chronic toxic effects in humans have a number of limitations. Some studies did not determine the body burden of compounds necessary to estimate dose.; The Yusho and Yu-Cheng poisoning episodes have uncertainty because exposure was to complex mixtures of halogenated aromatic hydrocarbons rather than to individual congeners. And epidemiological

studies and case studies have limitations in determining cause and effect relationships. Therefore, an animal study was chosen for determination of a NOAEL/LOAEL. The study chosen for use was that of Kociba *et al.* (1978), based on the duration of the study (2 years), the number of animals employed (50 per treatment group per sex), testing of both sexes, a dose range, which spanned from an apparent NOAEL to severe hepatic effects including carcinogenic effects, a complete histopathological examination of all organ systems, examination of urinary excretion of porphyrins and delta-aminolevulinic acid, and determination of serum activities of alkaline phosphatase, gamma-glutamyl transferase, and glutamic-pyruvic transaminase. The elevation of human serum values for gamma-glutamyl transferase is one of the consistently seen chronic responses in exposed human populations and reflects changes in liver biochemistry. Thus the examination of markers of liver toxicity also altered in animal models of chronic toxicity make the Kociba study an appropriate choice for detecting potential chronic toxic effects of 2,3,7,8-TCDD in humans. The NOAEL in the Kociba *et al.* (1978) study was determined to be 1 ng/kg body weight/day. For the purposes of determining the REL the 1 ng/kg/day dose was considered to be a NOAEL based upon the observations of Kociba *et al.* (1978).

VII. Data Strengths and Limitations for Development of the REL

NOAELs from a number of other studies compare favorably with the 1 ng/kg/day NOAEL. These include the NOAEL from the NTP (1982) study in B6C3F1 mice and the NOEL for enzyme induction in rats and marmosets calculated by Neubert (1991) of 1 ng/kg. Furthermore the 1 ng/kg/day NOAEL is lower than the LOAELs observed by Toth *et al.* (1979) of 7 ng/kg/day in mice and by Schantz *et al.* (1978) of 2.3 ng/kg/day in rhesus monkeys. Current exposure assessments for 2,3,7,8-TCDD and other dioxin-like compounds including the PCBs, PCDDs, and PCDFs estimate that the average daily background dose in the U.S. is 3-6 pg TEQ/kg/day (U.S. EPA 1994i) also placing the REL close to background exposures. The REL of 10 pg/kg/day should be protective of chronic effects on liver function and avoid significant increases in exposure over the background level of human exposure.

The strengths of the inhalation REL include the availability of chronic exposure data from a well-conducted study with histopathological analysis, the observation of a NOAEL, and the demonstration of a dose-response relationship. Major areas of uncertainty are the lack of adequate human exposure data and the lack of chronic inhalation exposure studies.

VIII. References

Ashe WF, and Suskind RR. 1950. Reports on chloracne cases, Monsanto Chemical Co., Nitro, West Virginia, October 1949 and April 1950. Cincinnati, OH: Department of Environmental Health, College of Medicine, University of Cincinnati (unpublished).

Birnbaum LS, Harris MW, Stocking LM, Clark AM, and Morrissey RE. 1989. Retinoic acid and 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD) selectively enhance teratogenesis in C57BL/6N mice. Toxicol. Appl. Pharmacol. 98: 487-500.

Bond GG, Ott MG, Brenner FE, and Cook RR. 1983. Medical and morbidity surveillance findings among employees potentially exposed to TCDD. Br. J. Ind. Med 40: 318-324.

CARB. 1999. Air toxics emissions data collected in the Air Toxics Hot Spots Program CEIDARS Database as of January 29, 1999.

Calvert GM, Hornung RW, Sweeney MH, Fingerhut MA, and Halperin WE. 1992. Hepatic and gastrointestinal effects in an occupational cohort exposed to 2,3,7,8-tetrachlorodibenzo-para-dioxin. JAMA 267: 2209-2214.

Caramaschi F, Del Caino G, Favaretti C, Giambelluca SE, Montesarchio E, and Fara GM. 1981. Chloracne following environmental contamination by TCDD in Seveso, Italy. Int. J. Epidemiol. 10: 135-143.

Centers for Disease Control Vietnam Experience Study. 1988. Health status of Vietnam veterans. II. Physical health. JAMA 259: 2708-2714.

Couture LA, Abbott BD, and Birnbaum LS. 1990a. A critical review of the developmental toxicity and teratogenicity of 2,3,7,8-tetrachlorodibenzo-p-dioxin: recent advances toward understanding the mechanism. Teratology 42: 619-627.

Egeland GM, Sweeney MH, Fingerhut MA, Wille KK, Schnorr TM, and Halperin WE. 1994. Total serum testosterone and gonadotropins in workers exposed to dioxin. Am. J. Epidemiol. 139: 272-281.

Funatsu I, Yamashih F, Yosikane T, Funatsu T, Ito Y, and Tsugawa S. 1971. A chlorobiphenyl induced fetopathy. Fukuoka Acta Med. 62: 139-149.

Giavini EM, Prati M, and Vismara C. 1982. Rabbit teratology studies with 2,3,7,8-tetrachlorodibenzo-p-dioxin. Environ. Res. 27: 74-78.

Goldman PJ. 1972. Critically acute chloracne caused by trichlorophenol decomposition products. Arbeitsmed. Sozialmed. Arbeitshygiene 7: 12-18.

Gray LE, Ostby JS, Kelce W, Marshall R, Diliberto JJ, and Birnbaum LS. 1993. Perinatal TCDD exposure alters sex differentiation in both female and male LE Hooded rats. Abstracts: Dioxin ‘93, 13th International Symposium on Chlorinated Dioxins and Related Compounds, Vienna, pp. 337-339.

HSDB. 1995. Hazardous Substances Data Bank. TOMES®. Vol 20. Denver, CO: Micromedex, Inc.

HSDB. 1999. Hazardous Substances Data Bank. Available online at http://sis.nlm.nih.gov

Hoffman RE, and Stehr-Green PA. 1989. Localized contamination with 2,3,7,8-tetrachlorodibenzo-p-dioxin: the Missouri episode. In: Kimbrough R.D, Jensen AA, eds. Halogenated biphenyls, terphenyls, naphthalenes, dibenzodioxins, and related products. New York, NY: Elsevier, pp. 471-483.

Hoffman RE, Stehr-Green PA, Wehb KB, Evans RG, Knutsen AP, Schram WF, Staake JL, Gibson BB, and Steinberg KK. 1986. Health effects of long-term exposure to 2,3,7,8-tetrachlorodibenzo-p-dioxin. JAMA 255: 2031-2038.

Hsu ST, Ma CI, Hsu SKH, Wu SS, Hsu NHM, Yeh CC, and Wu SB. 1985. Discovery and epidemiology of PCB poisoning in Taiwan: a four-year follow-up. Environ. Health Perspect. 59: 5-10.

Jirasek L, Kalensky K, Kubec K, Pazderova J, and Lukas E. 1974. Chronic poisoning by 2,3,7,8-tetrachlorodibenzo-p-dioxin. Ceskoslov. Dermatol. 49: 145-157.

Jones EL, and Krizek H. 1962. A technique for testing acnegenic potency in rabbits, applied to the potent acnegen, 2,3,7,8-tetrachlorodibenzo-p-dioxin. J. Invest. Dermatol. 39: 511-517.

Keys B, Hlavinka M, Mason G, and Safe S. 1985. Modulation of rat hepatic microsomal testosterone hydroxylases by 2,3,7,8-tetrachlorodibenzo-p-dioxin and related toxic isostereomers. Can. J. Pharmacol. 63: 1537-1542.

Kociba RJ, Keyes DG, Beyer JE, Carreon RM, Wade CE, Dittenber DA, Kalnins RP, Frauson LE, and Park CN. 1978. Results of a two-year chronic toxicity and oncogenicity study of 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD) in rats. Toxicol. Appl. Pharmacol. 46: 279-303.

Lan S-J, Yen Y-Y, Ko Y-C, and Chin E-R. 1989. Growth and development of permanent teeth germ of transplacental Yu-Cheng babies in Taiwan. Bull. Environ. Contam. Toxicol. 42: 931-934.

Law KL, Hwang BT, and Shaio IS. 1981. PCB poisoning in newborn twins. Clin. Med. (Taipei) 7: 83-91 (in Chinese).

Mably TA, Moore RW, Bjerke DL, and Peterson RE. 1991. The male reproductive system is highly sensitive to in utero and lactational 2,3,7,8-tetrachlorodibenzo-p-dioxin exposure. In: Gallo M A, Scheuplein RJ, van der Heijden CA, eds. Biological basis for risk assessment of dioxins and related compounds, Banbury Report 35. Cold Spring Harbor, NY: Cold Spring Harbor Laboratory; pp. 69-78.

Mably TA, Moore RW, and Peterson RE. 1992a. In utero and lactational exposure of male rats to 2,3,7,8-tetrachlorodibenzo-p-dioxin: 1. Effects on androgenic status. Toxicol. Appl. Pharmacol. 114: 97-107.

Mably TA, Moore RW, Goy RW, and Peterson RE. 1992b. In utero and lactational exposure of male rats to 2,3,7,8-tetrachlorodibenzo-p-dioxin: 2. Effects on sexual behavior and the regulation of luteinizing hormone secretion in adulthood. Toxicol. Appl. Pharmacol. 114: 108-117.

Mably TA, Bjerke DL, Moore RW, Gendron-Fitzpatrick A, and Peterson RE. 1992c. In utero and lactational exposure of male rats to 2,3,7,8-tetrachlorodibenzo-p-dioxin: 3. Effects on spermatogenesis and reproductive capability. Toxicol. Appl. Pharmacol. 114: 118-126.

Madhukar BV, Browstor DW, and Matsumura F. 1984. Effects of in vivo-administered 2,3,7,8-tetrachlorodibenzo-p-dioxin on receptor binding of epidermal growth factor in the hepatic plasma membrane of rat, guinea pig, mouse, and hamster. Proc. Natl. Acad. Sci. USA 81: 7407-7411.

Martin JV. 1984. Lipid abnormalities in workers exposed to dioxin. Br. J. Ind. Med. 41: 254-256.

Max SR, and Silbergeld EK. 1987. Skeletal muscle glucocorticoid receptor and glutamine synthetase activity in the wasting syndrome in rats treated with 2,3,7,8-tetrachlorodibenzo-p-dioxin. Toxicol. Appl. Pharmacol. 87: 523-527.

May G. 1982. Tetrachlorodibenzodioxin: a survey of subjects ten years after exposure. Br. J. Ind. Med. 39: 128-135.

Mittler JC, Ertel NH, Peng RX, Yang CS, and Kiernan T. 1984. Changes in testosterone hydroxylase activity in rat testis following administration of 2,3,7,8-tetrachlorodibenzo-p-dioxin. Ann. N.Y. Acad. Sci. 438: 645-648.

Mocarelli P, Marocchi A, Brambilla P, Gerthoux PM, Young DS, and Mantel N. 1986. Clinical laboratory manifestations of exposure to dioxin in children. A six year study of the effects of an environmental disaster near Seveso, Italy. JAMA 256: 2687-2695.

Moore JA, Gupta BN, Zinkl JG, and Voss JG. 1973. Postnatal effects of maternal exposure to 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD). Environ. Health Perspect. 5: 81-85.

Moore RW, and Peterson RE. 1985. Enhanced catabolism and elimination of androgens do not cause the androgenic deficiency in 2,3,7,8-tetrachlorodibenzo-p-dioxin-treated rats. Fed. Proc. 44: 518.

Moses M., Lilis R, Crow KD, Thornton J, Fischbein A, Anderson HA, and Selikoff IJ. 1984. Health status of workers with past exposure to 2,3,7,8-tetrachlorodibenzo-p-dioxin in the manufacture of 2,4,5-trichlorophenoxyacetic acid. Comparison of findings with and without chloracne. Am. J. Ind. Med. 5: 161-182.

Moses M, and Prioleau PG. 1985. Cutaneous histologic findings in chemical workers with and without chloracne with past exposure to 2,3,7,8-tetrachlorodibenzo-p-dioxin. J. Am. Acad. Dermatol. 12:497-506.

NTP 1982. National Toxicology Program. Carcinogenesis bioassay of 2,3,7,8-tetrachlorodibenzo-p-dioxin (CAS No. 1746-01-6) in Osborne-Mendel rats and B6C3F1 mice (gavage study). NTP Tech. Rept. Ser. 209. DHHS, PHS, NIH, Research Triangle Park, NC.

Neubert D. 1991. Animal data on the toxicity of TCDD and special aspects of risk assessment. Presented at a WHO consultation of tolerable daily intake of PCDDs and PCDFs from food, Bilthoven, The Netherlands, 1990.

Olson JR, McGarrigle BP, Tonucci DA, Schecter A, and Eichelberger H. 1990. Developmental toxicity of 2,3,7,8-TCDD in the rat and hamster. Chemosphere 20: 1117-1123.

Ott MG, Zober A, Messerer P, and German C. 1993. Laboratory results for selected target organs in 138 individuals occupationally exposed to TCDD. Presented at: 13th International Symposium on Chlorinated Dioxins and Related Compounds; September 20-24, 1993; Vienna, Austria.

Peterson RE, Seefeld MD, Christian BJ, Potter CL, Kelling K, and Keesey R. 1984. The wasting syndrome in 2,3,7,8-tetrachlorodibenzo-p-dioxin toxicity: basic features and their interpretation. In: Banbury report: biological mechanisms of dioxin action, Vol. 18. Poland A, Kimbrough R, eds. Plainview, NY: Cold Spring Harbor Laboratory, pp. 291-308.

Peterson RE, Theobold HM, and Kimmel GL. 1993. Developmental and reproductive toxicity of dioxins and related compounds: cross-species comparisons. Crit. Rev. Toxicol. 23(3):283-335.

Poland A, and Knutson JC. 1982. 2,3,7,8-Tetrachlorodibenzo-p-dioxin and related aromatic hydrocarbons: examination of the mechanism of toxicity. Annu. Rev. Pharmacol. Toxicol. 22: 517-554.

Quilley CP, and Rifkind AB. 1986. Prostaglandin release by the chick embryo heart is increased by 2,3,7,8-tetrachlorodibenzo-p-dioxin and by other cytochrome P-448 inducers. Biochem. Biophys. Res. Commun. 136(2): 582-589.

Reggiani G. 1980. Acute human exposure to TCDD in Seveso, Italy. J. Toxicol. Environ. Health 6: 27-43.

Reggiani GM. 1989. The Seveso accident: medical survey of a TCDD exposure. In: Kimbrough RD, Jensen AA, eds. Halogenated biphenyls, terphenyls, naphthalenes, dibenzodioxins and related products. 2nd ed. Amsterdam: Elsevier Science Publishers; pp. 445-470.

Reier SE, Martin DC, Bowman RE, Dmowski WP, and Becker JL. 1993. Endometriosis in rhesus monkeys (Macaca mulatta) following chronic exposure to 2,3,7,8-tetrachlorodibenzo-p-dioxin. Fundam. Appl. Toxicol. 21:433-441.

Rifkind AB, Gannon M, and Gross SS. 1990. Arachidonic acid metabolism by dioxin-induced cytochrome P450: a new hypothesis on the role of P-450 in dioxin toxicity. Biochem. Biophys. Res. Commun. 172(3): 1180-1188.

Roegner RH, Grubbs WD, Lustik MB, Brockman AS, Henderson SC, Williams DE, Wolfe WH, Michalek JE, and Miner JC. 1991. Air Force Health Study: an epidemiologic investigation of health effects in Air Force personnel following exposure to herbicides. Serum dioxin analysis of 1987 examination results. NTIS# AD A-237-516 through AD A-237-524.

Rogan WJ. 1982. PCBs and cola-colored babies: Japan 1968 and Taiwan 1979. Teratology 26: 259-261.

Rogan WJ, Gladen BC, Hung K-L, *et al.* 1988. Congenital poisoning by polychlorinated biphenyls and their contaminants in Taiwan. Science 241: 334-336.

Rogan W. 1989. Yu-Cheng. In: Halogenated biphenyls, terphenyls, naphthalenes, dibenzodioxins and related products. 2nd ed. Kimbrough RD, Jensen AA, eds. New York: Elsevier, pp. 401-415.

Rozman K, Rozman T, and Greim H. 1984. Effect of thyroidectomy and thyroxine on 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD) induced toxicity. Toxicol. Appl. Pharmacol. 72: 372-376.

Safe SH. 1986. Comparative toxicology and mechanism of action of polychlorinated dibenzo-p-dioxins and dibenzofurans. Annu. Rev. Pharmacol. Toxicol. 26: 371-398.

Schwetz BA, Norris JM, Sparschu GL, Rowe VK, Gehring PJ, Emerson JL, and Gehring CG. 1973. Toxicology of chlorinated dibenzo-p-dioxins. Environ. Health Perspect. 5: 87-99.

Schantz SL, Barsotti DA, and Allen JR. 1978. Toxicological effects produced in nonhuman primates chronically exposed to fifty parts per trillion 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD). Toxicol. Appl. Pharmacol. 48(1): A180.

Smith FA, Schwetz BA, and Nitschke KD. 1976. Teratogenicity of 2,3,7,8-tetrachlorodibenzo-p-dioxin in CF-1 mice. Toxicol. Appl. Pharmacol. 38: 517-523.

Sparschu GL, Dunn FL, and Rowe VK. 1971. Study of the teratogenicity of 2,3,7,8-tetrachlorodibenzo-p-dioxin in the rat. Food Cosmet. Toxicol. 9: 405-412.

Sunahara GI, Lucier G, McCoy Z, Bresnick EH, Sanchez ER, and Nelson KG. 1989. Characterization of 2,3,7,8-tetrachlorodibenzo-p-dioxin-mediated decreases in dexamethasone binding to rat hepatic cytosolic glucocorticoid receptor. Mol. Pharmacol. 36: 239-247.

Suskind R, Cholak J, Schater LJ, and Yeager D. 1953. Reports on clinical and environmental surveys at Monsanto Chemical Co., Nitro, West Virginia, 1953. Cincinnati, OH: Department of Environmental Health, University of Cincinnati (unpublished).

Suskind RR, and Hertzberg VS. 1984. Human health effects of 2,4,5-T and its toxic contaminants. JAMA 251:2372-2380.

Sweeney MH, Hornung RW, Wall DK, Fingerhut MA, and Halperin WE. 1992. Prevalence of diabetes and increased fasting serum glucose in workers with long-term exposure to 2,3,7,8-tetrachlorodibenzo-p-dioxin. Presented at: 12th International Symposium on Dioxins and Related Compounds; August 24-28; Tampere, Finland.

Taki I, Hisanaga S, and Amagase Y. 1969. Report on Yusho (chlorobiphenyls poisoning) pregnant women and their fetuses. Fukuoka Acta Med. 60: 471-474 (Japan).

Thunberg T, Ahlborg UG, and Johnsson H. 1979. Vitamin A (retinol) status in the rat after a single oral dose of 2,3,7,8-tetrachlorodibenzo-p-dioxin. Arch. Toxicol. 42: 265-274.

Toth K, Somfai-Relle S, Sugar J, and Bence J. 1979. Carcinogenicity testing of herbicide 2,4,5-trichlorophenoxyethanol containing dioxin and of pure dioxin in Swiss mice. Nature 278: 548-549.

U.S. EPA. 1989. Interim procedures for estimating risks associated with exposures to mixtures of chlorinated dibenzo-p-dioxins and dibenzofurans (CDDs and CDFs) and 1989 update. Washington, DC: Risk Assessment Forum.

U.S. EPA. 1994. Health Assessment Document for 2,3,7,8-Tetrachlorodibenzo-p-Dioxin (TCDD) and Related Compounds. Office of Health and Environmental Assessment, Office of Research and Development, United States Environmental Protection Agency, Washington, D.C. Vol 3:9-8 to 9-12.

U.S. EPA. 1994a. ibid. Vol 2:7-107.

U.S. EPA. 1994b. ibid. Vol 2:7-101.

U.S. EPA. 1994c. ibid Vol 2:7-238.

U.S. EPA. 1994d. ibid Vol 1:3-17.

U.S. EPA. 1994e. ibid Vol 1:3-14.

U.S. EPA. 1994f. ibid Vol 1:3-6.

U.S. EPA. 1994g. ibid Vol 1:3-25.

U.S. EPA. 1994h. ibid Vol 1:3-4-1.

U.S. EPA. 1994i. ibid Vol 3:9-86.

Webb KB, Evans RG, Knudsen DP, and Roodman S. 1989. Medical evaluation of subjects with known body levels of 2,3,7,8-tetrachlorodibenzo-p-dioxin. J. Toxicol. Environ. Health 28: 183-193.

WHO/IPCS. 1989. World Health Organization/International Programme on Chemical Safety. Polychlorinated dibenzo-p-dioxins and dibenzofurans. Environmental Health Criteria 88.

Wong KC, and Hwang MY. 1981. Children born to PCB poisoning mothers. Clin. Med. (Taipei) 7: 83-87 (in Chinese).

Yamaguchi A, Yoshimura T, and Kuratsune M. 1971. A survey on pregnant women having consumed rice oil contaminated with chlorobiphenyls and their babies. Fukuoka Acta Med. 62: 117-121 (in Japanese).

Yamashita F, and Hayashi M. 1985. Fetal PCB syndrome: clinical features, intrauterine growth retardation and possible alteration in calcium metabolism. Environ. Health Perspect. 59: 41-45.

CHRONIC TOXICITY SUMMARY

CHLORINE

CAS Registry Number: 7782-50-5

I. Chronic Toxicity Summary

Inhalation reference exposure level	**0.2 µg/m^3 (0.08 ppb)**
Critical effect(s)	Hyperplasia in respiratory epithelium in female rats
Hazard index target(s)	Respiratory system

II. Physical and Chemical Properties (HSDB, 1995; 1999 except as noted)

Description	Yellow/green gas
Molecular formula	Cl_2
Molecular weight	70.906 (Weast, 1989)
Density	2.9 g/L @ 25°C and 1 ATM
Boiling point	-34.04° C
Vapor pressure	5 atm @ 10.3°C; 5830 torr @ 25°C
Solubility	Slightly soluble in water (310 mL per 100 mL water at 10° C; 1.46 g per 100 mL water at 0° C)
Conversion factor	1 ppm = 2.9 mg/m^3 @ 25° C

III. Major Uses and Sources

In an industrial setting, chlorine is widely used as an oxidizing agent in water treatment and chemical processes. Chlorine is also used to disinfect swimming pool water. Chlorine gas is sometimes used at large public pools while household pools typically use hypochlorite solutions. Chlorine is an integral part of the bleaching process of wood pulp in pulpmills, although chlorine dioxide is replacing this use of chlorine. Chlorine as sodium hypochlorite is commonly used as a household cleaner and disinfectant (HSDB, 1995). The annual statewide industrial emissions from facilities reporting under the Air Toxics Hot Spots Act in California based on the most recent inventory were estimated to be 244,955 pounds of chlorine (CARB, 1999).

IV. Effects of Human Exposure

Shi and associates (1990) evaluated 353 workers from a diaphragm cell chlorine chemical plant. The workers ranged in age from 23-52 years with an average of 42.4 years. Two groups were compiled with respect to the workers' length of exposure in years. Group A consisted of 220 workers who were employed/ exposed for 10-25 years. Group B consisted of 133 workers

employed for less than 10 years. Both groups of workers were exposed to a range of 2.60-11.0 mg/m^3 (0.37-1.75 ppm) chlorine. The control group's average age was 39.7 years (ranging from 26-55 years), and it consisted of 192 workers not exposed to chlorine, but working within the same plant. For all the groups, respiratory symptoms and smoking habits were evaluated as well as clinical examinations, ENT examinations, chest x-rays and pulmonary function tests. Groups A and B showed 3-8 times higher incidence of upper airway complaints than the control workers. Current smokers in groups A and B experienced the highest incidence of pulmonary symptoms and group A workers had a higher prevalence of rhino-pharyngeal signs than the control workers. Abnormalities in chest x-rays were seen in 8.6% of group A workers and in 2.8% of group B workers, compared to 2.3% of the control workers. Groups A and B showed significantly impaired pulmonary function in tests of V50/H and FEF_{25-75} (forced expiratory flow between 25 and 75% of forced vital capacity (FVC), the total amount of air the subject can expel during a forced expiration) - compared with the control group, and group A showed reduced FEV_1 (forced expiratory volume in 1 second) results compared to the control group.

Kennedy *et al.* (1991) compared 321 pulpmill workers (189 of whom were exposed to chlorine or chlorine dioxide "gassings") to a control group of 237 rail yard workers in similar working conditions but not exposed to chlorine (79% and 84% respective participation rates). The workers had been employed for an average of 13 years at the pulpmill and 12.7 years at the rail yard. Chlorine gas and chlorine dioxide levels were measured together over a 4 week period during mainly a 12 hour shift. Time weighted averages (TWA) were <0.1 ppm, with the highest of <0.1-0.3 ppm. A significantly higher prevalence of wheezing was seen in pulpmill workers (both smokers and nonsmokers) who had reported more than one episode of chlorine "gassing" as compared to the rail yard workers and pulpmill workers with no chlorine gas exposure. More airflow obstruction was observed in exposed workers in spite of their nonsmoking and ex-smoking status, correlating to significantly lower average values for MMF (maximal mid-expiratory flow) and for the FEV_1 to FVC ratio. Comparison of pulpmill workers exposed to chlorine and /or chlorine dioxide with those pulpmill workers not exposed, suggests that chronic respiratory health impairment is associated with exposure to chlorine and/or chlorine dioxide. These researchers hypothesized that after the first high exposure incident, an inflammatory response occurred in small airways and that this reaction did not resolve in those workers who were continuously or repeatedly exposed to the irritant. It was also suggested that chronic airflow obstruction caused by repeated minor exposures led to chronic respiratory disability in some of the workers.

Patil *et al.* (1970) evaluated the exposure of 332 male diaphragm cell workers to 0.006-1.42 ppm chlorine gas (a range with a time-weighted average of 0.146 $\pm$0.287; most workers were exposed to less than 1 ppm). A control group consisting of 382 workers from 25 representative chlorine manufacturing plants was also studied. Both groups were comprised of men between the ages of 19-69 with a mean age of 31.2 $\pm$11.0 years. Physical examinations (blood and urine analysis, chest x-rays and electrocardiograms) were conducted, in most cases, within the first six months of the study year. At two month intervals, each plant was surveyed and chlorine levels were determined. Exposed employees were grouped according to job classification. Researchers found the average number of exposure years for the study group to be 10.9 $\pm$ 2.8 years and concluded that the exposure level had no correlation to the number of years exposure. Ninety-eight of the 332 workers were found to have abnormal teeth and gums, but no dose-response

relationship was concluded. Similarly, no dose-response relationships were shown with the symptoms of sputum production, cough, dyspnea, history of frequent colds, palpitation, chest pain, vital capacity, maximum breathing capacity and forced expiratory volume. Any deterioration in pulmonary function was shown to be age related. Of the 332 exposed workers, 9.4% experienced abnormal EKGs. 8.5% of the control group showed the same abnormalities, but this difference was not significant. Above 0.5 ppm, an increase appeared in the incidence of fatigue. No neurological defects developed and there was no noted prolonged anoxia as a result of the chlorine exposure. Also, no consistent gastrointestinal trouble or abnormal incidence of dermatitis was found. Exposed workers showed elevated white blood cell counts and decreased hematocrit values compared to the control group.

Bherer *et al.* (1994) conducted a follow up study of the Quebec pulp mill research done by Courteau and associates over a time interval of 18-24 months after the incidents of repeated exposures. Fifty-eight of the original 289 exposed workers from the moderate to high risk group were studied for developing reactive airways dysfunction syndrome (RADS). Workers at a moderate risk were defined as having shortness of breath after their most significant exposure, but not at the time of the initial study by Courteau *et al.* Moderate risk workers also had a record of other significant medical conditions and/or were 50 years of age or older. High risk workers were defined as those experiencing shortness of breath that continued one month after the exposure and/or abnormal lung sounds. Ninety percent of the follow up group completed questionnaires which revealed a 91% incidence of respiratory symptoms. Spirometry assessments and methacholine inhalation tests were conducted on 51 of the 58 workers. Twenty-three percent of the 58 workers still experienced bronchial obstruction and 41% continued to have bronchial hyper-responsiveness. Lower baseline FEV_1 was seen in those with a lower PC_{20}, and 52% of these workers showed an $FEV_1 < 80\%$ predicted.

Enarson *et al.* (1984) compared 392 pulpmill workers exposed to chlorine (unspecified duration) to a comparable group of 310 rail yard workers living in the same community, but not exposed to chlorine. In the pulpmill areas surveyed that predominantly had significant chlorine gas levels (machine room and bleach plant), workers were exposed to either an average of 0.02 ppm or 0.18 ppm Cl_2 respectively. Of the machine room workers, 23.2% experienced a cough as did 32.8% of those in the bleach plant, compared to 22.3% of the control rail yard workers. Chest tightness occurred in 31.5% of the machine room workers and 39.6% of the bleach plant workers as compared to 21.3% of the control. Only data from Caucasian subjects were reported.

Chester *et al.* (1969) evaluated 139 workers occupationally exposed to <1 ppm chlorine for an unspecified duration. Fifty-five of the 139 workers were exposed to additional accidental high concentrations of chlorine, which were severe enough to require oxygen therapy. Ventilation was affected by chlorine inhalation, with a decrease in the maximal midexpiratory flow (MMF). Smokers in this group had significantly reduced FVC, FEV_1 and MMF compared to nonsmokers. Fifty-six of the 139 subjects showed abnormal posteroanterior chest films, 49 of which had parenchyma and/or hilar calcifications consistent with old granulomatous disease and 11 of which had multiple, bilateral and diffuse calcifications. Researchers suggest that the first ventilation function affected in obstructive airway disease is MMF.

V. Effects of Exposure to Animals

Wolf *et al.* (1995) exposed male and female B6C3F1 mice and F344 rats to chlorine gas concentrations of 0 ppm, 0.4 ppm, 1.0 ppm and 2.5 ppm. The exposures were carried out for 104 weeks at 6 hr/day 3 days/week for female rats and 6 hr/day 5 days/week for mice and male rats. Based on previous studies, the authors determined that female rats could not tolerate 5 days/week exposure to chlorine. Each treatment group contained 320 male and 320 female mice. The rats were studied in groups of 70, yielding 280 per gender per species. For the first 13 weeks of observation, body weights and clinical observations were noted weekly, and for the remainder of the study, they were recorded once every two weeks. After 52 weeks, 10 rats were euthanized and autopsied. Organ weights were recorded, and hematologic and clinical chemistry parameters were determined. These same measurements were performed on all of the surviving mice and rats at the conclusion of the 104 weeks. Male mice exposed to 1.0 and 2.5 ppm Cl_2 showed decreased weight gain compared to controls while only female mice exposed to 2.5 ppm Cl_2 showed decreased weight gain. Male rats showed decreased weight gain at all levels of exposure while female rats showed the same result at only 1.0 and 2.5 ppm Cl_2 exposures. Various nonneoplastic nasal lesions were seen in all the airway epithelial types in the nose and at all levels of exposures for both species. These lesions were evaluated against background lesions found in the control animals. A statistically significant incidence of fenestration was seen in all three exposure concentrations of Cl_2. Statistically significant responses were seen in the traditional and respiratory epithelial regions of all exposed rats and mice. Statistically significant damage to olfactory epithelium occurred in all exposed rats and female mice and also in the 1.0 and 2.5 ppm exposed groups of male mice.

Klonne *et al.* (1987) exposed 32 male and female rhesus monkeys to chlorine gas for one year to measured concentrations of 0, 0.1, 0.5, and 2.3 ppm Cl_2. These monkeys were exposed to chlorine for 6 hours/day, 5 days/week. The monkeys were evaluated periodically on the basis of body weight, electrocardiograms, neurologic examinations, pulmonary function, hematologic parameters, serum chemistry, urinalysis, and blood gas and pH levels. Results were compared to the same test measurements recorded prior to the study. No significant difference was seen in body weight at any point in the experiment. Ocular irritation (tearing, rubbing of the eyes, reddened eyes) was observed after 6 weeks of exposure in the 2.3 ppm group. No exposure-related differences were seen in neurologic examinations, electrocardiograms, clinical chemistry, urinalysis, hematology or blood gas levels. Also, no exposure-related changes were observed in the parameters of ventilation distribution. Pulmonary function evaluations yielded a statistically significant trend for increasing pulmonary diffusing capacity and distribution of ventilation values for males and females in the 2.3 ppm exposure group. Both males and females of the 2.3 ppm group exhibited statistically significant increased incidence of respiratory epithelial hyperplasia. A mild form of the lesions was also seen in the 0.5 ppm group, 0.1 ppm group (females only) and one male in the control group. Two parasitic infections occurred, affecting the respiratory tract and resulting in 11 monkeys housing parasites and/or ova. Additionally, 16 monkeys displayed histologic changes characteristic of the presence of the parasites. However, the parasitic induced lesions were not associated with lesions in the respiratory epithelium.

VI. Derivation of Chronic Reference Exposure Level (REL)

Study	Wolf *et al.*, 1995
Study population	Female F344 rats (70 per group)
Exposure method	Discontinuous whole-body inhalation exposure (0, 0.4, 1.0 or 2.5 ppm)
Critical effects	Upper respiratory epithelial lesions (see following table)
LOAEL	0.4 ppm
NOAEL	Not established
BMC_{05}	0.14 ppm
Exposure continuity	6 hours/day, 3 days/week (MWF)
Average experimental exposure	0.015 ppm
Human equivalent concentration	0.0024 ppm (gas with extrathoracic respiratory effects, RGDR = 0.16 based on BW = 229 g, MV = 0.17 L/min, SA(ET) = 15 cm^2)
Exposure duration	2 years
LOAEL uncertainty factor	(not needed in the BMC approach)
Subchronic uncertainty factor	1
Interspecies uncertainty factor	3
Intraspecies uncertainty factor	10
Cumulative uncertainty factor	30
Inhalation reference exposure level	0.08 ppb (0.20 $\mu g/m^3$)

A benchmark dose analysis was performed using a log-normal probit analysis (Tox-Risk, version 3.5; ICF-Kaiser Inc., Ruston, LA) of the female rat data. Using the data for glandular epithelial eosinophilic proteinaceous accumulation (see Table 1 below) to derive the BMC_{05} resulted in a 3-fold lower value than the LOAEL of 0.4 ppm, or BMC_{05} = 0.14 ppm. (Adequate benchmark dose estimates could not be obtained for the other nasal lesions due to high background rates and shallow dose-response relationships.) A BMC_{05} is considered to be similar to a NOAEL in estimating a concentration associated with a low level of risk.

The Wolf *et al.* (1995) study of mice and rats was chosen as the key reference for the chlorine chronic REL for several reasons. First, the duration of the experiment was for a full lifetime of two years. Second, the sample sizes were large (280 per sex per species). Finally, appropriate sensitive endpoints of respiratory epithelial damage were examined. The mice and male rats were exposed to chlorine for 6 hours/day, 5 days/week, but the female rats were only exposed for 3 days/week as the authors observed the females to be more sensitive than the males. Table 1 shows the histological findings of the female rats. Statistically significant results ($p < 0.05$) were seen for all the tissues at 0.4 ppm chlorine exposure and above.

Table 1. Female Rat Epithelial Lesions following Chronic Chlorine Exposure (based on Table 5 of Wolf *et al.*, 1995)

Tissues	0 ppm	0.4 ppm	1.0 ppm	2.5 ppm
Goblet cell hyperplasia	3/70 (4%)	50/70 (71%)	63/70 (90%)	64/70 (91%)
Respiratory epithelium eosinophilic proteinaceous accumulation	49/70 (70%)	60/70 (85%)	59/70 (84%)	65/70 (93%)
Glandular epithelium eosinophilic proteinaceous accumulation	**16/70 (23%)**	**28/70 (40%)**	**52/70 (75%)**	**53/70 (76%)**
Olfactory epithelium eosinophilic proteinaceous accumulation	36/70 (52%)	64/70 (91%)	69/70 (99%)	69/70 (99%)

The Wolf *et al.* (1995) study was chosen over the Klonne *et al.* (1987) monkey study for the following reasons: the monkeys were exposed for only one year of their total 35 year lifetime, and the sample sizes were considerably smaller (4 monkeys per sex per group) than the mouse and rat groups (280 per sex per species). Although the exposure durations differed between the two studies, the histological results were similar, differing only slightly in the region of occurrence. The monkeys displayed both tracheal and nasal lesions. Both the rodents and the monkeys showed upper respiratory epithelial lesions, thus suggesting that the rodents may be an appropriate model for humans.

For comparison with the proposed REL of 0.08 ppb (0.2 $\mu g/m^3$) using the BMC approach, we estimated a REL of 0.02 ppb (0.06 $\mu g/m^3$) based on the same rat study but using the NOAEL/UF approach with a LOAEL of 0.4 ppm divided by a total UF of 300 (10 for LOAEL, 3 for interspecies, and 10 for intraspecies) and the RGDR of 0.16. As another comparison, using 0.1 ppm as a LOAEL for respiratory epithelial lesions in female monkeys, the LOAEL can be time-adjusted to an equivalent continuous value of 24 ppb. Applying a UF_L of 3 for a mild effect, a UF_S of 10 since it was only a 6 month study, an interspecies UF of 3 for monkeys, and an intraspecies UF of 10 results in an estimated REL of 0.02 ppb (0.06 $\mu g/m^3$).

The human studies were examined for possible use in the calculation of a REL. The studies were limited by very variable exposures (e.g., Patil *et al*. (1970)), the presence of serious adverse health effects in some workers (chest x-ray abnormalities in Shi (1990), abnormal teeth and gums in 98 of 332 workers in Paril *et al*. 1970)), exposure to other compounds such as chlorine dioxide (Kennedy *et al*. (1991)), multiple acute “gassings” with chlorine (Kennedy *et al*. (1991)), and absence of data on cigarette smoking, also a respiratory system irritant. As an illustration of what would be estimated, the study of Shi (1990) had a mean workplace exposure of 4.82 mg/m^3 (1.7 ppm). This LOAEL was time adjusted to an equivalent continuous exposure of 1.72 mg/m^3,

then divided by an uncertainty factor of 100 (10 for use of a LOAEL and 10 for intraspecies variability) to yield a REL of 20 $\mu g/m^3$ (7 ppb). However, the use of a LOAEL default uncertainty factor of 10 does not seem adequate for frank, possibly irreversible effects such as the chest x-ray abnormalities reported. There is currently no methodology to deal with such effects in REL development.

Adequate benchmark dose estimates could not be obtained for the other nasal lesions due to high background rates and shallow dose-response relationships.

VII. Data Strengths and Limitations for Development of the REL

The strengths of the inhalation REL for chlorine include the availability of chronic multiple-dose inhalation exposure data from a recent (1995), well-conducted animal study with histopathological analysis. Major areas of uncertainty are the lack of adequate human exposure data, the lack of observation of a NOAEL, and limited reproductive toxicity data.

VIII. References

Bherer L, Cushman R, Courteau JP, Quevillon M, Cote G, Bourbeau J, L'Archeveque J, Cartier A, and Malo JL. 1994. Survey of construction workers repeatedly exposed to chlorine over a three to six month period in a pulpmill: II. Follow up of affected workers by questionnaire, spirometry, and assessment of bronchial responsiveness 18 to 24 months after exposure ended. Occup. Environ. Med. 51:225-228.

CARB. 1999. Air toxics emissions data collected in the Air Toxics Hot Spots Program CEIDARS Database as of January 29, 1999.

Chang-Yeung M, Lam S, Kennedy SM, and Frew AJ. 1994. Persistent asthma after repeated exposure to high concentrations of gasses in pulpmills. Am. J. Respir. Crit. Care Med. 149:1676-1680.

Chester EH, Gillespie DG, and Krause FD. 1969. The prevalence of chronic obstructive pulmonary disease in chlorine gas workers. Am. Rev. Respir. Dis. 99(3):365-373.

Courteau JP, Cushman R, Bouchard F, Quevillon M, Chartrand A, and Bherer L. 1994. Survey of construction workers repeatedly exposed to chlorine over a three to six month period in a pulpmill: I. Exposure and symptomatology. Occup. Environ. Med. 51(4):219-224.

Donnelly SC, and Fitzgerald MX. 1990. Reactive airways dysfunction syndrome (RADS) due to chlorine gas exposure. Ir. J. Med. Sci. 159:275-277.

Enarson D, Johnson A, Block G, Schragg K, Maclean L, Dybuncio A, Chan-Yeung M, and Grzybowski S. 1984. Respiratory health at a pulpmill in British Columbia. Arch. Environ. Health 39:325-330.

Gautrin D, Boulet LP, Boutet M, Dugas M, Bherer L, L'Archeveque J, Laviolette M, Cote J, and Malo JL. 1994. Is reactive airways dysfunction syndrome a variant of occupational asthma? J. Allergy Clin. Immunol. 93:12-22.

HSDB. 1995. Hazardous Substances Data Bank. National Library of Medicine, Bethesda, MD (TOMES® CD-ROM version). Denver, CO: Micromedex, Inc. (edition expires 7/31/95).

HSDB. 1999. Hazardous Substances Data Bank. Available online at http://sis.nlm.nih.gov

Kennedy SM, Enarson DA, Janssen RG, and Chan-Yeung M. 1991. Lung health consequences of reported accidental chlorine gas exposures among pulpmill workers. Am. Rev. Respir. Dis. 143:74-79.

Klonne D, Ulrich C, Riley M, Hamm T, Morgan K, and Barrow C.1987. One-year inhalation toxicology study of chlorine in rhesus monkeys (Macaca mullata). Fundam. Appl. Toxicol. 9:557-572.

Patil L, Smith R, Vonwald A, and Mooney T. 1970. The health of diaphragm cell workers exposed to chlorine. Am. Ind. Hyg. Assoc. J. 31:678-686.

Rea W, Ross G, Johnson A, Smiley R, Sprague D, Fenyves E, and Samandi N. 1989. Confirmation of chemical sensitivity by means of double-blind inhalant challenge of toxic volatile chemicals. Clin. Ecol. 6:113-118.

Shi Z. 1990. Effects of long-term exposure to low concentration of chlorine on workers' health. In: Sakurai H; Okazaki I; Omae K, eds. Occupational Epidemiology, Proceedings of the seventh international symposium on epidemiology in occupational health. Tokyo, Japan. 1989. Pp. 173-177.

Weast RC. 1989. Handbook of Chemistry and Physics, 69th edition, Boca Raton, FL: CRC Press Inc.

Wolf DC, Morgan KT, Gross EA, Barrow C, Moss OR, James RA, and Popp JA. 1995. Two-year inhalation exposure of female and male B6C3F1 mice and F344 rats to chlorine gas induces lesions confined to the nose. Fundam. Appl. Toxicol. 24:111-131.

CHRONIC TOXICITY SUMMARY

CHLORINE DIOXIDE

(anthium dioxide; alcide; chlorine oxide; chlorine peroxide; chloryl radical; doxcide 50)

CAS Registry Number: 10049-04-4

I. Chronic Toxicity Summary

Inhalation reference exposure level	**0.6 μg/m^3** (0.2 ppb)
Critical effect(s)	Vascular congestion and peribronchiolar edema; hemorrhagic alveoli and congested capillaries in the lung in rats
Hazard index target(s)	Respiratory system

II. Physical and Chemical Properties (HSDB, 1994; CRC, 1994)

Description	Yellow to red liquid or gas
Molecular formula	ClO_2
Molecular weight	67.45 g/mol
Density	1.642 g/cm^3 @ 0°C (liquid)
Boiling point	9.9-11°C
Melting point	–59.5°C
Solubility	Soluble in water, alkaline and sulfuric acid solutions
Conversion factor	1 ppm = 2.76 mg/m^3

III. Major Uses or Sources

Chlorine dioxide is used directly as a bleaching agent for cellulose, textiles, flour, leather, oils, and beeswax. It is also used in the purification of water and as a bactericide and antiseptic (HSDB, 1994). The annual statewide industrial emissions from facilities reporting under the Air Toxics Hot Spots Act in California based on the most recent inventory were estimated to be 1136 pounds of chlorine dioxide (CARB, 2000).

IV. Effects of Human Exposures

Case reports of human occupational exposure to chlorine dioxide have shown that 19 ppm was fatal to one worker and 5 ppm was definitely irritating (Elkins, 1959). Seven out of 12 workers exposed regularly to chlorine dioxide at levels generally below 0.1 ppm (0.28 mg/m^3) reported symptoms of ocular and respiratory irritation leading to slight bronchitis (Gloemme and

Lundgren, 1957). However, the authors ascribed the bronchitis to occasional acute excursions of chlorine dioxide levels above 0.1 ppm due to technical problems such as equipment leakage. Concurrent exposure to chlorine and chlorine dioxide in pulp mill workers resulted in an increase in the reporting of subjective symptoms of irritation (Ferris *et al.*, 1967). In this study, the chlorine dioxide concentrations ranged from trace levels to 0.25 ppm (0.69 mg/m^3). No differences were found between these workers and controls by pulmonary function tests.

V. Effects of Animal Exposures

Eight rats (sex unspecified) were exposed for 5 hours/day, 5 days/week, for 2 months to 0 or 1 ppm (2.8 mg/m^3) chlorine dioxide (Paulet and Debrousses, 1972). The number of control animals was not specified. Microscopic evaluation of the lungs revealed vascular congestion and peribronchiolar edema in all animals exposed to chlorine dioxide. The subchronic LOAEL for respiratory effects was therefore 1 ppm (2.8 mg/m^3).

An earlier study by these researchers (Paulet and Debrousses, 1970) examined the effects of exposure to 2.5, 5, or 10 ppm chlorine dioxide for several hours/day for 30 days in rats and rabbits (n = 4-10 animals per group). Body weights, blood cell counts, and histopathological examination of the liver, lungs, and other tissues were measured in each group. At 10 ppm, nasal discharge, localized bronchopneumonia, and desquamated alveolar epithelium were observed. White and red blood cell counts were also increased with this exposure. Rats and rabbits exposed to 2.5 ppm for 7 hours/day for 30 days or for 4 hours/day for 45 days, respectively, showed significant respiratory effects, including hemorrhagic alveoli and inflammatory infiltration of the alveolar spaces.

Rats exposed to 5, 10, or 15 ppm (13.8, 27.6, or 41.4 mg/m^3) chlorine dioxide for 15 minutes, 2 or 4 times/day, for 1 month showed an increase in congested lungs, nasal discharge, and catarrhous lesions of the alveoli beginning at 10 ppm (Paulet and Debrousses, 1974). No significant changes in these parameters were seen at 5 ppm.

Dalhamn (1957) found that acute exposure to 260 ppm chlorine dioxide for 2 hours resulted in the death of 1 out of 4 rats. Five out of 5 rats died during exposures of 4 hours/day for 14 days. All exposed animals exhibited signs of respiratory distress and ocular discharge. No effects were seen in 5 rats exposed to 0.1 ppm for 5 hours/day, 7 days/week, for 10 weeks. Thus 0.1 ppm was a subchronic NOAEL.

VI. Derivation of Chronic Reference Exposure Level (REL)

Study	Paulet and Debrousses (1970, 1972)
Study population	Wistar rats (8 per exposure concentration)
Exposure method	Discontinuous whole-body inhalation (0 or 1 ppm)
Critical effects	Vascular congestion; peribronchial edema in all animals; lung alveolar damage
LOAEL	1 ppm (2.8 mg/m^3)
NOAEL	Not observed
Exposure continuity	5 hours/day, 5 days/week
Exposure duration	2 months (2/24 = 8.3% of lifetime)
Average experimental exposure	0.15 ppm for LOAEL group (1 x 5/24 x 5/7)
Human equivalent concentration	0.23 ppm for LOAEL group (gas with thoracic respiratory effects, RGDR = 1.57 based on MV = 0.17 m^3, SA(Th) = 3,460 cm^2)
LOAEL uncertainty factor	10
Subchronic uncertainty factor	3
Interspecies uncertainty factor	3
Intraspecies uncertainty factor	10
Cumulative uncertainty factor	1,000
Inhalation reference exposure level	0.0002 ppm (0.2 ppb, 0.0006 mg/m^3, 0.6 μg/m^3)

The U.S. EPA (1995) based its RfC of 0.2 μg/m^3 on the same study but included a Modifying Factor (MF) of 3 for database deficiencies. The criteria for use of modifying factors are not well specified by U.S. EPA. Such modifying factors were not used by OEHHA. In addition OEHHA assigned uncertainty factors according to its peer-reviewed, approved methodology (OEHHA, 2000).

OEHHA earlier developed a chronic REL for chlorine of 0.2 μg/m^3 (0.08 ppb) based on hyperplasia in respiratory epithelium in female rats. Based on chemical reactivity, the REL for chlorine dioxide might be expected to be lower than that for chlorine. However, there are much less toxicologic data available for chlorine dioxide than for chlorine.

VII. Data Strengths and Limitations for Development of the REL

The REL for chlorine dioxide had uncertainties in all areas of concern. Thus the best available study was still limited by lack of multiple exposure concentrations, by the relatively short duration of exposures, and by the small number of animals examined. Adequate human health effects information is lacking, although it appears likely that the proposed REL would be protective of the effects reported in the single limited human study available. Other limitations were the lack of dose-response information and the lack of comprehensive data on multi-organ effects.

VIII. References

CARB. 2000. California Air Resources Board. California Emissions Inventory Development and Reporting System. (CEIDARS). Data from Data Base Year 1998. February 12, 2000.

CRC. 1994. CRC Handbook of Chemistry and Physics, 75th edition. Lide DR, ed. Boca Raton, FL: CRC Press Inc.

Dalhamn T. 1957. Chlorine dioxide: Toxicity in experimental animals and industrial risks. AMA Arch. Ind. Hyg. 15:101-107.

Elkins HB. 1959. The Chemistry of Industrial Toxicology, 2nd ed. New York: Wiley and Sons, Inc. pp.89-90.

Ferris BG Jr, Burgess WA, and Worcester J. 1967. Prevalence of chronic respiratory disease in a pulp mill and a paper mill in the United States. Br. J. Ind. Med. 24(1):26-37.

Gloemme J, and Lundgren KD. 1957. Health hazards from chlorine dioxide. Arch. Ind. Health. 16: 169-176.

HSDB. 1994. Hazardous Substances Data Bank. TOMES®. Vol. 20. Denver, CO: Micromedex, Inc.

OEHHA. 2000. Office of Environmental Health Hazard Assessment. Air Toxics Hot Spots Program Risk Assessment Guidelines. Part III. Technical Support Document for the Determination of Noncancer Chronic Reference Exposure Levels. Available on-line at http://www.oehha.ca.gov

Paulet G, and Debrousses S. 1970. [On the action of ClO_2 at low concentrations on laboratory animals.] Arch. Mal. Prof. Med. Trav. Secur. Soc. 31(3):97-106. (in French)

Paulet G, and Debrousses S. 1972. [On the toxicology of chlorine dioxide.] Arch. Mal. Prof. Med. Trav. Secur. Soc. 33(1-2):59-61. (in French)

Paulet G, and Debrousses S. 1974. [Action of a discontinuous exposure to chlorine dioxide on the rat.] Arch. Mal. Prof. Med. Trav. Secur. Soc. 35(9):797-803. (in French)

U.S. EPA. 1994. U.S. Environmental Protection Agency. Integrated Risk Information System (IRIS), CD-ROM version. Denver, CO: Micromedex, Inc. (Edition expires 10/31/94).

CHRONIC TOXICITY SUMMARY

CHLOROBENZENE

(monochlorobenzene; benzene chloride; benzene monochloride; chlorbenzene; chlorbenzol; phenyl chloride)

CAS Registry Number: 108-90-7

I. Chronic Toxicity Summary

Inhalation reference exposure level	**1000 μg/m^3** (300 ppb)
Critical effect(s)	Increased liver weights, hepatocellular hypertrophy, renal degeneration and inflammation, and testicular degeneration in rats
Hazard index target(s)	Alimentary system; kidney; reproductive system

II. Physical and Chemical Properties Summary (HSDB, 1995; CRC, 1994)

Description	Colorless, neutral liquid
Molecular formula	C_6H_5Cl
Molecular weight	112.56 g/mol
Boiling point	132°C
Melting point	–45.2^{o}C
Vapor pressure	11.8 torr at 25°C
Solubility	Practically insoluble in water (0.049 g/100 ml); soluble in alcohol, benzene, chloroform, diethyl ether
Conversion factor	1 ppm = 4.60 mg/m^3 at 25 ^{o}C

III. Major Uses and Sources

As one of the most widely used chlorinated benzenes, mono-chlorobenzene has been a major chemical for at least 50 years. It was historically important in the manufacture of chlorinated pesticides, especially DDT, and in the production of phenol and aniline. Monochlorobenzene's principal current use is as a chemical intermediate in the production of chemicals such as nitrochlorobenzenes and diphenyl oxide. These chemicals are subsequently used in the production of herbicides, dyestuffs, and rubber chemicals. Additionally, monochlorobenzene is used as a solvent in degreasing processes (e.g., in metal cleaning operations), paints, adhesives, waxes and polishes (HSDB, 1995; NIOSH, 1993). The annual statewide industrial emissions from facilities reporting under the Air Toxics Hot Spots Act in California based on the most recent inventory were estimated to be 29,451 pounds of chlorobenzene (CARB, 2000).

IV. Effects of Human Exposure

Even though monochlorobenzene has been used industrially for many years, few epidemiologic and/or occupational studies have addressed the potential health status of workers chronically exposed to monochlorobenzene (NIOSH, 1993). A Russian occupational study (Rozenbaum *et al.*, 1947, as reported by the U.S. EPA, 1988) describes multiple central nervous system effects, including headache, numbness, dizziness, cyanosis, hyperesthesia, and muscle spasms, after intermittent exposure over 2 years to monochlorobenzene in a mixed chemical environment. No specific exposure levels or histopathologic data were reported.

Two small studies utilizing volunteers exposed to single doses of monochlorobenzene have reported central nervous system effects (Ogata *et al.*, 1991; Tarkhova, 1965). An exposure chamber study of five volunteers exposed up to 60 ppm monochlorobenzene (276 mg/m^3) for a single 7 hour exposure described acute subjective symptoms such as drowsiness, headache, eye irritation, and sore throat (Ogata *et al.*, 1991). One other human volunteer study described altered electrical activity of the cerebral cortex in four individuals exposed to 43.4 ppm monochlorobenzene vapors for 2.5 minutes (Tarkhova, 1965).

V. Effects of Animal Exposure

No chronic inhalation studies have evaluated the toxicity of monochlorobenzene. Only a single, oral chronic carcinogenicity study (NTP, 1985) has evaluated the long-term adverse affects of monochlorobenzene administration. However, a few subchronic inhalation studies have demonstrated adverse effects on the liver, the kidney, and, to a lesser extent, blood parameters following monochlorobenzene exposure over a period of weeks or months (Dilley, 1977; John *et al.*, 1984; Nair *et al.*, 1987).

One subchronic study evaluated Sprague-Dawley male rats and rabbits exposed to 0, 75, or 200 ppm of monochlorobenzene for 7 hr/day, 5 days/week, for up to 24 weeks (Dilley, 1977). In rats, monochlorobenzene-related toxicity included increased absolute and relative (to brain- or body-weight) organ weights (especially the liver) after 11 and 24 weeks of exposure (LOAEL 75 ppm). Male rabbits also demonstrated increases in liver weight after 24 weeks of exposure (LOAEL = 75 ppm). Some hematological changes were reported in rats including differences in platelet and reticulocyte counts between control and exposed animals; however, some changes observed at 11 weeks were variable and comparable to controls at 24 weeks (red blood cell count, hemoglobin, hematocrit, and white blood cell count). Pathological changes were observed in rats, with occasional focal lesions in the adrenal cortex, tubular lesions in the kidneys, and congestion in the liver and kidneys.

Two other subchronic inhalation studies reported adverse organ effects following monochlorobenzene exposure in rats and rabbits (John *et al.*, 1984; Nair *et al.*, 1987). In the first study, John *et al.* (1984) reported increased liver weights in rats and rabbits following short-term (10 or 13 day, 6 hours/day) monochlorobenzene exposure (LOAEL = 590 ppm in rats and 210 ppm in rabbits). Nair *et al.* (1987) exposed male and female Sprague-Dawley rats to 0, 50, 150, or 450 ppm monochlorobenzene vapors daily for 6 hours over 10-11 weeks prior to mating, and

up to day 20 of gestation for 2 generations. Nair *et al*. found dose-related changes in the livers, kidneys, and testes in both generations of males (F_0 and F_1). Hepatotoxicity occurred as hepatocellular hypertrophy and increased liver weights (mean and absolute) at concentrations greater than 50 ppm (LOAEL = 150 ppm). At this concentration (150 ppm), renal changes included tubular dilation, interstitial nephritis, and foci of regenerative epithelium. Testicular degeneration of the germinal epithelium occurred in both generations of exposed males, but no chlorobenzene-induced adverse effects on reproductive performance or fertility were seen.

VI. Derivation of Chronic Reference Exposure Level (REL)

Study	Nair *et al.* (1987)
Study population	Sprague-Dawley rats (30/sex/group)
Exposure method	Discontinuous inhalation exposures (0, 50, 150, and 450 ppm)
Critical Effects	Increases in absolute and relative liver weights (F_0 and F_1 both sexes), hepatocellular hypertrophy (F_0 and F_1 males), renal degeneration and inflammation (F_0 and F_1 both sexes), testicular degeneration (F_0 and F_1 males).
LOAEL	150 ppm
NOAEL	50 ppm
Exposure continuity	6 hours/day, 7 days/week
Exposure duration	11 weeks
Average experimental exposure	13 ppm for NOAEL group (50 x 6/24)
Human equivalent concentration	26 ppm (gas with systemic effects, based on RGDR = 2.0 for lambda (a) : lambda (h)) (Gargas *et al*., 1989)
LOAEL uncertainty factor	1
Subchronic uncertainty factor	3
Interspecies uncertainty factor	3
Intraspecies uncertainty factor	10
Cumulative uncertainty factor	100
Inhalation reference exposure level	0.3 ppm (300 ppb; 1.0 mg/m^3, 1000 µg/m^3)

Of the three inhalation studies available (Dilley, 1977; John *et al.*, 1984; Nair *et al.*, 1987), the Nair *et al.* (1987) two generational developmental study was selected for identifying a NOAEL and LOAEL. It best presented the histopathology of the adverse effects, and demonstrated a dose response relationship for these effects (statistically significant increases in mean liver weights, incidence of renal changes, and testicular degeneration).

Another subchronic inhalation study (Dilley, 1977) also observed increases in organ weights, including the liver, in rats after 11 and 24 weeks exposure to 75 and 250 ppm monochlorobenzene (LOAEL = 75 ppm), and in rabbits at 24 weeks. Similar adverse liver and kidney effects were found in subchronic oral bioassays (Kluwe *et al.*, 1985; NTP, 1985). These

include increases in liver weight and hepatocellular degeneration in rats (LOAEL = 125 mg/kg/day) and mice (LOAEL = 250 mg/kg/day), and renal necrosis and degeneration in rats (LOAEL = 500 mg/kg/day) and mice (LOAEL = 250 mg/kg/day) after 13 weeks oral exposure to chlorobenzene.

Uncertainty factors are appropriate due to the lack of chronic studies, both animal bioassay and human, and the limited number of subchronic inhalation studies, thereby requiring estimation of the chronic REL from this shorter term, single species study. The magnitude of interspecies variation remains unknown, as few species have been tested and human data for comparison are lacking. However, metabolic studies have demonstrated species variation in the urinary elimination of chlorobenzene metabolites (Ogata and Shimada 1983; Ogata *et al.*, 1991; Yoshida *et al.*, 1986). Humans metabolize and excrete chlorobenzene predominately as free and conjugated forms of 4-chlorocatechol and chlorophenols, while the main rodent urinary metabolite, p-chlorophenylmercapturic acid, is found in minor amounts (<0.5%). No information exists which identifies human subpopulations possibly susceptible to monochlorobenzene exposure.

For comparison with the proposed REL, a REL can be derived from the 24 week LOAEL of 75 ppm for liver effects (Dilley, 1977). The LOAEL is equivalent to a continuous exposure LOAEL of 15.6 ppm. Multiplying by the RGDR of 2 and dividing by a cumulative UF of 100 (3 for LOAEL, 3 for interspecies and 10 for intraspecies) also yields an estimate of 300 ppb.

VII. Data Strengths and Limitations for Development of the REL

The strengths of the inhalation REL for chlorobenzene include the observation of a NOAEL, the availability of subchronic inhalation exposure data from a well-conducted study with histopathological analysis, and the demonstration of a dose-response relationship. Major areas of uncertainty are the lack of adequate human exposure data and limited reproductive toxicity data.

VIII. References

CARB. 2000. California Air Resources Board. California Emissions Inventory Development and Reporting System. (CEIDARS). Data from Data Base Year 1998. February 12, 2000.

CRC. 1994. CRC Handbook of Chemistry and Physics, 75th edition. Lide DR, ed. Boca Raton, FL: CRC Press Inc.

Dilley JV. 1977. Final Report: Toxic evaluation of inhaled chlorobenzene (monochlorobenzene). Stanford Research Institute. Contract No. 210-76-0126. Prepared for: National Institute of Occupational Safety and Health, Division of Biomedical and Behavioral Sciences. Cincinnati, OH: NIOSH. NTIS No. PB-276623.

Gargas ML, Burgess RJ, Voisard DE, Cason GH, and Andersen ME. 1989. Partition coefficients of low-molecular-weight volatile chemicals in various liquids and tissues. Toxicol. Appl. Pharmacol. 98(1):87-99.

HSDB. 1995. Hazardous Substances Data Bank. TOMES® Denver, CO: Micromedex, Inc.

John JA, Hayes WC, Hanley TR, Johnson KA, Gushow TS, and Rao KS. 1984. Inhalation teratology study on monochlorobenzene in rats and rabbits. Toxicol. Appl. Pharmacol. 76:365-373.

Kluwe WM, Dill G, Persing R, and Peters A. 1985. Toxic responses to acute, subchronic, and chronic oral administrations of monochlorobenzene to rodents. J. Toxicol. Environ. Health 15(6):745-767.

Nair RS, Barter JA, Schroeder RE, Knezevich A, and Stack CR. 1987. A two-generation reproduction study with monochlorobenzene vapor in rats. Fundam. Appl. Toxicol. 9:678-686.

NTP. 1985. National Toxicology Program. Toxicology and carcinogenesis studies of chlorobenzenes (CAS No. 108-90-7) in F344/N rats and B6C3F1 mice (gavage studies). U.S. Dept. of Health and Human Services, Public Health Service, National Institutes of Health, NTP TR 261. NIH Pub. No. 86-2517, NTIS PB86-144714.

NIOSH. 1993. National Institute for Occupational Safety and Health. NIOH and NIOSH basis for an occupational health standard: Chlorobenzene. U.S. Department of Health and Human Services, Public Health Service, DHHS(NIOSH) Publication No. 93-102.

Ogata M, and Shimada Y. 1983. Differences in urinary monochlorobenzene metabolites between rats and humans. Int. Arch Occup. Environ. Health 53:51-57.

Ogata M, Taguchi T, Hirota N, Shimada Y, and Nakae S. 1991. Quantitation of urinary chlorobenzene metabolites by HPLC: Concentrations of 4-chlorocatechol and chlorophenols in urine and of chlorobenzene in biological specimens of subjects exposed to chlorobenzene. Int. Arch. Occup. Environ. Health 63:121-128.

Rozenbaum ND, Blekh RS, and Kremneva SN. 1947. [Use of chlorobenzene as a solvent from the standpoint of industrial hygiene.] Gig. I Sanit. 12:21-24. (Russian) [as cited in U.S. EPA, 1988.]

Tarkhova LP. 1965. Materials for determining the maximum permissible concentration of chlorobenzol in atmospheric air. Hygiene and Sanitation 30:327-333. (Jerusalem, Israel Program for Scientific Translation available for NTIS.)

U.S. EPA. 1988. United States Environmental Protection Agency. Health effects criteria document for chlorobenzene. Final draft. EPA 600/88-90/99. Washington DC: U.S. EPA. National Technical Information Service, PB89-192116.

Yoshida M, Sunaga M, and Hara I. 1986. Urinary metabolites levels in workers exposed to chlorobenzene. Ind. Health 24:255-258.

CHRONIC TOXICITY SUMMARY

CHLOROFORM

(trichloromethane; formyl trichloride; methenyl trichloride; methyl trichloride)

CAS Registry Number: 67-66-3

I. Chronic Toxicity Summary

Inhalation reference exposure level	**300 µg/m^3 (50 ppb)**
Critical effect(s)	Liver toxicity (degenerative, foamy vacuolization, and necrosis) in rats; increased liver weights in male rats Kidney toxicity (cloudy swelling and nephritis) in rats Developmental toxicity
Hazard index target(s)	Alimentary system; kidney; teratogenicity

II. Chemical Property Summary (HSDB, 1995; 1999; CRC, 1994)

Description	Colorless liquid
Molecular formula	$CHCl_3$
Molecular weight	119.49 g/mol
Boiling point	61.1^{o}C
Melting point	–63.6 ^{o}C
Vapor pressure	197-200 torr @ 25 ^{o}C
Solubility	Soluble in water (8220 mg/L); miscible in carbon tetrachloride, carbon disulfide, alcohols, benzene, ethers and oils
Conversion factor	4.9 µg/m^3 per ppb at 25ºC

III. Major Uses and Sources

Chloroform ($CHCl_3$) is used in industry and laboratory settings as a solvent for adhesives, pesticides, fats, oils and rubbers. It is also used as a chemical intermediate in the synthesis of fluorocarbon 22, dyes, pesticides, and tribromomethane. Chloroform is produced as a byproduct of water, sewage, and wood pulp chlorination (HSDB, 1995). In 1996, the latest year tabulated, the statewide mean outdoor monitored concentration of chloroform was approximately 0.037 ppb (CARB 1999a). The annual statewide industrial emissions from facilities reporting under the Air Toxics Hot Spots Act in California based on the most recent inventory were estimated to be 79,949 pounds of chloroform (CARB 1999b).

IV. Effects of Human Exposure

Limited information is available regarding possible adverse health effects in humans following chronic inhalation of chloroform. However, historical clinical reports from patients who underwent chloroform anesthesia indicate that acute inhalation exposure affects the central nervous system, cardiovascular system, stomach, liver, and kidneys (Schroeder, 1965; Smith *et al.*, 1973; Whitaker and Jones, 1965). Acute chloroform toxicity included impaired liver function (Smith *et al.*, 1973), toxic hepatitis (Lunt, 1953; Schroeder, 1965), cardiac arrhythmia (Payne, 1981; Schroeder, 1965; Whitaker and Jones, 1965), and nausea (Schroeder, 1965; Smith *et al.*, 1973; Whitaker and Jones, 1965), and caused central nervous system symptoms (Schroeder, 1965; Whitaker and Jones, 1965). Chronic inhalation studies are limited to a few occupational studies identifying the liver and the central nervous system as target organs (Challen *et al.*, 1958; Li *et al.*, 1993; Phoon *et al.*, 1983; Bomski *et al.*, 1967).

Challen *et al.* (1958) investigated workers manufacturing throat lozenges with exposure to chloroform vapors estimated in the range 77 to 237 ppm with episodes of >1100 ppm. Workers reported symptoms of fatigue, dull-wittedness, depression, gastrointestinal distress, and frequent and burning micturition. No evidence of liver dysfunction was found based on thymol turbidity, serum bilirubin, and urine urobilinogen levels.

Bomski *et al.* (1967) reported 17 cases of hepatomegaly in a group of 68 chloroform-exposed workers. Chloroform concentrations ranged from 2 to 205 ppm (duration 1 to 4 years). Three of the 17 workers with hepatomegaly had toxic hepatitis based on elevated serum enzymes. Additionally, 10 workers had splenomegaly. Workers exposed to chloroform had a 10-fold increased risk of contracting viral hepatitis compared to the general population. The study authors considered the chloroform induced liver toxicity as a predisposing factor for viral hepatitis, but the incidence of viral hepatitis in the workers is in itself a confounding factor.

Phoon *et al.* (1983) described two outbreaks of toxic jaundice in workers manufacturing electronics equipment in Singapore. One plant had 13 cases of jaundice, initially diagnosed as viral hepatitis, in a work area with >400 ppm chloroform. Blood samples from workers (five with jaundice, four without symptoms) contained between 0.10 and 0.29 mg chloroform/100 mL. A second factory reported 18 cases of hepatitis, all from a work area utilizing chloroform as an adhesive. Two samplings indicated air levels of 14.4 to 50.4 ppm chloroform. Due to a lack of fever and hepatitis B surface antigen in the patients, the authors attributed the jaundice to chloroform exposure rather than viral hepatitis.

More recently, Li et al.(1993) reported on 61 chloroform exposed workers from a variety of production factories. Exposure levels at 3 representative worksites varied widely, from 4.27 to 147.91 mg/m3 (0.9 to 30 ppm) (119 samples), with 45% of the samples below 20 mg/m3. The exposed workers were subclassified for some studies according to exposure levels into group 1 (mean level = 13.49 mg/m3 or 2.8 ppm) and group 2 (mean level = 29.51 mg/m3 or 6 ppm). Workers exposed to chloroform had slight liver damage indicated by higher (abnormal) levels of serum prealbumin (in group 2) and transferrin (in both groups) than those of control workers. Neurobehavioral functions were also affected, manifested as increases in scores of passive mood states and dose-related, negative changes in neurobehavioral testing.

These cross sectional studies are limited in their ability to establish chronic NOAEL/LOAEL values due to limited exposures, concurrent exposure to other chemicals, inadequate control groups and potential confounders. However, these studies indicate the potential for liver and central nervous system toxicity in humans exposed to chloroform via inhalation.

V. Effects of Animal Exposure

Exposure of experimental animals to chloroform for acute, subchronic or chronic durations results in toxicity to the liver and kidney, as well as to the respiratory and central nervous systems (USDHHS, 1993). The majority of chronic animal studies have used oral routes of chloroform administration (USDHHS, 1993), while only limited data are available on inhalation specific exposures. Both routes of exposure, however, appear to primarily affect the liver and kidney (Chu *et al.*, 1982; Heywood *et al.*, 1979; Jorgenson *et al.*, 1985; Miklashevshii *et al.*, 1966; Munson *et al.*, 1982; Roe *et al.*, 1979; Larson *et al*., 1996; Templin *et al*., 1996; Torkelson *et al.*, 1976).

Larson *et al*. (1996) exposed female and male B6C3F1 mice to atmospheric concentrations of 0, 0.3, 2, 10, 30, and 90 ppm chloroform 6 hr/day, 7 days/week for exposure periods of 4 days or of 3, 6, or 13 consecutive weeks. Additional exposure groups were exposed for 5 days/week for 13 weeks or for 5 days/week for 6 weeks and then examined at 13 weeks. Complete necropsy and microscopic evaluation revealed that chloroform treatment induced dose- and time-dependent lesions only in the livers and nasal passage of the female and male mice and in the kidneys of the male mice. Large increases in the liver cell labeling index were seen in the 90-ppm groups at all time points. The female mice were most sensitive. The no-observed-adverse-effect level (NOAEL) for induced hepatic cell proliferation was 10 ppm. The hepatic labeling indices in the 5 days/week groups were about half of those seen in the 7 days/week groups and returned to the normal baseline in the 6-week recovery groups. The NOAEL for increased liver weight (normalized to body weight) was 10 ppm in male mice. Histologic changes and regenerative cell proliferation were induced in the kidneys of male mice at 30 and 90 ppm with 7 days/week exposures and also at 10 ppm with the 5 days/week regimen. Nasal lesions were transient and occurred only in mice exposed to 10, 30, or 90 ppm for 4 days.

Templin *et al*. (1996) exposed male and female F-344 rats to airborne concentrations of 0, 2, 10, 30, 90, or 300 ppm chloroform 6 hr/day, 7 days/week for 4 days or 3, 6, or 13 weeks. Additional groups were exposed 5 days/week for 13 weeks, or 5 days/week for 6 weeks and held until Week 13. A “full-screen” necropsy identified the kidney, liver, and nasal passages as the only target organs. The primary target in the kidney was the epithelial cells of the proximal tubules of the cortex; significantly elevated increases in the cell labeling index were observed at concentrations of 30 ppm chloroform and above. However, only a marginal increase in the renal cell labeling index in the males was seen after exposures of 90 ppm, 5 days/week. Chloroform induced hepatic lesions in the midzonal and centrilobular regions with increases in the labeling index throughout the liver, but only at 300 ppm, an extremely toxic level. An additional liver lesion seen only at 300 ppm was numerous intestinal crypt-like ducts surrounded by dense connective tissue. Enhanced bone growth and hypercellularity in the lamina propria of the ethmoid turbinates of the nose occurred at the early time points at concentrations of 10 ppm and above.

At 90 days there was a generalized atrophy of the ethmoid turbinates at concentrations of 2 ppm (the lowest concentration tested) and above.

Torkelson and associates (1976) exposed rats (12/sex/group), rabbits (2-3/sex/group), and guinea pigs (8-12/sex/group) for 7 hours/day, 5 days/week over 6 months to 0, 25, 50 or 85 ppm chloroform vapor. Dogs were exposed to 25 ppm chloroform, for 7 hours/day, 5 days/week for 6 months. Dose and species-dependent pathological changes in the liver included mild to severe centrilobular granular degeneration, foamy vacuolization, focal necrosis, and fibrosis in both sexes of all species tested. Guinea pigs were the least sensitive and male rats the most sensitive to chloroform induced hepatotoxicity; the above adverse effects occurred at 25 ppm. Adverse kidney effects observed in all species included cloudy swelling of the renal tubular epithelium and interstitial and tubular nephritis. Pneumonitis was observed in the high (85 ppm) exposure groups of male rats, female guinea pigs, and male rabbits, and in the lower dose group of female rabbits (25 ppm). Clinical and blood parameters were also examined in rats and rabbits, but no alterations were attributable to chloroform exposure.

Effects on average body weight, and relative liver and kidney weights of rats due to chloroform exposure 7 hours/day for 6 months (Torkelson *et al*., 1976)

Sex	Parameter	Unexposed control	Air control	25 ppm	50 ppm	85 ppm
male	survival	11/12	10/12		9/10	6/10
	avg. bw	343	356		305*	316
	liver	2.45	2.52		2.48	2.76*
	kidney	0.69	0.70		0.81*	0.84*
male	survival	8/12	12/12	9/12		
	avg. bw	319	347	335		
	liver	2.67	2.41	2.65		
	kidney	0.75	0.70	0.83*		
female	survival	10/12	9/12		10/10	10/10
	avg. bw	202	223		203	206
	liver	2.92	2.99		3.00	3.12
	kidney	0.82	0.81		0.95	1.06
Female	survival	10/12	12/12	12/12		
	avg. bw	211	202	194		
	liver	3.02	2.93	3.08		
	kidney	0.83	0.84	0.94*		

* $p < 0.05$

VI. Derivation of Chronic Reference Exposure Level (REL)

Study	Torkelson *et al.*(1976)
Study population	Rats, unspecified strain (12/sex/group)
Exposure method	Discontinuous whole-body inhalation exposures (0, 25, 50, 85 ppm)
Critical effects	Pathological changes in liver (degenerative), and kidneys (cloudy swelling)
LOAEL	25 ppm
NOAEL	Not observed
Exposure continuity	7 hr/day for 5 days/week for 6 months
Average experimental exposure	5.3 ppm for LOAEL group (25 x 7/24 x 5/7)
Human equivalent concentration	15.9 ppm for LOAEL group (gas with systemic effects, based on RGDR = 3.0 for lambda (a) : lambda (h) (Gargas *et al.*, 1989))
Exposure duration	6 months
LOAEL uncertainty factor	10
Subchronic uncertainty factor	1
Interspecies uncertainty factor	3
Intraspecies uncertainty factor	10
Cumulative uncertainty factor	300
Inhalation reference exposure level	0.05 ppm (50 ppb; 0.30 mg/m^3; 300 µg/m^3)

In the study of Torkelson and associates (1976) rats were the most sensitive species and guinea pigs the least sensitive to chloroform vapors. Though of subchronic duration, this inhalation study still exposed rats discontinuously for 25% of a lifetime (25.8 weeks/104 weeks/lifetime). Pathological changes were observed in both sexes of rat at 50 and 85 ppm (244 or 415 mg/m^3) and in male rats at 25 ppm (122 mg/m^3) chloroform. These hepatic changes included mild to severe centrilobular granular degeneration, foamy vacuolization, focal necrosis, and fibrosis. Adverse effects in the kidney including cloudy swelling and nephritis were seen in all species tested at 25 ppm (122 mg/m^3) chloroform.

An unexpected finding in animals was the generalized atrophy of the ethmoid turbinates of F344 rats after a 90 day exposure at concentrations of 2 ppm chloroform and above (Templin *et al*., 1996). Nasal lesions have also been reported in F344 rats given chloroform by gavage (Larson *et al*., 1995). This severe and extensive chloroform-induced olfactory mucosal degeneration in rats is not associated with detectable olfactory deficit (Dorman et al., 1997). As the basis of the REL we have used the more usual chloroform organ targets of liver and kidney. However, confirmation of nasal effects in other rat strains and other species may require reassessing the basis of the REL for chloroform.

The human occupational studies have reported jaundice with or without alterations in liver enzymes at similar ambient concentrations: 2 to 204 ppm chloroform (10 to 995 mg/m^3) after at least 1 year (Bomski *et al.*, 1967) and 14 to 400 ppm chloroform (68 to 1952 mg/m^3) after 6 months or less (Phoon *et al.*, 1983). The presence of jaundice and hepatitis in these 2 reports

made them questionable for use in developing a REL. In the Li *et al*. (1993) study the workers were exposed for an average of 7.8 years (range = 1-15 years) and the air concentrations ranged from 4.27 to 141.25 mg/m^3 with a geometric average of 20.46 mg/m^3. The exposed workers were subdivided into higher (n=46) and lower (n=14) exposures, but the separation was not indicated for all results. If the lower exposure level of 2.8 ppm (13.49 mg/m^3) is classified as a mild LOAEL based on a significant difference from controls in one type of neurobehavioral test, the exposure level can be time adjusted to an equivalent continuous exposure of 1 ppm, then divided by a LOAEL UF of 3 and an intraspecies UF of 10 to yield a REL of 30 ppb, in good agreement with the proposed REL of 50 ppb (300 µg/m^3) based on animals (rats).

Chloroform is metabolized by the cytochrome P-450 dependent mixed function oxidase system, primarily in the liver, the respiratory epithelium, and the kidney. In the rat liver and kidneys, chloroform is metabolized to phosgene (Pohl *et al.*, 1984). The hepatotoxicity and nephrotoxicity of chloroform is thought to be due largely to phosgene (Bailie *et al.*, 1984). Individuals with concurrent exposure to certain chemical inducers of liver cytochrome P450 activity, including barbiturates, may be at potentially greater risk of chloroform toxicity (Cornish *et al.*, 1973). Others with possible higher sensitivity to chloroform include persons with underlying liver, kidney or neurological conditions.

VII. Data Strengths and Limitations for Development of the REL

Strengths of the chronic REL for chloroform derive from the critical effect being found in the liver, a well-established site of chloroform toxicity. Limitations in the data include the lack of a NOAEL in the key study, the less than lifetime duration of the key study, and the limited number of chronic inhalation studies available.

VIII. References

Bailie MB, Smith JH, Newton JF, and Hook JB. 1984. Mechanism of chloroform nephrotoxicity. IV. Phenobarbital potentiation of in vitro chloroform metabolism and toxicity in rabbit kidneys. Toxicol. Appl. Pharmacol. 74:285-292.

Bomski H, Sobolewska A, and Strakowski A. 1967. [Toxic damage to the livers of chemical plant workers by chloroform.] Int. Arch. Gewerbepathol. Gewerbehyg. 24:127-134.

CARB. 1999a. California Air Resources Board. Toxics Air Quality Data. Substance Chooser. Chloroform. Available online at http:/www.arb.ca.gov/aqd/toxics.htm

CARB. 1999b. Air toxics emissions data collected in the Air Toxics Hot Spots Program CEIDARS Database as of January 29, 1999.

Challen P, Hickish D, and Bedford J. 1958. Chronic chloroform intoxication. Br. J. Ind. Med. 15:243-249.

CRC. 1994. CRC Handbook of Chemistry and Physics, 75th edition. Lide DR, ed. Boca Raton, FL: CRC Press Inc.

Chu I, Villeneuve DC, Secours VE, Becking GC, and Valli VE. 1982. Toxicity of trihalomethanes: I. The acute and subacute toxicity of chloroform, bromodichloromethane, chlorodibromomethane and bromoform in rats. J. Environ. Sci. Health. B17:205-224.

Cornish HH, Ling B, and Barth M. 1973. Phenobarbital and organic solvent toxicity. Am. Ind. Hyg. Assoc. J. 34:487-492.

Dorman DC, Miller KL, D'Antonio A, James RA, Morgan KT. 1997. Chloroform-induced olfactory mucosal degeneration and osseous ethmoid hyperplasia are not associated with olfactory deficits in Fischer 344 rats.Toxicology 122(1-2):39-50.

Gargas ML, Burgess RJ, Voisard DE, Cason GH, and Andersen ME. 1989. Partition coefficients of low-molecular-weight volatile chemicals in various liquids and tissues. Toxicol. Appl. Pharmacol. 98(1):87-99

Heywood R, Sortwell RJ, Noel PRB, Street AE, Prentice DE, Roe FJC, Wadsworth PF, and Worden AN. 1979. Safety evaluation of toothpaste containing chloroform. III. Long-term study in beagle dogs. J. Environ. Pathol. Toxicol. 2:835-851.

HSDB. 1995. Hazardous Substances Data Bank. National Library of Medicine, Bethesda, Maryland (TOMES® CD-ROM Version). Denver, CO: Micromedex Inc.

Jorgenson TA, Meierhenry EF, Rushbrook CJ, Bull RJ, and Robinson M. 1985. Carcinogenicity of chloroform in drinking water to male Osborne-Mendel rats and female B6C3F1 mice. Fundam. Appl. Toxicol. 5:760-769.

Larson JL, Templin MV, Wolf DC, Jamison KC, Leininger JR, Mery S, Morgan KT, Wong BA, Conolly RB, Butterworth BE. 1996. A 90-day chloroform inhalation study in female and male B6C3F1 mice: implications for cancer risk assessment. Fundam. Appl. Toxicol. 30(1):118-137.

Larson JL, Wolf DC, Mery S, Morgan KT, Butterworth BE. 1995. Toxicity and cell proliferation in the liver, kidneys and nasal passages of female F-344 rats, induced by chloroform administered by gavage. Food Chem. Toxicol. 33(6):443-456.

Li LH, Jiang XZ, Laing YX, Chen ZQ, Zhou YF, and Wang YL. 1993. Studies on the toxicity and maximum allowable concentration of chloroform. Biomed. Environ. Sci. 6(2):179-186.

Lunt RL. 1953. Delayed chloroform poisoning in obstetric practice. Br. Med. J. 1:489-490.

Miklashevshii VE, Tugarinova VN, Rakhmanina NL, and Yakovleva GP. 1966. Toxicity of chloroform administered perorally. Hyg. Sanit. 31:320-323.

Munson AE, Sain LE, Sanders VM, Kauffmann BM, White KL, Page G, Barnes DW, and Borzelleca JF. 1982. Toxicology of organic drinking water contaminants: trichloromethane,

bromodichloromethane, dibromochloro-methane and tribromomethane. Environ. Health Perspect. 46:117-126.

Payne JP. 1981. Chloroform in clinical anaesthesis. Br. J. Anesth. 53:11s-15s.

Phoon W, Goh K, Lee L, Tan K, and Kwok S. 1983. Toxic jaundice from occupational exposure to chloroform. Med. J. Malaysia. 38(1):31-34.

Pohl L, Gorge J, and Satoh H. 1984. Strain and sex differences in chloroform-induced nephrotoxicity. Different rates of metabolism of chloroform to phosgene by the mouse kidney. Drug Metab. Disp. 12(3):304-3-8.

Roe FJC, Palmer AK, Worden AN, and Van Abbe NJ. 1979. Safety evaluation of toothpaste containing chloroform. I. Long-term studies in mice. J. Environ. Pathol. Toxicol. 2:799-819.

Schroeder HG. 1965. Acute and delayed chloroform poisoning. Br. J. Anaesth. 37:972-975.

Smith AA, Volpitto PP, Gramling ZW, DeVore MB, and Glassman AB. 1973. Chloroform, halothane, and regional anesthesia: A comparative study. Anesth. Analg. 52:1-11.

Templin MV, Larson JL, Butterworth BE, Jamison KC, Leininger JR, Mery S, Morgan KT, Wong BA, Wolf DC. 1996. A 90-day chloroform inhalation study in F-344 rats: profile of toxicity and relevance to cancer studies. Fundam. Appl. Toxicol. 32(1):109-125.

Torkelson T, Oyen F, and Rowe V. 1976. The toxicity of chloroform as determined by single and repeated exposure of laboratory animals. Am. Ind. Hyg. Assoc. J. 37:697-705.

USDHHS. 1993. Toxicology Profile for Chloroform. U.S. Department of Health and Human Services. Public Health Service. Agency for Toxic Substance and Disease Registry. April 1993.

Whitaker AM, and Jones CS. 1965. Report of chloroform anesthetics administered with a precision vaporizer. Anesth. Analg. 44:60-65.

CHRONIC TOXICITY SUMMARY

CHLOROPICRIN

(trichloronitromethane; nitrochloroform; nitrochloromethane)

CAS Registry Number: 76-06-2

I. Chronic Toxicity Summary

Inhalation reference exposure level	**0.4 µg/m^3** (0.05 ppb)
Critical effect(s)	Nasal rhinitis and bronchiectasis in mice
Hazard index target(s)	Respiratory system

II. Chemical Property Summary (from HSDB (1996) except as noted)

Description	Colorless to faint yellow liquid
Molecular formula	CCl_3NO_2
Molecular weight	164.4 g/mol
Boiling point	112°C
Melting point	–64°C (CRC, 1994)
Vapor pressure	5.7 torr @ 0°C (Fries and West, 1921); 3.2 kPa (24 torr) @ 25°C (Tomlin, 1994)
Solubility	1.6 g/L water @ 25°C; 2.272 g/L water @ 0°C 1.9 g/L water @ 20°C; miscible with benzene, ethanol, carbon disulfide, ether, carbon tetrachloride, acetone, methanol, acetic acid
Conversion factor	6.72 µg/m^3 per ppb at 25ºC

III. Major Uses and Sources

Chloropicrin is used primarily as a preplant soil fumigant against insects and fungi; it also kills weed and grass seeds when applied to soil. Chloropicrin is occasionally used as a fumigant in grain elevators and storage bins (HSDB, 1996). Chloropicrin is used as an indicator chemical in other fumigants such as methyl bromide because of its potent irritant properties. Chloropicrin was used in World War I as a chemical warfare agent because of its potent activity as a lachrymator. Chloropicrin has a minor use in the chemical synthesis of methyl violet. Chloropicrin can also form in drinking water as a result of chlorination processes (Duguet *et al.*, 1985; Merlet *et al.*, 1985). The annual statewide industrial emissions from facilities reporting under the Air Toxics Hot Spots Act in California based on the most recent inventory were estimated to be 1507 pounds of chloropicrin (CARB, 2000). This does not include emissions from its major use as a preplant soil fumigant, either alone or in combination with other

fumigants, because agricultural field applications are not covered under the Air Toxics Hot Spots program. Approximately 3,630,000 lbs. of chloropicrin were used in agriculture in California in 1999 (DPR, 2000).

IV. Effects of Human Exposure

No studies are available which describe toxic effects to humans from chronic exposure to chloropicrin. Human exposures to concentrations less than 1 ppm for very short periods of time are extremely irritating (ACGIH, 1992; Fries and West, 1921). The threshold of odor detection in humans is approximately 1 ppm (ACGIH, 1992).

V. Effects of Animal Exposure

Burleigh-Flayer and Benson (1995) conducted a chronic inhalation bioassay with CD rats (50-60 per sex per dose) exposed discontinuously to 0 (air), 0.1, 0.5, or 1.0 ppm 99.6% pure chloropicrin vapor 6 hours/day for 5 consecutive days/week over 107 weeks. Clinical signs (such as hypoactivity and decreased startle response) were increased in both sexes, primarily at 1.0 ppm. Increased mortality was noted in males at 0.5 and 1 ppm and in females at 1 ppm. Absolute and relative increased lung and liver weights and increased nasal rhinitis were reported in both sexes at the 1 ppm level. However, no effects were seen at 0.1 ppm. Thus this study yielded a NOAEL of 0.1 ppm (0.67 mg/m^3) for chronic non-cancer effects in rats.

Results from chronic inhalation of chloropicrin in rats (Burleigh-Flayer and Benson, 1995)

Chloropicrin	Lung wt., m	Lung wt., f	Rhinitis, m	Rhinitis, f	Mean survival, m
0	2.086 g	1.574 g	20/50	18/50	696 d
0.1 ppm	2.089 g	1.464 g	24/50	17/50	669 d
0.5 ppm	2.202 g	1.460 g	21/50	26/50	672 d*
1.0 ppm	2.448 g	1.633 g	35/50**	23/50	647 d**

*p<0.05; **p<0.01

A similar study in mice (Burleigh-Flayer *et al*., 1995) resulted in the same NOAEL. CD-1 mice (50/sex/dose) were exposed to chloropicrin (99.6% pure) vapor at 0 (air), 0.1, 0.5, or 1.0 ppm for 6 hours/day, 5 days/week for at least 78 weeks. Body weights and body weight gains were significantly decreased in both sexes at ≥ 0.5 ppm. Food consumption was decreased in males at 1.0 ppm and in females at ≥ 0.5 ppm. Absolute and relative lung weights were increased in a dose-related manner in both sexes at ≥ 0.5 ppm. Changes in pathology observed macroscopically in the 1.0 ppm males included increased numbers of lung nodules and increased numbers of kidney cysts. In females lung masses and kidney cysts were seen at 0.5 ppm. Microscopic pathology changes included increased nasal cavity lesions (including serous exudate, hyaline epithelial inclusions, rhinitis, olfactory and epithelial atrophy) and lung lesions (including alveolar protein deposits, alveolar histiocytosis, hemorrhage, peribronchiolar lymphocytic infiltrate, bronchiectasis, bronchial submucosal fibrosis, peribronchiolar smooth muscle hyperplasia), in addition to kidney cysts at ≥ 0.5 ppm (CDPR, 2000).

Results from chronic inhalation of chloropicrin in mice (Burleigh-Flayer *et al*., 1995)

Chloropicrin	Rhinitis, m	Rhinitis, f	Bronchiectasis, m	Bronchiectasis, f	
0	6/50	3/50	0/50	0/50	
0.1 ppm	7/50	6/50	3/50	5/50	
0.5 ppm	17/50**	18/50**	28/50**	28/50**	
1.0 ppm	35/50**	32/50**	41/50**	44/50**	

**p<0.01

Yoshida *et al*. (1987) exposed groups of 12 male Fischer 344 rats intermittently to 0, 0.37, 0.67, 1.58, or 2.93 ppm chloropicrin vapor 6 h/day, 5 days/week for 13 weeks. Mean body weights were reduced in the highest 2 exposure groups, and red blood cell count, hematocrit, and hemoglobin concentration were significantly increased in the 2.93 ppm group. The treatment-related histological lesions reported were degeneration and necrosis of the bronchial and bronchiolar epithelia at 2.93 ppm and hypertrophy of these epithelia at 1.58 ppm. Thus the primary target organ was the respiratory tract and the subchronic NOAEL was 0.67 ppm (4.5 mg/m^3). (Eyelid closure and decrease in motor activity were seen in all exposure groups only during exposure. No morphological changes were seen at 0.67 ppm, so the authors deemed the behavior changes minor and not toxicologically important.)

Male Swiss-Webster mice (group numbers ranging from 16-24) were exposed by inhalation to a single level of different sensory irritants including chloropicrin for 6 hours/day for 5 days; unexposed control groups had 8-10 mice (Buckley *et al.*, 1984). The exposure level for chloropicrin was 7.9 ppm, which approximated the level sufficient to cause a 50% decrease in respiratory rate in mice (RD_{50}) (Kane *et al.*, 1979). Half the exposed mice and half the control animals were terminated immediately after the exposures and the other half 72 hours after the last exposure. All were examined for respiratory tract lesions. Body weights of chloropicrin exposed animals were reduced 10-25% below controls, but increased to normal levels during the recovery period. Nasal exudate and distention of the abdomen were observed. "Moderate" lesions, characterized by exfoliation, erosion, ulceration, or necrosis, were observed in the respiratory and olfactory epithelium, and minimal inflammation and squamous metaplasia were observed in the respiratory epithelium alone. Moderate to severe damage to the lower respiratory tract was described as "fibrosing peribronchitis and peribronchiolitis". Exfoliation, hyperplasia, and squamous metaplasia were also noted.

Condie *et al.* (1994) conducted a study of the toxicity of chloropicrin by oral exposure in Sprague-Dawley rats. Ten and ninety-day studies were conducted by dosing animals daily with chloropicrin in vehicle (corn oil) at a volume of 1 ml/kg. Groups of 10 rats/sex/group were dosed with 0, 10, 20, 40, and 80 mg/kg for the 10-day study and with 0, 2, 8, and 32 mg/kg for the 90-day study. Parameters examined included mortality, body weight, food and water consumption, hematology, serum clinical chemistry, and gross pathology and histology of organs. Only the high-dose group and the control group animals from the 90-day study were examined histopathologically. In the 90-day study, 6 males and 2 females in the 32 mg/kg dose group and 1 male and 3 females in the 8 mg/kg dose group died before the scheduled termination time. The authors noted signs of pulmonary complications (inflammation and congestion) in the dead animals. Previously, the animals had shown signs of respiratory distress, including

wheezing and dyspnea. The deaths were considered to be exposure related and most likely due to aspiration of chloropicrin. Among the survivors, mean body weight, hemoglobin levels, and hematocrits were significantly reduced in males in the 32 mg/kg dose group. Absolute thymus weights were reduced in female rats at 32 mg/kg, and female rats in the 8 mg/kg dose group showed decreased white blood cell count. Most animals in the 32 mg/kg dose group (>60%) showed histopathological changes in the forestomach including chronic inflammation, acantholysis, and hyperkeratosis. The authors considered the NOAEL to be 8 mg/kg/day.

VI. Derivation of Chronic Reference Exposure Level (REL)

Study	Burleigh-Flayer and Benson (1995)
Study population	CD-1 mice (60 per sex per dose)
Exposure method	Discontinuous inhalation (0, 0.1, 0.5 or 1.0 ppm)
Critical effects	Nasal rhinitis; bronchiectasis
LOAEL	0.5 ppm
NOAEL	0.1 ppm
Exposure continuity	6 hours/day, 5 days/week
Exposure duration	107 weeks
BMC_{05}	0.042 ppm
Average experimental exposure	0.0075 ppm at the BMC_{05} (0.042 x 6/24 x 5/7)
Human equivalent concentration	0.0016 ppm at the BMC_{05} (gas with extrathoracic respiratory effects, RGDR = 0.21 based on MV = 0.044 L/min and SA(ET) = 3 cm^2)
LOAEL uncertainty factor	not needed in the BMC approach
Subchronic uncertainty factor	1
Interspecies uncertainty factor	3 (since RGDR adjustment was made)
Intraspecies uncertainty factor	10
Cumulative uncertainty factor	30
Inhalation reference exposure level	0.05 ppb (0.4 $\mu g/m^3$)

The data on bronchiecstasis incidence in male and female mice were combined and the chronic REL for chloropicrin was developed using the BMC approach. Of the several models tested, the Gamma MultiHit Model gave the best fit to the combined bronchiecstasis data (p = 0.9750). The MLE_{05} was 0.070 ppm and the BMC_{05} was 0.042 ppm. Use of time extrapolation to equivalent continuous exposure, an RGDR adjustment for the area of the respiratory tract affected, and a total uncertainty factor of 30 resulted in a chronic REL of 0.05 ppb (0.4 $\mu g/m^3$).

The chronic study in mice (Burleigh-Flayer *et al*., 1995) yielded the same NOAEL of 0.1 ppm as the chronic study in rats (Burleigh-Flayer and Benson, 1995). Use of the mouse data with the NOAEL/UF approach led to a cREL estimate of 0.1 ppb. Use of the rat data yielded a chronic REL estimate of 0.2 ppb by the NOAEL/UF approach.

As another comparison, the study of Yoshida *et al*. (1987) found a NOAEL in rats of 0.67 ppm for intermittent exposure for 13 weeks. This is equivalent to a continuous exposure of 120 ppb. Use of an RGDR of 0.25 for rats and a total uncertainty factor of 100 (3 for subchronic, 3 for interspecies, and 10 for intraspecies) results in a REL estimate of 0.03 ppb (0.2 $\mu g/m^3$).

VII. Data Strengths and Limitations for Development of the REL

Significant strengths in the REL for chloropicrin include the duration of exposure (lifetime) in the key study, the multiple dose study design with adequate sample sizes, and the demonstration of a NOAEL in rats and mice. Major areas of uncertainty are the lack of adequate human exposure data, limited reproductive toxicity data, and the appropriateness of time extrapolation of concentrations that cause irritative effects such as rhinitis.

VIII. Potential for Differential Impacts on Children's Health

Chloropicrin is a respiratory irritant. Respiratory irritants often have steep dose-response curves. Thus use of the human intraspecies factor of 10 should result in a REL that adequately protects children. Exacerbation of asthma, which has a more severe impact on children than on adults, is a known response to some respiratory irritants. However, there is no direct evidence in the literature to quantify such a response to chloropicrin, or to quantify a differential effect of chloropicrin on infants or children. We are currently evaluating our risk assessment methodologies, in particular the intraspecies uncertainty factor (UF_H), for adequacy in protecting infants and children. While we have not so far identified any indications that the currently used UF_H of 10 might be less than adequate to protect infants and children, this possibility should be considered in evaluating any exposure situation involving chronic exposures of infants or children to chloropicrin.

IX. References

ACGIH. 1992. American Conference of Governmental Industrial Hygienists, Inc. Documentation of the threshold limit values and biological exposure indices. Sixth edition. Cincinnati, OH: ACGIH.

Buckley LA, Jiang XZ, James RA, Morgan KT, and Barrow CS. 1984. Respiratory tract lesions induced by sensory irritants at the RD_{50} concentration. Toxicol. Appl. Pharmacol. 74:417-429.

Burleigh-Flayer HD, and Benson CL. 1995. Chloropicrin: Vapor inhalation oncogenicity study in CD rats. Bushy Run Research Center, July 29, 1995.

Burleigh-Flayer HD, Kintigh WJ, and Benson CL. 1995. Chloropicrin: Vapor inhalation oncogenicity study in CD-1 mice. Bushy Run Research Center, April 20, 1995.

CDPR. 2000. California Department of Pesticide Regulation. Review of Burleigh-Flayer et al. (1995) Chloropicrin: Vapor inhalation oncogenicity study in CD-1 mice.

CARB. 2000. California Air Resources Board. California Emissions Inventory Development and Reporting System. (CEIDARS). Data from Data Base Year 1998. February 12, 2000.

CRC. 1994. CRC Handbook of Chemistry and Physics, 75th edition. Lide DR, ed. Boca Raton, FL: CRC Press Inc.

Condie LW, Daniel FB, Olson GR, and Robinson M. 1994. Ten and ninety-day toxicity studies of chloropicrin in Sprague-Dawley rats. Drug Chem. Toxicol. 17:125-137.

DPR. 2000. California Department of Pesticide Regulation. Summary of Pesticide Use Report Data – 1999. Sacramento: DPR.

Duguet JP, Tsutsumi Y, Bruchet A, and Mallevialle J. 1985. Chloropicrin in potable water: conditions of formation and production during treatment processes. In: Water Chlorination: Chemistry, Environmental Impact and Health Effects. Jolley RL, Bull RJ, Davis WP, Katz S, Roberts MH, and Jacobs VA. (eds.) Chelsea, MI: Lewis Publishers, pp. 1201-1213.

Fries AA, and West CJ. 1921. Chapter VIII. Chloropicrin. In: Chemical Warfare. First edition. New York, NY: McGraw-Hill Book Company, Inc.

HSDB. 1996. Hazardous Substances Data Bank. National Library of Medicine, Bethesda, Maryland (TOMES® CD-ROM Version). Denver, CO: Micromedex, Inc. (Edition expires 7/31/96).

Kane LE, Barrow CS, and Alarie Y. 1979. A short-term test to predict acceptable levels of exposure to airborne sensory irritants. Am. Ind. Hyg. Assoc. J. 40:207-229.

Merlet N, Thibaud H, and Dore M. 1985. Chloropicrin formation during oxidative treatments in the preparation of drinking water. Sci. Total Environ. 47:223-228.

Tomlin, C.D.S. (ed.). 1994. The Pesticide Manual - World Compendium. 10th ed. Surrey, UK: The British Crop Protection Council. p. 192.

Yoshida M, Ikeda T, Iwasaki M, Ikeda M, Harada T, Ebino K, Tsuda S and Shirasu. 1987. Subchronic inhalation toxicity of chloropicrin vapor in rats. J. Pesticide Sci. 12:673-681.

CHRONIC TOXICITY SUMMARY

CHROMIUM, HEXAVALENT (SOLUBLE COMPOUNDS)

Molecular Formula	*Molecular Weight*	*Synonyms*	*CAS Registry Number*
CrO_3	99.99 g/mol	Chromic trioxide, chromium oxide, chromium trioxide, chromium (VI) oxide. (In acid aqueous solutions, exists as H_2CrO_4 – "chromic acid")	1333-82-0
K_2CrO_4	194.20 g/mol	Potassium chromate, dipotassium chromate, potassium (VI) chromate, dipotassium monochromate, chromate of potash	7789-00-6
Li_2CrO_4	129.87 g/mol	Lithium chromate, chromium lithium oxide, chromic acid dilithium salt, lithium chromate (VI)	14307-35-8
Na_2CrO_4	161.97 g/mol	Sodium chromate, chromic acid disodium salt, chromium disodium oxide, sodium chromate (VI), chromate of soda	7775-11-3
$K_2Cr_2O_7$	294.20 g/mol	Potassium dichromate, dichromic acid dipotassium salt, bichromate of potash	7778-50-9
$Na_2Cr_2O_7$	261.96 g/mol	Sodium dichromate, bichromate of sodium, dichromic acid disodium salt, chromium sodium oxide	10588-01-9

I. Chronic Toxicity Summary

A. Soluble Hexavalent Chromium Compounds (except chromic trioxide)

Inhalation reference exposure level	**0.2 µg Cr(VI)/m^3**
Critical effect(s)	Bronchoalveolar hyperplasia in lungs of rats
Hazard index target(s)	Respiratory system
Oral reference exposure level	**0.02 mg Cr(VI)/kg/day**
Critical effect(s)	Red blood cell effects (decreased mean corpuscular volume (MCV) and mean corpuscular hemoglobin (MCH)) in mice
Hazard index target(s)	Hematopoietic system

B. Chromic Trioxide (as chromic acid mist)

Inhalation reference exposure level	**0.002 µg Cr(VI)/m^3**
Critical effect(s)	Respiratory effects (nasal atrophy, nasal mucosal ulcerations, nasal septal perforations, transient pulmonary function changes) in human occupational study
Hazard index target(s)	Respiratory system

II. Physical and Chemical Properties (HSDB, 2000; CRC, 1994)

Description	CrO_3: dark red or brown crystals, flakes, or powder, exists as chromic acid (H_2CrO_4) in solution; K_2CrO_4, Na_2CrO_4: yellow crystals; $K_2Cr_2O_7$, $Na_2Cr_2O_7$: orange-red crystals; Li_2CrO_4: yellow crystalline powder
Molecular formula	See above
Molecular weight	See above
Density	CrO_3: 2.70 g/cm^3 @ 25°C
Boiling point	CrO_3: decomposes (temperature not available); $K_2Cr_2O_7$: 500 °C with decomposition; $Na_2Cr_2O_7$: 400 °C
Melting point	CrO_3: 197 °C; K_2CrO_4: 975 °C; Na_2CrO_4: 792 °C; $K_2Cr_2O_7$: 398 °C; $Na_2Cr_2O_7$: 356.7 °C
Vapor pressure	Not applicable
Solubility	CrO_3: soluble in water, ethyl alcohol, ethyl ether, sulfuric and nitric acid; K_2CrO_4, $K_2Cr_2O_7$, $Na_2Cr_2O_7$: soluble in water, insoluble in ethyl alcohol; Na_2CrO_4: soluble in water, slightly soluble in ethyl alcohol; Li_2CrO_4: soluble in water and ethyl alcohol
Conversion factor	Not applicable for particulates and mists

III. Major Uses or Sources

Hexavalent chromium (Cr(VI)) is considerably more toxic than trivalent chromium (Cr(III)), the form most commonly found naturally (ATSDR, 1993). Cr(VI) is generally produced by industrial processes. While more information is available on the toxicity of soluble Cr(VI)

compounds, information on poorly soluble Cr(VI) compounds has been included where applicable. In California, the major emission source of Cr(VI) results from the chrome plating industry (CARB, 1997). Chromic acid, used to electroplate metal parts, is the most common Cr(VI) compound produced in the U.S. (ATSDR, 1998). Chromic acid is also registered as a fungicide and pesticide in California for use in wood and lumber protection treatments (CDPR, 1998). Chromic acid solutions used for this purpose in the most recent year of reporting (1998) was 71,109 lbs. Minute emissions of Cr(VI) may result from lead chromate in paint used for road striping and from coatings in the aerospace and auto refinishing industries, although uses of Cr(IV)-containing coatings by these industries in California are decreasing (CARB, 1997 and 1988). Use of Cr(VI) as a corrosion inhibitor in cooling tower water is prohibited in California, and recently, in the remainder of the U.S. as well. Fuel combustion releases trace amounts of chromium (CARB, 1988). Most, if not all, of this emitted chromium is in the Cr(III) state. In the chromium ferroalloy industry, sodium chromate and dichromate can be produced from imported chromite (Cr(III)) ore. However, no such facilities in California have reported production or emission of these Cr(VI) compounds.

Primary routes of potential human exposure to chromium compounds are inhalation, ingestion, and dermal contact. Exposure to chromic acid is most often in the form of a mist; exposure to other soluble forms of Cr(VI) is as components of aerosols or particulate matter. The physical, chemical, and potency differences between Cr(VI) dusts and chromic acid mists necessitated the development of separate RELs for each. Environmental exposures would most likely occur through exposure to Cr(VI) dusts (U.S. EPA, 1998). Cr(VI) may persist in water as water-soluble complex anions. However, any Cr(VI) settling in the soil or water is expected to be eventually reduced to Cr(III) by organic matter. The South Coast Air Quality Management District (SCAQMD, 2000) detected ambient levels of hexavalent chromium ranging from 0.0001 to 0.0003 $\mu g/m^3$ at 10 stationary monitors placed throughout the South Coast Air Basin. The annual statewide industrial emissions from facilities reporting under the Air Toxics Hot Spots Act in California based on the most recent inventory were estimated to be 2311 pounds of hexavalent chromium (CARB, 2000).

IV. Effects of Human Exposure

Cr(VI) forms oxyanions at physiological pH (CrO_4^{-2}), which are quite similar to sulfate (SO_4^{-2}) and phosphate (HPO_4^{-3}) anions. Therefore, it is able to penetrate virtually every cell in the body because all cells transport sulfate and phosphate (Costa, 1997). Harmful effects are speculated to be related to the reduction of Cr(VI) to Cr(III) intracellularly when it crosses the cell membrane and forms complexes with intracellular macromolecules. Thus, Cr(VI) compounds have the potential to injure numerous organ systems. Toxicity following chronic Cr(VI) exposure has been reported in the respiratory tract, gastrointestinal system, eyes and conjunctiva, kidney, and hematopoietic system. Cr(VI) is corrosive and exposure to chromic acid mists may cause chronic skin ulcerations and upper respiratory lesions (U.S. EPA, 1998). In addition, allergic skin and respiratory reactions can occur with no relation to dose.

Nasal tissue damage has been frequently observed in chromium plating workers exposed chronically to chromic acid mists (Bloomfield and Blum, 1928; Vigliani and Zurlo, 1955;

Kleinfeld and Rosso, 1965; Gomes, 1972; Sorahan *et al*., 1998). However, workers in the chromate extraction and ferrochromium industry, exposed to particulates containing soluble Cr(VI) compounds, have also reported nasal lesions (Mancuso, 1951; Federal Security Agency, 1953; Machle and Gregorius, 1948; Wang *et al*., 1994; Walsh, 1953). Other less frequent mucous membrane injuries have been reported in workers exposed to chromate dust and chromic acid including sinusitis, laryngitis, conjunctivitis, and oral ulcerations (Mancuso, 1951; Federal Security Agency, 1953; Johansen *et al*., 1994). Nasal lesions include perforated septum, ulcerated septum, nasal atrophy, nosebleed, and inflamed mucosa following exposure to air chromium levels of about 0.1 to 5.6 mg/m^3. Exposure duration, when reported, ranged from 2 weeks to 25 years. However, there were problems in quantifying the effect for the above studies. The difficulties were primarily lack of adequate methods or data for determining exposure duration and/or exposure levels. The occupational studies summarized below provide the most reliable estimates of inhalation durations and concentrations resulting in chronic toxicity.

Workers exposed to $\geq$2 $\mu g/m^3$ Cr(VI) as chromic acid exhibited an increased incidence of nasal atrophy, nasal mucosal ulcerations, and nasal septal perforations as compared to controls (Lindberg and Hedenstierna, 1983). Workers exposed to less than 2 $\mu g/m^3$ (expressed as $\leq$ 1.9 $\mu g/m^3$) exhibited an increased incidence of irritated nasal mucosa and nasal atrophy compared to controls. The median exposure time of exposed workers was 2.5 years (range = 0.2-23.6 years). Frequency of throat and chest symptoms was similar to that of controls. The same study reported statistically significant decreases in forced expiratory volume in 1 second (FEV_1), forced vital capacity (FVC), and mean forced expiratory flow during the middle of the FVC in 1 second (FEF_{25-75}) measurements taken on a Thursday afternoon as compared to those taken on a Monday morning in nonsmoking workers exposed to 2 $\mu g/m^3$ Cr(VI) or more. Similar changes were observed in the smokers although only the difference in the FVC measured on a Thursday was statistically significant. No significant differences were observed between pulmonary function measurements of exposed and unexposed workers taken on a Monday morning (prior to a work week of exposure). Thus the authors infer that the observed pulmonary function changes are transient.

Nasal lesions were observed in 35 of 37 chrome platers exposed to a mean breathing zone concentration of 7.1 μg/m3 (range = 1.4-49.3 μg/m3) total chromium for an average of 2.2 years (range = 1.2 weeks-11 years) (Cohen et al., 1974). Actual exposure to Cr(VI) averaged 2.9 μg/m3 (range = 0.09-9.1 μg/m3). Workers employed more than one year had significantly greater nasal pathology than workers employed one year or less. Due to poor personal hygiene habits of the exposed workers, a 'direct contact' etiology may explain some of the nasal lesions.

Urinary levels of β_2-microglobulin in 24 chrome platers increased in dose-dependent fashion with increasing intensity of exposure to Cr(VI), indicating a nephrotoxic effect resulting from inhalation of Cr(VI) (Lindberg and Vesterberg, 1983). The 8-hr mean Cr(VI) levels ranged from 2 to 20 $\mu g/m^3$ and averaged 6 $\mu g/m^3$. Total exposure times ranged from 0.1 to 26 years and averaged 5.3 years. Most of the 24 chrome workers had irritation symptoms of the airways. As a group, the chrome platers had significantly higher levels of urinary β_2-microglobulin compared to a group of 27 referents. Comparison of 27 referents to a group of 27 ex-chrome-platers found no difference in urinary β_2-microglobulin levels, even though seven of the ex-chrome-platers had a permanent perforation of their nasal septum (indicating past exposure to high levels of Cr(VI)).

There was no correlation between total exposure time and urinary β_2-microglobulin levels. Urinary albumin levels remained unchanged in the Cr(VI)-exposed group. The results suggest that the nephrotoxic effects are reversible at the exposure levels studied.

Gastritis and duodenal ulcers, in addition to ulceration and perforation of the nasal septum, were observed in chrome platers exposed to a mean breathing zone concentration of 4 $\mu g/m^3$ chromic acid for an average of 7.5 years (Lucas and Kramkowski, 1975).

Male workers in the chromate and dichromate production industry, whose occupational exposures were 0.05-1.0 mg Cr(VI)/m^3 as chromium trioxide for a mean of 7 years, were reported to have elevated levels of low molecular weight proteins (retinol binding protein and tubular antigens) in the urine (Franchini and Mutti, 1988). The authors suggest that the presence of such proteins in the urine is an early indicator of kidney damage.

The respiratory health of workers exposed to low levels of dusts containing Cr(VI) was investigated at a stainless steel production plant (Huvinen *et al*., 1996). The data were presented as total chromium exposure and Cr(VI) exposure. A combined total of 109 exposed workers in the furnace department (median Cr(VI) exposure approximately 0.075-0.45 $\mu g/m^3$) and the steel smelting shop (average Cr(VI) exposure 0.5 $\mu g/m^3$) was compared to a control group of 95 workers that worked in the cold rolling mill. Total work exposure duration was 16.0 years (range: 8-26 years). No significant differences in lung function tests and radiological findings were observed between exposed and control workers. After controlling for age and smoking, no differences were observed for the prevalence of rhinitis, eye irritation, or respiratory symptoms between the two groups.

In a study summarized by U.S. EPA (1998), oral ulcers, diarrhea, stomach ache, indigestion, leukocytosis and vomiting were reported among a group of 155 Chinese villagers exposed to contaminated well-water containing 20 mg/L Cr(VI) in 1965 (Zhang and XiLin, 1987). However, precise exposure concentrations, exposure durations, and confounding factors were not provided. A follow-up study to assess cancer mortality reported that the average Cr(VI) concentration in 1965 from 170 wells of the most impacted village was only 2.6 ppm, and maximum levels did not exceed 5 ppm (Zhang and Li, 1997). Non-cancer effects were not presented and the apparent discrepancy in water levels of Cr(VI) with the earlier study was not discussed.

V. Effects of Animal Exposure

Exposure of C57BL/6 mice to 0 or 13 mg/m^3 $CaCrO_4$ dust (about 136 animals/sex/group) 5 hr/day, 5 days/wk for life resulted in emphysema-like changes of the lung, 'bronchiolarization' of the alveoli, and epithelial necrosis, marked hyperplasia, and atrophy of the bronchi in treated mice (Nettesheim *et al*., 1971). Other non-cancer histopathological findings in exposed mice included atrophy of the lymph nodes, spleen, and liver, and occasional small ulcerations of the stomach and intestinal mucosa. Cessation of body weight gain in both sexes was observed following the sixth month of exposure to the chromate dust.

Glaser *et al*. (1986) exposed 20 male Wistar rats/group to 25, 50, and 100 μg/m^3 aerosolized sodium dichromate solution and to 100 μg/m^3 of a pyrolyzed Cr(VI)/Cr(III) (3:2) oxide dust mixture 22-23 hr/day for 18 months. Observation in filtered air continued for another 12 months thereafter. A control group consisted of 40 rats. Mortality and body weights were unaffected by treatment. Lung chromium retention at the end of the study was 10-fold greater in rats exposed to the slightly water soluble chromium oxide mixture compared to high dose rats exposed to water-soluble sodium dichromate. No clinical signs of irritation were observed in any group. No hematological effects were noted in rats exposed to sodium dichromate. Rats exposed to the chromium oxide mixture had a significantly elevated white blood cell count at the 17th and 18th month, and significantly elevated red blood cells, hematocrits, and hemoglobin levels at the 27th month. Mean serum content of total immunoglobulin was significantly reduced in this group at 6 months exposure. Significantly increased lung weights were observed in chromium oxide-exposed rats, and for livers of sodium dichromate-exposed rats at the highest dose. Pigment-loaded macrophages were found in the sodium dichromate-exposed rats in a dose dependent manner and also in the chromium oxide group. Chromium oxide-exposed rats also developed focal thickened septa, partially combined with interstitial fibrosis and accumulation of eosinophilic substance in the alveolar lumens. The authors concluded that the hematological and pulmonary effects may be due to Cr-accumulation in the lungs and to depressed lung clearance function.

Rats exposed to 200 μg/m^3 Cr(VI) as aerosolized sodium dichromate by inhalation for 22 hours per day for 42 days exhibited decreased alveolar macrophage phagocytic activity; the lung clearance of inert iron oxide was significantly reduced in exposed rats compared to controls (Glaser *et al.*, 1985). Increased alveolar macrophage activity and a significantly elevated antibody response to injected sheep red blood cells were observed in rats exposed to 25 or 50 μg/m^3 Cr(VI) for 22 hours per day for 28 days. Ninety day exposure under the same exposure protocol resulted in increased rat lung and spleen weights at 50, 100 and 200 μg/m^3, but not 25 μg/m^3 (Glaser *et al.*, 1985). Histopathology of major organs was similar among all groups. Bronchoalveolar lavage fluid contained decreased macrophage cell counts above 25 μg/m^3. Increased antibody response to injected sheep red blood cells was observed in all treatment groups, while alveolar macrophage activity was elevated at 25 and 50 μg/m^3, but was significantly reduced at 200 μg/m^3.

A later experiment exposed male rats to 0, 50, 100, 200, or 400 μg Cr/m^3 22 hours per day, 7 days per week for 90 days (Glaser *et al*., 1990). Average measured concentrations were 0, 54, 109, 204, and 403 μg Cr/m^3, respectively. Subacute respiratory dyspnea and reduction in body weight gain were observed at the two highest exposures. Mean white blood cell count increased in a dose-dependent manner among treated rats, but returned to normal 30 days following cessation of exposure. Histopathological examination revealed histiocytosis (macrophage accumulation) in all treatment groups (Table 1). Bronchoalveolar lavage fluid (BALF) contained elevated levels of albumin, lactate dehydrogenase (LDH), and total protein in all exposed groups. Statistically significant elevations in these parameters were observed mainly in the 200 and 400 μg/m^3 exposure groups. At necropsy, a statistically significant increase in lung weight (g dry wt/kg body wt) was observed in rats exposed to 100, 200, and 400 μg/m^3 as compared to controls. Lung weights were still significantly elevated in the three highest exposure groups 30 days following cessation of exposure. An analysis of the data (Malsch *et al.*, 1994) determined a

benchmark dose (95% confidence interval with dose associated with a 10% elevation in the parameter) for each of these endpoints. The analysis also examined changes in lung and spleen weight reported in Glaser *et al.* (1985). The most sensitive endpoint was LDH in BALF.

Table 1. Key bronchoalveolar lavage fluid (BALF) and histopathological findings after 90 days exposure to sodium dichromate (Glaser et al., 1990).

μg Cr/m^3	*Total Protein in BALF*[a] *(mg/L)*	*Albumin in BALF (mg/L)*	*LDH in BALF (U/L)*	*Broncho-alveolar Hyperplasia*	*Lung Histiocytosis*	*Right lung dry weight (g/kg BW)*
0	226±30	77±13	29±5	0/10	2/10	0.44± 0.03
50	396±79**	115+23**	34±3*	3/10	9/10	0.48±0.05
100	326±35**	86±13	31±4	2/10	10/10	0.50±0.06*
200	703±178**	117±20**	63±11**	3/10	9/10	0.55±0.04**
400	975±246**	184±59**	83±17**	7/10	10/10	0.65±0.05**

a All BALF parameters are mean + SD, n = 10/group
* $p < 0.05$; ** $p < 0.001$: comparison of exposed groups vs. controls

Cohen *et al*. (1998) investigated the immunotoxicologic effects of inhaled chromium by exposing F-344 rats (10/group/exposure duration) nose-only to 0 and 360 μg/m^3 potassium chromate 5 hr/day, 5 days/week for 2 or 4 weeks. Exposed rats had greater levels of total recoverable cells, neutrophils, and monocytes in bronchopulmonary lavage compared to controls at 2 and/or 4 weeks. Pulmonary macrophages (PM) were reduced, although total PM levels remained unaffected. Four-week exposure to potassium chromate also resulted in modulated PM-inducible interleukins-1 and –6, and tumor necrosis factor-α, and increased PM basal nitric oxide production and interferon-γ-primed/zymosan-stimulated reactive oxygen intermediate production.

Nasal septal perforation, hyperplastic and metaplastic changes in the larynx, trachea, and bronchus, and emphysema were observed in mice exposed two days per week for 12 months to CrO_3 mist (Adachi, 1987; Adachi *et al.*, 1986). Chromic acid concentrations were either 3.63 mg/m^3 for 30 minutes per day or 1.81 mg/m^3 for 120 minutes per day. An additional 20 mice exposed to 1.81 mg/m^3 were necropsied 6 months after the last exposure. Lesions of the nasal septum, trachea, and lungs were still evident in some mice.

The investigators of the toxicity studies summarized below administered soluble Cr(VI) compounds to experimental animals by the oral route.

Groups of eight male and eight female Sprague-Dawley rats were supplied with drinking water containing 0-11 ppm (0-11 mg/L) Cr(VI), as K_2CrO_4, for 1 year (Mackenzie *et al.*, 1958). The control group (10/sex) received distilled water. A second experiment involved three groups of 12 male and 9 female rats. One group was given 25 ppm (25 mg/L) Cr(VI); a second received 25 ppm chromium in the form of chromic chloride; and the controls received distilled water. For rats treated with 0-11 ppm (in the diet), hematological determinations (red and white blood cell counts, differential white cell counts, and hemoglobin) were performed monthly, and tissues (livers, kidneys and femurs) were examined at 6 months and 1 year. Spleens were also examined

at 1 year. The 25 ppm groups (and corresponding controls) were examined similarly, except that no animals were killed at 6 months. No significant adverse effects were seen in appearance, weight gain, or food consumption, and there were no treatment-related effects regarding hematological parameters or other tissues in any treatment group. The rats receiving 25 ppm Cr(VI) showed an approximate 20% reduction in water consumption. This dose corresponds to 2.4 mg Cr(VI)/kg/day based on actual body weight and water consumption data. An abrupt rise in tissue chromium concentrations was noted in rats treated with greater than 5 ppm. The authors stated that "apparently, tissues can accumulate considerable quantities of chromium before pathological changes result." In the 25 ppm treatment groups, tissue concentrations of chromium were approximately 9 times higher for those treated with hexavalent chromium than for the trivalent group.

Anwar *et al.* (1961) observed no significant effects in groups of female dogs (2/dose group) given 0, 0.45, 2.25, 4.5, 6.75, or 11.2 ppm Cr(VI) (as K_2CrO_4) in drinking water for 4 years. The calculated doses ranged from 0.012-0.30 mg/kg of Cr(VI).

Numerous rodent studies have been recently undertaken to investigate the reproductive and developmental effects of Cr(VI) exposure via the drinking water (Trivedi *et al*., 1989; Junaid *et al*., 1995; Murthy *et al*., 1996; Junaid *et al*., 1996a; Junaid *et al*., 1996b; Kanojia *et al*., 1996; Elbetieha and Al-Hamood, 1997; Al-Hamood *et al*., 1998; Kanojia *et al*., 1998). Exposure concentrations ranged from 250 to 5000 ppm for durations as short as five days during gestation to as long as 3 months pre-gestational exposure. In general, the longer exposures resulted in more serious reproductive and developmental effects.

Kanojia *et al*. (1998) administered 0, 250, 500, and 750 ppm potassium dichromate via drinking water to female Druckrey strain rats for 90 days prior to gestation. Based on daily water intake and final body weights, the estimated daily Cr(VI) intake was 33, 68, and 98 mg/kg-day, respectively. Ten to 15% mortality, hair loss, lethargy, aggressiveness and a significant reduction in body weight gain were observed in rats at the two highest doses. While not statistically significant, weight of the low dose rats were 32% lower than controls. All treated rats were acyclic at the end of the 90 day exposure period and an additional 15-20 days without Cr(VI) exposure were needed for the estrus cycle to start. Mating and fertility indexes decreased with increasing Cr(VI) intake. Ten rats/group were sacrificed on day 19 of gestation for fetotoxicity assessment. Significantly reduced fetal weight and increased pre- and post-implantation loss occurred at all dose levels. Gross and skeletal abnormalities in low dose fetuses included subdermal hemorrhagic patches, drooping wrists, and reduced caudal bone ossification. No gross visceral abnormalities were seen in treated groups.

Administration of potassium dichromate to rats (Kanojia *et al*., 1996) and mice (Junaid *et al*., 1996a) in drinking water at concentrations of 250, 500, and 750 ppm for 20 days prior to gestation resulted in increased post-implantation loss and decreased placental weight in both species at the lowest dose. Also at this dose level, decreased fetal weight and crown-rump length were observed in mice, and increased resorptions and decreased number of live fetuses were observed in rats. Gross and skeletal abnormalities were observed in both species beginning at the 500 ppm dose level.

Groups of Sprague-Dawley rats (NTP, 1996a) and BALB/C mice (NTP, 1996b) were administered potassium dichromate in their diet at 0, 15, 50, 100, or 400 ppm for 9 weeks (24 males and 48 females/species/group) followed by a recovery period of 8 weeks. Average Cr(VI) consumption for male/female rats were 1/1, 3/3, 6/7, and 24/28 mg/kg-day, respectively. Average Cr(VI) consumption for male/female mice were 3/5, 10/16, 21/34, and 92/137 mg/kg-day, respectively. Six males and 12 females of both species were necropsied after 3, 6, or 9 weeks of treatment or after the full recovery period. There was no treatment-related histopathology observed in kidneys, ovaries, and testes in either species. Hematological analysis revealed slight decreases in mean corpuscular volume (MCV) and mean corpuscular hemoglobin (MCH) at the highest dose in both species, which is indicative of iron deficiency. MCV and MCH were normal in these groups following the 8-week recovery period. Microscopic evaluation of the livers of mice noted cytoplasmic vacuolization of hepatocytes in treated animals beginning at 50 ppm. Also in mice, there was a slight decrease in mean body weights in the 400 ppm males (5-9%) and females (4%) and the 100 ppm females (2-4%) during the dosing periods. Feed consumption by mice was generally increased in all treated groups, particularly the 400 ppm males and females. During the recovery period, feed consumption was comparable across groups.

The NTP (1997) investigated the potential reproductive toxicity of Cr(VI) in mice using the Reproductive Assessment by Continuous Breeding protocol. Groups of 20 male and female pairs of BALB/c mice (F_0) were exposed to 0, 100, 200, and 400 ppm potassium dichromate in their diet during the continuous breeding phase (approximately 12 weeks). F_1 generation litters received the same concentration of Cr(VI) in their diet as their F_0 parents and were used for assessment of second generation reproductive toxicity at sexual maturity. There were no treatment-related changes in any of the reproductive parameters in this study. In F_1 mice, the MCV was slightly decreased in males at the two highest doses, and slightly decreased in females in all dose groups. MCH and hemoglobin were slightly reduced in high dose males and high dose females, respectively. Mean body weights of the high dose F_0 and F_1 animals were slightly decreased, and mean food consumption in the F_1 mice was elevated. Reduced mean absolute liver weights were observed in 400 ppm F_0 mice of both sexes. The mean calculated doses were 19.4, 38.6, and 85.7 mg/kg-day for F_0 males and females and 22.4, 45.5, and 104.9 mg/kg-day for F_1 males and females in the 100, 200, and 400 ppm dose groups, respectively.

In an investigation of the spermatogenic and steroidogenic effects of Cr (VI), Chowdhury and Mitra (1995) administered 0, 20, 40, and 60 mg/kg-day sodium dichromate by oral gavage to male rats for 90 days. Reduced Leydig cell population, reduced body and testicular weight, and degeneration of testicular tissue was observed at the two highest doses. Biochemical measures of spermatogenic and steroidogenic impairment, including decreased testicular DNA, RNA, protein, serum testosterone, and 3β-Δ^5-hydroxy steroid dehydrogenase (3β-Δ^5-HCH), were also reduced at the two highest doses. Only relatively small reductions in testicular protein, 3β-Δ^5-HCH, and serum testosterone were seen in the 20 mg/kg rats.

VI. Derivation of Chronic Reference Exposure Levels (RELs)

A. Derivation of Chronic Inhalation Reference Exposure Level for Soluble Hexavalent Chromium Compounds other than Chromic Trioxide

Study	Glaser *et al*., 1990
Study population	Male Wistar rats (30 per group)
Exposure method	Discontinuous whole-body inhalation (0, 54, 109, 204, or 403 µg Cr(VI)/m^3 as sodium dichromate aerosol)
Critical effects	Bronchoalveolar hyperplasia
LOAEL	50 µg/m^3
NOAEL	Not observed
BMC_{05}	12.50 µg/m^3
Exposure continuity	22 hr/day, 7 days/week
Exposure duration	90 days
Average exposure	11.46 µg/m^3 Cr(VI) (12.50 x 22/24)
Human equivalent concentration	24.47 µg/m^3 Cr(VI) (2.1355 [RDDR] x 11.46)
LOAEL uncertainty factor	Not needed in the BMC approach
Subchronic uncertainty factor	3
Interspecies uncertainty factor	3
Intraspecies uncertainty factor	10
Cumulative uncertainty factor	100
Inhalation reference exposure level	0.2 µg/m^3 (0.0002 mg/m^3)

The study by Glaser *et al*. (1990) provides the best available inhalation data that demonstrate a dose-response relationship for various pulmonary toxicity endpoints. The BMC_{05} of 12.50 µg/m^3 was derived from quantal data for bronchoalveolar hyperplasia. The presence of bronchoalveolar hyperplasia in exposed rats is supported by other indicators of lung inflammation, including increased total protein, LDH, and albumin in BALF (see Table 1). A quantal-linear model analysis (U.S. EPA, National Center for Environmental Assessment, benchmark dose software, version 1.20) of the quantal data provided the most reasonable line fit and resulted in the lowest BMC_{05}. A BMC_{05} is considered to be similar to a NOAEL in estimating a concentration associated with a low level of risk. Lung histiocytosis (macrophage accumulation) was present in nearly all exposed animals, but this quantal data set was only suitable for a NOAEL/LOAEL approach and was not considered as direct an indicator of lung injury as bronchoalveolar hyperplasia.

Based on OEHHA methodology, a comparison REL developed using the NOAEL/LOAEL approach would yield 0.3 µg/m^3. Adjustment of the LOAEL of 50 µg/m^3 (a NOAEL was not observed) to the human equivalent concentration uses the same parameters as shown in the REL derivation above. However, a LOAEL UF of 3 is added to the existing UFs to result in a cumulative UF of 300.

The U.S. EPA (1998) RfC of 0.1 µg/m^3 is also based on data from Glaser et al. (1990), but derived a BMC_{10} (16 µg/m^3), as developed by Malsch *et al*. (1994), from continuous data of

LDH in BALF. Using a polynomial model provided by a different benchmark software package (*THC*, Clement International Corp., Ruston LA), increasing LDH concentration in BALF with increasing dose provided the lowest BMC_{10} among the various BALF endpoints. OEHHA is currently not developing BMCs for RELs based on continuous data. A BMC_{05} derived from quantal data and a BMC_{05} derived from continuous data may not have the same meaning. Conceivably, depending on the standard deviations of the data points, the BMC_{05} based on continuous data could still be above the statistically significant effect level. OEHHA believes that further evaluation of BMC's based on continuous data is needed prior to their application to RELs.

OEHHA and U.S. EPA also diverge on the assignment of the Subchronic UF. The Glaser *et al*. (1990) study indicated that chromium was still acculmulating in lung tissue at the end of 90 days. This evidence and the fact that the study did not investigate upper airway effects and other extra-pulmonary effects led U.S. EPA to assign a subchronic UF of 10 (U.S. EPA, 1998). Based on OEHHA methodology, OEHHA used a subchronic UF of 3. In support of a UF of 3, the 18-month sodium dichromate exposure study performed by Glaser *et al*. (1986), under similar exposure conditions used in the key 90-day study, did not find histopathological evidence of lung inflammation or major organ effects, or suggest severe chromium accumulation in exposed rats. However, BALF analysis was not performed in the chronic study.

For comparison with the proposed REL, the occupational study by Huvinen et al. (1996) established a NOAEL of 0.5 µg/m3 for lack of pulmonary findings. However, this study is deficient for REL purposes due to the lack of a LOAEL. Unfortunately, other occupational studies suffered from lack of adequate methods or data for determining exposure duration and/or exposure levels. Use of an occupational time adjustment (10/20 m3 inhaled/day, 5/7 days/week) and an interspecies UF of 10 for the Huvinen et al. (1996) study would result in an estimated REL of 0.02 µg/m3. Average exposure duration was 16 years, so a subchronic UF of 1 was sufficient.

B. Derivation of Chronic Inhalation Reference Exposure Level for CrO3 as Chromic Acid

Study	Lindberg and Hedenstierna, 1983
Study population	Human workers (100 exposed workers, 119 unexposed controls)
Exposure method	Occupational exposure to chromic acid mist
Critical effects	Nasal atrophy, nasal mucosal ulcerations, nasal septal perforations, transient pulmonary function changes
LOAEL	1.9 µg/m^3 established as "low exposure" group (8-hr mean $\leq$ 1.9 µg/m^3)
NOAEL	Not observed
Exposure continuity	8 hr/day (10 m^3 per 20 m^3 day), 5 days/week
Exposure duration	Mean of 2.5 years (range = 0.2 - 23.6 years)
Average exposure	0.68 µg/m^3 Cr(VI) (1.9 x 10/20 x 5/7)
Human equivalent concentration	0.68 µg/m^3 Cr(VI)
LOAEL uncertainty factor	3
Subchronic uncertainty factor	10
Interspecies uncertainty factor	1
Intraspecies uncertainty factor	10
Cumulative uncertainty factor	300
Inhalation reference exposure level	0.002 µg/m^3 (0.000002 mg/m^3)

The occupational exposure study of Lindberg and Hedenstierna (1983) was selected as the best available human study. A 3-fold LOAEL to NOAEL uncertainty factor (UF) was applied due to the low incidence of nasal atrophy at the LOAEL (4 out of 19) and the apparent reversibility of the lesion at this exposure level. While Lindberg and Hedenstierna (1983) did not follow-up on any of the active cases of nasal ulcerations, which occurred only in workers in the 'high exposure' group, they did note that one worker, who exhibited nasal atrophy, had no visible nasal lesions 4 months after termination of exposure.

U.S. EPA (1998) based its RfC of 0.008 µg/m^3 for exposure to chromic acid mists and dissolved Cr(VI) aerosols on the same study but established the LOAEL at 2 µg/m^3 and applied a total UF of 90 (3 each for the LOAEL to NOAEL and subchronic to chronic extrapolation, and 10 for intraspecies extrapolation). It was unclear why U.S. EPA (1998) chose UFs of 3 for LOAEL and subchronic extrapolations. It was also unclear why the total uncertainty factor was 90, rather than 100, which would be obtained by following the usual convention (that the value for uncertainty factors of "3" is actually 3.16, the square root of 10, although it is usually only quoted to 1 significant figure).

For comparison, a REL can be estimated from the Adachi *et al*. (1987) study in which mice were exposed to 1.81 mg/m^3 chromic acid mist 2 hr/day, twice a week for 12 months. Lesions were observed in treated mice throughout the respiratory tract; a NOAEL was not determined. Application of the exposure continuity adjustment (2/24 hr/day x 2/7 days/week), an RDDR of 2.26 (MMAD and sigma g roughly estimated at 5 and 3 µm, respectively), and a total UF of 300 (10 for LOAEL to NOAEL, 3 for interspecies, and 10 for intraspecies) yields a REL of 0.3 µg/m^3.

In addition to being inhaled, airborne hexavalent chromium can settle onto crops and soil and enter the body by ingestion. Thus, an oral chronic reference exposure level for soluble salts of metallic chromium(VI) is also required for assessing risks from stationary sources in the Air Toxics Hot Spots program.

C. Derivation of Chronic Oral Reference Exposure Level for Chromium VI (Based on U.S. EPA RfD)

Study	Mackenzie *et al.*, 1958
Study population	8 male and 8 female Sprague-Dawley rats
Exposure method	Drinking water
Critical effects	No adverse effects seen
LOAEL	None
NOAEL	2.4 mg/kg-day (converted from 25 mg/L of chromium as K_2CrO_4)
Exposure continuity	Continuous
Exposure duration	1 year
Average experimental exposure	2.4 mg/kg-day (0.11 ppm Cr(VI))
LOAEL uncertainty factor	1
Subchronic uncertainty factor	1
Interspecies uncertainty factor	10
Intraspecies uncertainty factor	10
Cumulative uncertainty factor	100
Oral reference exposure level	0.02 mg/kg bw-day

The oral REL (0.02 mg/kg bw-day) and U.S. EPA's oral Reference Dose (RfD) of 0.003 mg/kg-day (U.S. EPA, 1998) are based on the same study by MacKenzie *et al*. (1958). No adverse effects were reported at any dose in the study. The highest dose group (25 mg/L) was selected for derivation of the oral REL and RfD based on the reported body weight of the rat (0.35 kg) and the reported average daily drinking water consumption for the rat (0.035 L/day). Because a LOAEL was not observed in the primary study, the subchronic NTP studies provide supporting evidence to justify a REL based on MacKenzie *et al*. (1958). Cr(VI) was administered in the diet of rats for 9 weeks and a NOAEL of 6 mg/kg-day was observed for slightly depressed MCV and MCH values (NTP, 1996a). The LOAEL was 24 mg/kg-day. The NTP (1996b, 1997) also observed slightly depressed MCV and MCH values in mice, but at higher Cr(VI) concentrations. While the changes are small and may be a mild adverse effect at best, the NTP (1997) noted that decreased MCV and MCH are indicators of iron deficiency and suggested that an interaction between chromium and iron is altering erythrocyte formation. The liver effects noted in female mice in the 9 week study (NTP, 1996b) were not observed in the mouse reproductive study (NTP, 1997). Therefore, the toxicological significance of this finding is uncertain.

U.S. EPA (1998) applied UFs of 3 for subchronic, 10 for intraspecies, 10 for interspecies, and a modifying factor of 3 (to account for concerns raised by the study of Zhang and XiLin (1987)) to the NOAEL for an RfD of 0.003 mg/kg-day. The criteria for use of modifying factors are not

well specified by U.S. EPA. Such modifying factors were not used by OEHHA. Because the exposure duration in the primary study was greater than 12% of the estimated lifespan of rats, OEHHA applied UF of 1 for extrapolation to chronic exposure.

U.S. EPA stated its confidence in the RfD as: Study - Low; Data Base - Low; and RfD - Low. Confidence in the chosen study is low because of the small number of animals tested, the small number of parameters measured, and the lack of toxic effect at the highest dose tested. Confidence in the database is low because the supporting studies are of equally low quality, and teratogenic and reproductive endpoints are not well studied. Low confidence in the RfD follows.

OEHHA notes that more reproduction/developmental studies have been published that support the RfD and oral REL since U.S. EPA published its findings (U.S. EPA, 1998). In general, these studies indicate that reproductive and developmental effects occur at doses greater than an order of magnitude above the NOAEL established by MacKenzie *et al*. (1958) and the NTP (1996a,b, 1997). However, the dose levels used were relatively high such that a NOAEL was typically lacking.

VII. Data Strengths and Limitations for Development of the REL

The major strength of the inhalation REL for chromic acid mist is the use of human data. The major uncertainties for this inhalation REL is the lack of controlled and quantified exposure data and the lack of a NOAEL in the key chromic acid study.

The suitably thorough analysis of lower airway effects and the development of a BMC from continuous data are strengths for the Cr(VI) dust inhalation REL. Limitations include the lack of comprehensive data on multi-organ effects, the lack of chronic studies, the lack of upper airway analysis in the key study, and the lack of quantified exposure data in humans. The animal studies by Glaser *et al*. (1990, 1986) suggest that the lower respiratory airway is a primary target for Cr(VI) dusts. However, occupational studies (Mancuso, 1951; Federal Security Agency, 1953; Machle and Gregorius, 1948; Wang *et al*., 1994; Walsh, 1953) indicate that nasal lesions result from exposure to Cr(VI) dusts and may, in fact, be the most sensitive indicator of human toxicity resulting from exposure to soluble Cr(VI) dusts. However, this finding is attenuated by the fact that dermal exposure to chromic acid and Cr(VI) dusts due to poor hygienic practices of workers may overestimate the airborne concentrations necessary to result in nasal lesions.

The major strength for the oral REL is the consistency of the doses resulting in NOAELs and/or LOAELs among the major and supporting studies. The major limitations for the oral REL, other than the ones noted above by U.S. EPA, are the lack of lifetime exposure studies in experimental animals and the lack of adequate oral human exposure data.

VIII. References

Adachi S, Yoshimura H, Katayama H, Takemoto K. 1986. [Effects of chromium compounds on the respiratory system. IV. Long term inhalation of chromic acid mist in electroplating to ICR female mice.] Sangyo Igaku (Jpn. J. Ind. Health) 28:283-287. [cited in ATSDR, 1993].

Adachi S. 1987. [Effect of chromium compounds on the respiratory system. V. Long term inhalation of chromic acid mist in electroplating by C57BL female mice and recapitulation on our experimental studies.] Sangyo Igaku (Jpn. J. Ind. Health) 29:17-33. [cited in ATSDR, 1993].

Al-Hamood MH, Elbetieha A, and Bataineh H. 1998. Sexual maturation and fertility of male and female mice exposed prenatally and postnatally to trivalent and hexavalent chromium compounds. Reprod. Fertil. Dev. 10:179-183.

ATSDR. 1993. Agency for Toxic Substances and Disease Registry. Toxicological Profile for Chromium. Prepared by Syracuse Research Corporation under subcontract to Clement International Corporation under Contract No. 205-88-0608. Prepared for U.S. Department of Health and Human Services, Public Health Service, ATSDR.

ATSDR. 1998. Agency for Toxic Substances and Disease Registry. Toxicological Profile for Chromium. Prepared by Sciences International under subcontract to Research Triangle Institute under Contract No. 205-93-0606. Prepared for U.S. Department of Health and Human Services, Public Health Service, ATSDR.

Anwar RA, Langham, FF, Hoppert CA, Alfredson BV, and Byerrum RU. 1961. Chronic toxicity studies. III. Chronic toxicity of cadmium and chromium in dogs. Arch. Environ. Health 3:456-460.

Bloomfield JJ, and Blum W. 1928. Health hazards in chromium plating. Public Health Rep 43:2330-2351.

CARB. 1988. California Air Resources Board. Proposed Hexavalent Chromium Control Plan. Staff Report. Date of Release: January 1988.

CARB. 1997. California Air Resources Board. Toxic Air Contaminant Identification List (Summaries). Date of release: September 1997.

CARB. 2000. California Air Resources Board. California Emissions Inventory Development and Reporting System (CEIDARS). Data from Data Base Year 1998. February 12, 2000.

CDPR. 1998. California Department of Pesticide Regulation. Summary of Pesticide Use Report Data, 1998, Indexed by Chemical. http://www.cdpr.ca.gov/docs/pur/pur98rep/chmrpt98.pdf

Chowdhury AR, and Mitra C. 1995. Spermatogenic and steroidogenic impairment after chromium treatment in rats. Indian J. Exp. Biol. 33:480-484.

Cohen MD, Zelikoff JT, Chen L-C, and Schlesinger RB. 1998. Immunotoxicologic effects of inhaled chromium: Role of particle solubility and co-exposure to ozone. Toxicol. Appl. Pharmacol. 152:30-40.

Cohen SR, Davis DM, and Kramkowski PE. 1974. Clinical manifestations of chromic acid toxicity: Nasal lesions in electroplate workers. Cutis 13:558-568.

Costa M. 1997. Toxicity and carcinogenicity of Cr(VI) in animal models and humans. Crit. Rev. Toxicol. 27(5):431-442.

CRC. 1994. CRC Handbook of Chemistry and Physics, 75th edition. Lide DR, ed. Boca Raton, FL: CRC Press Inc.

Elbetieha A, and Al-Hamood MH. 1997. Long-term exposure of male and female mice to trivalent and hexavalent chromium compounds: effect on fertility. Toxicology 116:39-47.

Federal Security Agency. 1953. Health of workers in chromate producing industry. A study. U.S. Public Health Service Publication No. 192. Washington, DC. U.S. Government Printing Office.

Franchini I, and Mutti A. 1988. Selected toxicological aspects of chromium (VI) compounds. Sci. Total Env. 71:379-387. [cited in ATSDR, 1993].

Glaser U, and Hochrainer H. 1988. Report on the subchronic inhalation toxicity of sodium dichromate in rats with attachment and cover letter dated 112089. Industrial Health Foundation Inc. Available from: EPA\OTS Doc#FYI-OTS-1289-0729; Fiche No. OD-NTIS/OTS 0000729.

Glaser U, Hochrainer D, Kloppel H, and Oldiges H. 1986. Carcinogenicity of sodium dichromate and chromium (VI/III) oxide aerosols inhaled by male Wistar rats. Toxicology 42:219-232.

Glaser U, Hochrainer D, Kloppel H, and Kuhnen H. 1985. Low level chromium (VI) inhalation effects on alveolar macrophages and immune functions in Wistar rats. Arch. Toxicol. 57:250-256.

Glaser U, Hochrainer D, Steinhoff, D. 1990. Investigation of irritating properties of inhaled Cr(VI) with possible influence on its carcinogenic action. In: Environmental Hygiene II. Seemayer NO, Hadnagy W. (eds.) Berlin/New York: Springer-Verlag.

Gomes ER. 1972. Incidence of chromium-induced lesions among electroplating workers in Brazil. Ind. Med. 41(12);21-25.

HSDB. 2000. Hazardous Substances Data Bank. National Library of Medicine, Bethesda, MD. Available online through Toxicology Data Network at http://toxnet.nlm.nih.gov

Huvinen M, Uitti J, Zitting A, Roto P, Virkola K, Kuikka P, Laippala P, and Aitio A. 1996. Respiratory health of workers exposed to low levels of chromium in stainless steel production. Occup. Environ. Med. 53:741-747.

Johansen M, Overgaard E, and Toft A. 1994. Severe chronic inflammation of the mucous membranes in eyes and upper respiratory tract due to work-related exposure to hexavalent chromium. J. Laryngol. Otol. 108:591-592.

Junaid M, Murthy RC, and Saxena DK. 1995. Chromium fetotoxicity in mice during late pregnancy. Vet. Hum. Toxicol. 37(4):320-323.

Junaid M, Murthy RC, and Saxena DK. 1996a. Embryo- and fetotoxicity of chromium in pregestationally exposed mice. Bull. Environ. Contam. Toxicol. 57:327-334.

Junaid M, Murthy RC, and Saxena DK. 1996b. Embryotoxicity of orally administered chromium in mice: Exposure during the period of organogenesis. Toxicol. Lett. 84:143-148.

Kleinfeld M, and Rosso A. 1965. Ulcerations of the nasal septum due to inhalation of chromic acid mist. Ind. Med. Surg. 34:242-243.

Kanojia RK, Junaid M, and Murthy RC. 1996. Chromium induced teratogenicity in female rat. Toxicol. Lett. 89:207-213.

Kanojia RK, Junaid M, and Murthy RC. 1998. Embryo and fetotoxicity of hexavalent chromium: a long-term study. Toxicol. Lett. 95:165-172.

Lindberg E, and Hedenstierna G. 1983. Chrome plating: symptoms, findings in the upper airways, and effects on lung function. Arch. Environ. Health 38(6):367-374.

Linberg E, and Vesterberg O. 1983. Urinary excretion of proteins in chromeplaters, exchromeplaters and referents. Scand. J. Work. Environ. Health 9:505-510.

Lucas JB, and Kramkowski RS. 1975. Health hazard evaluation/ toxicity determination report H.H. 74-87-221 Industrial Platers Inc. Columbus, OH. Cincinnati, OH: National Institute for Occupational Safety and Health. NTIS #PB 249 401.

Machle W, and Gregorius F. 1948. Cancer of the respiratory system in the United States chromate-producing industry. Public Health Rep 63(35):1114-1127.

MacKenzie RD, Byerrum RU, Decker CF, Hoppert CA, and Langham RF. 1958. Chronic toxicity studies. II. Hexavalent and trivalent chromium administered in drinking water to rats. AMA Arch. Ind. Health. 18:232-234.

Malsch PA, Proctor DM, and Finley BL. 1994. Estimation of a chromium inhalation reference concentration using the benchmark dose method: A case study. Regul. Toxicol. Pharmacol. 20:58-82.

Mancuso TF. 1951. Occupational cancer and other health hazards in a chromate plant: A medical appraisal. II. Clinical and toxicologic aspects. Ind. Med. Surg. 20(9):393-407.

Murthy RC, Junaid M, and Saxena DK. 1996. Ovarian dysfunction in mice following chromium (VI) exposure. Toxicol. Lett. 89:147-154.

Nettesheim P, Hanna MG Jr., Doherty DG, Newell RF, and Hellman A. 1971. Effect of calcium chromate dust, influenza virus, and 100 R whole-body x radiation on lung tumor incidence in mice. J. Natl. Cancer Inst. 47(5):1129-1144.

NTP. 1996a. National Toxicology Program. Final report on the reproductive toxicity of potassium dichromate (hexavalent) (CAS No. 7778-50-9) administered in diet to SD rats. Research Triangle Park, NC. December 16, 1996.

NTP. 1996b. National Toxicology Program. Final report on the reproductive toxicity of potassium dichromate (hexavalent) (CAS No. 7778-50-9) administered in diet to BALB/c mice. Research Triangle Park, NC. January 10, 1997.

NTP. 1997. National Toxicology Program. Final report on the reproductive toxicity of potassium dichromate (hexavalent) (CAS No. 7778-50-9) administered in diet to BALB/c mice. Research Triangle Park, NC. February 25, 1997.

Sorahan T, Burges DCL, Hamilton L, and Harrington JM. 1998. Lung cancer mortality in nickel/chromium platers, 1946-95. Occup. Environ. Med. 55:236-242.

SCAQMD. 2000. South Coast Air Quality Management District. MATES-II. Multiple Air Toxics Exposure Study in the South Coast Air Basin. Final Report. Diamond Bar, CA: SCAQMD. March. p. 3-10.

Trivedi B, Saxena DK, Murthy RC, and Chandra SV. 1989. Embryotoxicity and fetotoxicity of orally administered hexavalent chromium in mice. Reprod. Toxicol. 3:275-278.

U.S. EPA. 1984. United States Environmental Protection Agency. Health Assessment Document for Chromium. Environmental Criteria and Assessment Office, Office of Research and Development. Research Triangle Park, NC: U.S. EPA.

U.S. EPA. 1996. Integrated Risk Information System (IRIS). Chromium (VI).

U.S. EPA. 1998. United States Environmental Protection Agency. Toxicological Review of Hexavalent Chromium. In Support of Summary Information on the Integrated Risk Information System (IRIS). Washington, DC.

Vigliani EC, and Zurlo N. 1955. Erfahrung der Clinica del Lavoro mit einigen maximalen Arbeitsplatzkonzentrationen (MAK) von Industriegiften. Arch. Gewerbepath. Gewerbhyg. 13:528-534. (Ger.)

Walsh EN. 1953. Chromate hazards in industry. JAMA 153(14):1305-1308.

Wang X, Qin Q, Xu X, Xu J, Wang J, Zhou J, Huang S, Zhai W, Zhou H, and Chen J. 1994. Chromium-induced early changes in renal function among ferrochromium-producing workers. Toxicology 90:93-101.

Zhang J, and XiLin L. 1987. Chromium pollution of soil and water in Jinzhou. J. Chinese Prevent. Med. 21:262-264.

Zhang J, and Li S. 1997. Cancer mortality in a Chinese population exposed to hexavalent chromium in water. J. Occup. Environ. Med. 39(4):315-319.

CHRONIC TOXICITY SUMMARY

CRESOL MIXTURES

Compounds	*Synonyms*	*CAS Reg. No.*
cresols	cresylic acid; tricresol; hydroxytoluene; methylphenol	1319-77-3
o-cresol	1-hydroxy-2-methylbenzene; 2-hydroxytoluene; 2-methylphenol	95-48-7
m-cresol	1-hydroxy-3-methylbenzene; 3-hydroxytoluene; 3-methylphenol	108-39-4
p-cresol	1-hydroxy-4-methylbenzene; 4-hydroxytoluene; 4-methylphenol	106-44-5

I. Chronic Toxicity Summary

Inhalation reference exposure level	**600 µg/m^3** (100 ppb)
Critical effect(s)	Neurotoxicity
Hazard index target(s)	Nervous system

II. Chemical Property Summary (HSDB, 1995; CRC, 1994, unless otherwise noted)

Description	Colorless in pure form; yellowish, brownish-yellow, or pinkish liquid
Molecular formula	C_7H_8O
Molecular weight	108.14 g/mol
Boiling point	191.0°C (o-cresol) 202°C (m-cresol) 201.9°C (p-cresol)
Melting point	29.8°C (o-cresol) 11.8°C (m-cresol) 35.5°C (p-cresol)
Solubility	Soluble in 50 parts water; miscible with alcohol, benzene, ether, glycerol, petroleum ether; soluble in vegetable oils, glycol
Conversion factor	4.42 µg/m^3 per ppb at 25ºC

III. Major Uses and Sources

Cresol compounds (mixtures of the ortho-, meta- and para-isomers) can be obtained from coal tar and petroleum or synthesized by sulfonation or oxidation of toluene (HSDB, 1995). Crude cresol (commercial grade) contains approximately 20% o-cresol, 40% m-cresol, and 30% p-

cresol. Phenol and xylenols are present in small amounts as contaminants. Cresylic acid compounds are called cresol when the boiling point is below 204°C.

Cresols have a wide variety of uses including the manufacture of synthetic resins, tricresyl phosphate, salicylaldehyde, coumarin, and herbicides. Cresols also serve as components of degreasing compounds in textile scouring and paintbrush cleaners as well as fumigants in photographic developers and explosives. Cresols also function as antiseptics, disinfectants, and parasiticides in veterinary medicine. An approximate breakdown of cresol and cresylic acid use is 20% phenolic resins, 20% wire enamel solvents, 10% agricultural chemicals, 5% phosphate esters, 5% disinfectants and cleaning compounds, 5% ore flotation, and 25% miscellaneous and exports.

Any combustion process, which results in the generation of phenolic compounds (such as automobile exhaust or coal, wood, or trash smoke), may be a potential source of exposure to cresols. Cresols are also formed from the atmospheric photooxidation of toluene. However, under normal conditions low vapor pressure limits the inhalation hazard presented by cresols (HSDB, 1995). The annual statewide industrial emissions from facilities reporting under the Air Toxics Hot Spots Act in California based on the most recent inventory were estimated to be 8407 pounds of mixtures of cresols (cresylic acid), 3 pounds of m-cresol, and 3 pounds of o-cresol (CARB, 2000).

IV. Effects of Exposures to Humans

Brief exposure to 6 mg cresol/m^3 resulted in irritation of the throat and nose, nasal constriction, and dryness in 8 of 10 subjects (Uzhdavini *et al.*, 1972).

Chemical burns may result from exposure to cresols (Pegg and Campbell, 1985). The lungs of humans exposed to cresols have shown signs of emphysema, edema, bronchopneumonia, and small hemorrhages (Clayton and Clayton, 1982). Skin contact has resulted in the development of white patches and blistering, eventually turning brown or black (Lefaux, 1968). Other reported effects include turbidity, inflammation, and fatty degeneration of the liver, nephritis, and hemorrhage of the epicardium and endocardium. An infant fatally exposed to ~20 ml of a 90% cresol solution dermally showed widespread edema of the internal organs, especially the brain and kidney (Green, 1975). The liver showed signs of centrilobular and midzonal necrosis.

Chronic systemic poisoning by any route of exposure may produce symptoms of vomiting, dysphagia, salivation, diarrhea, loss of appetite, headache, fainting, dizziness, and mental disturbances (Sittig, 1981). Skin rash and discoloration may also result from prolonged or repeated exposure of the skin. Death may result from severe damage to the liver and kidneys. Oral poisoning has resulted in kidney problems (likely from the direct action of cresol) and pancreatitis (from constriction of the pancreatic ducts) (Klimkiewicz *et al.*, 1974, as reported in HSDB, 1995).

V. Effects of Exposures to Animals

The effects of inhaled o-cresol were examined in several species (Uzhdavini *et al.*, 1972, as reported in ATSDR, 1992 and U.S. EPA, 1982). Cats exposed for 30 minutes to 5-9 mg o-cresol/m^3 showed signs of respiratory irritation as indicated by increased parotid gland secretions. Exposure of mice for 2 hrs/day for 1 month to 50 mg o-cresol/m^3 did not have an effect on mortality, however, heart muscle degeneration and degeneration of nerve cells and glial elements were observed.

Uzhdavini *et al.* (1972) exposed rats (both sexes, numbers not stated) by inhalation to 9.0 ± 0.9 mg o-cresol/m^3, first for 2 months (6 hours/day, 5 days/week), then for 2 more months (4 hours/day, 5 days/week). Endpoints examined in rats included elementary conditioned defensive reflex, white blood cell levels, bone marrow elements, and liver function (as indicated by increased susceptibility to hexobarbital narcosis). Both cresol-exposed and control animals showed some loss of the defensive reflex; the effect occurred in all exposed animals before the end of the second month and in control animals at later times. White blood cell counts were elevated in male animals, peaked at the end of the exposure period, and returned to normal one month after cessation of exposure. Exposed animals also showed a statistically significant change in the leukoid-to-erythroid ratio in the bone marrow. Liver toxicity was suggested by an extension in the duration of hexobarbital narcosis in treated animals. Although guinea pigs were similarly evaluated for changes in blood cell counts and ECG, scant reporting of experimental detail limits the usefulness of this portion of the study.

NR rats were exposed by inhalation to 0.0052 or 0.05 mg tricresol/m^3 for 3 months (Kurliandskii *et al.*, 1975; as described by U.S. EPA, 1982). The proportional composition of the compound was not specified. Effects observed in the high-dose group included decreased weight gain, increased central nervous system excitability, increased oxygen consumption, and histological changes in the lung and liver. Serum gamma-globulin levels were also reduced. No effects were observed in the low-dose group. Rats (6/group, sex unspecified) were also exposed for 24 hours to 0.01, 0.1, and 2.4 mg tricresol/m^3 with a control group of 6 rats for each exposure group. The absorption of neutral red dye by lung tissue was used as an indicator of protein denaturation in the tissue. Significantly increased dye absorption over control animals was observed at both 2.4 and 0.1 mg tricresol/m^3. The degree of dye absorption in the low-dose group was not significantly increased over controls.

In a 90-day subchronic toxicity study (U.S. EPA, 1986), 30 Sprague-Dawley rats/sex/dose were gavaged daily with 0, 50, 175, or 600 mg/kg/day p-cresol. Body and organ weights, food consumption, mortality, clinical signs of toxicity, and clinical pathology were evaluated. At 600 mg/kg/day, o-cresol showed 47% combined mortality (9/30 males, 19/30 females), and a 30% reduction in body weight at week 1 and 10% at necropsy. Kidney-to-body weight ratio was 13% higher than that of the control value at the end of the study. CNS effects such as lethargy, ataxia, coma, dyspnea, tremor, and convulsions were seen within 15 to 30 minutes after dosing; but recovery occurred within 1 hour post-gavage. At 450 mg/kg/day, combined mortality was 10% (1/10 male, 1/10 female). In the 175 mg/kg/day group, two animals exhibited tremors on day 1 of the study during the hour following gavage administration, and one of the two became comatose. At 50 mg/kg/day, no significant adverse effects were observed (USEPA, 1999a,b).

In a 90-day neurotoxicity study (U.S. EPA, 1987), 10 Sprague-Dawley rats/sex/dose were gavaged daily with o-cresol at 0, 50, 175, 450, or 600 mg/kg/day. In addition to the parameters evaluated above, various signs of neurotoxicity were monitored. The lowest dose of o-cresol caused clinical signs of CNS-stimulation post-dosing, such as salivation, rapid respiration, and hypoactivity; however, these symptoms were low in incidence and sporadic in nature. Higher doses of o-cresol (greater than 450 mg/kg/day) produced significant neurological events, such as increased salivation, urination, tremors, lacrimation, palpebral closure, and rapid respiration. Animals given high doses also showed abnormal patterns in the neurobehavioral tests. The NOAEL based on systemic toxicity was 50 mg/kg/day (USEPA, 1999a,b).

Dermal exposure of rats to 1.0-1.7 ml cresol/kg body weight for 1-2 hours resulted in skin discoloration and death of the animals (Campbell, 1941).

Exposure to high concentrations of toluene vapors, or to intravenous o-cresol, a toluene metabolite, at about 0.9 mg/min, caused excitation of the somatosensory evoked potential (SEP) and electroencephalograph (EEG) of Fischer 344 rats (Mattsson *et al*., 1989). Both substances induced an increase in EEG beta activity and caused a large increase in activity at 5 Hz. Toluene exposed rats were lightly anesthetized, while o-cresol rats were conscious but hyperreactive. When exposure was continued, both sets of rats had involuntary muscle movements and tremors. Neither benzoic acid and hippuric acid, also metabolites of toluene, caused neuroexcitation. The authors concluded that metabolically derived cresols are plausible candidates for the neuroexcitatory properties of toluene.

In rat liver slices at equimolar concentrations, p-cresol was 5- to 10-times as toxic as the o- or m-isomers for cell killing (Thompson *et al*., 1994). p-Cresol rapidly depleted intracellular glutathione levels, while the o- and m-isomers depleted it to a lesser extent. p-Cresol was metabolized to a reactive intermediate which bound covalently to protein. The reaction was inhibited by N-acetylcysteine.

The National Toxicology Program (NTP) sponsored reproductive toxicity tests of cresol isomers in Swiss CD-1 mice using the risk assessment by continuous breeding (RACB) protocol (Heindel *et al*., 1997a, 1997b). For o-cresol the exposure concentrations in the continuous cohabitation task were 0.05%, 0.2%, and 0.5% in feed (approximately 60, 220, and 550 mg/kg/day (Heindel *et al*., 1997a). At these doses o-cresol was not a reproductive toxicant. When a m-/p-cresol mixture was used at concentrations of 0.25, 1.0 and 1.5% in feed (approximately 370, 1500, and 2100 mg/kg/day), the m/p mixture was a reproductive toxicant, since (1) fewer F_1 pups per litter were produced, (2) both generations showed reduced pup weights, and (3) reproductive organs showed weight reductions. Unfortunately the responses were not dose-dependent and the mixture was judged not to be a selective reproductive toxicant. Oral gavage administration of o-, m-, or p-cresol, separately, in rats did not produce selective reproductive toxicity; i.e., for each of the cresol isomers, in the absence of parental toxicity, there was no reproductive toxicity. The NOEL for reproductive toxicity for each isomer was 175 mg/kg/day (Tyl 1989a, 1989b, 1989c).

VI. Derivation of Inhalation Chronic Reference Exposure Level

Study	U.S. EPA, 1987
Study population	Sprague-Dawley rats
Exposure method	Gavage at 0, 50, 175, 450, or 600 mg/kg-day
Critical effects	Decreased body weights and neurotoxicity (tremors, salivation, lacrimation, etc.)
LOAEL	175 mg/kg-day
NOAEL	50 mg/kg-day
Exposure continuity	Daily gavage
Exposure duration	90 days
LOAEL uncertainty factor	1
Subchronic uncertainty factor	3 (90 day study)
Interspecies uncertainty factor	10
Intraspecies uncertainty factor	10
Cumulative uncertainty factor	300
U.S. EPA Reference Dose (RfD)	0.17 mg/kg/day
Route-to-route extrapolation factor	3500 $\mu g/m^3$ per mg/kg/day
Inhalation chronic REL	600 $\mu g/m^3$ (100 ppb)

An RfD of 0.05 mg/kg/day was derived by the USEPA for both o-cresol and m-cresol (USEPA 1998a, 1998b; listed as 2-methylphenol and 3-methylphenol). The RfD for p-cresol was withdrawn by the USEPA. U.S EPA used a subchronic uncertainty factor of 10 for a 90 day study in rats. In accordance with its approved methodology (OEHHA, 2000), OEHHA used a factor of 3.

The available literature on the observed toxicity of cresol compounds and cresol mixtures to humans by inhalation indicates that at high concentrations these compounds are initially toxic due to their ability to cause chemical burns and are therefore of concern at the site of contact. In humans occupationally exposed, inhalation exposure is reported to cause respiratory effects including the development of pneumonia, pulmonary edema, and hemorrhage (Clayton and Clayton, 1982). Other case reports of cresol toxicity to humans are confounded by the presence of other compounds, such as phenol, formaldehyde, and ammonia (Corcos, 1939; NIOSH, 1974). The only quantitative information from inhalation exposures to humans, however, comes from acute exposure studies showing irritation at 6 mg cresol/m^3 (Uzhdavini *et al.*, 1972, as reported in ATSDR, 1992). Toxic effects reported in animals include bone marrow and liver toxicity in rats from 4 month exposure to 9 mg cresol/m^3 (Uzhdavini *et al.*, 1972, as reported in U.S. EPA, 1982). Other animal studies have shown more systemic effects from inhalation exposure to cresols. Uzhdavini *et al.*, 1972 reported cardiac and nerve cell degeneration in mice exposed for 2 hour/day for 1 month to 50 mg o-cresol/m^3. Kurliandskii *et al.* (1975) (as reported in HSDB, 1995) observed decreased weight gain with histological changes in the liver and lungs of rats exposed for 3 months to 0.05 mg tricresol/m^3. Although this study reports adverse effects at levels below those observed in the Uzhdavini *et al.* (1972) study, limited experimental detail precludes the use of these data in the development of the chronic REL.

The only useful inhalation data for the development of a chronic REL are those showing hematological toxicity to the bone marrow of rats exposed for 4 months to o-cresol (Uzhdavini *et al*. (1972) as reported in U.S. EPA, 1982). These authors report a LOAEL of 9 mg tricresol/m^3. OEHHA staff decided not to use this study. (1) A complete translation from the original Russian was not available so that only the interpretations of others were available. (2) Some endpoints tested are not commonly used in toxicology. And (3) some of the results reported were unusual (e.g., elevation of white blood cells in male but not female rats).

As noted above, the inhalation study conducted by Kurliandskii *et al.* (1975) suggests that adverse health effects occur in experimental animals at exposure levels considerably below those reported by Uzhdavini *et al.* (1972) (9 mg/m^3 vs. 0.05 mg/m^3). The report from which the lower level is drawn has limitations. Human subjects exposed briefly to levels below the LOAEL have reported respiratory irritation.

VII. Data Strengths and Limitations for Development of the REL

The strengths of the REL for cresols include the use of measured exposure data of animals exposed over a significant fraction of their lifetime. Major areas of uncertainty are route-to-route extrapolation, the lack of chronic human data, and the paucity of reproductive and developmental toxicity studies. Additional inhalation studies of cresols will be useful.

VIII. References

ATSDR. 1992. Agency for Toxic Substances and Disease Registry. Toxicological profile for cresols: o-cresol, p-cresol, and m-cresol. Atlanta, GA: ATSDR. NTIS No. PB93-110732.

CARB. 2000. California Air Resources Board. California Emissions Inventory Development and Reporting System (CEIDARS). Data from Data Base Year 1998. February 12, 2000.

Campbell I. 1941. Petroleum cresylic acids. A study of their toxicity and the toxicity of cresylic disinfectants. Soap Sanitation Chem. 17:103.

Chao J, Lin CT, and Chung TH. 1983. Vapor pressure of coal chemicals. J. Chem. Refer. Data 12:1033-1063.

CRC. 1994. CRC Handbook of Chemistry and Physics, 75th edition. Lide DR, ed. Boca Raton, FL: CRC Press Inc.

Clayton GD, and Clayton FE (eds). 1982. Patty's Industrial Hygiene and Toxicology. Third, revised ed. New York: John Wiley Sons, p. 2133.

Corcos A. 1939. [Contribution to the study of occupational poisoning by cresols]. Dissertation. Paris (Vigot Freres Editeurs) (in French).

Daubert TE, and Danner RD. 1985. Data compilation tables of properties of pure compounds. In: Design Institute for Physical Property Data. New York: American Institute for Chemical Engineers.

Green MA. 1975. A household remedy misused - fatal cresol poisoning following cutaneous absorption (a case report). Med. Sci. Law 15:65-66.

Heindel J, George J, Fail P, Izard MK, and Grizzle T. 1997a. o-Cresol. Environ. Health Perspect. 105(Suppl 1):293-294.

Heindel J, Izard MK, George J, Fail P, and Grizzle T. 1997b. m-/p-Cresol. Environ. Health Perspect. 105(Suppl 1):295-296.

HSDB. 1995. Hazardous Substances Data Bank. National Library of Medicine, Bethesda, Maryland (CD-ROM Version). Denver, CO: Micromedex, Inc. (Edition expires 7/31/96).

Klimkiewicz E, Klinger M, Kurbiel A, and Soltys W. 1974. [Case of liquid lysol poisoning complicated by acute pancreatitis and acute renal failure]. Wiad. Lek. 27:1211-1214.

Kurliandskii BA, Partsef DI, and Chernomorskii AR. 1975. [A method of determining the daily average maximum permissible concentration of tricresol in the atmosphere]. Gig. Sanit. 5:85-87.

Lefaux R. (ed.) 1968. Practical Toxicology of Plastics. Cleveland: CRC Press, Inc.

Mattsson JL, Albee RR, and Gorzinski SJ. 1989. Similarities of toluene and o-cresol neurotoxication in rats. Neurotoxicol. Teratol. 11:71-75.

NIOSH. 1974. National Institute for Occupational Safety and Health. Health hazard evaluation / Toxicity determination report no. 73-5-110. Prepared by Cummins Northeastern Incorporated. Dedham, MA.

OEHHA. 2000. Office of Environmental Health Hazard Assessment. Air Toxics Hot Spots Program Risk Assessment Guidelines. Part III. Technical Support Document for the Determination of Noncancer Chronic Reference Exposure Levels. Available on-line at http://www.oehha.ca.gov

Pegg SP, and Campbell DC. 1985. Children's burns due to cresol. Burns Incl. Therm. Inj. 11(4):294-296.

Sittig M. (ed) 1981. Handbook of Toxic and Hazardous Chemicals. Park Ridge, NJ: Noyes Data Corporation.

Thompson DC, Perera K, Fisher R, and Brendel K. 1994. Cresol isomers: comparison of toxic potency in rat liver slices. Toxicol. Appl. Pharmacol. 125(1):51-58

Tyl RW. 1989a. Two Generation Reproduction Study of o-Cresol (CAS No. 95-48-7) Administered by Gavage to Sprague-Dawley Rats. Bushy Run Research Center, Export, PA. Project Report 51-614.

Tyl RW. 1989b. Two Generation Reproduction Study of m-Cresol (CAS No. 108-39-4) Administered by Gavage to Sprague-Dawley Rats. Bushy Run Research Center, Export, PA. Project Report 51-634.

Tyl RW. 1989c. Two Generation Reproduction Study of p-Cresol (CAS No. 106-44-5) Administered by Gavage to Sprague-Dawley Rats. Bushy Run Research Center, Export, PA. Project Report 52-512.

U.S. EPA 1982. United States Environmental Protection Agency. Health effects assessment for cresols. Office of Research and Development, Office of Health and Environmental Assessment, Environmental Criteria and Assessment Office. EPA/540/1-86/050. Cincinnati, OH: US EPA. NTIS. PB86-134616.

U.S. EPA. 1986. o, m, p-Cresol. 90-Day oral subchronic toxicity studies in rats. Office of Solid Waste. Washington, DC: U.S. EPA.

U.S. EPA. 1987. o, m, p-Cresol. 90-Day oral subchronic neurotoxicity study in rats. Office of Solid Waste. Washington, DC: U.S. EPA.

U.S. EPA. 1999a. 2-Methyphenol. Integrated Risk Information System (IRIS).

U.S. EPA. 1999b. 3-Methylphenol. Integrated Risk Information System (IRIS).

Uzhdavini ER, Astaf'yeva K, Mamayeva AA, and Bakhtizina GZ. 1972. [Inhalation toxicity of o-cresol]. Trudy Ufimskogo Nauchno-Isseldovatel'skogo Instituto Gigiyeny Profzabolevaniya 7:115-119. (in Russian)

CHRONIC TOXICITY SUMMARY

1,4-DICHLOROBENZENE

(p-dichlorobenzene; di-chloricide; p-dichlorobenzol; Paradow; Paramoth; Parazene; p-chlorophenyl chloride)

CAS Registry Number: 106-46-7

I. Chronic Toxicity Summary

Inhalation reference exposure level	**800 µg/m^3** (100 ppb)
Critical effect(s)	General effects (reduced body weights and food consumption) in rats CNS effects (tremors) in rats Respiratory/dermal effects (nasal and ocular discharge) in rats Liver effects (increased liver weight) in rats, and Kidney effects (increased kidney weight) in rats.
Hazard index target(s)	Nervous system; respiratory system; alimentary system; kidney

II. Chemical Property Summary (HSDB, 1997; CRC, 1994)

Description	White crystals, monoclinic prisms
Molecular formula	$C_6H_4Cl_2$
Molecular weight	147.01 g/mol
Boiling point	174°C
Melting point	52.7^{o}C
Vapor pressure	10 torr @ 54.8^{o}C
Solubility	Soluble in chloroform, carbon disulfide, alcohol, ether, acetone, benzene
Conversion factor	1 ppm = 6.0 mg/m^3 at 25ºC

III. Major Uses and Sources

Commercial grade 1,4-dichlorobenzene (1,4-DCB) is available in the USA as a technical grade liquid, typically containing a small percentage (>0.1% by weight) of meta (1,3-DCB) and ortho (1,2-DCB) isomers; as a solution in solvent or oil suspension; or as crystalline material pressed into various forms (HSDB, 1997). Besides its role as an intermediate in the synthesis of various organics, dyes and pharmaceuticals, 1,4-dichlorobenzene is used as a space or garbage deodorizer for odor control. The insecticidal and germicidal properties of 1,4-dichlorobenzene are used to control fruit borers and ants, moths, blue mold in tobacco seed beds, and mildew and mold on leather or fabrics. In 1996, the latest year tabulated, the statewide mean outdoor

monitored concentration of 1,4-DCB was approximately 0.15 ppb (CARB, 1999). The annual statewide industrial emissions from facilities reporting under the Air Toxics Hot Spots Act in California based on the most recent inventory were estimated to be 30,577 pounds of dichlorobenzene (CARB, 2000).

IV. Effects of Human Exposure

Case reports of human exposure to 1,4-DCB include malaise, nausea, hepatic manifestations (yellow atrophy and cirrhosis), proteinuria, bilirubinuria, hematuria, and anemia. A woman exposed to 1,4-DCB for 6 years developed central nervous system effects, including severe cerebellar ataxia, dysarthria, weakness in all limbs, and hyporeflexia (U.S. EPA, 1985).

No epidemiologic studies of 1,4-DCB exposures were located.

V. Effects of Animal Exposure

Rats, rabbits and guinea pigs were exposed to 0, 96, 158, 341 or 798 ppm (0, 577, 950, 2050 or 4800 mg/m^3) 1,4-DCB by inhalation 7 hours/day, 5 days/week for 6-7 months (Hollingsworth *et al.*, 1956). High dose animals showed marked tremors, weakness, loss of weight, eye irritation and unconsciousness. Liver and kidney changes included cloudy swelling and centrilobular cellular degeneration (liver). In another inhalation study in rats animals were exposed to 0, 75 or 500 ppm (0, 451 or 3006 mg/m^3) for 5 hours/day, 5 days/week for 76 weeks (Riley *et al.*, 1980). The authors found increased kidney and liver weights in the high dose group. Thus 75 ppm was a NOAEL. Studies with oral exposure to 1,4-DCB, including the NTP (1987) chronic bioassay study (maximum dose of 300 mg/kg-day), have also found an increased incidence of renal and hepatic lesions (cellular degeneration and focal necrosis).

Three inhalation reproductive studies, one in rabbits (Hayes *et al.*, 1985), one in mice (Anderson and Hodge, 1976), and one in rats (Chlorobenzene Producers Assn., 1986), found minimal reproductive effects. In rabbits exposed on days 6-18 of gestation to 100, 300, and 800 ppm 1,4-DCB, only the differences in percentage of implantations resorbed and in percentage of litters with resorptions were significantly increased and only in the 300 ppm group (Hayes *et al.*, 1985). No reduction in reproductive performance was observed in mice exposed to 0, 75, 225, or 450 ppm 1,4-DCB for 6 hours/day for 5 days (Anderson and Hodge, 1976).

In a two-generation reproductive study (Chlorobenzene Producers Association, 1986), Sprague-Dawley rats P1 (28/sex/group) were exposed to 0, 50, 150 or 450 ppm (0, 301, 902, or 2705 mg/m^3) of 1,4-DCB vapor, 6 hours/day, 7 days/week for 10 weeks, and then mated for 3 weeks. The second generation F1 weanlings were exposed to 1,4-DCB for 11 weeks and then mated. No developmental abnormalities were observed in pups examined. At 450 ppm significant decreases in live births, pup weights, and pup survival were seen in both the F1 and F2 generations. Non-reproductive effects observed in the parental males in the 150 and 450 ppm groups included significantly increased liver and kidney weights. All dose levels caused hyaline droplet nephrosis in post-pubescent males; but this change was associated with the formation of

alpha-2u-globulin, an abnormality considered specific for male rats with no relative human significance (U.S. EPA, 1991). The Chlorobenzene Producers Association reproductive study was chosen by the U.S. EPA to derive the RfC.

VI. Derivation of Chronic Reference Exposure Level

Study	Chlorobenzene Producers Association, 1986
Study population	Sprague-Dawley rats (28 rats/sex/group)
Exposure method	Discontinuous whole-body inhalation exposures (0, 50, 150 or 450 ppm)
Critical effects	Reduced body weights and food consumption; tremors; nasal and ocular discharge; increased liver and kidney weights
LOAEL	150 ppm
NOAEL	50 ppm
Exposure continuity	6 hr/day for 7 days/week
Average experimental exposure	13 ppm for NOAEL group (50 x 6/24)
Human equivalent concentration	13 ppm for NOAEL group (gas with systemic effects, based on RGDR = 1.0 using default assumption that lambda (a) = lambda (h))
Exposure duration	10 weeks
LOAEL uncertainty factor	1
Subchronic uncertainty factor	3
Interspecies uncertainty factor	3
Intraspecies uncertainty factor	10
Cumulative uncertainty factor	100
Inhalation reference exposure level	0.1 ppm (100 ppb, 0.8 mg/m^3, 800 µg/m^3)

The chronic REL for 1,4-dichlorochlorobenzene is also the U.S. EPA RfC. OEHHA agrees with the U.S.EPA analysis. A 3-fold subchronic uncertainty factor (instead of 10) was used by U.S. EPA because of data suggesting limited progression of hepatic lesions (Riley *et al.*, 1980). Ten weeks are also greater than 8% of a rat's two-year lifetime and thus in accord with OEHHA's use of a subchronic UF of 3 (OEHHA, 2000).

For comparison, Riley *et al*. (1980) found a chronic NOAEL of 75 ppm for kidney and liver effects in rats, which is equivalent to 11.2 ppm continuous exposure. Use of an RGDR of 1 and a total UF of 30 (3 for interspecies and 10 for intraspecies) results in a REL estimate of 0.4 ppm.

VII. Data Strengths and Limitations for Development of the REL

The major strengths of the REL for 1,4-dichlorochlorobenzene are the observation of a NOAEL and the demonstration of a dose-response relationship. The major uncertainties are the lack of human data and the lack of chronic, multiple-species health effects data.

VIII. References

Anderson D, and Hodge MCE. 1976. Paradichlorobenzene: Dominant lethal study in the mouse. ICI Report No. CTL/P/296. November.

CARB. 1999. California Air Resources Board. Toxics Air Quality Data. Substance Chooser. Para-Dichlorobenzene. Available online at http://www.arb.ca.gov/aqd/toxics.htm

CARB. 2000. California Air Resources Board. California Emissions Inventory Development and Reporting System (CEIDARS). Data from Data Base Year 1998. February 12, 2000.

CRC. 1994. CRC Handbook of Chemistry and Physics, 75th edition. Lide DR, ed. Boca Raton, FL: CRC Press Inc.

Chlorobenzene Producers Association. 1986. Parachlorobenzene: Two-generation reproduction study in Sprague-Dawley rats. Study 86-81-90605. MRID No. 411088-1.

Hayes WC, Hanley TR, Gushow TS, Johnson KA, and John JA. 1985. Teratogenic potential of inhaled dichlorobenzenes in rats and rabbits. Fundam. Appl. Toxicol. 5(1): 190-202.

HSDB. 1997. Hazardous Substances Data Bank. TOMES® Vol. 33. Denver, CO: Micromedex, Inc. (edition expires 7/31/97)

Hollingsworth RL, Rowe VK, Oyen F, Hoyle HR, and Spencer HC. 1956. Toxicity of paradichlorobenzene: Determinations of experimental animals and human subjects. AMA Arch. Ind. Health. 14: 138-147.

NTP. 1987. National Toxicology Program. Toxicology and carcinogenesis studies of 1,4-dichlorobenzene in F344/N rats and B6C3F1 mice (gavage studies). NTP TR 319. NIH Publ. No. 87-2575.

OEHHA. 2000. Office of Environmental Health Hazard Assessment. Air Toxics Hot Spots Program Risk Assessment Guidelines. Part III. Technical Support Document for the Determination of Noncancer Chronic Reference Exposure Levels. Available on-line at http://www.oehha.ca.gov

Riley RA, Chart IS, Doss A, Gore CW, Patton D, and Weight TM. 1980. Para-dichlorobenzene: Long-term inhalation study in the rat. ICI Report No. CTL/P/447. August, 1980.

U.S. EPA. 1985. U.S. Environmental Protection Agency. Health Assessment Document for Chlorinated Benzenes. EPA/600/8-84/015F. Prepared by the Office of Health and Environmental Assessment, Environmental Criteria and Assessment Office. Cincinnati, OH: U.S. EPA.

U.S. EPA. 1991. U.S. Environmental Protection Agency. Alpha-2u-Globulin: Association with Chemically Induced Renal Toxicity and Neoplasia in the Male Rat. EPA/625/3-91/019F.

Prepared for the Risk Assessment Forum, U.S. EPA, Washington, DC 20460. [as cited in U.S. EPA, 1994.]

U.S.EPA. 1994. U.S. Environmental Protection Agency. 1994. Integrated Risk Information System (IRIS) Database. Reference concentration (RfC) for 1,4-Dichlorobenzene. Available online at http://www.epa.gov/ngispgm3/iris

CHRONIC TOXICITY SUMMARY

1,1-DICHLOROETHYLENE

(DCE; 1,1-dichloroethene; VDC; vinylidene chloride)

CAS Registry Number: 73-35-4

I. Chronic Toxicity Summary

Inhalation reference exposure level	**70 μg/m^3** (20 ppb)
Critical effect(s)	Increased mortality; hepatic effects (mottled livers and increases in liver enzymes) in guinea pigs
Hazard index target(s)	Alimentary system

II. Physical and Chemical Properties (HSDB, 1994; CRC, 1994)

Description	Colorless liquid
Molecular formula	$C_2H_2Cl_2$
Molecular weight	96.95 g/mol
Boiling point	31.7^{o}C
Melting point	–122.5^{o}C
Vapor pressure	500 torr @ 20°C
Solubility	Soluble in water (2.5 g/L); miscible in organic solvents
Conversion factor	3.97 μg/m^3 per ppb at 25 ^{o}C

III. Major Uses and Sources

1,1-Dichloroethylene (1,1-DCE) is used in the production of polyvinylidene chloride copolymers (HSDB, 1994). 1,1-DCE containing copolymers include other compounds such as acrylonitrile, vinyl chloride, methacrylonitrile, and methacrylate. These copolymers are used in flexible packaging materials; as flame retardant coatings for fiber, carpet backing, and piping; as coating for steel pipes; and in adhesive applications. Flexible films for food packaging, such as SARAN and VELON wraps, use such polyvinylidene chloride copolymers. The annual statewide industrial emissions from facilities reporting under the Air Toxics Hot Spots Act in California based on the most recent inventory were estimated to be 2458 pounds of vinylidene chloride (CARB, 2000).

IV. Effects of Human Exposure

Limited information exists regarding the human health effects following exposure to 1,1-DCE. A few case reports and mortality studies have reported hepatotoxicity and nephrotoxicity after repeated, low-level exposures (USEPA, 1976; Ott *et al.*, 1976). However, these investigations were conducted in industrial settings with the possibility of mixed chemical exposures. In preliminary clinical findings reported by the EPA (1976), workers exposed to 1,1-DCE for 6 years or less had a high incidence of hepatotoxicity, with liver scans and measurements of liver enzymes revealing 50% or greater loss in liver function in 27 of 46 exposed workers. Unfortunately, no follow-up study was reported.

V. Effects of Animal Exposure

Several studies have reported on the subchronic or chronic toxicity of 1,1-DCE in laboratory animals exposed either via oral or inhalation routes. The liver is the primary target organ of 1,1-DCE toxicity following acute or chronic inhalation exposure. Such exposure is marked by both biochemical changes (alterations in serum enzyme levels) and histological changes (e.g., midzonal and centrilobular swelling, degeneration, and necrosis) (Gage, 1970; Lee *et al.*, 1977; Plummer *et al.*, 1990; Quast, 1976; Quast *et al.*, 1986). Unfortunately, these longer-term studies used only one or two doses or a limited number of animals.

Male and female rats exposed intermittently (6 hours/day, 5 days/week) to 125 or 200 ppm 1,1-DCE over 30 days exhibited centrilobular fatty degeneration or hepatocellular necrosis (Quast 1976, as cited by USDHHS, 1994). Two other studies identified hepatic changes in rats at lower concentrations of 1,1-DCE (6 hours/day, 5 days/week): cytoplasmic vacuolation after 30- or 90-day exposure to 25 or 75 ppm 1,1-DCE (Balmer *et al.*, 1976, as cited by USDHHS, 1994), and fatty changes after 6 months at 25 ppm 1,1-DCE (Quast *et al.*, 1986).

Laboratory animals appear less tolerant of continuous exposure (23-24 hours per day) than intermittent exposure. Beagle dogs exposed to 100 ppm 1,1-DCE for 8 hours/day, 5 days/week for 42 days had no evidence of hepatotoxicity, but continuous exposure to 48 ppm for 90 days caused liver changes (Prendergast *et al.*, 1967). Similarly, monkeys continuously exposed to 48 ppm for 90 days exhibited focal necrosis and hemosiderin deposition, while no liver toxicity was apparent following 42 days of intermittent exposure to 100 ppm 1,1-DCE (Prendergast *et al.*, 1967). Guinea pigs exposed to 1,1-DCE for 24 hours per day for 90 days (0, 5, 15, 25, or 48 ppm) displayed mottled livers at 15 ppm, and increased liver enzyme levels (serum glutamic-pyruvic transaminase (SGPT) and alkaline phosphatase (AP)) at 48 ppm. A NOAEL of 5 ppm based on liver changes (Prendergast *et al.*, 1967) is indicated by the results.

Data on continuously exposed guinea pigs from Prendergast *et al*. (1967)

ppm 1,1-DCE (mg/m^3)	*Survival*	*Body weight change*	*Liver AP*	*SGPT*
0	312/314	+69.0%	0.08±0.03	10±5
5 (20)	43/45	+58.6%	0.08±0.03	11±3
15 (61)	12/15	+55.3%	Not reported	Not reported
25 (101)	12/15	+74.0%	Not reported	Not reported
48 (191)	8/15	+50.3%	0.19±0.04	>70

Additional adverse effects observed to a lesser extent in laboratory animals include respiratory and renal toxicity. Nephrotoxicity observed following chronic 1,1-DCE exposure included gross organ (increases in kidney weight) (Klimisch *et al.*, 1979; Quast *et al.*, 1986) and histological changes (tubular swelling, degeneration, and necrosis) (Klimisch *et al.*, 1979; Lee *et al.*, 1977; Prendergast *et al.*, 1967). Continuous exposure of rats to 48 ppm 1,1-DCE for 90 days caused nuclear hypertrophy of the renal tubular epithelium (Prendergast *et al.*, 1976). Mice exposed to 25 ppm 1,1-DCE 4 hours/day, 4 or 5 days/week, for 52 weeks displayed severe tubular nephrotoxicity (Maltoni *et al.*, 1985 as cited by USDHHS, 1994). Nasal irritation was observed in rats exposed to 200 ppm for 4 weeks (Gage 1970). But no respiratory effects were attributed to 1,1-DCE exposure in rats, monkeys, dogs, rabbits, or guinea pigs exposed to 100 ppm intermittently for 6 weeks (Prendergast *et al.*, 1967) or in rats exposed to 75 ppm for 18 months (Quast *et al.*, 1986).

Toxicokinetic studies in laboratory animals have demonstrated that 1,1-DCE is readily absorbed and rapidly distributed following inhalation exposure (Dallas *et al.*, 1983; McKenna *et al.*, 1978b). Following inhalation exposure to radioactively labeled 1,1-DCE, rats preferentially accumulate radioactivity in the kidney and liver (McKenna *et al.*, 1978b; Jaeger *et al.*, 1977). Glutathione (GSH) conjugation appears to be the major detoxification route for 1,1-DCE intermediates, and GSH-depleting experimental states, such as drugs and fasting, may tend to increase 1,1-DCE toxicity (Jaeger *et al.*, 1977; McKenna *et al.*, 1978; Reichert *et al.*, 1978). One study greatly increased 1,1-DCE induced lethality and hepatotoxicity in rats by pretreatment with acetaminophen (Wright and Moore, 1991).

VI. Derivation of Chronic Reference Exposure Level (REL)

Study	Prendergast *et al.* (1967)
Study population	Guinea pigs (15 per group, except 45 animals in 20 mg/m^3 group)
Exposure method	Continuous whole body inhalation (0, 20, 61, 101, or 189 mg/m^3)
Critical effects	Increased mortality at 61, 101, and 189 mg/m^3; hepatic effects (mottled livers and increases in SGPT and AP enzymes) noted at 189 mg/m^3
LOAEL	61 mg/m^3 (15 ppm)
NOAEL	20 mg/m^3 (5 ppm)
Exposure continuity	Continuous
Exposure duration	90 days
Average experimental exposure	20 mg/m^3 for NOAEL group
Human equivalent concentration	20 mg/m^3 for NOAEL group (gas with systemic effects, based on default assumption that RGDR = 1 using default assumption that lambda (a) = lambda (h))
LOAEL uncertainty factor	1
Subchronic uncertainty factor	10 (since guinea pig life-span is approx. 6 years)
Interspecies uncertainty factor	3
Intraspecies uncertainty factor	10
Cumulative uncertainty factor	300
Inhalation reference exposure level	0.07 mg/m^3 (70 μg/m^3; 0.02 ppm; 20 ppb)

The principal study (Prendergast *et al.*, 1967) identified adverse hepatic and/or renal effects in rats (15 or 45/group), guinea pigs (15 or 45/group), dogs (2 or 6/group), and monkeys (3, 9, or 21/group) exposed to inhaled 1,1-DCE. Continuous exposure to 1,1-DCE, 24 hours/day over 90 days, demonstrated more severe effects than intermittent exposure, 6 hours/day, 5 days/week for 6 weeks, in the species tested. Unlike the other available subchronic and chronic studies, this principal study included multiple exposure levels of 0, 5, 15, 25 and 48 ppm (0, 20, 61, 101, and 189 mg/m^3). Mortality, hematologic and body weight data were well tabulated and presented in this study. Histopathologic evaluation was conducted on the heart, lung, liver, spleen and kidneys. Following continuous exposure, adverse hepatic effects included focal necrosis in monkeys (LOAEL = 189 mg/m^3, NOAEL = 101 mg/m^3), in dogs (LOAEL = 189 mg/m^3, NOAEL = 101 mg/m^3), and in rats (LOAEL = 189 mg/m^3, NOAEL = 101 mg/m^3); and altered lipid content and increases in SGPT and alkaline phosphatase in guinea pigs (LOAEL = 189 mg/m^3, NOAEL = 20 mg/m^3). Additionally, renal alterations were observed in rats as nuclear hypertrophy in the tubular epithelium (LOAEL = 189 mg/m^3, NOAEL = 61 mg/m^3). Monkeys exposed to 1,1-DCE also displayed a greater than 25% decrease in body weight (LOAEL 189 mg/m^3, NOAEL 20 mg/m^3). The subchronic study by Prendergast *et al.* (1967) was chosen over the chronic studies because of its better design, its use of continuous exposure, and its exhibition of toxic effects below the LOAELs reported in the other studies.

Although limited in number, the other chronic and subchronic studies available consistently demonstrate adverse hepatic effects following 1,1-DCE exposure (Lee *et al.*, 1977; Maltoni *et*

al., 1985; Plummer *et al.*, 1990; Quast *et al.*, 1986). Hepatocellular fatty change was observed in rats exposed to 25 ppm or 75 ppm 1,1-DCE intermittently (6 hrs/d, 5 d/wk) for 18 months. This mid-zonal fatty change was also observed at the 12-month interim sacrifice, but did not appear to progress in severity or incidence over time (Quast *et al.*, 1986). A more severe hepatocellular necrosis and renal tubular necrosis were observed in mice exposed to 55 ppm 1,1-DCE 6 hr/d, 5 d/week for 1 year (Lee *et al.*, 1977).

For comparison, Quast *et al.* (1986) determined a LOAEL of 25 ppm for liver effects of minimal severity in rats after 18 months exposure. Use of continuous time adjustment to 4.5 ppm, multiplication by an RGDR of 1, and division by a total UF of 100 (3 for LOAEL to NOAEL, 3 for interspecies, and 10 for intraspecies) results in an estimate of 45 ppb (200 $\mu g/m^3$).

VII. Data Strengths and Limitations for Development of the REL

Uncertainty factors are appropriate due to the limited number of subchronic and chronic inhalation studies (greater than 1 year duration) in laboratory animals. In addition, few industrial surveys and epidemiological studies are available on the adverse effects of 1,1-DCE in humans; these are limited by small sample size, short follow-up, and/or brief exposure periods. But this limited evidence does suggest an association between repeated exposure to 1,1-DCE and liver damage in humans (EPA, 1976), and the key study is an animal study which found adverse hepatic effects. No toxicokinetic data regarding the absorption, distribution, metabolism or excretion of 1,1-DCE in humans are available.

VIII. References

Balmer MF, Smith FA, Leach LJ, and Yuile CL. 1976. Effects in the liver of methylene chloride inhaled alone and with ethyl alcohol. Am. Ind. Hyg. Assoc. J. 37(6):345-352.

CARB. 2000. California Air Resources Board. California Emissions Inventory Development and Reporting System (CEIDARS). Data from Data Base Year 1998. February 12, 2000.

CRC. 1994. CRC Handbook of Chemistry and Physics, 75th edition. Lide DR, ed. Boca Raton, FL: CRC Press Inc.

Dallas CE, Weir FW, Feldman S, Putcha L, and Bruckner JV. 1983. The uptake and disposition of 1,1-dichloroethylene in rats during inhalation exposure. Toxicol. Appl. Pharmacol. 68(1):140-151.

Gage JC. 1970. The subacute inhalation toxicity of 109 industrial chemicals. Br. J. Ind. Med. 27:1-18.

HSDB. 1994. Hazardous Substances Data Bank. TOMES®. Denver, CO: Micromedex.

Jaeger RJ, Shoner LG, and Coffman LJ. 1977. 1,1-Dichloroethylene hepatotoxicity: Proposed mechanism of action and distribution and binding of [^{14}C] radioactivity following inhalation exposure in rats. Environ. Health Perspect. 21:113-119.

Klimisch HJ, Deckardt K, and Mitea D. 1979. [Subchronic toxicity of vinylidene chloride in rats after 6 weeks exposure.] BASF Aktiengesellschaft, Ludwigshafen: 31. (German). HSE Translation No. 9Q. [as cited in USDHHS, 1994.]

Lee CC, Bhandari JC, Winston JM, House WB, Peters PJ, Dixon RL, and Woods JS. 1977. Inhalation toxicity of vinyl chloride and vinylidene chloride. Environ. Health Perspect. 21:25-32.

Maltoni C, Lefemine P, Chieco P, Cotti G, and Patella V. 1985. Experimental research on vinylidene chloride carcinogenesis. In: Archives of Research on Industrial Carcinogenesis. Vol. 3. Maltoni C, Mehlman MA, eds. Princeton, NJ: Princeton Scientific Publishers. [as cited in USDHHS, 1994.]

McKenna MJ, Zempel JA, Madrid EO, and Gehring PJ. 1978. The pharmacokinetics of [14C] vinylidene chloride in rats following inhalation administration. Toxicol. Appl. Pharmacol. 45:599-610.

Ott MG, Fishbeck WA, Townsend JC, and Schneider EJ. 1976. A health study of employees exposed to vinylidene chloride. J. Occup. Med. 18:735-738.

Plummer JL, de la Hall PM, Ilsley AH, Jenner MA, and Cousins MJ. 1990. Influence of enzyme induction and exposure profile on liver injury due to chlorinated hydrocarbon inhalation. Pharmacol. Toxicol. 67:329-335.

Prendergast JA, Jones RA, Jenkins LJ, and Siegel J. 1967. Effects on experimental animals of long-term inhalation of trichloroethylene, carbon tetrachloride, 1,1,1-trichloroethane, dichlorodifluoromethane, and 1,1-dichloroethylene. Toxicol. Appl. Pharmacol. 10:270-289.

Quast JF. 1976. Pathology report on male and female rats exposed to vinylidene chloride vapors for 6 hours per day, 5 days per week during a 30-day period. Toxicology Research Laboratory, Health and Environmental Sciences, Dow Chemical U.S.A., Midland, MI. [as cited in USDHHS, 1994.]

Quast JF, McKenna MJ, Rampy LW, and Norris JM. 1986. Chronic toxicity and oncogenicity study on inhaled vinylidene chloride in rats. Fundam. Appl. Toxicol. 6:105-144.

Reichert D, Werner HW, and Henschler D. 1978. Role of liver glutathione in 1,1-dichloroethylene metabolism and hepatotoxicity in intact rats and isolated perfused rat liver. Arch. Toxicol. 41:169-179.

USDHHS. 1994. United States Department of Health and Human Services. Toxicological profile for 1,1-dichloroethene (update). U.S. Department of Health & Human Services. Public Health Service. Agency for Toxic Substances and Disease Registry. TP-93/07.

U.S. EPA. 1976. United States Environmental Protection Agency. Summary characteristics of selected chemicals of near-term interest. EPA-56014-76-004. Washington, DC: U.S. Environmental Protection Agency. [as cited in USDHHS, 1994.]

Wright PB, and Moore L. 1991. Potentiation of the toxicity of model hepatotoxicants by acetaminophen. Toxicol. Appl. Pharmacol. 109:327-335.

CHRONIC TOXICITY SUMMARY

DIETHANOLAMINE

(DEA; 2,2'-iminodiethanol; 2,2'-iminobisethanol; diethylolamine; 2,2'-aminodiethanol; 2,2'-dihydroxydiethylamine)

CAS Registry Number: 111-42-2

I. Chronic Toxicity Summary

Inhalation reference exposure level	**3 μg/m^3** (0.6 ppb)
Critical effect(s)	Laryngeal lesions in rats
Hazard index target(s)	Respiratory system; cardiovascular system

II. Physical and Chemical Properties (Melnick and Thomaszewski, 1990; Dow, 1980; CRC, 1994)

Description	Colorless crystals
Molecular formula	$C_4H_{11}NO_2$
Molecular weight	105.14 g/mol
Density	1.097 g/cm^3 @ 20°C
Boiling point	268.8°C
Melting point	28°C
Vapor pressure	0.00014 torr @ 25°C
Solubility	Soluble in alcohol, water, acetone
Conversion factor	1 ppm = 4.3 mg/m^3 @ 25°C

III. Major Uses and Sources

Diethanolamine is used in the formation of soaps, emulsifiers, thickeners, wetting agents, and detergents in cosmetic formulations (Melnick and Thomaszewski, 1990; Knaak *et al*., 1997). It is used as a dispersing agent in some agricultural chemicals, as an absorbent for acidic gases, as a humectant, as an intermediate in the synthesis of morpholine, as a corrosion inhibitor, and as a component in textile specialty agents (Beyer *et al.*, 1983). Diethanolamine is permitted in articles intended for use in production, processing, or packaging of food (CFR, 1981; cited in Melnick and Thomaszewski, 1990). It is also found in adhesives, sealants, and cutting fluids (Melnick and Thomaszewski, 1990). The annual statewide industrial emissions from facilities reporting under the Air Toxics Hot Spots Act in California based on the most recent inventory were estimated to be 1520 pounds of diethanolamine (CARB, 2000).

IV. Effects of Chronic Exposures to Humans

There have been no controlled or epidemiological studies of chronic diethanolamine exposure in humans. There is a single case report of occupational asthma determined to be due to the patient's handling of a cutting fluid containing diethanolamine (Piipari *et al*., 1998). Specific bronchial provocation tests were done with the cutting fluid containing DEA and with DEA aerosol at two concentrations (0.75 mg/m^3 and 1.0 mg/m^3) below the occupational limit of 2.0 mg/m^3. DEA caused asthmatic airway obstruction at both concentrations, but IgE-antibodies specific for DEA were not found.

V. Effects of Exposures in Animals

Diethanolamine replaces choline in phospholipids (Blum *et al*., 1972). DEA also reversibly inhibits phosphatidylcholine synthesis by blocking choline uptake and competing for utilization in the CDP-choline pathway (Lehman-McKeeman and Gamsky, 1999). Systemic toxicity occurs in many tissue types including the nervous system, liver, kidney, and blood system.

Gamer *et al*. (1996*)* exposed groups of 26 Wistar rats (13 male and 13 female) head-nose to a liquid aerosol of DEA for six hours per working day for 90 days at target concentrations of 15, 150, and 400 mg/m^3. Three of each sex were used for whole animal perfusion studies and the remaining 20 animals were examined for pathology. The study found no functional or morphological evidence of neurotoxicity. Retardation of body weight increase was observed in animals exposed to high concentrations. No systemic effects occurred at the low dose, but systemic effects in the liver, kidney, male reproductive system, and red blood cell occurred in the high concentration dose group. In the mid-dose group, mild liver and kidney effects were present. Local irritation of the larynx and trachea was found in the high and mid dose groups; irritating laryngeal effects were also detected in the low dose group. Based on this study 15 mg/m^3 is a NOAEL for liver and kidney effects and a LOAEL for irritation of the larynx. The equivalent continuous exposure at the LOAEL is 2.7 mg/m^3 (15 x 6/24 x 5/7).

Incidence of laryngeal lesions (Gamer *et al*., 1996)

Aerosolized diethanolamine	Chronic inflammation of the larynx	Squamous hyperplasia	Focal squamous metaplasia of laryngeal epithelium at base of the epiglottis
0	None*	None	None
15 mg/m^3	4/20	0/20	20/20
150 mg/m3	20/20	13/20	20/20
400 mg/m^3	20/20	17/20	20/20

* The report does not give control incidences. Assumed 0/20.

In an abstract Hartung *et al*. (1970) reported that inhalation by male rats of 6 ppm (25.8 mg/m^3) DEA vapor 8 hours/day, 5 days/week for 13 weeks resulted in depressed growth rates, increased lung and kidney weights, and even some mortality. Rats exposed continuously for 216 hours (nine days) to 25 ppm (108 mg/m^3) DEA showed increased liver and kidney weights, elevated

blood urea nitrogen (BUN), and increased serum glutamate oxaloacetate transferase (SGOT), an indicator of liver damage (Hartung et al., 1970). In studies at lower DEA levels, Eastman Kodak (1967) exposed dogs, weanling and adult rats, and guinea pigs to 0.26 ppm (1.1 mg/m^3) DEA for 90 days and found no pathology attributable to DEA. In a 45-day study with 0.5 ppm (2.2 mg/m^3) DEA they also found no pathology attributable to DEA except for a possible slight retardation in rat growth rate.

Gamer *et al*. (1993) exposed groups of 25 pregnant Wistar rats on gestation days 6-15 to a (nose-only) liquid aerosol of DEA at 10, 50 and 200 mg/m^3. Maternal toxicity, indicated by vaginal hemorrhage in 8 of the dams on gestation day 14, and fetotoxicity, evidenced by a statistically significant ($p<0.05$) increased incidence of total fetal skeletal variations, were observed at 200 mg/m^3. No teratogenic effects were seen at any level. Thus 50 mg/m^3 was a NOAEL for maternal toxicity and for embryo-fetal effects.

A 13-week drinking water study in rats (10 per sex per group) showed significant dose-dependent hematological changes following exposure to DEA at all concentrations tested: 320, 630, 1250, 2500, and 5000 ppm in males, and 160, 320, 630, 1250, and 2500 ppm in females. Hematological effects included decreased hemoglobin and mean corpuscular volume (Melnick *et al.*, 1994a). Similar hematological changes were observed following daily topical treatment. In addition to the hematological effects, female rats also showed dose-dependent spinal cord and medullary demyelination beginning at a drinking water concentration of 1250 ppm DEA. Male rats displayed demyelination beginning at 2500 ppm. Female rats gained significantly less weight than controls beginning at 63 mg/kg/day topical treatment. In a companion drinking water study (Melnick *et al.*, 1994b), mice (10 per sex per group) were exposed to concentrations of 0, 630, 1250, 2500, 5000, and 10,000 ppm DEA and displayed dose-dependent hepatotoxicity, nephrotoxicity, and cardiac toxicity. Daily topical treatment in a separate study resulted in skin lesions in mice. Significant hepatic toxicity was observed at all drinking water concentrations, and skin lesions were observed at all topical doses.

Data from female rats exposed to diethanolamine by Melnick *et al*. (1994)

Dose (ppm)	mg/kg/day DEA consumed	Survival	Mean bw change (g)	Hgb (g/dL)	Mean cell volume	Mean cell Hgb (pg)
0	0	10/10	120±6a	15.1±0.3	56±0.2	17.9±0.2
160	14	9/10	106±3	15.2±0.1	55±0.2**	17.8±0.1*
320	32	10/10	98±3**	13.8±0.1**	54±0.2**	17.7±0.1**
630	57	10/10	95±4**	13.0±0.1**	53±0.3**	17.2±0.1**
1250	124	10/10	85±4**	11.3±0.2**	51±0.3**	16.7±0.1**
2500	242	10/10	63±4**	10.50±.2**	49±0.2**	16.30±.1**

a Values are means±SEM; * $p<0.05$ or ** $p<0.01$ versus control group

Barbee and Hartung (1979a) found that repeated treatment of rats with 330 mg DEA/kg/day significantly inhibited formation of phosphatidyl choline and phosphatidyl ethanolamine in the liver as compared with control rats. In a subsequent study, Barbee and Hartung (1979b) noted changes in liver mitochondrial activity in rats (4 per group) following exposure to DEA in

drinking water for up to 5 weeks. Mitochondrial changes were observed at 42 mg/kg/day after 2 weeks.

Daily oral treatment of male rats with 0, 250, 500, or 750 mg/kg/day for 5 days, or 100 mg/kg/day for 14 days resulted in reduced activities of the liver enzymes microsomal hydroxylase and N-demethylase (Foster *et al.*, 1971).

In a developmental study Marty *et al.* (1999) administered DEA cutaneously to pregnant CD rats during gestation days 6-15 at doses of 0, 150, 500, and 1500 mg/kg/day. Dams exhibited reduced body weight at the highest dose, skin irritation and increased kidney weights at both 500 and 1500 mg/kg/day, and a slight microcytic anemia with abnormal red blood cell morphology at all 3 dose levels. The blood results are consistent with the results of topical application of DEA by Melnick *et al*. (1994b). Rat fetuses had increased incidences of six skeletal variations at 1500 mg/kg/day. Lower doses were without effect on the fetuses. Marty *et al*. (1999) also administered DEA cutaneously to pregnant New Zealand White rabbits on days 6-18 of gestation at 0, 35, 100, and 350 mg/kg/day. Dams administered the highest dose exhibited various skin lesions, reduced food consumption, and color changes in the kidneys, but no hematological changes. Body weight gain was reduced at ≥ 100 mg/kg/day. There was no evidence of maternal toxicity at 35 mg/kg/day and no evidence of developmental toxicity in rabbits at any dose. Developmental toxicity was observed only in the rat and only at doses causing significant maternal toxicity, including hematological effects. Due to a dose discrepancy, the authors adjusted the no observable effect level (NOEL) for DEA developmental toxicity to 380 mg/kg/day for rats. In rabbits, the embryonal/fetal NOEL was 350 mg/kg/day.

VI. Derivation of Chronic Reference Exposure Level (REL)

Study	Gamer *et al*. (1996)
Study population	Wistar rats (male and female)
Exposure method	Inhalation 6 h/day, 5 d/wk
Critical effects	Chronic inflammation and squamous hyperplasia and metaplasia of the larynx
LOAEL	15 mg/m^3
NOAEL	Not observed
Exposure duration	90 days
Average experimental exposure	2700 μg/m^3 for LOAEL group (15 mg/m^3 x 6h/24h x 5d/7d x 1000 μg/mg)
LOAEL uncertainty factor	3 (see below)
Subchronic uncertainty factor	3
Interspecies uncertainty factor	10
Intraspecies uncertainty factor	10
Cumulative uncertainty factor	1000
Inhalation reference exposure level	3 μg/m^3 (0.6 ppb)

No chronic inhalation studies with diethanolamine were located in the peer-reviewed literature. Thus the 90 day study by Gamer *et al*., which found a LOAEL of 15 mg/m^3 for irritation of the

rat larynx, was used to derive the REL. All 20 of the rats in the 15 mg/m^3 exposure group showed focal squamous metaplasia of the laryngeal epithelium at the base of the epiglottis, and 4 of the 20 had inflammatory cells present in the larynx. The former lesion seemed to be very limited and did not justify use of the full LOAEL uncertainty factor of 10.

For comparison, the BASF (1993) developmental study by the inhalation route found a LOAEL of 200 mg/m^3 DEA and a NOAEL of 50 mg/m^3 for fetotoxic effects. The equivalent continuous exposure at the NOAEL is 12.5 mg/m^3. Multiplying by an RGDR of 1 and dividing by an interspecies uncertainty factor (UF_A) of 3 and an intraspecies uncertainty factor (UF_H) of 10 results in a REL estimate of 40 μg/m^3.

As another comparison, the study by Melnick *et al.* (1994a) shows dose-dependent adverse hematological and CNS effects in rats exposed to DEA in drinking water. Similar systemic effects were observed following dermal exposure. The Melnick *et al.* subchronic study was of the longest duration and was the most comprehensive report of the systemic effects of DEA in the literature. However, portal-of-entry effects of DEA have not been examined and should be addressed in future studies since this compound has irritant properties. The data from female rats were used since females were more sensitive than males to the hematologic effects of DEA. The LOAEL was 160 mg/L, or 14 mg/kg-day based on water consumption rates. Dividing by a LOAEL UF of 3, a subchronic UF of 3, an interspecies UF of 10, and an intraspecies UF of 10 (cumulative UF = 1000) results in a oral REL of 0.014 mg/kg-day. Using route-to-route extrapolation and assuming that a 70 kg person inhales 20 m^3 of air per day leads to an inhalation REL estimate of 50 μg/m^3 (10 ppb) DEA.

VII. Data Strengths and Limitations for Development of the REL

The diethanolamine database is relatively weak. Major areas of uncertainty are the lack of adequate human exposure data, the absence of a NOAEL in the major study, the lack of reproductive and developmental toxicity studies, and the lack of chronic inhalation, multiple-species, health effects data.

VIII. Potential for Differential Impacts on Children's Health

Since the proposed chronic REL of 3 μg/m^3 based on laryngeal effects is much lower than the comparison REL of 40 μg/m^3 based on fetotoxic effects, the REL should adequately protect infants and children. Diethanolamine is a respiratory irritant and thus might exacerbate asthma, which has a more severe impact on children than on adults. The large uncertainty factor of 1000 should protect against that potential hazard. However, there is no direct evidence in the literature to demonstrate that DEA exacerbates asthma or to quantify a differential effect of diethanolamine on the larynx or on other organs in infants and children.

IX. References

Barbee SJ, and Hartung R. 1979a. The effect of diethanolamine on hepatic and renal phospholipid metabolism in the rat. Toxicol. Appl. Pharmacol. 47:421-430.

Barbee SJ, and Hartung R. 1979b. Diethanolamine-induced alteration of hepatic mitochondrial function and structure. Toxicol. Appl. Pharmacol. 47:431-440.

Beyer KH Jr, Bergfeld WF, Berndt WO, Boutwell RK, Carlton WW, Hoffman DK, and Schroeter AL. 1983. Final report on the safety assessment of triethanolamine, diethanolamine, and monoethanolamine. J. Am. Coll. Toxicol. 2:183-235.

Blum K, Huizenga CG, Ryback RS, Johnson DK, and Geller I. 1972. Toxicity of diethanolamine in mice. Toxicol. Appl. Pharmacol. 22:175-185.

CARB. 2000. California Air Resources Board. California Emissions Inventory Development and Reporting System (CEIDARS). Data from Data Base Year 1998. February 12, 2000.

CFR. 1981. Code of Federal Regulations. Title 21, Parts 175.105, 175.300, 176.170, 176.180, 176.200, 176.210, 177.1680, 177.2600, 177.2800, 178.3910. U.S. Printing Office, Washington, D.C. [as cited in Melnick and Thomaszewski, 1990.]

CRC. 1994. CRC Handbook of Chemistry and Physics, 75th edition. Lide DR, ed. Boca Raton, FL: CRC Press Inc.

Dow Chemical Co. 1980. Alkanolamines Handbook. Midland, MI: Dow Chemical Company.

Eastman Kodak. Co. 1967. Health and safety studies for diethanolamine, Laboratory tests to determine effect of inhalation of two ethanolamines - diethanolamine (DEA), methylaminoethanol (MAE), Formulation 485K - Histological addendum to Final Report. TSCA 8d Submission 86-890000205, Microfiche Number OTS0516742. Washington, D.C.: OPPT, U.S. EPA.

Foster GV Jr, Hartung R, and Cornish HH. 1971. Inhibition of hepatic microsomal enzymes by N-substituted ethanolamines. Toxicol. Appl. Pharmacol. 19:847-855.

Gamer AO, Hellwig J, and Hildebrand B. 1993. Study of the prenatal toxicity of diethanolamin in rats after inhalation. BASF Project No. 31RO233/90010. Ludwigshafen, FRG: BASF

Gamer AO, Mellert W, Leibold E, Deckardt K, Kaufman W, and Hildebrand B. 1996. Diethanolamin – Subchronic inhalation toxicity and neurotoxicity study in Wistar rats. 90-day liquid aerosol exposure-. BASF Project No. 50I0075/93011. Heidelberg, FRG: BASF.

Hartung R, Rigas LK, and Cornish HH. 1970. Acute and chronic toxicity of diethanolamine. Toxicol. Appl. Pharmacol. 17:308 (abstract).

Knaak JB, Leung HW, Stott WT, Busch J and Bilsky J. 1997. Toxicology of mono-, di-, and triethanolamine. Rev. Environ. Contam. Toxicol. 49:1-86.

Lehman-McKeeman LD, and Gamsky EA. 1999. Diethanolamine inhibits choline uptake and phosphatidylcholine synthesis in Chinese hamster ovary cells. Biochem. Biophys. Res. Commun. 262(3):600-604.

Marty MS, Neeper-Bradley TL, Neptun DA, and Carney EW. 1999. Developmental toxicity of diethanolamine applied cutaneously to CD rats and New Zealand White rabbits. Regul. Toxicol. Pharmacol. 30(3):169-181.

Melnick RL, and Thomaszewski KE. 1990. Diethanolamine. In: Ethel Browning's Toxicity and Metabolism of Industrial Solvents. 2nd ed. Buhler DR, and Reed DJ. (eds.) Vol 2: Nitrogen and Phosphorus Solvents. New York: Elsevier. pp. 401-410.

Melnick RL, Mahler J, Bucher JR, Thompson M, Hejtmancik M, Ryan MJ, and Mezza LE. 1994a. Toxicity of diethanolamine. 1. Drinking water and topical application exposures in F344 rats. J. Appl. Toxicol. 14(1):1-9.

Melnick RL, Mahler J, Bucher JR, Hejtmancik M, Singer A, and Persing R. 1994b. Toxicity of diethanolamine. 2. Drinking water and topical application exposures in B6C3F1 mice. J. Appl. Toxicol. 14(1):11-19.

Piipari R, Tuppurainen M, Tuomi T, Mantyla L, Henriks-Eckerman ML,Keskinen H, and Nordman H. 1998. Diethanolamine-induced occupational asthma, a case report. Clin. Exp. Allergy 28(3):358-362.

CHRONIC TOXICITY SUMMARY

N,N-DIMETHYLFORMAMIDE

(N-formyldimethylamine)

CAS Registry Number: 68-12-2

I. Chronic Toxicity Summary

Inhalation reference exposure level	**80 µg/m^3** (30 ppb)
Critical effect(s)	Liver dysfunction and respiratory irritation in humans
Hazard index target(s)	Alimentary system, respiratory system

II. Chemical Property Summary (HSDB, 1994)

Description	Colorless to very slightly yellow liquid
Molecular formula	C_3H_7NO
Molecular weight	73.09 g/mol
Boiling point	153°C
Melting point	–61°C
Vapor pressure	3.7 torr @ 25°C
Solubility	Soluble in alcohol, ether, acetone, benzene, and chloroform; miscible with water
Conversion factor	2.99 µg/m^3 per ppb at 25ºC

III. Major Uses and Sources

Dimethylformamide (DMF) is primarily used as a solvent in the production of polyurethane products and acrylic fibers. It is also used in the pharmaceutical industry, in the formulation of pesticides, and in the manufacture of synthetic leathers, fibers, films, and surface coatings (Howard, 1993; Gescher, 1993; Redlich *et al.*, 1988). DMF may be emitted to the environment as a result of its use in a variety of petrochemical industries (Howard, 1993). The annual statewide industrial emissions from facilities reporting under the Air Toxics Hot Spots Act in California based on the most recent inventory were estimated to be 18,249 pounds of DMF (CARB, 2000).

IV. Effects of Human Exposure

Among 100 workers occupationally exposed to DMF for at least one year (mean exposure of 5 years; range = 1-15 years), a statistically significant incidence of hepatic impairment, as indicated by elevated gamma-glutamyl transpeptidase levels and digestive disturbances, was

noted (Cirla *et al.*, 1984). Other changes, that were not statistically significant, included increased SGOT and SGPT and enlarged livers. The mean time-weighted average concentration of DMF was 22 mg/m^3 (range = 8-58 mg/m^3). Symptoms of irritation occurring only during work at statistically significantly higher incidences included watery eyes, dry throat, and coughing. Also, the exposed workers reported a reduced sense of smell and dry coughs at home with a statistically significant difference as compared to controls. Several of the DMF exposed workers also reported alcohol intolerance characterized by a disulfiram-type reaction (facial flushing and palpitations following alcohol ingestion). Alcohol consumption, a potential confounder, was controlled for in the study design.

A similar study was conducted on workers who had been employed in an acrylic acid fiber plant for more than 5 years (Cantenacci *et al.*, 1984). Concentrations to which the workers were exposed were characterized as either an 8-hour TWA of 18 mg/m^3 or an 8-hour TWA of 3 mg/m^3. Measures of liver function including SGOT, SGPT, gamma-glutamyl transferase, and alkaline phosphatase levels were not significantly different between exposed and unexposed workers. However, the U.S. EPA cautions that because only 54 matched pairs of workers were examined, the power of this study was not high enough to reliably detect a difference in enzyme levels.

Redlich *et al*. (1988) characterized a plant-wide outbreak of liver disease among workers in a factory coating fabric with polyurethane. Fifty-eight of 66 (88%) workers participated and each had standard liver screening function tests done at least once. At the work site DMF was being used in poorly ventilated areas without appropriate skin protection. No other major known hepatotoxic exposure was identified. Overall, 36 of 58 (62%) workers tested had elevations of either aspartate aminotransferase (AST) or alanine aminotransferase (ALT) levels. Enzyme abnormalities occurred almost exclusively in production workers (35 out of 46 abnormal). Only 1 of 12 non-production workers showed elevations in enzyme levels ($p < 0.0001$). Serologic tests excluded known infectious causes of hepatitis in all but 2 workers. Changes, characteristic of liver injury, were confirmed by histologic examination of biopsy specimens from 4 workers. Improvement in liver enzyme abnormalities and symptoms in most patients were seen, after modification of work practices and removal of workers most severely affected from exposure. However, some patients showed persistent elevations of enzyme levels. No measurements or estimates of DMF exposure levels were reported.

Wang *et al*. (1991) investigated the prevalence of liver injury associated with DMF exposure in 183 of 204 (76%) employees of a synthetic leather factory by performing medical examinations, liver function tests, and creatine phosphokinase (CPK) determinations. Air concentrations were measured with personal samplers and gas chromatography. The concentration of DMF in air to which each worker was exposed was categorized as high (DMF exposure index 2: 25-60 ppm; 75-180 mg/m^3), medium (index 1: 10-40 ppm), and low (index 0: <10 ppm). High exposure concentrations were significantly associated with elevated alanine aminotransferase (ALT) levels (i.e., greater than or equal to 35 International Units/liter), a result that did not change after stratification by hepatitis B carrier status. Logistic regression analysis indicated that exposure to high DMF levels was associated with elevated ALT ($p = 0.01$), whereas hepatitis B surface antigen (HBsAg) was slightly but independently associated with elevated ALT ($p = .07$). Workers with normal ALT values had significantly higher mean ALT and aspartate

aminotransferase (AST) activities, especially among those who were not HBsAg carriers. A significant association existed between elevated CPK levels and exposure to DMF. However, an analysis of the CPK isoenzyme among 143 workers did not reveal any specific damage to muscles. Thus the authors ascribed the liver injury to DMF.

U.S. EPA (1994) states that subjective evidence of liver toxicity, such as digestive impairment and alcohol intolerance, is often observed at exposures below those that cause clinical changes in liver enzymes. Thus, the symptoms may be more sensitive indicators of hepatic impairment.

Three unexplained cases of small-for-date third trimester intrauterine deaths were observed in a group of women working as quality control analysts in the pharmaceutical industry (Farquhason *et al.*, 1983). This represented a 30% stillbirth rate as compared with the average for the general population of about 0.26%. While the authors concluded that the occurrence of stillbirth in these women was not likely due to chance, the effects cannot be solely attributed to DMF because the women were exposed to other agents in addition to DMF.

V. Effects of Animal Exposure

Malley *et al*. (1994) exposed male and female Crl:CD rats and mice to 0, 25, 100, or 400 ppm DMF for 6 hr/day, 5 days/week for 18 months (mice) or 2 years (rats). No compound-related effects on clinical observations or survival were observed. Body weights of rats exposed to 100 (males only) and 400 ppm were reduced, while body weights were increased in 400 ppm mice. No hematologic changes were observed in either species. Serum sorbitol dehydrogenase activity was increased in rats exposed to 100 or 400 ppm. DMF-related morphological changes were observed only in liver. Exposure of rats to 100 and 400 ppm produced increased relative liver weights, centrilobular hepatocellular hypertrophy, lipofuscin/hemosiderin accumulation in Kupffer cells, and centrilobular single cell necrosis (400 ppm only). In mice, increased liver weights (100 ppm males, 400 ppm both sexes), centrilobular hepatocellular hypertrophy, accumulation of lipofuscin/hemosiderin in Kupffer cells, and centrilobular single cell necrosis were observed in all exposure groups. These observations occurred in a dose-response fashion and were minimal at 25 ppm. No increase in hepatic cell proliferation was seen in mice or female rats. Slightly higher proliferation was seen in male rats exposed to 400 ppm at 2 weeks and 3 months but not at 12 months. Thus 25 ppm was a chronic NOAEL for both rats and mice.

A developmental toxicity study using three species (mice, rabbits, and rats) and four routes of administration (oral, inhalation, dermal, and intraperitoneal) identified the rabbit as the most sensitive of the three species. Groups of 15 pregnant rabbits were exposed for 6 hours per day on days 8-20 of gestation to 50, 150, or 450 ppm (150, 449, or 1350 mg/m^3) DMF (Hellwig *et al.*, 1991). Slight maternal toxicity, as indicated by non-statistically significant decreases in maternal body weight gain, was observed in the 450 ppm exposure group. An increased number of total malformations per litter was observed in the 450 ppm exposure group. Malformations observed at statistically higher incidences compared to controls included hernia umbilicalis, external variations, pseudoankylosis of the forelimbs, and skeletal variation and retardation. The authors conclude that there was a clear teratogenic effect in rabbits following maternal exposure to 450 ppm DMF and a marginal effect following exposure to 150 ppm DMF. A NOAEL of

50 ppm for fetal and maternal effects was reported. Inhalation exposure to 150 ppm was calculated by the authors to approximate a daily dose of 45 mg/kg/day, which coincides with previous work on this compound in this species.

VI. Derivation of Chronic Reference Exposure Level

Study	Cirla *et al.*, 1984; Catenacci *et al.*, 1984
Study population	Occupationally exposed workers
Exposure method	Discontinuous inhalation exposures
Critical effects	Digestive disturbances and slight hepatic changes
LOAEL	22 mg/m^3
NOAEL	Not observed
Exposure continuity	8 hr/day (10 m^3/day), 5 days/week (assumed)
Average occupational exposure	7.9 mg/m^3 for LOAEL group (22 x 10/20 x 5/7)
Human equivalent concentration	7.9 mg/m^3
Exposure duration	5 years (mean exposure duration)
LOAEL uncertainty factor	3
Subchronic uncertainty factor	3
Interspecies uncertainty factor	1
Intraspecies uncertainty factor	10
Cumulative uncertainty factor	100
Inhalation reference exposure level	0.08 mg/m^3 (80 µg/m^3, 0.03 ppm, 30 ppb)

The U.S. EPA (1994) based its RfC of 30 µg/m^3 on the same study but included a Modifying Factor (MF) of 3 due to lack of reproductive toxicity data in the DMF database. The criteria for use of modifying factors are not well specified by U.S. EPA. Such modifying factors were not used by OEHHA. Intermediate uncertainty factors were used for LOAEL to NOAEL and subchronic to chronic extrapolation because of the mild nature of the effects observed and the less than chronic exposure duration.

For comparison Hellwig *et al*. (1991) found a developmental NOAEL of 50 ppm in rabbits exposed 6 hours per day on gestation days 8-20, equivalent to continuous exposure of 12.5 ppm. Multiplication by an RGDR of 1 and division by a UF of 30 (3 for interspecies and 10 for intraspecies) results in a REL estimate of 400 ppb. The NOAEL of 25 ppm for rats and mice in the chronic study of Malley *et al*. (1994) leads to a REL estimate of 150 ppb.

VII. Data Strengths and Limitations for Development of the REL

The major strength of the REL for N,N-dimethylformamide is the availability of human health effects data over several years of exposure. The major uncertainties are the difficulty in estimating exposure patterns and magnitude, the lack of a NOAEL observation, and the lack of complete reproductive and developmental toxicity data.

VIII. References

CARB. 2000. California Air Resources Board. California Emissions Inventory Development and Reporting System (CEIDARS). Data from Data Base Year 1998. February 12, 2000.

Catenacci G, Grampella D, Terzi R, Sala A, and Pollini G. 1984. Hepatic function in subjects exposed to environmental concentrations of DMF lower than the actually proposed TLV. G. Ital. Med. Lav. 6(3-4):157-158.

Cirla AM, Pisati G, Invernizzi E, and Torricelli P. 1984. Epidemiological study on workers exposed to low dimethylformamide concentrations. G. Ital. Med. Lav. 6(3-4):149-156.

Farquharson RG, Hall MH, and Fullerton WT. 1983. Poor obstetric outcome in three quality control laboratory workers. Lancet 1(8331):983-984. [cited in U.S. EPA, 1994].

Gescher A. 1993. Metabolism of N,N-dimethylformamide: key to the understanding of its toxicity. Chem. Res. Toxicol. 6(3):245-251.

HSDB. 1994. Hazardous Substances Data Bank. National Library of Medicine, Bethesda, MD (TOMES® CD-ROM Version). Denver, CO: Micromedex, Inc. (Edition expires 11/31/94).

Hellwig J, Merkle J, Klimisch HJ, and Jackh R. 1991. Studies on the prenatal toxicity of N,N-dimethylformamide in mice, rats and rabbits. Food Chem. Toxicol. 29(3):192-201.

Howard PH. (ed) 1993. Handbook of Environmental Fate and Exposure Data for Organic Chemicals. Vol. IV: Solvents 2. Chelsea, MI: Lewis Publishers Inc.

Malley LA, Slone TW Jr, Van Pelt C, Elliott GS, Ross PE, Stadler JC, and Kennedy GL Jr. 1994. Chronic toxicity/oncogenicity of dimethylformamide in rats and mice following inhalation exposure. Fundam. Appl. Toxicol. 23(2):268-279.

Redlich C, Beckett WS, Sparer J, Barwick KW, Riely CA, Miller H, Sigal SL, Shalat SL, and Cullen MR. 1988. Liver disease associated with occupational exposure to the solvent dimethylformamide. Ann. Intern. Med. 108:680-686.

U.S. EPA. 1994. U.S. Environmental Protection Agency. IRIS. 1994. Integrated Risk Information System (IRIS). Reference concentration for chronic inhalation exposure (RfC) for N,N,-Dimethylformamide. Available online at http://www.epa.gov/ngispgm3/iris

Wang JD, Lai MY, Chen JS, Lin JM, Chiang JR, Shiau SJ, and Chang WS. 1991. Dimethylformamide-induced liver damage among synthetic leather workers. Arch. Environ. Health 46(3):161-166.

CHRONIC TOXICITY SUMMARY

1,4-DIOXANE

(Synonym: dihydro-p-dioxin, diethylene dioxide, p-dioxane, glycolethylene ether)

CAS Registry Number: 123-91-1

I. Chronic Toxicity Summary

Inhalation reference exposure level	**3,000 μg/m^3 (800 ppb)**
Critical effects	Liver, kidney, hematologic changes in rats
Hazard index target(s)	Alimentary system; kidney; circulatory system

II. Chemical Property Summary (HSDB, 1995; 1999; CRC, 1994)

Description	Colorless liquid with a faint, pleasant odor
Molecular formula	$C_4H_8O_2$
Molecular weight	88.10 g/mol
Boiling point	101.5 ^oC
Melting point	11.8^oC
Vapor pressure	37 torr @ 25^oC
Solubility	Miscible with water, aromatic solvents, and oils
Kow	0.537
Conversion factor	3.60 μg/m^3 per ppb at 25^oC

III. Major Uses and Sources

1,4-Dioxane (dioxane), a cyclic ether, is used as a degreasing agent, as a component of paint and varnish removers, and as a wetting and dispersion agent in the textile industry. Dioxane is used as a solvent in chemical synthesis, as a fluid for scintillation counting, and as a dehydrating agent in the preparation of tissue sections for histology (Grant and Grant, 1987; HSDB, 1995). The annual statewide emissions from facilities reporting under the Air Toxics Hot Spots Act in California based on the most recent inventory were estimated to be 155,549 pounds of 1,4-dioxane (CARB, 1999).

IV. Effects of Human Exposure

Dioxane is absorbed by all routes of administration (HSDB, 1995). In humans, the major metabolite of dioxane is β-hydroxyethoxyacetic acid (HEAA) and the kidney is the major route of excretion (Young *et al.*, 1976). The enzyme(s) responsible for HEAA formation has not been studied, but data from Young *et al.* (1977) indicate saturation does not occur up to an inhalation exposure of 50 ppm for 6 hours. Under these conditions the half-life for dioxane elimination is

59 min (plasma) and 48 min (urine). Although physiologically based pharmacokinetic (PBPK) modeling suggests HEAA is the ultimate toxicant in rodents exposed to dioxane by ingestion, the same modeling procedure does not permit such a distinction for humans exposed by inhalation (Reitz *et al.*, 1990).

Several anecdotal reports have appeared in which adverse health effects due to chronic dioxane exposure are described. Barber (1934) described dioxane exposed factory workers, some of whom exhibited signs of liver changes, increased urinary protein and increased white blood cell counts, and some of whom died from apparent acute exposures. Although the kidney and liver lesions were considered manifestations of acute exposure, the author suggested a chronic component that was manifested by increased white blood cells. A case was reported in which a worker, who died following exposure by inhalation and direct skin contact to high (unspecified) dioxane levels, exhibited lesions in the liver, kidneys, brain and respiratory system, but the effects could not be easily separated from the effects due to high intake of alcohol (Johnstone, 1959).

In a German study (Thiess *et al.*, 1976 / in German, described in NIOSH, 1977) 74 workers exposed to dioxane in a dioxane-manufacturing plant (average potential exposure duration - 25 years) underwent evaluation for adverse health effects. Air measurements indicated dioxane levels varied from 0.01 to 13 ppm. Clinical evaluations were applied to 24 current and 23 previous workers. Evidence of increased (i.e., abnormal) aspartate transaminase (also known as serum glutamate-oxalacetic transaminase or SGOT), alanine transaminase (serum glutamate pyruvate transaminase or SGPT), alkaline phosphatase, and gamma glutamyltransferase activities (liver function) was noted in these workers, but not in those who had retired. The indicators of liver dysfunction, however, could not be separated from alcohol consumption or exposure to ethylene chlorohydrin and/or dichloroethane.

A follow-up mortality study was conducted on 165 chemical plant manufacturing and processing workers who were exposed to dioxane levels ranging from less than 25 to greater than 75 ppm between 1954 and 1975 (Buffler *et al.*, 1978). Total deaths due to all causes, including cancer, did not differ from the statewide control group, but the data were not reanalyzed after removing the deaths due to malignant neoplasms. The study is limited by the small number of deaths and by the small sample number. The study did not assess hematologic or clinical parameters that could indicate adverse health effects in the absence of mortality.

Yaqoob and Bell (1994) reviewed human studies on the relationship between exposure to hydrocarbon solvents - including dioxane - and renal failure, in particular rare glomerulonephritis. The results of their analysis suggest that such solvents may play a role in renal failure, but dioxane was not specifically discussed. Of interest to the discussion on chronic exposure to dioxane is the suggestion that the mechanism of the disease process involves local autoimmunity with decreased circulating white blood cells (see below).

V. Effects of Animal Exposure

In rats, the major metabolite of dioxane is HEAA, which is excreted through the kidneys (Braun and Young, 1977). Exposure to dioxane by ingestion results in saturation of metabolism above

100 mg/kg given in single dose. Saturation of metabolism was also observed as low as 10 mg/kg if dioxane was administered in multiple doses. Dioxane itself is not cleared through the kidney. A decrease in metabolic clearance with increasing dose (iv) has been interpreted as the saturation of metabolism at the higher doses (Young *et al.*, 1978).

For Sprague-Dawley rats, the metabolic fate of inhaled dioxane (head only exposure) was based on one air concentration (50 ppm). At this level, nearly all the dioxane was metabolized to HEAA since HEAA represented 99 percent of the total dioxane + HEAA measured. The plasma half-life for dioxane under these conditions was 1.1 hours. The absorption of dioxane through the inhalation pathway could not be exactly determined, because of a high inhalation rate (0.24 liters/min), calculated on the basis of complete absorption (Young *et al.*, 1978; U.S. EPA, 1988). Although the high inhalation rate could be dioxane-related, another explanation may be the stress incurred when the jugular veins were cannulated as part of the experiment. Extensive absorption by inhalation is also inferred from the high tissue/air partition coefficients (Reitz *et al.*, 1990).

Although the PBPK modeling suggests that in rat the parent dioxane is a better dose surrogate than HEAA for exposure by ingestion, the inhalation modeling did not use more than one inhalation dose. No studies were located on the biological or biochemical properties of HEAA or the properties of the enzyme(s) that are responsible for the transformation of dioxane into HEAA.

Rats (Wistar) were exposed by inhalation to dioxane (111 ppm; 7 hours/day, 5 days/week) for 2 years (Torkelson *et al.*, 1974). Increased mortality and decreased body weight gains, compared to unexposed control rats, were not observed. Among the male rats, decreased blood urea nitrogen (kidney function), decreased alkaline phosphatase (cholestatic liver function), increased red blood cells, and decreased white blood cells were observed. According to the authors, exposure-related, non-cancerous tissue lesions were not observed during the 2-year period.

In another inhalation study, rats were exposed to dioxane at levels of 0.15, 1.3, and 5.7 ppm (Pilipyuk *et al.*, 1978). Frequency was not specified, but the duration is given as “90 successive days”. At the end of the 3-month exposure, increased SGOT activity at the two highest doses and increased SGPT activity at all doses were measured in the sera of the exposed rats. Rats exposed to the highest dose also exhibited increased urinary protein and chloride levels, each of which returned to control levels during an unspecified recovery period. Pilipyuk *et al.* (1978) also report changes in the minimum time (ms) required for an electric stimulus to result in excitation of extensor and flexor muscles. Although Pilipyuk *et al.* (1978) consider the changes to be a reflection of adverse effects due to exposure to dioxane, Torkelson *et al.* (1974) do not consider the hematologic and clinical changes of toxicologic importance. In particular, toxic manifestations are usually associated with increased blood urea nitrogen and alkaline phosphatase levels, whereas these levels decreased in the Torkelson *et al.* (1974) investigation. The reason for the discrepancies between the two studies, in particular the extremely low dioxane exposure levels in the Pilipyuk *et al.* (1978) study, is unknown. One explanation could be the purity of the dioxane used, which was not described in the latter study, although such contamination would be unlikely to account for the large difference in exposure levels.

Kociba *et al.* (1974) exposed rats (Sherman) to dioxane by ingestion of drinking water for up to 2-years. The drinking water levels were 0, 0.01, 0.1, and 1.0 percent, which were converted to daily intake according to measured rates of water consumption during exposure. Exposure to the highest level resulted in decreased body weight gain and increased deaths. According to the authors, exposure related hematologic changes did not occur. Histopathologic examination revealed evidence of regeneration of hepatic and kidney tissues in rats exposed to 1.0 or 0.1 percent, but not in rats exposed to 0.01 percent dioxane. On the assumption of total absorption of dioxane from the gastrointestinal tract, the exposure levels in female and male rats is as follows: 0.01%-18 ppm/F, 9.3 ppm/M; 0.1% -144 ppm/F, 91 ppm/M.

The teratogenic potential of dioxane was studied in rats (Giavini *et al.*, 1985). Dioxane was administered by gavage at doses of 0, 0.25, 0.5, and 1.0 ml/kg-day, on gestation days 6-15, and observations continued through day 21. Dams exposed to the highest dose exhibited nonsignificant weight loss and a significant decrease in food consumption during the first 16 days. During the remaining 5 days, food consumption increased, but the weight gain reduction in the presence of dioxane continued. At the 1.0 ml/kg-day dose, mean fetal weight and ossified sternebrae were also reduced. The inability to separate the developmental toxicity from maternal or embryotoxicity renders these data inconclusive as to the developmental toxicity of dioxane. If toxicity to the dam and/or embryo exists, the NOAEL for dioxane (based on density = 1.03 gm/ml) is 517 mg/kg-day.

VI. Derivation of Chronic Reference Exposure Level (REL)

Study	Torkelson *et al.* (1974)
Study populations	Rats
Exposure method	Discontinuous inhalation
Critical effects	No effects on liver, kidney, or hematologic function were noted in this study. Such dysfunctions, however, were observed in rats exposed to dioxane by ingestion (Kociba *et al.* 1974) and humans (Thiess, *et al.*, 1976, described by NIOSH, 1977).
LOAEL	Not observed in inhalation studies
NOAEL	111 ppm
Exposure continuity	7 h/d x 5 days/wk
Average experimental exposure	23 ppm (111 x 7/24 x 5/7)
Human equivalent concentration	23 ppm (gas with systemic effects, based on RGDR = 1.0 using default assumption that lambda (a) = lambda (h))
Exposure duration	2 years
LOAEL uncertainty factor	1
Subchronic exposure	1
Interspecies uncertainty factor	3
Intraspecies uncertainty factor	10
Cumulative uncertainty factor	30
Inhalation reference exposure level	0.8 ppm (800 ppb; 2.8 mg/m^3; 3000 µg/m^3)

The lifetime rat inhalation study of Torkelson *et al.* (1974) is the only detailed inhalation study available in the literature. The Pilipyuk *et al.* (1977) study contains useful and consistent data, but the absence of necessary details prevents the use of these results for the determination of a chronic reference exposure level (REL). Although the ingestion study (Kociba *et al.*, 1974) shows unequivocal toxic responses (liver and kidney) of the rat to dioxane by ingestion, exposure to 111 ppm by inhalation leads to equivocal results (Torkelson *et al.*, 1974). In particular, serum markers for liver and kidney dysfunction decrease in value, whereas toxic responses are associated with increased levels. The lack of toxic hematologic endpoints observed in the ingestion study suggests that toxicity of dioxane may be route-of-exposure specific. Hematologic changes were also observed in the early worker study wherein changes in white blood cell count occurred (Barber, 1934), but the directions are different. The studies on humans and rodents therefore suggest inhalation of dioxane may lead to adverse biologic effects, but good dose-response data are not available. A partial explanation may lie in the dose-response characteristic of the metabolism of dioxane, wherein toxicity may be a function of the saturation of metabolism. For inhalation, neither the point of saturation nor the mechanism has been established. Importantly, the end-point for dioxane chronic exposure may not be established.

VII. Data Strengths and Limitations for Development of the REL

Although a free-standing NOAEL is not a desirable parameter to use for the development of a chronic REL, other studies support the conclusion that exposure to dioxane leads to adverse health effects. These observations have been documented among experimental animals (Kociba *et al.*, 1974; Pilipyuk *et al.*, 1977) and humans (Thiess *et al.*, 1976, described in NIOSH, 1977). Until additional data from inhalation dose-response studies become available, a chronic REL based on the free-standing NOAEL is considered the best available.

The strength of the REL for 1,4-dioxane is that it is based on a full lifetime study, with a large number of toxic endpoints and a good sample size. The weaknesses include use of a free standing NOAEL, the limited human data, and the lack of developmental studies.

VIII. References

Barber H. 1934. Haemorrhagic nephritis and necrosis of the liver from dioxane poisoning. Guy's Hospital Report. 84:267-280.

Braun WH, and Young JD. 1977. Identification of β-hydroxyethoxyacetic acid as the major urinary metabolite of 1,4-dioxane in the rat. Toxicol. Appl. Pharmacol. 39:33-38.

Buffler PA, Wood SM, Suarez MS, and Kilian DJ. 1978. Mortality follow-up of workers exposed to 1,4-dioxane. J. Occup. Med. 20:255-259.

CARB. 1999. Air toxics emissions data collected in the Air Toxics Hot Spots Program CEIDARS Database as of January 29, 1999.

CRC. 1994. CRC Handbook of Chemistry and Physics, 75th edition. Lide DR, ed. Boca Raton, FL: CRC Press Inc.

Giavini W, Vismara C, and Broccia ML. 1985. Teratogenesis study of dioxane in rats. Toxicol. Lett. 26:85-88.

Grant R, and Grant C. 1987. Grant and Hackh's Chemical Dictionary. R. Grant and C. Grant, eds. 5th ed. New York: McGraw-Hill Book Co. p. 189.

HSDB. 1995. Hazardous Substances Data Bank. National Library of Medicine, Bethesda, MD (TOMES® CD-ROM version). Denver, CO: Micromedex, Inc. (edition expires 7/31/95).

HSDB. 1999. Hazardous Substances Data Bank. Available online at http://sis.nlm.nih.gov

Johnstone RT. 1959. Death due to dioxane? AMA Arch. Ind. Health. 20:445-447.

Kociba RJ. 1974. Chronic toxicity study of dioxane in the drinking water of Sherman rats. Midland,MI: Dow Chemical Company.

NIOSH. 1977. Criteria for a Recommended Standard. Occupational Exposure to Dioxane. National Institute for Occupational Safety and Health, Centers for Disease Control, Public Health Service, Department of Health Education and Welfare. Publication No. 77-226.

Pilipyuk ZI, Gorban GM, Solomin GI, and Gorshunova AI. 1977. Toxicology of 1-4-dioxane. Space Biology and Aerospace Medicine. 11:70-74. (translated from Russian).

Reitz RH, McCroskey PS, Park CN, Andersen ME, and Gargas ML. 1990. Development of a physiologically based pharmacokinetic model for risk assessment with 1,4-dioxane. Toxicol. Appl. Pharmacol. 105:37-54.

Thiess AM, Tress E, Fleig I. 1976. [Industrial-medical investigation results in the case of workers exposed to dioxane.] (Ger.) Arbeitsmed. Sozialmed. Praventivmed. 11:35-46.

Torkelson TR, Leong BKJ, Kociba RJ, Richter WA, and Gehring PJ. 1974. 1.4-Dioxane. II. Results of a 2-year inhalation study in rats. Toxicol. Appl. Pharmacol. 30:287-298.

U.S. EPA (U.S. Environmental Protection Agency). 1988. Recommendations for and Documentation of Biological Values for Use in Risk Assessment. Chapter 4. Cincinnati, OH: United States Environmental Protection Agency.

Yaqoob M, and Bell GM. 1994. Occupational factors and renal disease. Renal Failure. 16:425-434.

Young JD, Braun WH, and Gehring PJ. 1978. Dose-dependent fate of 1,4-dioxane in rats. J. Toxicol. Environ. Health. 4:709-726.

Young JD, Braun WH, Gehring PJ, Horvath BS, and Daniel RL. 1976. 1,4-Dioxane and β-hydroxyethoxyacetic acid excretion in urine of humans exposed to dioxane vapors. Toxicol. Appl. Pharmacol. 38:643-646.

Young JD, Braun WH, Rampy LW, Chenoweth MB, and Blau GE. 1977. Pharmacokinetics of 1,4-dioxane in humans. J. Toxicol. Environ. Health. 3:507-520.

CHRONIC TOXICITY SUMMARY

CHRONIC TOXICITY SUMMARY

EPICHLOROHYDRIN

(1-chloro-2,3-epoxy-propane)

CAS Registry Number: 106-89-8

I. Chronic Toxicity Summary

Inhalation reference exposure level	**3 μg/m^3** (0.8 ppb)
Critical effects	Histological changes in nasal turbinates in rats
Hazard index target(s)	Respiratory system; eyes

II. Physical and Chemical Properties (HSDB, 1997; CRC, 1994)

Description	Colorless liquid
Molecular formula	C_3H_5ClO
Molecular weight	92.52 g/mol
Density	1.181 g/cm^3 @ 20° C
Boiling point	117° C
Melting point	–26°C
Vapor pressure	13 torr @ 20° C
Solubility	Slightly soluble in water, soluble in most organic solvents
Conversion factor	1 ppm = 3.78 mg/m^3 @ 25° C

III. Major Uses and Sources

Epichlorohydrin is a major raw material used in the manufacture of epoxy and phenoxy resins. It is also used as a solvent and in the synthesis of glycerol. Other uses include that of insect fumigation and as a chemical intermediate for the formation of glycidyl acrylate derivatives such as those used in the formation of eyeglass lenses (HSDB, 1994). The annual statewide industrial emissions from facilities reporting under the Air Toxics Hot Spots Act in California based on the most recent inventory were estimated to be 4841 pounds of epichlorohydrin (CARB, 2000).

IV. Effects of Exposures to Humans

Studies of male reproductive function have shown no evidence of decreased sperm counts in populations occupationally exposed to epichlorohydrin (Milby *et al.*, 1981).

V. Effects of Exposures in Animals

Rats were exposed for 136 weeks (6 hours/day, 5 days/week) to 0, 10, 30, or 100 ppm (0, 38, 113, or 380 mg/m^3) epichlorohydrin (Laskin *et al.*, 1980). Kidney damage in the form of renal tubular degeneration and dilatation was observed in rats exposed to 30 ppm or greater. The observation of severe inflammation in the nasal passages of 90% of the control animals, as well as in the treated animals, prevented comparison of this effect between the two groups.

A subchronic exposure of rats to 9, 17, 27, 56, or 120 ppm (34, 64, 102, 212, or 454 mg/m^3) for 6 hours/day, 5 days/week for 11-19 exposures showed evidence of extrarespiratory effects. These included liver congestion and necrosis and tubular atrophy in the kidneys at the highest concentration (Gage, 1959). Lethargy and weight loss were observed at 56 ppm.

A study on the effects of epichlorohydrin exposure for 10 weeks (6 hours/day, 5 days/week) on male and female fertility in rats and rabbits showed that male rats, exposed to 50 ppm (189 mg/m^3), were significantly less fertile than controls, as measured by successful matings to unexposed females (John *et al.*, 1979; 1983a). No histological changes were observed in the testes of the male rats at the end of exposure. No significant effects on fertility occurred in the exposed female rats. Degenerative changes in the nasal epithelium were observed in the female rats exposed to 25 ppm (94.5 mg/m^3), and in both sexes at 50 ppm.

A teratology study was carried out in rats and rabbits exposed to 0, 2.5, or 25 ppm (0, 9.5, or 95 mg/m^3) epichlorohydrin 7 hours/day during the critical days of gestation. There were no significant differences between controls and treated animals in the incidence of developmental defects, in maternal toxicity, or in histopathology of the lungs, nasal turbinates, or trachea (John *et al.*, 1983b).

Mice and rats (10/sex/concentration/strain) were exposed to 0, 5, 25, or 50 ppm (0, 19, 95, or 190 mg/m^3) epichlorohydrin for 6 hours/day, 5 days/week for 90 days (Quast *et al.*, 1979). Animals were observed for clinical signs of toxicity and were measured biweekly for body weight changes. Body weight measurements, clinical chemistry, hematology, and urinalysis were conducted. Gross and histopathological examinations were performed at the end of the experiment. Exposures of rats to 25 and 50 ppm epichlorohydrin resulted in inflammation, focal erosions, hyperplasia, and metaplasia in the nasal turbinates. No adverse effects were observed in rats exposed to 5 ppm (19 mg/m^3). Mice similarly showed focal erosion, hyperplasia and metaplasia in the epithelium of the nasal turbinates when exposed to 25 ppm epichlorohydrin or greater.

VI. Derivation of Chronic Reference Exposure Level

Study	Quast *et al.* (1979)
Study population	Rats and mice (10 per sex per concentration)
Exposure method	Discontinuous whole-body inhalation
Critical effects	Inflammation, focal erosions, hyperplasia, and metaplasia in the nasal turbinates
LOAEL	25 ppm (94.5 mg/m^3)
NOAEL	5 ppm (19 mg/m^3)
Exposure continuity	6 hours/day, 5 days/week
Exposure duration	90 days
Average experimental exposure	0.89 ppm (5 x 6/24 x 5/7)
Human equivalent concentration	0.083 ppm (gas with extrathoracic respiratory effects, RGDR = 0.093, based on MVa = 0.14 m^3/day , MVh = 20 m^3/day, SAa(ET) = 15 cm^2), SAh(ET) = 200 cm^2
LOAEL uncertainty factor	1
Subchronic uncertainty factor	3
Interspecies uncertainty factor	3
Intraspecies uncertainty factor	10
Cumulative uncertainty factor	100
Inhalation reference exposure level	0.0008 ppm (0.8 ppb; 0.003 mg/m^3; 3 μg/m^3)

The U.S. EPA (1994) based its RfC of 1 μg/m^3 on the same study but used a subchronic UF of 10 for a 90 day study instead of 3 (OEHHA, 2000).

VII. Data Strengths and Limitations for Development of the REL

The strengths of the inhalation REL for epichlorohydrin include the availability of controlled exposure inhalation studies in multiple species at multiple exposure concentrations and with adequate histopathogical analysis and the observation of a NOAEL. Major areas of uncertainty are the lack of adequate human exposure data, the lack of chronic inhalation exposure studies, the limited reproductive toxicity data, and the small groups tested in the study.

VIII. References

CARB. 2000. California Air Resources Board. California Emissions Inventory Development and Reporting System (CEIDARS). Data from Data Base Year 1998. February 12, 2000.

CRC. 1994. CRC Handbook of Chemistry and Physics, 75th edition. Lide DR, ed. Boca Raton, FL: CRC Press Inc.

Gage JC. 1959. The toxicity of epichlorohydrin vapour. Br. J. Ind. Med. 16:11-14.

HSDB. 1997. Hazardous Substances Data Bank. TOMES® Vol. 33. Denver, CO: Micromedex, Inc. (edition expires 7/31/97)

John JA, Quast JF, Murray FJ, *et al.* 1979. The effects of inhaled epichlorohydrin on the semen of rabbits and on the fertility of male and female rats. Toxicology Research Laboratory, Dow Chemical, Midland, MI. July 1979. NIEHS Contract No. NO1-ES-2102 (Unpublished).

John JA, Gushow TS, Ayres JA, Hanley TR, Quast JF, and Rao KS. 1983a. Teratologic evaluation of inhaled epichlorohydrin and allyl chloride in rats and rabbits. Fundam. Appl. Toxicol. 3:437-442.

John JA, Quast JF, Murray FJ, Calhoun LG, and Staples RE. 1983b. Inhalation toxicity of epichlorohydrin: effects on fertility in rats and rabbits. Toxicol. Appl. Pharmacol. 68:415-423.
/
Laskin S, Sellakumar AR, Kuschner M, Nelson N, La Mendola S, Rusch GM, Katz GV, Dulak NC, and Albert RE. 1980. Inhalation carcinogenicity of epichlorohydrin in noninbred Sprague-Dawley rats. J. Natl. Cancer Inst. 65(4):751-757.

Milby TH, Whorton MD, Stubbs HA, Ross CE, Joyner RE, and Lipshultz LI. 1981. Testicular function among epichlorohydrin workers. Br. J. Ind. Med. 38:372-377.

OEHHA. 2000. Office of Environmental Health Hazard Assessment. Air Toxics Hot Spots Program Risk Assessment Guidelines. Part III. Technical Support Document for the Determination of Noncancer Chronic Reference Exposure Levels. Available on-line at http://www.oehha.ca.gov

Quast JF, Henck JW, Postma BJ, Schuetz DJ, and McKenna MJ. 1979. Epichlorohydrin - subchronic studies. I. A 90-day inhalation study in laboratory rodents. Toxicology Research Laboratory, Dow Chemical U.S.A., Midland, MI (unpublished).

U.S. EPA. 1994. U.S. Environmental Protection Agency. Reference concentration for epichlorohydrin. Integrated Risk Information System (IRIS), CD-ROM version. Denver, CO: Micromedex, Inc. (Edition expires 10/31/94).

CHRONIC TOXICITY SUMMARY

1,2-EPOXYBUTANE

(1-butene oxide; 1,2-butene oxide; 1,2-butylene oxide; 1,2-epoxybutane; 2-ethyloxirane; ethylethylene oxide; NCI-C55527)

CAS Registry Number: 106-88-7

I. Chronic Toxicity Summary

Inhalation reference exposure level	**20 μg/m^3** (6 ppb)
Critical effect(s)	Degenerative lesions of the nasal cavity in mice
Hazard index target(s)	Respiratory system; cardiovascular system

II. Physical and Chemical Properties (HSDB, 1997)

Description	Colorless liquid with disagreeable odor
Molecular formula	C_4H_8O
Molecular weight	72.12 g/mol
Density	0.837 g/cm^3 @ 17°C
Boiling point	63.3°C
Melting point	Not available (CRC, 1994)
Vapor pressure	176 torr @ 25°C
Solubility	Soluble in ethanol, ether, acetone, water
Odor threshold	Unknown
Conversion factor	1 ppm = 2.95 mg/m^3

III. Major Uses or Sources

1,2-Epoxybutane is used as a chemical intermediate, acid scavenger, and stabilizer for chlorinated solvents (Reprotext, 1994). It is highly reactive, flammable, and undergoes exothermic polymerization reactions in the presence of acids, bases, and some salts. It is less volatile than ethylene oxide or propylene oxide (Reprotext, 1994). The annual statewide industrial emissions from facilities reporting under the Air Toxics Hot Spots Act in California based on the most recent inventory were estimated to be 6105 pounds of 1,2-epoxybutane (CARB, 2000).

IV. Effects of Human Exposure

No human toxicological data were found for 1,2-epoxybutane.

V. Effects of Animal Exposure

F344/N rats (50/sex) were exposed to 0, 200, or 400 ppm EBU for 6 hours/day, 5 days/week for 2 years (NTP, 1988). Survival was impaired and concentration-related increases of inflammation, respiratory epithelial hyperplasia, olfactory sensory epithelial atrophy, and hyperostosis of the nasal turbinate bone cavity were observed in male and female rats exposed to either concentration.

B6C3F1 mice (50/sex) were exposed to 0, 50, or 100 ppm EBU for 6 hours/day, 5 days/week for 2 years (NTP, 1988). Survival and body weight gain were reduced significantly at 100 ppm in both sexes. Significant concentration-related increases in incidence of chronic inflammation, epithelial hyperplasia, and erosion of the nasal cavity were noted in both sexes at either concentration. Increases in granulocytic hyperplasia and splenic hematopoiesis were noted at both concentrations in female mice.

Number of mice with lesions in the nasal cavity and olfactory sensory epithelium (NTP, 1988)

Sex		**Males**			**Females**	
EBU concentration	0 ppm	50 ppm	100 ppm	0 ppm	50 ppm	100 ppm
Number of mice studied	49	49	50	50	50	48
Nasal cavity						
Chronic inflammation	0	33	40	0	39	44
Erosion	0	7	17	0	16	24
Regeneration	0	15	17	0	14	15
Epithelial hyperplasia	0	32	45	1	34	35
Squamous metaplasia	1	24	41	0	34	41
Squam. cell papilloma	0	0	1	0	0	0
Olfactory sensory epithelium – atrophy	0	13	32	0	25	35

Male and female mice exposed to 800 ppm (2360 mg/m^3) EBU for 6 hours/day, 5 days/week, for 13 weeks were listless after the first exposure (NTP, 1988). Animals from this group all died by the end of the 13-week exposure. Renal tubular necrosis, and thymic and splenic atrophy were seen in mice exposed to 800 ppm; decreased liver weights were observed following exposure of mice to 400 ppm (1180 mg/m^3) or more. Inflammation of the nasal turbinates was seen in female mice exposed to 100 ppm (295 mg/m^3) or more. No inflammation was observed in controls.

Miller *et al*. (1981) exposed rats and mice of either sex to 0, 75, 150, or 600 ppm (0, 221, 442, or 1770 mg/m^3) EBU 6 hours/day, 5 days/week, for 13 weeks. In this study, no treatment-related effects were noted except for histological lesions in the nasal mucosal epithelium and reduced specific gravity in the urine of rats treated with 600 ppm.

Wolf (1961) observed increased lung weights in rats exposed to 800 ppm of a mixture of epoxybutane isomers. No increase in lung weight was seen at 400 ppm.

Sikov *et al.* (1981) conducted experiments to determine the reproductive toxicity of EBU in rats and rabbits. Rats were exposed to 0, 250, or 1000 ppm (0, 738, or 2950 mg/m^3) 1,2-epoxybutane for 7 hours/day, 5 days/week for 3 weeks prior to gestation, or for 7 hours/day on days 1-19 of gestation. Maternal toxicity in the form of 10% weight loss was observed in rats exposed to 1000 ppm. One death out of 42 occurred in the dams exposed to 1000 ppm. No adverse histological, reproductive, or developmental effects were seen at any concentration. Exposure of rabbits on days 1-24 of gestation to the same concentrations as in the rat experiment showed more severe effects at lower concentrations than those observed in rats. In the rabbits, 6 out of 48 dams died during exposure to 250 ppm, and 14 out of 24 died at 1000 ppm. Extensive maternal mortality in this study prevented evaluation of the reproductive and developmental effects.

VI. Derivation of Chronic Reference Exposure Level

Study	National Toxicology Program (NTP, 1988)
Study population	Rats and mice
Exposure method	Discontinuous inhalation to 0, 50, or 100 ppm EBU
Critical effects	Damage to the upper respiratory epithelium was observed in both species at all concentrations. Mice also showed an increased incidence of granulocytic hyperplasia and splenic hematopoiesis at both concentrations, possibly due to inflammation in the upper respiratory tract.
LOAEL	50 ppm (mice)
NOAEL	Not observed
Exposure continuity	6 hours/day, 5 days/week
Exposure duration	2 years
Average experimental exposure	8.9 ppm for LOAEL group (50 x 6/24 x 5/7)
Human equivalent concentration	1.8 ppm for LOAEL group (gas with extrathoracic respiratory effects, RGDR = 0.20, based on MVa = 0.06 m^3/day, MVh = 20 m^3/day, SAa(ET) = 3.0 cm^2, SAh(ET) = 200 cm^2)
LOAEL uncertainty factor	10 (high incidence of adverse effects)
Subchronic uncertainty factor	1
Interspecies uncertainty factor	3
Intraspecies uncertainty factor	10
Cumulative uncertainty factor	300
Inhalation reference exposure level	0.006 ppm (6 ppb; 0.02 mg/m^3; 20 µg/m^3)

The chronic REL is also the U.S. EPA RfC (U.S. EPA, 1994). OEHHA staff reviewed and agreed with U.S. EPA's analysis of the data.

VII. Data Strengths and Limitations for Development of the REL

The strengths of the inhalation REL for 1,2-epoxybutane include the availability of controlled exposure inhalation studies in multiple species at multiple exposure concentrations and with adequate histopathological analysis. Major areas of uncertainty are the lack of adequate human exposure data and the lack of observation of a NOAEL in the key study.

VIII. References

CARB. 2000. California Air Resources Board. California Emissions Inventory Development and Reporting System (CEIDARS). Data from Data Base Year 1998. February 12, 2000.

CRC. 1994. CRC Handbook of Chemistry and Physics, 75th edition. Lide DR, ed. Boca Raton, FL: CRC Press Inc.

HSDB. 1997. Hazardous Substances Data Bank. National Library of Medicine, Bethesda, MD (TOMES® CD-ROM version). Denver, CO: Micromedex, Inc. (edition expires 7/31/97).

Miller RR, Quast JF, Ayres JA, and McKenna MJ. 1981. Inhalation toxicity of butylene oxide. Fundam. Appl. Toxicol. 1(4):319-324. [as cited in: Integrated Risk Information System (IRIS). U.S. Environmental Protection Agency, Washington, DC. (CD-ROM version). Denver, CO: Micromedex, Inc. (Edition expires 10/31/94).]

NTP. 1988. National Toxicology Program. Technical Report Series No. 329. Toxicology and Carcinogenesis Studies of 1,2-Epoxybutane (CAS No. 106-88-7) in F344 Rats and B6C3Fl Mice. U.S. Department of Health and Human Services, Public Health Service, National Institutes of Health. Research Triangle Park, NC: NTP.

REPROTOX. 1994. The REPROTOX(R) System: Reproductive Reviews of Drugs, Chemicals, Physical and Biological Agents. (TOMES® CD-ROM Version). Denver, CO: Micromedex, Inc.

Sikov MR, Cannon WC, Carr DB, Miller RA, Montgomery LF, and Phelps DW. 1981. Teratologic assessment of butylene oxide, styrene oxide, and methyl bromide. Division of Biomedical and Behavioral Science, NIOSH. NIOSH/000993 14. NIOSH Technical Report No. 81-124.

U.S. EPA. 1994. U.S. Environmental Protection Agency. Integrated Risk Information System (IRIS) database. Reference concentration (RfC) for 1,2-epoxybutane. Available online at http://www.epa.gov/ngispgm3/iris

Wolf MA. 1961. Results of repeated exposures of laboratory animals to the vapors of butylene oxide(s) (Mixed isomers). Dow Chemical Biochemical Research Department. EPA/OTS Document No. 878211232. [as cited in: Integrated Risk Information System (IRIS). U.S. Environmental Protection Agency, Washington, D.C. (CD ROM version). Denver, CO: Micromedex, Inc. (Edition expires 10/31/94]

CHRONIC TOXICITY SUMMARY

ETHYL CHLORIDE

(Chloroethane; monochloroethane; ether hydrochloric)

CAS Registry Number: 75-00-3

I. Chronic Toxicity Summary

Inhalation reference exposure level	**30,000 µg/m^3 (10,000 ppb)**
Critical effect(s)	Delayed fetal ossification in mice
Hazard index target(s)	Teratogenicity; alimentary system

II. Physical and Chemical Properties (HSDB, 1995; 1999)

Description	Colorless gas
Molecular formula	C_2H_5Cl
Molecular weight	64.52
Density	0.9214 g/cm^3 @ 0°C
Boiling point	12.3 °C
Melting point	-138.7 °C
Vapor pressure	1000 torr @ 20 °C
Conversion factor	1 ppm = 2.64 mg/m^3 @ 25°C

III. Major Uses or Sources

Ethyl chloride has been used as a starting point in the production of tetraethyl lead and as a refrigerant, solvent and alkylating agent (HSDB, 1995). It is also used as a topical anesthetic (Clayton and Clayton, 1994). The annual statewide industrial emissions from facilities reporting under the Air Toxics Hot Spots Act in California based on the most recent inventory were estimated to be 291,300 pounds of ethyl chloride (CARB, 1999).

IV. Effects of Human Exposure

Neurological symptoms have been observed in human case studies in instances of ethyl chloride abuse. Cerebellar-related symptoms including ataxia, tremors, speech difficulties, and hallucinations were observed in a 28-year old female who had sniffed 200-300 ml ethyl chloride off her sleeve daily for 4 months (Hes *et al.* 1979). The patient's liver was enlarged and tender. Four weeks following cessation of exposure, all symptoms were absent.

V. Effects of Animal Exposure

Pregnant mice were exposed to 1300, 4000, or 13000 mg/m^3 ethyl chloride in air for 6 hours per day on days 6-15 of gestation (Scortichini *et al.*, 1986). No effects on fetal resorption rates, litter size, body weight or maternal health were observed. A statistically significant increase in the incidence of delayed ossification of the skull bones was observed in fetuses from the 13,000 mg/m^3 (4900 ppm) ethyl chloride exposed group. This skull effect was accompanied by a non-significant increased incidence of cervical ribs (a supernumerary rib is considered to be a malformation). No significant adverse effects were observed in fetuses from the 4000 mg/m^3 (1500 ppm) exposure group.

No significant adverse effects were observed in rats and mice exposed to 0 or 15,000 ppm ethyl chloride for 6 hours per day, 5 days per week for 102 weeks (rats) or 100 weeks (mice) (NTP, 1989). At necropsy, a complete histopathologic examination (approximately 35 tissues) failed to identify evidence of non-cancer toxicity. The same study also exposed rats and mice to 2500, 5000, 10,000 or 19,000 ppm ethyl chloride 6 hours per day, 5 days per week for 13 weeks. No exposure-related clinical signs of toxicity or histological changes were observed in exposed animals. Thus the subchronic NOAEL for mice and rats is 19,000 ppm, which is equivalent to a continuous exposure of 3400 ppm, and a free-standing chronic NOAEL is 15,000 ppm, which is equivalent to a continuous exposure of 2700 ppm (7100 mg/m^3).

Increased relative liver weights and a slight increase in hepatocellular vacuolation were observed in mice exposed to 5000 ppm ethyl chloride 23 hours per day for 11 days (Landry *et al.*, 1989). No effects were observed in mice exposed to 0, 250, or 1250 ppm ethyl chloride for the same period.

Following acclimatization to an inhalation chamber, two groups of 10 female mice were exposed to 0 or 15,000 ppm (40,000 mg/m^3) ethyl chloride 6 hours per day for 2 weeks (Breslin *et al.*, 1988). Groups of five male mice were housed in each inhalation chamber to synchronize and promote regular cyclicity. The mean length of the estrous cycle in control mice remained constant at 4.5 days during both pre-exposure and exposure periods. Mice in the 15,000 ppm exposure group showed a 0.6 day increase in the mean cycle length during exposure (5.6 days) when compared to the pre-exposure period (5.0 days). The authors attribute this increase in estrous cycle length to a general stress response although they note that it does not preclude direct effects on neuroendocrine function.

VI. Derivation of Reference Exposure Level

Study	Scortichini *et al.*, 1986
Study population	Mice
Exposure method	Discontinuous whole-body inhalation (on days 6-15 of gestation)
Critical effects	Delayed ossification of skull foramina
LOAEL	13,000 mg/m^3
NOAEL	4,000 mg/m^3
Exposure continuity	6 hours per day
Exposure duration	Days 6-15 of gestation
Average experimental exposure	1,000 mg/m^3 for NOAEL group (4000 x 6/24)
Human equivalent concentration	1,000 mg/m^3 for NOAEL group (gas with systemic effects, based on RGDR = 1.0 using default assumption that lambda (a) = lambda (h))
LOAEL uncertainty factor	1
Subchronic uncertainty factor	1
Interspecies uncertainty factor	3
Intraspecies uncertainty factor	10
Cumulative uncertainty factor	30
Inhalation reference exposure level	30 mg/m^3 (30,000 µg/m^3; 10 ppm; 10,000 ppb)

To develop the chronic REL OEHHA used the same study on which U.S. EPA based its RfC of 10,000 µg/m^3. The REL is based on a developmental toxicity study. In accordance with U.S. EPA methodology, a time-weighted average concentration for the discontinuous exposure experiment is not used by U.S. EPA when the key effect is developmental toxicity. However, OEHHA prefers to make a time adjustment to equivalent continuous exposure because the chronic REL assumes continuous exposure. U.S. EPA also used a Modifying Factor (MF). The database deficiencies leading U.S. EPA to employ a modifying factor include the lack of a multigenerational reproductive study. The criteria for use of such modifying factors are not well described. Such MFs were not used by OEHHA.

As a comparison to the proposed REL of 10 ppm, NTP (1989) found a free-standing NOAEL of 15,000 ppm in rats and mice exposed to ethyl chloride for 6 hours per day, 5 days per week for 2 years. Time adjusting to continuous exposure results in an adjusted NOAEL of 2679 ppm. Applying an RGDR of 1, a UF_A of 3 and a UF_H of 10 results in an estimated REL of 90 ppm.

VII. Data Strengths and Limitations for Development of the REL

The strengths of the inhalation REL for ethyl chloride include the availability of controlled exposure inhalation studies in multiple species at multiple exposure concentrations and with adequate histopathogical analysis, and the observation of a NOAEL. Major areas of uncertainty are the lack of adequate human exposure data, and the lack of a multigenerational reproductive study.

VIII. References

Breslin JW, Berdasco NM, Phillips JE, and Johnson KA. 1988. Ethyl Chloride (EtCl): Effects on Estrous Cycling in B6C3F1 Mice. Final Report with cover letter dated 11/21/88. Dow Chemical Company. EPA Document # 86-890000040.

CARB. 1999. Air toxics emissions data collected in the Air Toxics Hot Spots Program CEIDARS Database as of January 29, 1999.

Clayton GD, and Clayton FE. (eds.) 1994. Patty's Industrial Hygiene and Toxicology. Vol II Part E. New York: John Wiley and Sons, Inc. pp. 4082-4087.

HSDB. 1995. Hazardous Substances Data Bank. National Library of Medicine, Bethesda, MD (TOMES® CD-ROM Version). Micromedex, Inc., Denver, Colorado (Edition expires 1/31/95).

HSDB. 1999. Hazardous Substances Data Bank. Available online at http://sis.nlm.nih.gov

Hes JPh, Cohn DF, Streifler M. 1979. Ethyl chloride sniffing and cerebellar dysfunction (case report). Isr. Ann. Psychiatr. Relat. Discip. 17(2):122-125. [cited in U.S. EPA, 1995].

Landry TD, Johnson KA, Phillips JE, Weiss SK. 1989. Ethyl chloride: 11-Day continuous exposure inhalation toxicity study in B6C3F1 mice. Fundam. Appl. Toxicol. 13:516-522.

NTP. 1989. National Toxicology Program. Toxicology and Carcinogenesis Studies of Chloroethane (Ethyl Chloride) (CAS No. 75-00-3) in F344/N Rats and B6C3F1 Mice. NTP, US Department of Health and Human Services, Public Health Service, National Institutes of Health. NTP Technical Report Number 346.

Scortichini BH, Johnson KA, Momany-Pfruender JJ, Hanley TR. 1986. Ethyl Chloride: Inhalation Teratology Study in CF-1 Mice. Dow Chemical Company. EPA Document #86-870002248.

U.S. EPA (U. S. Environmental Protection Agency). 1995. Integrated Risk Information System (IRIS). Cincinnati, OH: Office of Health and Environmental Assessment, Environmental Criteria Assessment Office (CD-ROM Version).

CHRONIC TOXICITY SUMMARY

ETHYLBENZENE

(Phenylethane; NCI-C56393)

CAS Registry Number: 100-41-4

I. Chronic Toxicity Summary

Inhalation reference exposure level	**2000 µg/m^3 (400 ppb)**
Critical effect(s)	Liver, kidney, pituitary gland in mice and rats
Hazard index target(s)	Alimentary system (liver); kidney; endocrine system

II. Physical and Chemical Properties (HSDB, 1994)

Description	colorless liquid
Molecular formula	C_8H_{10}
Molecular weight	106.16 g/mol
Boiling point	136.2°C
Melting point	-95°C
Vapor pressure	10 torr @ 25.9°C
Density	0.867 g/cm^3 @ 20°C
Solubility	Soluble in ethanol and ether, low solubility in water (0.014 g/100 ml at 15°C)
Conversion factor	1 ppm = 4.35 mg/m^3

III. Major Uses or Sources

Ethylbenzene is used as a precursor in the manufacture of styrene (HSDB, 1994). It is also used in the production of synthetic rubber, and is present in automobile and aviation fuels. It is found in commercial xylene (Reprotext, 1994). In 1996, the latest year tabulated, the statewide mean outdoor monitored concentration of ethylbenzene was approximately 0.4 ppb (CARB, 1999a). The latest annual statewide emissions from facilities reporting under the Air Toxics Hot Spots Act in California, based on the most recent inventory, were estimated to be 161,846 pounds of ethylbenzene (CARB, 1999b).

IV. Effects of Human Exposure

Studies on the effects of workplace exposures to ethylbenzene have been complicated by concurrent exposures to other chemicals, such as xylenes (Angerer and Wulf, 1985). Bardodej

and Cirek (1988) reported no significant hematological or liver function changes in 200 ethylbenzene production workers over a 20-year period.

V. Effects of Animal Exposure

Rats and mice (10/sex/group) were exposed to 0, 100, 250, 500, 750, and 1000 ppm (0, 434, 1086, 2171, 3257, and 4343 mg/m^3) ethylbenzene 6 hours/day, 5 days/week for 90 days (NTP, 1988; 1989; 1990). Rats displayed significantly lower serum alkaline phosphatase in groups exposed to 500 ppm or higher. Dose-dependent increases in liver weights were observed in male rats beginning at 250 ppm, while this effect was not seen until 500 ppm in the females. An increase in relative kidney weights was seen in the 3 highest concentrations in both sexes. Minimal lung inflammation was observed in several of the treatment groups, but this phenomenon was attributed to the presence of an infectious agent rather than to ethylbenzene exposure. The mice in this study did not show any treatment-related effects except for elevated liver and kidney weights at 750 and 1000 ppm, respectively.

Rats and mice were exposed to ethylbenzene (greater than 99% pure) by inhalation for 2 years (NTP, 1999; Chan *et al*., 1998). Groups of 50 male and 50 female F344/N rats were exposed to 0, 75, 250, or 750 ppm, 6 hours per day, 5 days per week, for 104 weeks. Survival of male rats in the 750 ppm group was significantly less than that of the chamber controls. Mean body weights of 250 and 750 ppm males were generally less than those of the chamber controls beginning at week 20. Mean body weights of exposed groups of females were generally less than those of chamber controls during the second year of the study. In addition to renal tumors, the incidence of renal tubule hyperplasia in 750 ppm males was significantly greater than that in the chamber controls. The severity of nephropathy in 750 ppm male rats was significantly increased relative to the chamber controls. Some increases in incidence and severity of nephropathy were noted in all exposed female rats, but these were statistically significant only at 750 ppm.

Groups of 50 male and 50 female B6C3F1 mice were exposed to 0, 75, 250, or 750 ppm ethylbenzene by inhalation, 6 hours per day, 5 days per week, for 103 weeks. Survival of exposed mice was similar to controls. Mean body weights of females exposed to 75 ppm were greater than those of the chamber controls from week 72 until the end of the study. In addition to lung and liver tumors, the incidence of eosinophilic liver foci in 750 ppm females was significantly increased compared to that in the chamber controls. There was a spectrum of nonneoplastic liver changes related to ethylbenzene exposure in male mice, including syncytial alteration of hepatocytes, hepatocellular hypertrophy, and hepatocyte necrosis. The incidences of hyperplasia of the pituitary gland pars distalis in 250 and 750 ppm females and the incidences of thyroid gland follicular cell hyperplasia in 750 ppm males and females were significantly increased compared to those in the chamber control groups. Based on an evaluation of all the non-cancer data in mice and rats OEHHA staff selected 75 ppm as the NOAEL for the NTP (1999) study.

Rats (17-20 per group) were exposed to 0, 600, 1200, or 2400 mg/m^3 for 24 hours/day on days 7 to 15 of gestation (Ungvary and Tatrai, 1985). Developmental malformations in the form of "anomalies of the uropoietic apparatus" were observed at the 2400 mg/m^3 concentration.

Skeletal retardation was observed in all exposed groups compared with controls. The incidence of skeletal abnormalities increased with higher concentrations of ethylbenzene.

Rabbits exposed by these investigators to the same concentrations as the rats on days 7 to 15 of gestation, exhibited maternal weight loss with exposure to 1000 mg/m^3 ethylbenzene. There were no live fetuses in this group for which abnormalities could be evaluated. No developmental defects were observed in the lower exposure groups.

Rats (78-107 per group) and rabbits (29-30 per group) were exposed for 6 or 7 hours/day, 7 days/week, during days 1-l9 and 1-24 of gestation, respectively, to 0, 100, or 1000 ppm (0, 434, or 4342 mg/m^3) ethylbenzene (Andrew *et al.*, 1981; Hardin *et al.*, 1981). No effects were observed in the rabbits for maternal toxicity during exposure or at time of necropsy. Similarly, no effects were seen in the fetuses of the rabbits. The only significant effect of ethylbenzene exposure in the rabbits was a reduced number of live kits in the 1000 ppm group. A greater number and severity of effects were seen in rats exposed to 1000 ppm ethylbenzene. Maternal rats exposed to 1000 ppm exhibited significantly increased liver, kidney, and spleen weights compared with controls. Fetal rats showed an increase in skeletal variations at the 1000 ppm concentration, but the results of the 100 ppm exposure were not conclusive.

Clark (1983) found no significant effects on body weight, food intake, hematology, urinalysis, organ weights or histopathology in rats (18 per group) exposed to 100 ppm (434 mg/m^3) ethylbenzene for 6 hours/day, 5 days/week, for 12 weeks.

Degeneration of the testicular epithelium was noted in guinea pigs and a rhesus monkey exposed to 600 ppm (2604 mg/m^3) for 6 months (Wolf *et al.*, 1956). No effects were reported for female monkeys exposed to the same conditions.

Cragg *et al.* (1989) exposed mice and rats (5/sex/group) to 0, 99, 382, and 782 ppm (0, 430, 1659, and 3396 mg/m^3) 6 hours/day, 5 days/week for 4 weeks. Some evidence of increased salivation and lacrimation was seen in the rats exposed to 382 ppm. No other gross signs of toxicity were observed. Both male and female rats had significantly enlarged livers following exposure to 782 ppm. Female mice also showed a significant increase in liver weight at this concentration. No histopathological lesions were seen in the livers of these mice.

Dose-dependent induction of liver cytochrome P450 enzymes in rats by ethylbenzene was observed by Elovaara *et al.* (1985). Rats (5 per group) were exposed to 0, 50, 300, or 600 ppm (0, 217, 1302, or 2604 mg/m^3) ethylbenzene for 6 hours/day, 5 days/week for 2, 5, 9, or 16 weeks. Cytochrome P450 enzyme induction, and microscopic changes in endoplasmic reticulum and cellular ultrastructure were evident at all ethylbenzene concentrations by week 2, and persisted throughout the exposure. Liver weights were not elevated in these studies.

VI. Derivation of the Chronic Reference Exposure Level

Study	NTP, 1999; Chan *et al*., 1998
Study population	Male and female rats and mice (50 per group)
Exposure method	Discontinuous inhalation
Critical effects	Nephrotoxicity, body weight reduction (rats) hyperplasia of the pituitary gland; liver cellular alterations and necrosis (mice)
LOAEL	250 ppm
NOAEL	75 ppm
Exposure continuity	6 hours/day, 5 days/week
Exposure duration	103 weeks.
Average experimental exposure	13 ppm for NOAEL group
Human equivalent concentration	13 ppm for NOAEL group (gas with systemic effects, based on RGDR = 1.0 using default assumption that lambda (a) = lambda (h))
LOAEL uncertainty factor	1
Subchronic uncertainty factor	1
Interspecies uncertainty factor	3
Intraspecies uncertainty factor	10
Cumulative uncertainty factor	30
Inhalation reference exposure level	0.4 ppm (400 ppb; 2 mg/m^3; 2,000 µg/m^3)

The REL is based on a lifetime toxicity/carcinogenesis study. The NOAEL for non-neoplastic effects in the study was 75 ppm, and the LOAEL was 250 ppm. Some shorter duration studies discussed above (e.g. NTP, 1988, 1989, 1990) identify higher concentrations as NOAELs, but the study used (NTP 1999) is the most recent available and is considered the most reliable for assessing chronic effects.

U.S. EPA based its RfC on developmental toxicity studies in rats and rabbits (Andrew *et al.*, 1981; Hardin *et al.*, 1981; U.S. EPA, 1994). The NOAEL in the studies was 100 ppm, and the LOAEL was 1000 ppm. In accordance with its methodology, U.S. EPA did not use a time-weighted average concentration for the discontinuous exposure experiment since the key effect was developmental toxicity. If OEHHA methodology is followed (which includes the time-weighted averaging of the exposure concentrations, and uncertainty factors of 3 (interspecies, with RGDR = 1) and 10 (intraspecies), this study would indicate a REL of 0.6 ppm (3 mg/m^3). The study by Ungvary and Tatrai (1985) reported a NOAEL of 600 mg/m^3 for developmental and maternal effects in several species. However, the reporting and general quality of this paper create less confidence in its results.

For comparison to the proposed REL of 0.4 ppm, Clark (1983) found no significant effects in rats exposed to 100 ppm ethylbenzene 6 h/day, 5 d/week, for 12 weeks. This NOAEL can be time-adjusted to 18 ppm, then divided by a subchronic UF of 3, an interspecies UF of 3, and an intraspecies UF of 10 which results in a REL of 0.2 ppm. (The default value of 1 for RGDR was used). It appears that the proposed REL provides a sufficient margin of safety to provide

protection against the reported developmental effects (Andrew *et al.,* 1981; Hardin *et al.,* 1981; Ungvary and Tatrai, 1985)

VII. Data Strengths and Limitations for Development of the REL

The strengths of the inhalation REL for ethylbenzene include the availability of controlled exposure inhalation studies in multiple species at multiple exposure concentrations and with adequate histopathogical analysis, and the observation of a NOAEL in lifetime chronic inhalation exposure studies. The major area of uncertainty is the lack of adequate human exposure data.

VIII. References

Andrew FD, Buschbom RL, Cannon WC, Miller RA, Montgomery LF, Phelps DW, *et al.* 1981. Teratologic assessment of ethylbenzene and 2-ethoxyethanol. Battelle Pacific Northwest Laboratory, Richland, WA. PB 83-208074, p. 108. [as cited in: U.S.EPA's Integrated Risk Information System (IRIS) database. 1994.]

Angerer J, and Wulf H. 1985. Occupational chronic exposure to organic solvents. XI. Alkylbenzene exposure of varnish workers: Effects on hematopoietic system. Int. Arch. Occup. Environ. Health. 56(4):307-321. [as cited in U.S.EPA's Integrated Risk Information System (IRIS) database. 1994.]

Bardodej Z, and Cirek A. 1988. Long-term study on workers occupationally exposed to ethylbenzene. J. Hyg. Epidemiol. Microbiol. Immunol. 32(1):1-5. [As cited in U.S.EPA's Integrated Risk Information System (IRIS) database. 1994.]

CARB. 1999a. California Air Resources Board. Toxics Air Quality Data. Substance Chooser. Ethyl Benzene. Available online at http:/www.arb.ca.gov/aqd/toxics.htm

CARB. 1999b. Air toxics emissions data collected in the Air Toxics Hot Spots Program CEIDARS Database as of January 29, 1999.

Clark DG. 1983. Ethylbenzene hydroperoxide (EBHP) and ethyl benzene (EB): 12-week inhalation study in rats. (Group research report with attachments and cover sheet.) EPA OTS Public Files. Shell Oil Co. Document No. 86870001629. Fiche Number 0516206. [as cited in U.S.EPA's Integrated Risk Information System (IRIS) database. 1994.]

Chan PC, Haseman JK, Mahleri J, Aranyi C. 1998. Tumor induction in F344/N rats and B6C3F1 mice following inhalation exposure to ethylbenzene. Toxicol. Lett. 99(1):23-32.

Cragg ST, Clarke EA, Daly IW, Miller RR, Terrill JB, and Quellette RE. 1989. Subchronic inhalation toxicity of ethylbenzene in mice, rats, and rabbits. Fundam. Appl. Toxicol. 13(3):399-408. [as cited in U.S.EPA's Integrated Risk Information System (IRIS) database. 1994.]

Elovaara E, Engstrom K, Nickels J, Aito A, and Vainio H. 1985. Biochemical and morphological effects of long-term inhalation exposure of rats to ethylbenzene. Xenobiotica. 15(4):299-308. [as cited in U.S.EPA's Integrated Risk Information System (IRIS) database. 1994.]

Hardin BD, Bond GP, Sikov MR, Andrew FD, Beliles RP, and Niemeier RW. 1981. Testing of selected workplace chemicals for teratogenic potential. Scand. J. Work Environ. Health. 7(suppl 4): 66-75.

HSDB. 1994. Hazardous Substances Data Bank. National Library of Medicine, Bethesda, MD (CD-ROM version) Denver, CO: Micromedex, Inc. (Edition expires 4/30/94).

NTP. 1988. National Toxicology Program. Subchronic and chronic toxicity study of ethylbenzene. 90-Day subchronic study report on inhalation exposure of F344/N rats and B6C3F1 mice. Chicago, IL: IIT Research Institute. [as cited in U.S.EPA's Integrated Risk Information System (IRIS) database. 1994.]

NTP. 1989. National Toxicology Program. Chairperson's report. Pathology Working Group (PWG) review of subchronic toxicity testing on ethylbenzene administered by inhalation in F344 rats and B6C3FI mice. [as cited in U.S.EPA's Integrated Risk Information System (IRIS) database. 1994.]

NTP. 1990. National Toxicology Program. Draft NTP Technical Report on the Toxicity Studies of Ethylbenzene in F344 Rats and B6C3F1 Mice (inhalation Studies). NTP TOX 10, U.S. DHHS. [as cited in U.S.EPA's Integrated Risk Information System (IRIS) database. 1994.]

NTP. 1999. National Toxicology Program. Toxicology and Carcinogenesis Studies of Ethylbenzene (CAS No. 100-41-4) in F344/N Rats and B6C3F1 Mice (Inhalation Studies). TR-466.

Reprotext ® database. (CD-ROM version) Denver, CO: Micromedex, Inc. (Edition expires 4/30/94).

Ungvary G, and Tatrai E. 1985. On the embryotoxic effects of benzene and its alkyl derivatives in mice, rats, and rabbits. Arch. Toxicol. Suppl. 8:425-430.

U.S.EPA. 1994. Ethylbenzene. Integated Risk Information System (IRIS) on-line database.

Wolf MA, Rowe VKL, McCollister DD, Hollingsworth RL, and Oyen F. 1956. Toxicological studies of certain alkylated benzenes and benzene. Arch. Ind. Health 14: 387-398.

CHRONIC TOXICITY SUMMARY

ETHYLENE DIBROMIDE

(1,2-dibromoethane; dibromoethane; alpha, beta-dibromoethane; EDB; ethylene bromide; glycol bromide)

CAS Registry Number: 106-93-4

I. Chronic Toxicity Summary

Inhalation reference exposure level	**0.8 μg/m^3** (0.1 ppb)
Critical effect(s)	Decreased sperm count/ejaculate, decreased percentage of viable and motile sperm, increased semen pH, and increased proportion of sperm with specific morphological abnormalities in human males
Hazard index target(s)	Reproductive system

II. Chemical Property Summary (HSDB, 1995; CRC, 1994)

Description	Colorless, heavy, nonflammable liquid with a mildly sweet, chloroform-like odor.
Molecular formula	$C_2H_4Br_2$
Molecular weight	187.88 g/mol
Boiling point	131-132°C
Melting point	9.9°C
Vapor pressure	0.11 torr at 20°C
Solubility	Slightly soluble in water (3400 mg/L water at 20°C). Miscible with most organic solvents.
Conversion factor	7.68 μg/m^3 per ppb at 25°C

III. Major Uses and Sources

Ethylene dibromide (EDB) is used as a solvent for resins, gums, and waxes, and as a chemical intermediate in the synthesis of dyes and pharmaceuticals (HSDB, 1995). EDB was once widely used as a fumigant for the control of pests in the U.S. Because of concerns regarding its carcinogenicity, the agricultural uses of EDB were banned in 1983 (RECT, 1988). EDB was also commonly used as a gasoline additive to scavenge inorganic lead compounds. The transition to the use of lead-free gasoline has drastically curtailed the use of EDB in this country (REPROTOX, 1995). EDB is now used mainly in industry. EDB may be formed naturally in

the ocean as a result of macro algae growth. Exposure to the general population, via inhalation, may occur in the vicinity of industries and in industrial settings where this compound is manufactured and used. The annual statewide industrial emissions from facilities reporting under the Air Toxics Hot Spots Act in California based on the most recent inventory were estimated to be 1179 pounds of EDB (CARB, 2000).

IV. Effects of Human Exposures

Pharmacokinetic studies of EDB in humans could not be found in the literature. However, *in vitro* studies of EDB metabolism in human liver samples have been performed (Wiersma *et al.*, 1986). These experiments have shown that the enzyme systems known to metabolize EDB in rodent liver also metabolize EDB in the human liver. EDB was metabolized by human liver cytosolic glutathione S-transferases (GST), microsomal GST, and microsomal mixed function oxidases (MFO). MFO activity resulted in adducts irreversibly bound to protein, while GST activity was mostly responsible for adducts irreversibly bound to DNA. Rodent liver enzymes similarly activate EDB to metabolites that bind to cellular macromolecules. In human fetal liver (16-18 weeks gestation) cytosolic GST was also found to metabolize EDB with high efficiency (Kulkarni *et al.*, 1992). Since detoxification via MFO activity may be limited at this stage of development, the results suggest that the human fetus and neonate may be at greater risk from EDB toxicity than adults.

A study of mortality from cancer and respiratory diseases was conducted among 161 employees exposed to EDB in 2 production units operated from 1942 to 1969 and from the mid-1920s to 1976, respectively (Ott *et al.*, 1980). No apparent connection was found between mortality due to respiratory diseases and exposure to EDB, when compared to U.S. white male mortality figures.

Due to the structural similarity of EDB to dibromochloropropane (DBCP), a known toxic agent in human male reproductive organs, a number of epidemiological studies concerning male reproduction and spermatogenesis were conducted.

In a study of 59 employees exposed to EDB at the Ethyl Corporation plant in Magnolia, Arkansas, the sperm counts of the exposed men were divided into 2 groups depending on estimated exposure (Ter Haar, 1980). Twenty percent of the low exposure group (<0.5 ppm) had sperm counts below 40 million, whereas 42% of the high exposure group (0.5 to 5 ppm) had sperm counts below this figure. The sperm counts were intermediate between counts reported for 2 types of U.S. samples (for normal men). The observed births among the two exposure groups were found to be similar to the number of expected births. The author determined that EDB had no effect on sterility or reproduction in the workers. Weaknesses of this study include the small population of exposed workers and the lack of a concurrent unexposed control group. Taking these defects of the study into account, Dobbins (1987) concluded that the results provide evidence that EDB exposure between 0.5 and 5.0 ppm is associated with lower sperm counts.

A comparison of observed marital fertility with expected fertility (based on U.S. fertility rates) was conducted among 297 men working at 4 U.S. plants that manufacture EDB (Wong *et al.*,

1979). Fertility was 20% below expected for the four plants combined. This was largely due to one plant (plant D), which was 49% below the expected level. After omitting the incidence of vasectomies and hysterectomies among married couples, observed fertility was still 39% below the expected figure for plant D but was now no longer statistically significant. Exposure levels of EDB at plant D were not known but were estimated to be no more than 5 ppm. Later review determined that expected (control) levels of fertility and the power of the study were too low, resulting in the inability to identify a possible adverse effect (Dobbins, 1987). The lower fertility at plant D indicates that EDB has the potential to reduce fertility, but the extent of the reduction cannot be estimated from this study. Further treatment of the data by a method that uses the proper statistical adjustments of reproductive experience in the U.S. population (used as the control) suggests borderline significance for reduced fertility among the combined workers at the four plants (Wong *et al.*, 1985). The fertility evaluation indicates that more in-depth epidemiologic or physiologic studies are needed.

Semen analysis of 83 pineapple workers at two plantations was performed by Rogers and associates (1981). EDB-exposed workers were removed from each group and placed in a separate group. The remaining two groups of workers acted as control groups. Sperm counts, motility, and morphology were similar among the three groups. However, 43.8% of exposed workers had abnormally low counts (<40 million/ml), while abnormally low sperm counts of controls were 34.2% and 17.8%. Of the four exposed workers that had fertility tests done, all tested in the infertile range. Forty percent or less tested in the infertile range among the control groups. The results suggest that workers exposed to EDB had reduced sperm counts, but exposure levels were not known.

Semen analysis among 46 men employed in the papaya fumigation industry was conducted to determine if EDB affected semen quality (Ratcliff *et al.*, 1987; Schrader *et al.*, 1987). Average duration of exposure was 5 years and the geometric mean breathing zone exposure to airborne EDB was 88 ppb (8 hr time weighted average) with peak exposures of up to 262 ppb. The comparison group consisted of 43 unexposed men from a nearby sugar refinery. Following consideration of confounding factors, statistically significant decreases in sperm count/ejaculate, the percentage of viable and motile sperm, and increases in the proportion of sperm with specific morphological abnormalities (tapered heads, absent heads, and abnormal tails) were observed among exposed men. Semen pH was significantly more alkaline than that of unexposed workers. Other measured sperm quality parameters were unchanged. This study suggests that EDB can result in reproductive impairment. However, no measurement of male fertility was conducted.

In a study that examined similar indices of semen quality, 6 week exposure of 10 forestry workers to EDB (60 ppb time weighted average, with peak exposures of up to 2165 ppb) resulted in decreased semen volume and slower sperm velocity (Schrader *et al.*, 1988). Six unexposed men were used as controls. The researchers suggest that short-term exposure to EDB results in decreased sperm velocity, while long-term exposure, as in the previous study of EDB-exposed papaya workers, results in sperm immotility and cell death.

V. Effects of Animal Exposures

EDB is readily and rapidly absorbed from the lung when breathed as a vapor, from the GI tract when taken orally, or through the skin when applied dermally (HSDB, 1995). In rats, the rate of absorption of EDB from the respiratory tract reached a plateau within 10 to 20 minutes following exposure to 75 ppm EDB for up to 2 hours (Stott and McKenna, 1984). About 58% of the EDB was absorbed. Intraperitoneal injection of [^{14}C]EDB into guinea pigs resulted in the highest concentrations in liver, kidneys, and adrenals (Plotnick and Conner, 1976). Sixty-five percent of the dose was excreted as metabolites in urine, 3% in feces, and 12% excreted unchanged in expired air. In rats, the highest concentrations of [^{14}C]EDB label were found in liver, kidney and spleen following an oral dose of 15 mg/kg body wt (Plotnick *et al.*, 1979). Studies with rats have provided evidence that 2 pathways of metabolic bioactivation exist for EDB (RECT, 1988). The oxidative pathway yields the metabolite 2-bromo-acetaldehyde, which is associated with cell macromolecule binding and liver damage. The conjugative pathway principally yields glutathione products, such as *S*-(2-bromoethyl)-glutathione, which are mainly responsible for DNA binding and mutagenesis. In rats, orally administered EDB is excreted primarily in the urine as mercapturic acid derivatives (Jones and Edwards, 1968). The biologic half-life for elimination of [^{14}C]EDB in rats is 5.1-5.6 hours (Watanabe *et al.*, 1978) and less than 48 hours in mice and guinea pigs (HSDB, 1995). Besides the small amount irreversibly bound to cell macromolecules and DNA, EDB shows little, if any, bioaccumulation in mammalian systems.

In a subchronic toxicity study of experimental animals, rats and guinea pigs were given EDB by oral administration for about 4 months (Aman *et al.*, 1946). Body weights and mortality of animals at or below an average daily dose of 40-50 mg/kg body wt-day were unaffected. However, only one control animal/species was used, the dosing regimen was not well described, and pathologic examination was apparently not performed.

Subchronic exposure of rats (20/sex/group) to 50 ppm EDB for as many as 63 seven-hour exposures in 91 days resulted in no significant change in body weights (Rowe *et al.*, 1952). Liver and kidney weights were increased in both sexes while testis weights were decreased in males. Also, lung weights in males were elevated and spleen weights in females were decreased. Histopathological examination revealed no changes. Guinea pigs (8/sex/group) subjected to as many as 57 seven-hour exposures of 50 ppm EDB in 80 days exhibited reduced body weights. Organ weights were unchanged, but microscopic examination of the livers showed slight central fatty degeneration. In kidneys, slight interstitial congestion and edema with slight parenchymatous degeneration of the tubular epithelium were observed. Four rabbits exposed to 59 seven-hour sessions at 50 ppm in 84 days showed no signs of adverse effects. Clinical signs of monkeys exposed to 50 ppm EDB (49 seven-hour exposures in 70 days) included an ill, unkempt appearance and nervousness. Slight central fatty degeneration in livers was observed, but pathology was not seen in other tissues. Exposure of the same four species to 25 ppm EDB for up to 220 days (145 to 156 seven-hour exposures) showed no signs of adverse effects.

In a 13-week inhalation study, 5 Fischer 344 albino rats/group/sex and 10 B6C3F1 mice/group/sex were exposed to 0, 3, 15, or 75 ppm EDB for 6 hr/day, 5 days/week (Reznik *et al.*, 1980). At 75 ppm, rats and mice exhibited severe necrosis and atrophy of the olfactory epithelium in the nasal cavity. Squamous metaplasia, hyperplasia and cytomegaly of the

epithelium were also seen in nasal turbinates, larynx, trachea, bronchi, and bronchioles. Minor alterations were seen in the nasal cavity of only a few male and female rats at 15 ppm. No compound-related lesions were observed in the olfactory and respiratory epithelium at 3 ppm. No lesions were seen in other tissues at any dose.

In another 13-week inhalation study, 40 male and 20 female CDF(F344) rats/group were exposed to 0, 3, 10, or 40 ppm EDB 6 hr/day, 5 days/week (Nitschke *et al.*, 1981). Male rats in the 40 ppm group exhibited decreased weight gain throughout most of the exposure period. However, reduced weight gain was never more than 6-8% below control levels. With the exception of decreased specific gravity of urine in females of the 40 ppm group, no treatment-related changes were observed in any rat group with respect to urinalysis, hematology, and clinical chemistry. At the end of 13 weeks, relative liver and kidney weights of males exposed to 40 ppm EDB were significantly elevated, while relative liver weights of females in the two highest exposure groups were significantly elevated. Absolute liver weight of females in the 40 ppm group was also significantly elevated. Histopathological examination revealed lesions primarily confined to the anterior sections of the nasal turbinates. Hyperplasia and nonkeratinizing squamous metaplasia of the respiratory epithelium were observed in nasal turbinates of rats exposed to 40 ppm EDB. Only slight epithelial hyperplasia of nasal turbinates was noted at 10 ppm. No treatment related effects were seen at 3 ppm. Livers of females in the 40 ppm group showed a slight increase in fat. After an 88 day recovery period, there was a reversion to normal of the nasal turbinates in all but one rat.

In what was originally scheduled to be a lifetime exposure study, 50 Osborne-Mendel rats/group/sex and 50 B6C3F1 mice/group/sex were administered EDB 5 days/week by gastric lavage over a substantial portion of their life-span (NCI, 1978). Twenty untreated controls/sex and 20 vehicle controls/sex of each species were included in the study. Rats received initial doses of 80 and 40 mg/kg body wt-day for the first 17 weeks. Due to high mortality, dosing of high dose rats was discontinued for 13 weeks and resumed on week 30 at 40 mg/kg body wt-day. In week 42, all intubations of low and high dose rats ceased for 1 week followed by 4 weeks of dose administration. All surviving, treated male rats were necropsied in week 49; all surviving, treated female rats were necropsied in week 61. The resulting time-weighted average dose over the test period was 38 and 41 mg/kg body wt-day for low and high dose males, respectively, and 37 and 39 mg/kg body wt-day for low and high dose females, respectively. Mice received initial doses of 120 and 60 mg/kg body wt-day. In weeks 11-13, high and low doses were increased to 200 and 100 mg/kg body wt-day, respectively. Original dose levels were resumed after week 13. At week 40, administration of EDB was decreased to 60 mg/kg body wt-day for high dose mice. EDB administration was discontinued at week 54 with necropsy occurring at week 78 for males and high dose females. Low dose female mice were observed for 37 weeks after intubation ceased. The resulting time-weighted average dose over the test period was 62 and 107 mg/kg body wt-day for low and high dose mice, respectively. In rats, clinical signs by week 5 included reddened ears and hunched back in all treatment groups. By week 10, all treated rats had reduced body weights ($\geq$10%). Both female and male rats exhibited dose-dependent mortality. Many of the deaths occurred during or shortly after intubation, suggesting an acute toxic reaction. Pathology revealed hyperkeratosis and acanthosis of the forestomach in high dose males and females and in one low dose female. A small number of rats in both treatment groups showed adrenal cortex degeneration and peliosis of the liver (hepatitis). Dosed males showed

early development of testicular atrophy. In mice, dose-related body weight reduction and mortality were observed. Clinical signs included alopecia, thin, hunched appearance, soft feces and body sores. Hyperkeratosis and acanthosis of the forestomach were seen in high dose male and female mice. One incidence each of hyperkeratosis (in a female) and acanthosis (in a male) was seen at the low dose. Splenic changes were present in high dose mice and testicular atrophy was present in high dose males.

In a long-term inhalation exposure study, F344 rats and $B6C3F_1$ mice were exposed to 0, 10, or 40 ppm EDB 6 hr/day, 5 days/week for up to 103 weeks (NTP, 1982). In male and female rats, the high dose groups had reduced body weights and increased mortality that began at about week 60. The treatment-related non-neoplastic pathology included hepatic necrosis (both sexes), epithelial hyperplasia and suppurative inflammation throughout the respiratory system (both sexes), and nephropathy (males only). Toxic nephropathy and mineralization were also seen in high dose female rats. Testicular degeneration and atrophy occurred with greater frequency in exposed rats and may be related to observed testicular tumors. Spermatic granulomas were also more frequently seen in high-dose males. Degeneration of the adrenal cortex appeared to be dose-related in females, but only one incidence each was seen in low and high dose males. Increased incidence of retinal atrophy was observed in exposed females. In mice, body weights were reduced at the high dose in both males and females. Many of the high dose animals exhibited a progressive weakness of the limbs or body during the second year. Increased mortality occurred in a dose-related manner in females and was significantly greater in low dose males. Non-neoplastic pathology included epithelial hyperplasia throughout the respiratory system and serous and suppurative inflammation of the nasal cavity in exposed mice. In all male mice, the principal cause of death was urinary bladder inflammation. However, bladder epithelial hyperplasia was only seen in exposed animals. An increased incidence of suppurative inflammation of the prostate was present but was also seen in controls. Dose-related spleen hematopoiesis was observed in females.

Another long-term inhalation study investigated the effects of 0 or 20 ppm EDB (7 hr/day, 5 days/week) on 48 Sprague-Dawley rats/sex/group for 18 months (Wong *et al.*, 1982). Significantly lower body weight gains (>10% difference from controls) occurred by the 15th month in males, and by the 18th month in females. Significantly reduced food consumption was not apparent. Increased mortality rates in both sexes occurred beginning in the 12th month of EDB exposure. All hematological findings were within normal ranges. The only recorded non-neoplastic gross or microscopic finding was atrophy of the spleen in males, which may be related to tumor formation (hemangiosarcoma). The nasal cavity was not examined.

In a study of the effect of EDB on sperm production in bulls (Isreal-Friesian breed), 4 calves were fed 2 mg/kg body wt-day for 12 months (Amir and Volcani, 1965). The bulls were then given EDB in gelatin capsules every other day for 2-4 months longer. EDB did not appear to affect the growth, health, and libido of the bulls. However, semen density and motility were significantly lower compared to untreated control bulls of the same age. Many abnormal spermatozoa were also present in treated bulls. A NOAEL for this effect was apparently not determined. Cessation of EDB administration resulted in normal sperm within 10 days to 3 months. Further studies confirmed that EDB adversely affected sperm production without any other apparent effects on bulls (Amir and Volcani, 1967; Amir and Ben-David, 1973). However,

feeding rams 2-5 mg/kg body wt-day for 120 days did not result in any effect on sperm or on the health of the animal (Amir, 1991).

Female B6C3F1 mice (10/group) were given 31.25, 62.5, or 125 mg/kg EDB by gastric lavage 5 days/week for 12 weeks (Ratajzak *et al*., 1995). At the highest dose, EDB significantly prolonged intervals between estrus, decreased hemoglobin and hematocrit levels, and increased cholesterol, triglycerides, total protein, and albumin. The highest dose also caused an immunosuppressive effect by lowering the *in vitro* splenic lymphocyte response to T- and B-cell mitogens.

In a developmental toxicity study, 15-17 pregnant Charles River CD rats and 17-19 pregnant CD mice were exposed to 0, 20, 38, and 80 ppm EDB by inhalation 23 hr/day during days 6 to 16 of gestation (Short *et al.*, 1978). A significant increase in mortality occurred in adult rats exposed to 80 ppm EDB and in adult mice exposed to 38 and 80 ppm EDB. Mice exposed to the highest dose experienced 100% mortality. Reduced body weights and feed consumption occurred in both species at all doses tested. Fetal mortality was increased in rats at the highest dose and in mice at 38 ppm. Reduced fetal body weights occurred at 38 ppm in rats and at all exposure levels in mice. No anomalies were seen in rat fetuses. An increase in runts at 38 ppm and a dose-dependent increase in skeletal anomalies were observed among mouse fetuses. However, these anomalies were characteristic of delayed development and occurred at doses that adversely affected maternal welfare. Therefore, these effects are indicative of fetal toxicity rather than teratogenicity.

Male reproductive toxicity of EDB has been evaluated in some other experimental animals. New Zealand white rabbits, dosed subcutaneously with 0, 15, 30, or 45 mg/kg body wt-day, showed adverse effects at the highest dose (Williams *et al.*, 1991). Increased mortality, increased serum enzymes, and liver damage were observed at this dose level. With respect to sperm quality, sperm velocity, motility, and motion parameters were reduced at the highest dose. A dose related decrease in semen pH was also noted. However, male fertility and fetal structural development were unaffected.

A dominant lethal assay in mice was negative following a single intraperitoneal injection of 100 mg EDB/kg body wt (Barnett *et al.*, 1992). Germ cell tests did not indicate that EDB was a germ cell mutagen in male mice.

VI. Derivation of Chronic Reference Exposure Level (REL)

Study	Ratcliff *et al.*, 1987
Study population	46 exposed men, 43 unexposed men; 89 total
Exposure method	Variable workplace breathing zone airborne exposure (88 ppb geometric mean 8-hour time weighted average (TWA) exposure with peak exposures up to 262 ppb)
Critical effects	Reproductive toxicity; decreased sperm count/ejaculate, decreased percentage of viable and motile sperm, increased semen pH, and increased proportion of sperm with specific morphological abnormalities (tapered heads, absent heads, and abnormal tails) in human males
LOAEL	88 ppb
NOAEL	Not observed
Exposure continuity	8 hr/day (10 m^3/day occupational inhalation exposure rate), 5 days/week
Exposure duration	Average, 4.9 years (with standard deviation of 3.6 years)
Average experimental exposure	31 ppb for LOAEL group (88 x 10/20 x 5/7)
Human equivalent concentration	31 ppb
LOAEL uncertainty factor	10
Subchronic uncertainty factor	3
Interspecies factor	1
Intraspecies factor	10
Cumulative uncertainty factor	300
Inhalation reference exposure level	0.1 ppb (0.0008 mg/m^3, 0.8 μg/m^3)

The primary study by Ratcliff and associates (1987) found significant changes in sperm quality indices of papaya workers exposed to EDB vapors for an average of nearly 5 years. No other health effects were apparent. A level of EDB at which no toxicity was observed (NOAEL) was not determined.

In addition to the primary study of Ratcliff *et al.* (1987), several other epidemiological studies together strongly suggest a correlation between EDB exposure and male reproductive toxicity (Ter Haar, 1980; Wong *et al.*, 1979; Wong *et al.*, 1985; Rogers *et al.*, 1981; Schrader *et al.*, 1988). This lesion appears to occur in humans at concentrations at which other toxic effects are not seen. EDB also shares some structural similarity to dibromochloropropane (DBCP), a known reproductive toxicant in human males. The evidence for male reproductive toxicity of EDB is not as strong as that for DBCP, probably because EDB is not as potent as DBCP in producing this toxic effect. However, animal studies demonstrate testicular toxicity and the number of studies indicating a connection between male reproductive toxicity and EDB exposure cannot be ignored for the development of the REL.

Chronic oral exposure of bulls to EDB results in similar toxic effects at low concentrations (equivalent to 0.9 ppm) without affecting the general health of the animal (Amir and Volcani, 1965; Amir, 1991). However, the small sample size and the lack of a dose-response effect and an observed NOAEL limits the usefulness of this study. Long-term studies of EDB toxicity in other experimental animals also lack the determination of a NOAEL (NCI, 1978; NTP, 1982). Evidence of testicular atrophy was found in other long-term studies with experimental animals, but at concentrations that also produced toxic effects in other organ systems.

For comparison with the proposed REL based on a human study, the NTP (1982) chronic inhalation study established a LOAEL (10 ppm) for liver, kidney, eyes, and the respiratory, male reproductive, and endocrine system in rats. A LOAEL was established in mice for mortality, spleen changes in females, and respiratory system toxicity. A NOAEL was not established for either species. Use of a time adjustment (6/24 hr/day, 5/7 day/week), an RGDR of 1, and a total uncertainty factor of 300 (an interspecies UF of 3, a LOAEL to NOAEL UF of 10, and an intraspecies UF of 10) resulted in an estimated REL of 6 ppb (50 $\mu g/m^3$).

VII. Data Strengths and Limitations for Development of the REL

The strengths of the inhalation REL for ethylene dibromide include the use of human exposure data from workers exposed over a period of years, and the presence of the toxic endpoint (male reproductive system) in several experimental animal species. Major areas of uncertainty are the lack of observation of a NOAEL, the uncertainty in estimating occupational exposure, the potential variability in occupational exposure concentration, and the limited nature of the study (fertility was not actually tested). The database for chronic toxicity of EDB in experimental animals would be enhanced if the proper doses were chosen to determine a NOAEL.

VIII. Potential for Differential Impacts on Children's Health

Little fetal toxicity was observed when pregnant rats and mice were exposed to 20 ppm EDB during gestation (Short *et al.*, 1978). Thus the REL of 0.1 ppb should adequately protect infants and children. However, we do not know if adolescent boys would be more sensitive than men to this alkylating agent. Differences in metabolic capability between infants and older children and adults may result in either more or less toxicity of EDB. Both oxidative and conjugated metabolites are toxic. Infants may produce proportionately more conjugate than oxidized metabolite relative to adults.

IX. References

Aman J, Farkas L, and Ben-Shamai MH. 1946. Experiments on the use of ethylene dibromide as a fumigant for grain and seed. Annals Appl. Biol. 33:389-395.

Amir D. 1991. The spermicidal effect of ethylene dibromide in bulls and rams. Molec. Repro. Develop. 28:99-109.

Amir D, and Ben-David E. 1973. The pattern of structural changes induced in bull spermatozoa by oral or injected ethylene dibromide (EDB). Ann. Biol. Anim. Biochem. Biophys. 13(2):165-170.

Amir D, and Volcani R. 1965. Effect of dietary ethylene dibromide on bull semen. Nature 206(4979):99-100.

Amir D, and Volcani R. 1967. The effect of dietary ethylene dibromide (EDB) on the testes of bulls. Fertil. Steril. 18(1):144-148.

Barnett LB, Lovell DP, Felton CF, Gibson BJ, Cobb RR, Sharpe DS, Shelby MD, and Lewis SE. 1992. Ethylene dibromide: Negative results with the mouse dominant lethal assay and the electrophoretic specific-locus test. Mutat. Res. 282:127-133.

CARB. 2000. California Air Resources Board. California Emissions Inventory Development and Reporting System. (CEIDARS). Data from Data Base Year 1998. February 12, 2000.

CRC. 1994. CRC Handbook of Chemistry and Physics, 75th edition. Lide DR, ed. Boca Raton, FL: CRC Press Inc.

Dobbins JG. 1987. Regulation and the use of “negative” results from human reproductive studies: The case of ethylene dibromide. Am. J. Ind. Med. 12:33-45.

HSDB. 1995. Hazardous Substances Data Bank. National Library of Medicine, Bethesda, MD (TOMES® CD-ROM Version). Denver, CO: Micromedex, Inc. (Edition expires 11/31/95).

Jones AR, and Edwards K. 1968. The comparative metabolism of ethylene dimethane-sulphonate and ethylene dibromide. Experientia 24:1100-1101.

Kulkarni AP, Edwards J, and Richards IS. 1992. Metabolism of 1,2-dibromoethane in the human fetal liver. Gen. Pharmacol., 23(1):1-5.

NCI. 1978. National Cancer Institute. Bioassay of 1,2-dibromoethane for possible carcinogenicity. Technical Report Series No. 86, DHEW Publ. no. (NIH) 78-1336.

Nitschke KD, Kociba RJ, Keyes DG, and McKenna MJ. 1981. A thirteen week repeated inhalation study of ethylene dibromide in rats. Fundam. Appl. Toxicol. 1:437-442.

NTP. 1982. National Toxicology Program. Carcinogenesis bioassay of 1,2-dibromoethane in F344 rats and B6C3F$_1$ mice (inhalation study). Technical Report Series No. 210, NIH Publ. no. 82-1766.

Ott MG, Scharnweber HC, and Langner RR. 1980. Mortality experience of 161 employees exposed to ethylene dibromide in two production units. Br. J. Ind. Med. 37:163-168.

Plotnick HB, and Conner WL. 1976. Tissue distribution of ^{14}C-labeled ethylene dibromide in the guinea pig. Res. Commun. Chem. Path. Pharmacol. 13(2):251-258.

Plotnick HB, Weigel WW, Richards DE, and Cheever KL. 1979. The effect of dietary disulfiram upon the tissue distribution and excretion of ^{14}C-1,2-dibromoethane in the rat. Res. Commun. Chem. Pathol. Pharmacol. 26(3):535-545.

Ratajczak HV, Thomas PT, Gerhart J, and Sothern RB. 1995. Imunotoxicologic effects of ethylene dibromide in the mouse and their modulation by the estrus cycle. In Vivo 9:299-304.

Ratcliff JM, Schrader SM, Steenland K, Clapp DE, Turner T, and Hornung RW. 1987. Semen quality in papaya workers with long term exposure to ethylene dibromide. Br. J. Ind. Med. 44:317-326.

RECT. 1988. Reviews of Environmental Contamination and Toxicology. Ethylene dibromide. 104:115-129.

REPROTOX. 1995. The REPROTOX(R) System: Reproductive Reviews of Drugs, Chemicals, Physical and Biological agents. Denver, CO: Micromedex, Inc. (Edition expires 7/31/95).

Reznik G, Stinson SF, and Ward JM. 1980. Respiratory pathology in rats and mice after inhalation of 1,2-dibromo-3-chloropropane or 1,2 dibromoethane for 13 weeks. Arch. Toxicol. 46:233-240.

Rogers BJ, Fujita JS, Najita L, and Hale RW. 1981. Reduction of sperm concentration in a population exposed to ethylene dibromide (EDB). J. Androl. 2:35-36.

Rowe VK, Spencer HC, McCollister DD, Hollingsworth RL, and Adams EM. 1952. Toxicity of ethylene dibromide determined on experimental animals. Arch. Ind. Hyg. Occup. Med. 6(2):158-173.

Schrader SM, Ratcliff JM, Turner TW, and Hornung RW. 1987. The use of new field methods of semen analysis in the study of occupational hazards to reproduction: The example of ethylene dibromide. J. Occup. Med. 29(12):963-966.

Schrader SM, Turner TW, and Ratcliff JM. 1988. The effects of ethylene dibromide on semen quality: A comparison of short-term and chronic exposure. Repro. Toxicol. 2:191-198.

Short RD, Minor JL, Winston JM, Seifter J, and Lee C. 1978. Inhalation of ethylene dibromide during gestation by rats and mice. Toxicol. Appl. Pharmacol. 46:173-182.

Stott WT, and McKenna MJ. 1984. The comparative absorption and excretion of chemical vapors in the upper, lower, and intact respiratory tract of rats. Fundam. Appl. Toxicol. 4:594-602.

Ter Haar G. 1980. An investigation of possible sterility and health effects from exposure to ethylene dibromide. In: Banbury Report 5-Ethylene Dibromide: A Potential Health Risk? Ames B, Infante P, and Reitz R. (eds). Cold Spring Harbor, NY: Cold Spring Harbor Laboratory. pp. 167-188.

Watanabe P, Young J, Schlachter M, Zempel J, and Karbowski R. 1978. Fate of inhaled ethylene dibromide in rats. Toxicol. Appl. Pharmacol. 45:224(abstract).

Wiersma DA, Schnellmann RG, and Sipes IG. 1986. The in vitro metabolism and bioactivation of 1,2-dibromoethane (ethylene dibromide) by human liver. J. Biochem. Toxicol. 1(3):1-11.

Williams J, Gladen BC, Turner TW, Schrader SM, and Chapin RE. 1991. The effects of ethylene dibromide on semen quality and fertility in the rabbit: Evaluation of a model for human seminal characteristics. Fundam. Appl. Toxicol. 16:687-700.

Wong LCK, Winston JM, Hong CB, and Plotnick H. 1982. Carcinogenicity and toxicity of 1,2-dibromoethane in the rat. Toxicol. Appl. Pharmacol. 63:155-165.

Wong O, Utidjian HMD, and Karten VS. 1979. Retrospective evaluation of reproductive performance of workers exposed to ethylene dibromide (EDB). J. Occup. Med. 21(2):98-102.

Wong O, Morgan RW, and Whorton MD. 1985. An epidemiologic surveillance program for evaluating occupational reproductive hazards. Am. J. Ind. Med. 7:295-306.

CHRONIC TOXICITY SUMMARY

ETHYLENE DICHLORIDE

(1,2-dichloroethane)

CAS Registry Number: 107-06-2

I. Chronic Toxicity Summary

Inhalation reference exposure level	**400 µg/m^3** (100 ppb)
Critical effect(s)	Hepatotoxicity; elevated liver enzyme levels in serum of rats.
Hazard index target(s)	Liver

II. Physical and Chemical Properties (HSDB, 2000; CRC, 1994)

Description	Clear, colorless, oily liquid
Molecular formula	$C_2H_4Cl_2$
Molecular weight	98.97 g/mol
Density	1.2351 g/cm^3 @ 20ºC
Boiling point	57.4ºC
Melting point	–96.9ºC
Vapor pressure	64 torr @ 20ºC
Solubility	Slightly soluble in water (0.869 g/100 ml at 20ºC); miscible with alcohol; soluble in ordinary organic solvents
Conversion factor	1 ppm = 4.05 mg/m^3

III. Major Uses or Sources

Ethylene dichloride (EDC) is used primarily in the production of vinyl chloride monomer (HSDB, 2000). It is also an intermediate in the manufacture of trichloroethane and fluorocarbons and is used as a solvent. In California, EDC is also used as a reactant carrier in the production of solid fuel (CARB, 1997). EDC was commonly used as a gasoline additive to scavenge inorganic lead compounds. The transition to the use of lead-free gasoline has essentially eliminated the use of EDC as a fuel additive in this country. EDC was also used as a soil fumigant but is no longer registered for this use on agricultural products in the United States. The annual statewide industrial emissions from facilities reporting under the Air Toxics Hot Spots Act in California based on the most recent inventory were estimated to be 24,935 pounds of ethylene dichloride (CARB, 2000).

IV. Effects of Human Exposure

Toxicological data resulting solely from long-term exposure to EDC in humans are lacking. Nausea, vomiting, dizziness, and unspecified blood changes were reported in a study of workers exposed to levels of 10-37 ppm EDC (Brzozowski *et al.*, 1954). Kozik (1957) reported adverse central nervous system and liver effects in workers occupationally exposed to concentrations of 16 ppm EDC and below. Rosenbaum (1947) also reported nervous system effects in a study of 100 Russian workers exposed for less than 5 years to concentrations of EDC less than 25 ppm.

Immediately following a 30-minute exposure to an unknown concentration of EDC, a 51 year-old male was somnolent and experienced vomiting (Nouchi *et al.*, 1984). Delirious and trembling, the worker was admitted to the hospital 20 hours post-exposure. The liver was palpable, but serum liver enzymes were normal. The patient lapsed into a coma 3.5 hours following admission to the hospital. A marked elevation in serum liver enzymes was noted on the second day of hospitalization, 35 hours post-exposure. Multiple organ failure occurred on the fourth day of hospitalization and the patient died of arrhythmia. At autopsy, the lungs were congested and edematous. Diffuse degenerative changes were observed in the myocardium. Extensive centrilobular necrosis was observed in the liver, and acute centrilobular necrosis was observed in the kidney. Nerve cells in the brain, including Purkinje cells, appeared shrunken with pyknotic nuclei. The latency period for hepatotoxicity of approximately 20 hours suggests that metabolism of the compound yields the reactive agent (see below).

V. Effects of Animal Exposure

As with humans, the absorption and distribution of EDC in rats following ingestion or inhalation is rapid and complete (IARC, 1999). Metabolism in rats and mice is extensive with 85% of the metabolites appearing in urine. Metabolism occurs predominantly via two pathways, one catalyzed by cytochrome P450 and one by glutathione S-transferase. The direct conjugation with glutathione catalyzed by glutathione S-transferase may ultimately result in the putative alkyating agent (episulfonium ion) primarily responsible for toxicity and carcinogenicity. Evidence for DNA-damaging metabolites resulting via the P450 pathway exists (IARC, 1999). However, this pathway appears to be a minor route for toxic metabolite formation.

Acute exposure in mice resulted in toxic effects similar to those seen in the human case study presented above, including liver and kidney damage (Francovitch *et al*., 1986). Acute EDC exposure exhibits a steep dose-response curve with respect to mortality. However, the long-term exposure studies were notable for the limited organ toxicity and mortality observed in comparison to acute studies (IARC, 1999).

Male and female rats (50 per sex) were exposed to 50 ppm EDC 7 hours per day, 5 days per week for 2 years (Cheever *et al.*, 1990). Absolute and relative liver weights were not significantly different from controls. Daily observations, gross pathology, and extensive histopathology revealed no differences from controls other than a slight increase in unspecified testicular lesions in the EDC group. Additional rats were exposed to 50 ppm EDC with 0.05% disulfiram (a non-carcinogen used extensively in the rubber industry and as a treatment

(Antabuse) for alcoholism) in the diet. Disulfiram treatment resulted in increased number of tumors, increased blood levels of EDC, and increased liver (primarily bile duct cysts) and kidney (chronic nephropathy) lesions. It was concluded that some pathways responsible for metabolism of EDC were inhibited by disulfiram, resulting in increased EDC blood levels and bioactivation to toxic metabolites via other metabolic pathways.

Rats (8-10 per sex per group) were exposed to 0, 5, 10, 50, and 150-250 ppm EDC 7 hours per day, 5 days per week for up to 18 months (Spreafico *et al.*, 1980). Serum chemistry measurements were taken after 3, 6, 12, and 18 months of exposure. Rats to be examined after 3, 6 and 18 months of exposure were 3 months of age at the beginning of the experiment, and rats to be examined after 12 months of exposure were 14 months of age at the beginning of the experiment. Complete histological exams were conducted but non-cancer effects were not discussed. No consistent treatment-related changes in serum chemistry parameters were observed at 3, 6, or 18 months of exposure. However, rats exposed to higher levels of EDC for 12 months exhibited changes in serum chemistry indicative of chronic liver damage, primarily increased alanine aminotransferase (ALT) levels at the two highest exposures. Lactate dehydrogenase (LDH) and aspartate aminotransferase (AST) levels were significantly decreased, but did not appear to be dose-related. γ-Glutamyl transpeptidase levels were elevated but at non-significant levels. Indicators of kidney toxicity included increased blood urea nitrogen levels in the 150 ppm group and increased uric acid levels at the two highest exposures. However, the control values for both of these parameters were significantly lower than that seen in rats tested at other times in this study. Thus, the toxicological significance is questionable. Cholesterol was reduced significantly at the higher exposure levels but the toxicological significance of this finding was unknown. The marked difference between serum chemistry parameters following 12 months of exposure, compared to those following 3, 6, and 18 months of exposure, may be due to the considerable difference in the age of the rats at the start of exposure. This study identifies a 12-month LOAEL of 50 ppm and a NOAEL of 10 ppm in rats.

A study examining the interaction between 1,2-dichloroethane and disulfiram (DSF) exposed rats to EDC concentrations of 150, 300, or 450 ppm 5 days per week for 30 days (Igwe *et al.*, 1986a; Igwe *et al*., 1986b). Increased liver weights and increased 5-nucleotidase (5-NT) activity were observed in rats following exposure to 450 ppm EDC (the LOAEL for this study). This study also determined that the interaction between DSF and EDC greatly increased the toxicity of EDC (i.e., increased serum activities of SDH, APT, and 5-NT, bilateral testicular atrophy, periportal necrosis and cytoplasmic swelling of hepatocytes, and bile duct proliferation). Therefore, any person exposed to DSF either occupationally or therapeutically is likely to be more susceptible to the effects of EDC toxicity.

Rats, rabbits, guinea pigs, dogs, cats, and monkeys were used in exposures ranging from approximately 100 to 1000 ppm EDC (Heppel *et al.*, 1946). At the highest experimental concentration of 963 ppm, high mortality was observed in rats, rabbits, and guinea pigs following exposure 7 hours per day, 5 days per week for two weeks or less. At 963 ppm guinea pigs exhibited lacrimation and inactivity during exposure; pulmonary congestion was noted at autopsy. Rats exposed to this concentration exhibited degenerative proliferative changes in the renal tubular epithelium and splenitis. Pulmonary congestion and focal hemorrhage were also noted in 2 of 4 rats examined. While 4 of 6 cats exposed to this concentration survived until

sacrifice 11 weeks following termination of exposure, congestion and fatty infiltration of the liver were observed at necropsy. Due to high mortality in the rodents at the higher concentration, a subsequent experiment exposed rats and guinea pigs 7 hours per day, 5 days per week to 100 ppm EDC for four months. No increase in mortality or effects on growth was observed in rats exposed to this concentration. The rats were successfully bred and their pups were exposed with the dams. No significant findings were observed upon gross and histological examinations of 10/39 exposed and 10 control rats. This study is severely limited by the methods used to determine the exposure concentration and by the lack of quantitative measurements of toxicity other than death. This study does, however, indicate that fatty infiltration of the liver is one indication of toxicity following multiple exposures to EDC.

In developmental toxicity studies summarized by Zhao *et al.* (1997), rats were exposed to 0, 24.8, and 207.6 mg/m^3 (equivalent to 0, 6, and 51 ppm) EDC for 6 hr/day from two weeks before mating and throughout gestation. Statistically significant increases in pre-implantation loss and decreased male pup weights were observed at the highest dose. Gross skeletal and visceral malformations were not found.

In a developmental study by Payan *et al.* (1995), Sprague-Dawley rats were exposed to 150, 200, 250, or 300 ppm EDC for 6 hrs/day from day 6 to 20 of gestation. Maternal toxicity (reduced body weight gain; death of two females) was observed at the highest exposure. Statistically significant evidence of altered growth and teratogenic effects were not observed at any concentration.

Rao *et al*. (1980) exposed rats and rabbits to 100 or 300 ppm EDC for 7 hr/day on days 6 through 15 (rats) or 6 through 18 (rabbits) of gestation. Maternal toxicity (mortality) was observed in rabbits at 100 ppm, and both species at 300 ppm. One rat exhibited resorption of all implantations at the maternally-toxic dose. Otherwise, no fetotoxic or teratogenic effects were observed in either species. In a reproduction study, rats were exposed to 25, 75, or 150 ppm EDC 6 hr/day, 5 days/week for 60 days before breeding. Exposure following this period was 6 hr/day, 7 days/week. Maternal animals were not exposed to EDC from gestational day 21 through day 4 postpartum. EDC had no effect on reproduction over one generation within two litters.

In a two-generation study conducted by Lane *et al.* (1982), ICR Swiss mice were administered 30, 90, or 290 mg/L EDC in drinking water (equivalent to about 5, 15, or 50 mg/kg bw/day) starting five weeks before mating of the F_0 generation. No treatment-related effects on fertility, gestation, viability, weight gain, or lactation indices were noted. EDC exposure did not result in teratogenic or dominant lethal effects.

No gross or histopathological indications of hepato- or nephrotoxicity were observed in Osborn-Mendel rats (47 or 95 mg/kg bw/day, 5 days/week for both sexes) or B6C3F1 mice (97 or 195 mg/kg bw/day, 5 days/week for males; 149 or 299 mg/kg bw/day, 5 days/week for females), which were given EDC via gavage for 78 weeks (NCI, 1978). However, rats of each sex and female mice had significantly reduced survival at the highest dose.

In a comparative study of the toxicity of EDC, Morgan *et al.* (1990) administered 0, 500, 1000, 2000, 4000, and 8000 ppm in drinking water to several species of rats for 13 weeks. A statistically significant increase in kidney weight was observed in male and female Fischer 344/N rats administered 1000 ppm or greater in drinking water. However, minimal histological damage was observed only in the kidney of female Fischer 344/N rats. A statistically significant decrease in body weight was observed in rats administered 8000 ppm. Significant decreases in absolute and relative kidney weight were observed in male and female rats administered concentrations of 1000 ppm EDC. A significant increase in relative liver weight was observed in male rats administered 2000 ppm EDC and greater and female rats administered 4000 ppm EDC and greater. Similar but less marked toxicity was observed in the Sprague-Dawley and Osborne-Mendel rats administered 1000 ppm. Additionally, rats were administered EDC in corn oil by gavage at doses of 0, 30, 60, 120, 240, and 480 mg/kg for 13 weeks (Morgan *et al.*, 1990). Rats administered EDC by gavage exhibited high mortality in the higher dose groups. Statistically significant increases in kidney weights were observed in surviving male rats administered EDC and in female rats administered 120 or 240 mg/kg. However, no histological damage to the liver or kidney was observed.

VI. Derivation of Chronic Reference Exposure Level (REL)

Study	Spreafico *et al.*, 1980.
Study population	Rats (8-10 per sex/group)
Exposure method	Discontinuous whole-body inhalation exposures (0, 5, 10, 50, or 150-250 ppm)
Critical effects	Significant elevation in liver enzymes
Exposure duration	12 months
Exposure continuity	7 hours/day, 5 days/week
LOAEL	50 ppm
NOAEL	10 ppm
Average experimental exposure	2.1 ppm for NOAEL group (10 x 7/24 x 5/7)
Human equivalent concentration	3.2 ppm for NOAEL group (gas with systemic effects, based on RGDR = 1.5 for lambda (a) : lambda (h)) (Gargas *et al*., 1989)
LOAEL uncertainty factor	1
Subchronic uncertainty factor	1
Interspecies uncertainty factor	3
Intraspecies uncertainty factor	10
Cumulative uncertainty factor	30
Inhalation reference exposure level	0.1 ppm (100 ppb; 0.4 mg/m^3; 400 μg/m^3)

Cheever *et al*. (1990) and Spreafico *et al.* (1980) were the only chronic inhalation exposure studies found in the literature that presented non-cancer effects. No reproductive and developmental effects were observed in studies published in peer-reviewed journals. The study by Spreafico *et al*. (1980) was chosen for REL development based on the utilization of multiple exposure levels and the observation of a NOAEL and a LOAEL for liver effects.

The Agency for Toxic Substances and Disease Registry (ATSDR) calculated a chronic inhalation minimal risk level (MRL) for EDC of 0.2 ppm (ATSDR, 1994). The calculation was based on the study by Cheever *et al*. (1990), which determined a free-standing NOAEL of 50 ppm for lack of liver effects. A LOAEL was not determined. To derive the MRL, the ATSDR applied uncertainty factors (UFs) of 10 each for intraspecies and interspecies variability, and a modifying factor of 3 to account for database deficiencies, to the NOAEL of 50 ppm. The criteria for use of modifying factors are not well specified by ATSDR. Such modifying factors were not used by OEHHA. A continuity correction for discontinuous exposure was not applied. The resulting MRL was 0.2 ppm (0.7 mg/m^3).

For comparison to the proposed REL, a REL developed by OEHHA based on the free-standing NOAEL of 50 ppm determined in rats by Cheever *et al*. (1990) would include a continuity correction (50 ppm x 7/24 x 5/7) resulting in an equivalent continuous level of 10.42 ppm.. Application of an RGDR = 1.5 and UFs of 3 for interspecies and 10 for intraspecies differences result in a REL of 0.5 ppm (2 mg/m^3).

VII. Data Strengths and Limitations for Development of the REL

The strengths of the inhalation REL for ethylene dichloride include the availability of chronic inhalation exposure data, the relatively large number of exposure levels at lower concentrations (allowing for better elucidation of the dose-response relationship for hepatotoxicity), and the observation of a NOAEL. Major areas of uncertainty are the lack of adequate human exposure data, the small groups tested in the key study, and the lack of health effects data from multiple species.

The small number of animals per group and the relatively modest clinical chemistry findings observed in the Spreafico *et al.* (1980) study may have resulted in false-positives, false-negatives, and lack of clear dose-response relationships. Repeating the study in one or more experimental animal species with full histopathological examination of organs and a greater number of animals/dose would significantly enhance the chronic toxicity database for EDC.

VIII. References

ATSDR. 1994. Agency for Toxic Substances and Disease Registry. Toxicological Profile for 1,2-Dichloroethane (Update). U.S. Department of Health and Human Services, Public Health Service.

Brzozowski J, Czajka J, Dutkiewicz T, Kesy I, and Wojcik J. 1954. Work hygiene and the health condition of workers occupied in combating the *Leptinotarsa decemlineata* with HCH and dichloroethane. Med. Pr. 5: 89-98. [cited in U.S. EPA, 1985].

CARB. 1997. California Air Resources Board. Ethylene Dichloride. In: Toxic Air Contaminant Identification List Summaries. California Environmental Protection Agency, pp. 483-487.

CARB. 2000. California Air Resources Board. California Emissions Inventory Development and Reporting System (CEIDARS). Data from Data Base Year 1998. February 12, 2000.

CRC. 1994. CRC Handbook of Chemistry and Physics, 75th edition. Lide DR, ed. Boca Raton, FL: CRC Press Inc.

Cheever KL, Cholakis JM, El-Hawari AM, Kovatch RM, and Weisburger EK. 1990. Ethylene dichloride: The influence of disulfiram or ethanol on oncogenicity, metabolism, and DNA covalent binding in rats. Fundam. Appl. Toxicol. 14:243-261.

Francovitch RJ, Schor NA, and George WJ. 1986. Effects of SKF-525A, phenobarbitol, and 3-methylcholanthrene on ethylene dichloride toxicity following inhalation exposure. J. Am. Coll. Toxicol. 5(2):117-126.

Gargas ML, Burgess RJ, Voisard DE, Cason GH, and Anderson ME. 1989. Partition coefficients of low-molecular weight volatile chemicals in various liquids and tissues. Toxicol. Appl. Pharmacol. 98:87-99.

HSDB. 2000. Hazardous Substances Data Bank. National Library of Medicine, Bethesda, MD. Available online through Toxicology Data Network at http://toxnet.nlm.nih.gov

Heppel LA, Neal PA, Perrin TL, Endicott KM, and Porterfield VT. 1946. The toxicology of 1,2-dichloroethane (ethylene dichloride). V. The effects of daily inhalations. J. Ind. Hyg. Toxicol. 28(4):113-120.

IARC. 1999. International Agency for Research on Cancer. Re-evaluation of some organic chemicals, hydrazine and hydrogen peroxide. IARC Monogr. Eval. Carcinog. Risks Hum. 71(Pt.2):501-529.

Igwe OJ, Que Hee SS, and Wagner WD. 1986a. Interaction between 1,2-dichloroethane and disulfiram. I. Toxicological effects. Fundam. Appl. Toxicol. 6:733-746.

Igwe OJ, Que Hee SS, and Wagner WD. 1986b. Interaction between 1,2-dichloroethane and tetraethylthiuram disulfide (disulfiram). II. Hepatotoxic manifestations with possible mechanism of action. Toxicol. Appl. Pharmacol. 86:286-297.

Kozik I. 1957. Problems of occupational hygiene in the use of dichloroethane in the aviation industry. Gig. Tr. Prof. Zabol. 1:31-38. [cited in U.S. EPA, 1985].

Lane RW, Riddle BL, and Borzelleca JF. 1982. Effects of 1,2-dichloroethane and 1,1,1-trichloroethane in drinking water on reproduction and development in mice. Toxicol. Appl. Pharmacol. 63:409-421.

Morgan DL, Bucher JR, Elwell MR, Lilja HS, and Krishna Murthy AS. 1990. Comparative toxicity of ethylene dichloride in F344/N, Sprague-Dawley and Osborne-Mendel rats. Food Chem. Toxicol. 28(12):839-845.

NCI. 1978. United States National Cancer Institute. Bioassay of 1,2-dichloroethane for possible carcinogenicity CAS No. 107-06-2 (NCI-CG-TR-55), Washington DC.

Nouchi T, Miura H, Kanayama M, Mizuguchi O, and Takano T. 1984. Fatal intoxication by 1,2-dichloroethane - a case report. Int. Arch. Occup. Environ. Health 54:111-113.

Payan JP, Saillenfait AM, Bonnet P, Fabry JP, Langonne I, and Sabate JP. 1995. Assessment of the developmental toxicity and placental transfer of 1,2-dichloroethane in rats. Fundam. Appl. Toxicol. 28:187-198.

Rao KS, Murray JS, Deacon MM, John JA, Calhoun LL, and Young JT. 1980. Teratogenicity and reproduction studies in animals inhaling ethylene dichloride. In: Banbury Report 5. Ethylene Dichloride: A Potential Health Risk? Ames B, Infante P, and Reitz R. (eds). Cold Spring Harbor, NY: Cold Spring Harbor Laboratory. pp. 149-166.

Rosenbaum ND. 1947. Ethylene dichloride as an industrial poison. Gig. Sanit. 12(2): 17-21. [cited in U.S. EPA, 1985].

Spreafico F, Zuccato E, Marcucci F, Sironi M, Paglialunga S, Madonna M, and Mussini E. 1980. Pharmacokinetics of ethylene dichloride in rats treated by different routes and its long-term inhalatory toxicity. In: Banbury Report 5. Ethylene Dichloride: A Potential Health Risk? Ames B, Infante P, and Reitz R. (eds). Cold Spring Harbor, NY: Cold Spring Harbor Laboratory. pp. 107-129.

U.S. EPA. 1985. U.S. Environmental Protection Agency. Health Assessment Document for 1,2-Dichloroethane (Ethylene Dichloride). U.S. EPA, Office of Research and Development, Office of Health and Environmental Assessment, Environmental Criteria and Assessment Office. Research Triangle Park, NC: U.S. EPA.

Zhao S-F, Zhang X-C, Zhang L-F, Zhao S-S, Zhang F, Wang Q-F, Wang Y-L, and Bao Y-S. 1997. The evaluation of developmental toxicity of chemicals exposed occupationally using whole embryo culture. Int. J. Dev. Biol. 41:275-282.

CHRONIC TOXICOLOGY SUMMARY

ETHYLENE GLYCOL

(1,2-dihydroxyethane; 1,2-ethanediol)

CAS Registry Number: 107-21-1

I. Chronic Toxicity Summary

Chronic reference exposure level	**400 μg/m^3 (200 ppb)**
Critical effects	Respiratory irritation in human volunteers
Hazard index target(s)	Respiratory system; kidney; teratogenicity

II. Physical and Chemical Properties (HSDB, 1996; 1999)

Description	Clear, colorless, odorless liquid
Molecular formula	$C_2H_6O_2$
Molecular weight	62.07 g/mol
Density	1.1088-1.1135 g/cm^3 @ 20° C
Boiling point	197.6° C
Melting point	–13° C (CRC, 1994)
Vapor pressure	0.06 torr @ 20°C; 0.092 torr @ 25°C
Solubility	Soluble in water and ethanol; slightly soluble in ether. Insoluble in benzene and petroleum ether.
Conversion factor	1 ppm = 2.5 mg/m^3 @ 25° C

III. Major Uses and Sources

Ethylene glycol is used as an antifreeze agent in cooling and heating systems (HSDB, 1996). It is used in hydraulic brake systems; as an ingredient in electrolytic condensers; as a solvent in the paint and plastics industries; and in inks for ball-point pens and printer's inks. It is used in the manufacture of some synthetic fibers (Terylene and Dacron), and in synthetic waxes. It is used in some skin lotions and flavoring essences. Also, it is used in asphalt emulsion plants, in wood stains and adhesives, and in leather dyeing. It has been used as a de-icing fluid for airport runways. The annual statewide emissions from facilities reporting under the Air Toxics Hot Spots Act in California based on the most recent inventory were estimated to be 66,636 pounds of ethylene glycol (CARB, 1999).

IV. Effects of Human Exposure

Laitinen *et al.* (1995) found that 10 motor servicing workers had significantly higher urinary levels of ethylene glycol and ammonia, and decreased urinary glycosaminoglycan levels, compared with 10 controls. The ethylene glycol levels in air were undetectable in the workers' breathing zones (i.e. below 1.9 ppm), therefore dermal absorption appeared to be the primary route of exposure. Because the dermal absorption rate is high, airborne ethylene glycol concentrations in workplaces likely underestimate the total exposure.

In a study of 20 volunteer male prisoners in Alabama, 20 hour/day exposure to aerosolized ethylene glycol concentrations varying up to a mean of 20 ppm (49 mg/m^3) for 30 days was without effect (Wills *et al.*, 1974). The actual concentrations measured in the exposure chamber were:

	Concentration of ethylene glycol in air, mg/m^3		
Days	Low	High[a]	Mean
1-7	3.6	75.0	37
8-14	18.8	44.8	29
15-21	0.8	41.6	17
22-28	3.5	49.2	23
29-35	20.6	66.8	49
36-37	14.4	39.0	31

[a] does not include the very high concentrations maintained for comparatively brief periods.

Respiratory irritation was noted after 15 minutes at an exposure concentration of 75 ppm (188 mg/m^3), and became quickly intolerable at 123 ppm (308 mg/m^3). No effects were observed in normal clinical chemistry, clinical serum enzyme levels for liver and kidney toxicity (including SGOT and serum alkaline phosphatase), hematotoxicity (including % hematocrit and gm hemogloin per 100 ml blood), or psychological responses (including simple reaction time, weight discrimination, and depth perception). The respiratory irritation at 75 ppm resolved soon after exposure with no long term effects noted after a 6-week follow-up period.

V. Effects of Animal Exposure

A chronic feeding study in rats and mice was conducted by DePass *et al.* (1986a). In this study, rats (130 per sex per group) and mice (80 per sex per group) were exposed to 0, 0.04, 0.2, or 1 g/kg/day for up to 2 years. All male rats in the high dose group died by 475 days. A large number of effects were observed in this group, including: reduced body weight, increased water intake, increased blood urea nitrogen and creatinine, reduced erythrocyte counts, reduced hematocrit and hemoglobin, increased neutrophil count, and increased urine volume. Heart, kidney, lung, parathyroid, stomach, and other vascular mineralization and hyperplasia were observed histologically in the high dose group of the male rats. Female rats exhibited fatty changes and granulomas in the liver at the high dose. Liver effects were not reported for the

males. The NOAEL in rats for chronic oral ethylene glycol toxicity was 200 mg/kg/day. No effects were observed in mice. Therefore, the NOAEL for mice was 40 mg/kg/day.

Coon *et al*. (1970) exposed groups of rats (as well as guinea pigs, rabbits, dogs, and monkeys) to ethylene glycol intermittently 8 hours/day, 5 days per week for 6 weeks (30 exposures) to 10 or 57 mg/m^3 or continuously to 12 mg/m^3 for 90 days. At 10 mg/m^3 2 rabbits had conjunctivitis and liver changes were noted in a few animals of the other species. At 57 mg/m^3 no signs of toxicity were seen during the exposure. Nonspecific inflammatory changes were noted in some lungs and hearts of all species. A few livers also showed necrotic areas. Continuous exposure to 12 mg/m^3 led to moderate to severe eye irritation in rats and rabbits. Edema in the rabbits led to eye closure. Two rats developed corneal opacities. All hematologic parameters and various enzymes assayed were within normal limits. At necropsy organs appeared normal. Histopathological analysis revealed inflammatory changes in the lungs of all species, but the controls also showed a lesser degree of inflammation. Several guinea pigs showed foci of inflammatory cells in the kidney.

Mortality in Coon et al. (1970)			*Number died/number exposed*				
Ethylene glycol mg/m^3	Exposure duration	Equivalent continuous concentration	Rat	Guinea pig	Rabbit	Dog	Monkey
0 (control)	90 days	0	4/123	0/73	0/12	0/12	0/8
10±1	6 wk	2.4	0/15	0/15	0/3	0/2	0/2
57±14	6 wk	13.6	0/15	0/15	0/3	0/2	0/2
12±2	90 days	12.0	1/15	3/15	1/3	0/2	0/3

Studies on the effects of inhaled ethylene glycol on reproduction and development of rats and mice were conducted by Tyl *et al.* (1995a, 1995b). In a study using whole-body exposure of rats and mice to ethylene glycol at analyzed concentrations of 0, 119, 888, or 2090 mg/m^3 for 6 hours/day on days 6-15 of gestation, mice were found to be the more sensitive species. Maternal toxicity in rats included a significant increase in absolute and relative liver weight at 2090 mg/m^3. No effects on weight gain, organ weights other than liver, fecundity, live fetuses per litter, or pre- or post-implantation loss were observed in rats. In addition, terata were not observed at any concentration. Reduced ossification in the humerus, zygomatic arch, and the metatarsals and proximal phalanges of the hindlimb was present in fetuses exposed to 888 or 2090 mg/m^3. The NOAEL for maternal toxicity in rats was 888 mg/m^3, while the NOAEL for fetotoxicity was 119 mg/m^3.

In mice, reduced body weight and gravid uterine weight during and after the exposure were observed at the 888 and 2090 mg/m^3 concentrations. Increased nonviable implants per litter and reduced fetal body weights were also observed in groups exposed to 888 or 2090 mg/m^3. External, visceral, skeletal, and total malformations were increased in the 888 and 2090 mg/m^3 groups. The NOAEL for these effects in mice was 119 mg/m^3.

A similar experiment in mice using nose-only exposures was conducted by these researchers (Tyl *et al.*, 1995a) to determine the role of dermal absorption and/or ingestion on the effects

observed with the whole-body exposure. Nose-only exposures to ethylene glycol were for 6 hours/day, on gestational days 6 through 15 at concentrations of 0, 500, 1000, and 2000 mg/m^3. The NOAEL for maternal effects (increased kidney weight) was 500 mg/m^3, and the NOAEL for fetal toxicity (skeletal variations and fused ribs) was 1000 mg/m^3. Thus, secondary dermal and/or oral exposures appear to have contributed significantly to the developmental and maternal toxicity in mice exposed to ethylene glycol aerosol. The nose-only inhalation exposure study by Tyl *et al.* (1995a) was conducted in addition to the whole-body inhalation study since extensive adsorption of ethylene glycol onto the fur of the animals was demonstrated in the whole-body experiment. Normal grooming behavior would have resulted in significantly larger doses of ethylene glycol than that expected by inhalation only.

A 3-generation study on the effects of ethylene glycol on reproductive performance and gross health of offspring in rats was conducted by DePass *et al.* (1986b). Rats were exposed orally to 40, 200, or 1000 mg/kg/day ad libitum in the feed through 3 generations. No effects on pup survivability or pup body weight were observed. Total and viable implants were also not affected. Teratogenic effects were not examined in this study.

Tyl *et al.* (1993) studied the reproductive and developmental effects of ethylene glycol in rabbits exposed by gavage on days 6 to 19 of gestation. Dams were exposed to 0, 100, 500, 1000, or 2000 mg/kg/day. Exposure to 2000 mg/kg/day resulted in 42% mortality, and abortion or early delivery in 4 does. No evidence of embryotoxicity or teratogenicity was observed in the groups exposed to 1000 mg/kg/day or less. The NOAEL for maternal toxicity was determined to be 1000 mg/kg/day.

VI. Derivation of Chronic Reference Exposure Level

Study	Wills *et al.* (1974)
Study population	Human volunteer prisoners
Exposure method	Discontinuous whole-body inhalation
Critical effects	Respiratory tract irritation
LOAEL	75 ppm
NOAEL	20 ppm
Exposure continuity	20 hours/day
Exposure duration	30 days
Average exposure	16.7 ppm for NOAEL group (20 x 20/24)
Human equivalent concentration	16.7 ppm
LOAEL uncertainty factor	1
Subchronic uncertainty factor	10
Interspecies factor	1
Intraspecies factor	10
Cumulative uncertainty factor	100
Inhalation reference exposure level	0.2 ppm (200 ppb; 0.4 mg/m^3; 400 μg/m^3)

The subchronic study by Wills *et al.* (1974) represents the only human inhalation data for ethylene glycol toxicity. The experiment showed a concentration-response relationship, with onset of irritation occurring at 188 mg/m^3 and intense and intolerable irritation occurring at 308

mg/m^3. The volunteers were followed for 6 weeks without any apparent long-term effects from the exposures. Although the irritation experienced in the human subjects appears to be an acute phenomenon and not a cumulative lasting effect, the subchronic uncertainty factor of 10 was retained to protect against other systemic effects associated with ethylene glycol such as kidney damage which may occur over a long-term exposure.

The chronic feeding study in rats by DePass *et al.* (1986a) showed significant chronic effects including reduced body weight, increased water intake, increased blood urea nitrogen and creatinine, reduced erythrocyte counts, reduced hematocrit and hemoglobin, increased neutrophil counts, increased urine volume, and reduced urine specific gravity and pH in rats exposed to a concentration of 1000 mg/kg/day. However, no effects were reported in mice. In contrast, reproductive and developmental toxicity studies in mice, rats, and rabbits have shown the mouse to be the most sensitive species for both terata and maternal toxicity endpoints (Tyl *et al.*, 1995a; Tyl *et al.*, 1993; Neeper-Bradley *et al.*, 1995). In addition, the 3-generation reproductive toxicity study by DePass *et al.* (1986b) showed no significant effects on rat pup survival or body weight at concentrations up to 1000 mg/kg/day. However, developmental endpoints were not reported in this study. From the available data, the toxicity of ethylene glycol is apparently greatest in the maternal mouse. The estimated equivalent air concentrations (assuming a 70 kg human inhales 20 m^3/day) from the feed in the 3-generation study by DePass *et al.* (1986b) are 700 mg/m^3 and 3500 mg/m^3 for the NOAEL and LOAEL, respectively.

For comparison with the proposed REL of 400 μg/m^3 based on a one month human study, the inhalation NOAEL of 48 ppm, obtained by Tyl *et al*. (1995) in mice discontinuously exposed for 10 days on gestation days 6-15, was used to estimate a REL based on animal data. Use of a time adjustment from 6 to 24 hours/day, an RGDR of 1, an interspecies UF of 3, and an intraspecies UF of 10 resulted in an estimated REL of 0.4 ppm (1000 μg/m^3) for ethylene glycol.

VII. Data Strengths and Limitations for Development of the REL

The strengths of the inhalation REL for ethylene glycol include the use of human exposure data, the use of controlled, nearly continuous inhalation exposures, the observation of a NOAEL, and the similar REL value estimated from an animal study. Major areas of uncertainty are the short length of the key study and the lack of chronic inhalation exposure studies in both animals and man (LaKind *et al*., 1999).

VIII. References

CARB. 1999. Air toxics emissions data collected in the Air Toxics Hot Spots Program CEIDARS Database as of January 29, 1999.

CRC. 1994. CRC Handbook of Chemistry and Physics, 75th edition. Lide DR, ed. Boca Raton, FL: CRC Press Inc.

Coon RA, Jones RA, Jenkins LJ Jr, and Siegel J. 1970. Animal inhalation studies on ammonia, ethylene glycol, formaldehyde, dimethylamine, and ethanol. Toxicol. Appl. Pharmacol. 16(3):646-655.

DePass LR, Garman RH, Woodside MD, Giddens WE, Maronpot RR, and Weil CS. 1986a. Chronic toxicity and oncogenicity studies of ethylene glycol in rats and mice. Fundam. Appl. Toxicol. 7:547-565.

DePass LR, Woodside MD, Maronpot RR, and Weil CS. 1986b. Three-generation reproduction and dominant lethal mutagenesis studies of ethylene glycol in the rat. Fundam. Appl. Toxicol. 7:566-572.

HSDB. 1996. Hazardous Substances Data Bank. National Library of Medicine, Bethesda, MD (CD-ROM version) Denver, CO: Micromedex, Inc. (Edition expires 4/30/96).

HSDB. 1999. Hazardous Substances Data Bank. Available online at http://sis.nlm.nih.gov

Laitinen J, Liesivuori J, and Savolainen H. 1995. Exposure to glycols and their renal effects in motor servicing workers. Occup. Med. 45(5):259-262.

LaKind JS, McKenna EA, Hubner RP, and Tardiff RG. 1999. A review of the comparative mammalian toxicity of ethylene glycol and propylene glycol. Crit. Rev. Toxicol. 29(4):331-365.

Neeper-Bradley TL, Tyl RW, Fisher LC, Kubena MF, Vrbanic MA, and Losco PE. 1995. Determination of a No-Observed-Effect level for developmental toxicity of ethylene glycol administered by gavage to CD rats and CD-1 mice. Fundam. Appl. Toxicol. 27:121-130.

Tyl RW, Ballantyne B, Fisher LC, Fait TA, Dodd DE, Klonne DR, Pritts IM, and Losco PE. 1995a. Evaluation of the developmental toxicity of ethylene glycol aerosol in CD-1 mice by nose-only exposure. Fundam. Appl. Toxicol. 27:49-62.

Tyl RW, Ballantyne B, Fisher LC, Fait DL, Savine TA, Dodd DE, Klonne DR, and Pritts IM. 1995b. Evaluation of the developmental toxicity of ethylene glycol aerosol in the CD rat and CD-1 mouse by whole-body exposure. Fundam. Appl. Toxicol. 24:57-75.

Tyl RW, Price CJ, Marr MC, Myers CB, Seely JC, Heindel JJ, and Schwetz BA. 1993. Developmental toxicity evaluation of ethylene glycol by gavage in New Zealand white rabbits. Fundam. Appl. Toxicol. 20:402-412.

Wills JH, Coulston F, Harris ES, McChesney EW, Russell JC, and Serrone DW. 1974. Inhalation of aerosolized ethylene glycol by man. Clin. Toxicol. 7:463-476.

CHRONIC TOXICITY SUMMARY

ETHYLENE GLYCOL MONOETHYL ETHER

(2-ethoxyethanol; EGEE)

CAS Registry Number: 110-80-5

I. Chronic Toxicity Summary

Inhalation reference exposure level	**70 µg/m^3 (20 ppb)**
Critical effect(s)	Testicular degeneration and decreased hemoglobin in rabbits
Hazard index target(s)	Reproductive system; hematopoietic system

II. Chemical Property Summary (from HSDB, 1996; 1999)

Description	Colorless liquid; sweet, pleasant, ether-like odor
Molecular formula	$C_4H_{10}O_2$
Molecular weight	90.12
Boiling point	135°C
Vapor pressure	3.8 torr @ 20°C; 5.31 torr at 25°C
Solubility	Miscible with water and organic solvents
Conversion factor	3.69 µg/m^3 per ppb at 25ºC

III. Major Uses and Sources

Ethylene glycol monoethyl ether (EGEE) is a widely used solvent for nitrocellulose, dyes, inks, resins, lacquers, paints, and varnishes (HSDB, 1996). It is also a component of many cleaning agents, epoxy coatings, paints, hydraulic fluid, and is an anti-icing fuel additive in aviation. EGEE is also a chemical intermediate in the production of another solvent, ethylene glycol monoethyl ether acetate. The specific annual statewide industrial emissions of EGEE from facilities reporting under the Air Toxics Hot Spots Act in California based on the most recent inventory were estimated to be 443,748 pounds (CARB, 1999). (Many industries did not report emissions of specific glycol ethers. Thus there were also emitted 2,922,744 pounds of the general category glycol ethers, which can include EGEE.)

IV. Effects of Human Exposure

Sperm quality was examined in 37 workers exposed to EGEE by skin contact and/or inhalation in two buildings (Clapp *et al.*, 1987; Ratcliffe *et al.*, 1989). Exposure levels ranged from undetectable to 24 ppm with an average exposure level of 6 ppm in one building and 11 ppm in the other. A statistically significant difference in mean sperm count was observed between the

37 exposed male workers and 39 unexposed male workers. Semen volume and pH, viability, motility, velocity, and morphology were not significantly different between the two groups. The primary metabolite of EGEE, ethoxyacetic acid, was identified in the urine of exposed but not control workers. Both exposed and control subjects had significantly lower sperm counts than historical controls. Furthermore, members of both groups may have been exposed to other compounds including metals, solvents, heat, and vibration.

Welch and Cullen (1988) evaluated shipyard painters exposed to ethylene glycol ethers (EGEE and EGME). Air concentrations at the workplace were estimated based on 102 samples over six shifts in Sparer *et al*. (1988). Time –weighted average (TWA) exposures to EGEE ranged from 0 to 80.5 mg/m^3 with a mean of 9.9 mg/m^3. TWA exposures to EGME ranged from 0 to 17.7 mg/m^3 (mean = 2.6 mg/m^3). The authors note that during the time period of measurement, painting activities were unusually low and previous NIOSH analyses indicated considerably higher exposures. Ninety-four painters and 55 controls answered a medical and environmental exposure questionnaire including work history and provided blood, urine, and in some cases semen samples. Mean hemoglobin levels, total cell counts and differential counts did not differ between exposed and control. However, the authors found that the lowest quartile of hemoglobin was mostly painters and the lowest polymorphonuclear leukocyte counts were in painters. Nine painters were considered anemic and five were considered granulocytopenic. The authors note that the absence of a significant difference in the group as a whole and the inability to detect a dose-response pattern in the exposed group make a strong conclusion unwarranted.

Welch *et al*. (1988) evaluated the semen samples from the workers in the cohort from Welch and Cullen (1988). Sperm concentration, velocity, motility, morphology, morphometry, and viability were measured. Although not statistically significant, the measures of sperm count tended to be lower in the painters with a $p = 0.10$ for density and $p = 0.11$ for count. When nonsmokers were analyzed separately from smokers, the number of oligospermic painters was larger than that in controls at $p = 0.05$. There was no difference between controls and exposed men who were smokers. The authors state that although mean values of sperm count did not differ significantly between controls and exposed groups, biologically important differences were seen when the proportion of men with oligospermia was examined. The proportion of painters with azoospermia was 5% with only 1% expected based on other population surveys. The authors note that to create a dose-response model for an effect of glycol ethers on semen parameters would require description of the exposure of each individual 3 to 6 months prior to sampling. The painters moved frequently from one exposure area to the next, making exposure assessment particularly difficult in this cohort.

Cullen *et al*. (1992) conducted a histopathologic analysis of the bone marrow and circulating blood cells in the workers previously examined in Welch *et al*. (1988). The objects of the study included : 1) to exclude other causes for granulocytopenia and depressed hemoglobin levels noted in some painters exposed to ethylene glycol ethers, 2) to determine if subclinical evidence of hematologic damage is present in healthy coworkers, and 3) to identify host or exogenous factors which may increase the risk of hematologic damage in glycol ether exposed painters. Workers were grouped as follows: Group I consisted of those painters that had anemia or granulocytopenia in the Welch and Cullen (1988) study; Group II consisted of exposed painters with normal hematology; Group III consisted of unexposed controls. A battery of hematologic

and biochemical parameters were measured and a questionnaire was completed to determine occupational exposure status, health status and drug and alcohol consumption. All hematologic parameters were normal in all groups. Tests of liver, renal, and thyroid function were normal in all groups. Bone marrow histology showed no differences between groups. One biochemical parameter, pyruvate kinase activity, was lower in Group I than Groups II and III ($p = 0.05$). Depression of red cell pyruvate kinase did not vary by race and was lower in every subject in Group I by more than one standard deviation. Low pyruvate kinase is the most consistent red cell enzyme defect noted in acquired hematologic disorders.

V. Effects of Animal Exposure

Sprague-Dawley rats (15/sex/group) and New Zealand white rabbits (10/sex/group) were exposed to 0, 25, 103, or 403 ppm EGEE by inhalation for 6 hours/days, 5 days/week, for 13 weeks (Barbee *et al.*, 1984). Animals were physically examined weekly and, at the end of the study, hematology, clinical chemistry, and histopathological examination were performed. No histopathological changes in the respiratory tract were found. Among rabbits, body weight was reduced in the high-dose group males and females. In the 25 ppm dose group, adrenal weight was reduced significantly among males, although this effect was not found to be dose-related. Among males in the high-dose group, testes weights were significantly reduced with a corresponding degenerative change to the seminiferous tubule epithelium. No effect on spermatogenic activity was found, however. Significant hematological effects observed at the high-dose included decreased hemoglobin, hematocrit, and erythrocyte count.

Teratologic effects in pregnant rats from the inhalation of EGEE were reported (Tinston *et al.*, 1983a). The results of this study were presented in summary form (Doe, 1984). Wistar rats (24/group) were exposed to target concentrations of 0, 10, 50, or 250 ppm EGEE for 6 hours/day during gestational days 6-15 and the animals were sacrificed on day 21. Maternal toxicity was observed in the high-dose group with decreased hemoglobin, hematocrit, and mean corpuscular volume. Significant increases in preimplantation loss occurred in the 10 and 50 ppm dose groups, however the absence of this effect at 250 ppm indicated a poor dose-response, and because implantation occurred on the first day of exposure, the relatedness of the effect to exposure is in question. Post-implantation loss was also increased in the mid-dose group, however, no corresponding decrease in intrauterine death was observed in this group. Minor skeletal defects, particularly delayed ossification, were widely observed in the fetuses of mothers exposed to 250 ppm EGEE. Delayed ossification of the cervical vertebrae and sternebrae and the presence of extra ribs was significantly increased in both the 50 and 250 ppm dose groups.

Teratologic effects on pregnant rabbits from inhalation exposure to EGEE were also reported (Tinston *et al.*, 1983b; also summarized by Doe, 1984). Dutch rabbits (24/group) were exposed to 0, 10, 50, or 175 ppm EGEE for 6 hours/day during gestational days 6-18, with sacrifice occurring on gestational day 29. There were no indications of maternal toxicity or litter effects. A statistically significant increase in minor defects and skeletal variants was found in fetuses in the 175 ppm dose group. Other slightly increased incidences of defects in the lower dose groups alone, including extra ribs and partial ossification of the vertebrae, were not considered treatment-related.

Behavioral teratogenic effects were examined in pregnant Sprague-Dawley rats (14 or 15/dose group) exposed to 0 or 100 ppm EGEE for 7 hours/day through gestational days 7-13 (early) or days 14-20 (late) (Nelson *et al.*, 1981). No maternal toxicity was observed and fetal weights were unchanged, although mean gestational length was increased in rats exposed on gestational days 14-20. Six tests (ascent, rotorod, open field, activity wheel, avoidance conditioning, and operant conditioning) were selected to measure motor, sensory, and cognitive function at several stages of development. The offspring of the rats exposed during days 7-13 exhibited impaired performance on the rotorod test (a test of neuromuscular ability) and increased latency in an open field test (a test of exploratory activity) as compared to controls. The offspring of rats exposed during days 14-20 of gestation exhibited decreased activity on an activity wheel (a test of circadian activity). Also, avoidance conditioning revealed that these pups received shocks of a greater number and duration than controls. Neurochemical differences between the prenatally exposed and control pups were measured in newborns and in pups 21 days of age. In newborns from both EGEE-exposed groups, total brain norepinephrine was decreased. In 21-day old pups of both groups, norepinephrine and dopamine levels in the cerebrum were increased. Serotonin level was increased in the cerebrum of the late exposure group only. The authors concluded that there were behavioral and neurochemical alterations in offspring of rats following prenatal exposure to 100 ppm EGEE, however the study design was inadequate to detect gross teratologic anomalies. In a dose range-finding study, two sets of pregnant rats (3-4/group) were exposed during the gestational days 7-13 or 14-20 to 0, 200 (late group only), 300, 600, 900, or 1200 ppm EGEE for 7 hours/day. Increased fetal and pup mortality was observed in all groups exposed to EGEE.

Behavioral and neurochemical effects on the offspring of pregnant S-D rats exposed to 0 or 200 ppm EGEE on gestational days 7-13 were reported (Nelson *et al.*, 1982a; Nelson *et al.*, 1982b). Pregnancy duration was significantly increased in exposed dams. Significantly increased levels of norepinephrine and dopamine were observed in the 21-day old offspring of EGEE-exposed animals. Behavioral changes in pups of treated dams included decreased neuromotor ability and decreased activity.

An investigation into teratologic effects of EGEE was conducted by exposing pregnant rats and rabbits to EGEE by inhalation on gestational days 0-19 (Andrew *et al.*, 1981). Rats (37/group) were exposed to 0, 202, or 767 ppm EGEE for 7 hours/day. All fetuses were resorbed and maternal weight gain was reduced in the high-dose group. In the mid-dose group, a decrease in fetal weight and size (crown-rump length) was observed. Minor skeletal defects and variants and cardiovascular defects were increased in the mid-dose group. Rabbits (29/group) were exposed to 0, 16, or 617 ppm EGEE for 4 hours/day. Maternal weight gain and food intake were decreased in exposed animals. The incidence of fetal resorptions was increased in both the mid- and high-dose group animals. Major cardiovascular defects and minor skeletal defects (extra ribs, delayed ossification) were significantly increased in the mid-dose group. Andrew *et al.* (1981) also examined reproductive effects by exposing female Wistar rats (37/group) to 1, 150, or 649 ppm EGEE 7 hours/day, 5 days/week for 3 weeks before mating with untreated males. No significant effects were observed.

VI. Derivation of Reference Exposure Level

Study	Barbee *et al.*, 1984
Study population	Rabbits
Exposure method	Discontinuous inhalation
Critical effects	Testicular degeneration and decreased hemoglobin levels
LOAEL	403 ppm
NOAEL	103 ppm
Exposure continuity	6 hr/day, 5 days/week
Exposure duration	13 weeks
Average experimental exposure	18.4 ppm (68 mg/m^3) for the NOAEL group
Human equivalent concentration	18.4 ppm (68 mg/m^3) for the NOAEL group (gas with systemic effects, based on RGDR = 1.0 using default assumption that lambda (a) = lambda (h))
Subchronic uncertainty factor	10
LOAEL uncertainty factor	1
Interspecies factor	10 (see explanation below)
Intraspecies factor	10
Cumulative uncertainty factor	1000
Inhalation reference exposure level	0.02 ppm (20 ppb, 0.07 mg/m^3 , 70 µg/m^3)

The reproductive effects observed in the subchronic inhalation study of Barbee *et al.* (1984) were determined by the US EPA (U.S. EPA, 1990) to be the most sensitive endpoints due to EGEE exposure and resulted in a reference concentration (RfC) of 0.2 mg/m^3 (0.06 ppm). OEHHA staff concurred regarding the basis of the U.S. EPA RfC but differed in the application of the interspecies uncertainty factor. Reduced testes weight and testicular degeneration were found in rabbits exposed to EGEE at 403 ppm for 13 weeks. Changes in hematological parameters including decreased hemoglobin, hematocrit, and erythrocyte count were also observed at this dose. A gas:extrarespiratory effect ratio of 1.0 was used to calculate a human equivalency concentration (HEC) in the absence of information relating the effect in rabbits relative to humans.

For a comparison with the proposed REL of 60 ppb (200 µg/m^3) based on testicular degeneration, a REL can be calculated from the LOAEL of 202 ppm observed in the teratology study of Andrew *et al*. (1981). The 7 h exposure to 202 ppm is time-adjusted to a continuous exposure of 59 ppm. Using a RGDR of 1 for a systemic effect, a UF_L of 10, a UF_A of 3 and a UF_H of 10 results in an estimated REL of 200 ppb (700 µg/m^3). Nelson *et al*. (1981) found a LOAEL of 100 ppm for neurobehavioral developmental toxicity in rats exposed 7 hours per day on days 7 to 13 of gestation. The equivalent continuous exposure is 29 ppm. Using an RGDR of 1, a LOAEL UF of 10, an interspecies UF of 3, and an intraspecies UF of 10 results in a REL of 100 ppb (400 µg/m^3).

Although reproductive toxicity has been reported in male workers occupationally exposed to EGEE (Clapp *et al.*, 1987; Ratcliffe *et al.*, 1989), potential confounding factors, particularly

exposure to other compounds, make the study inadequate for the development of the reference exposure level. However, for another comparison with the proposed REL of 60 ppb, if only EGEE caused the adverse reproductive effect, use of a mean concentration between the 2 buildings of 8 ppm for workplace exposure, extrapolation to an equivalent continuous exposure of 3 ppm, and division by 10 for a LOAEL (serious effect) and 10 for intraspecies variability result in a REL of 30 ppb (100 $\mu g/m^3$).

Another comparison with the proposed REL of 60 ppb can be made using the study of Welch *et al*. (1988), who studied shipyard painters exposed to both EGEE and EGME. The authors examined the semen of 73 painters and 40 non-exposed shipyard employees. The men supplied demographic characteristics, medical conditions, personal habits, and reproductive history; underwent a physical examination; and provided a semen sample. An industrial hygiene survey showed that the painters were exposed to EGEE at a time-weighted average (TWA) concentration varying from 0 to 80.5 mg/m^3 (mean = 9.9 mg/m^3, and to EGME at a TWA concentration varying from 0 to 17.7 mg/m^3 (mean = 2.6 mg/m^3). The painters had an increased prevalence of oligospermia and azoospermia and an increased odds ratio for a lower sperm count per ejaculate. (The results were controlled for smoking.) Adding the mean levels together results in a total glycol ether concentration of 12.5 mg/m^3, which is equivalent to a continuous exposure of 4.5 mg/m^3. Division by a UF of 10 for a LOAEL and by another of 10 for intraspecies variability results in a REL of 40 $\mu g/m^3$ (10 ppb). A similar REL would be calculated using the report by Cullen *et a*l. (1992) of depression in red cell pyruvate kinase among anemic and granulocytopenic painters. Since exposure in these studies was to both EGEE and EGME, and exposure assessment was made difficult by frequent job movement and other factors, these studies were not deemed suitable for developing a REL. However, the possibility that humans are more susceptible to EGEE toxicity is raised by the series of studies by Welch *et al*. (1988) and Welch and Cullen (1988) such that we have deviated from the RfC and opted to use an interspecies uncertainty factor of 10 rather than 3 as would usually be the case with an HEC adjustment.

VII. Data Strengths and Limitations for Development of the REL

The strengths of the inhalation REL for EGEE include the availability of subchronic inhalation exposure data from a well-conducted study with histopathological analysis, and the observation of a NOAEL. The observation in several studies noted above of both hematological abnormalities and sperm abnormalities in exposed workers, although difficult to use in a quantitative risk assessment, provide support for the REL developed from animals. In addition, several comparative calculations indicate that RELs based on other studies are generally in agreement with that based on Barbee *et al*. (1984). Major areas of uncertainty are the lack of adequate human exposure data and the lack of chronic inhalation exposure studies.

VIII. References

Andrew FD, Buschbom RL, Cannon WC, *et al.* 1981. Teratologic assessment of ethylbenzene and 2-ethoxyethanol. Prepared for the National Institute for Occupational Safety and Health under Contract 210-79-0037, Battelle Pacific Northwest Laboratories, Richland WA 99352. Microfiche #OTS0513150.

Barbee SJ, Terrill JB, DeSousa DJ, and Conaway CC. 1984. Subchronic inhalation toxicology of ethylene glycol monoethyl ether in the rat and the rabbit. Environ. Health Perspect 57:157-163.

CARB. 1999. Air toxics emissions data collected in the Air Toxics Hot Spots Program CEIDARS Database as of January 29, 1999.

Clapp DE, Smallwood AW, Moseley C, and DeBord KE. 1987. Workplace assessment of exposure to 2-ethoxyethanol. Appl. Ind. Hyg. 2:183-187.

Cullen MR, Rado T, Waldron JA, Sparer J, Welch LS. 1983. Bone marrow injury in lithographers exposed to glycol ethers and organic solvents used in multicolor offset and ultraviolet curing printng processes. Arch. Environ. Health 38(6):347-354.

Cullen MR, Solomon LR, Pace PE, Buckley P, Duffy TP, McPhedran P, Kelsey KT, Redlich CA. 1992. Morphologic, biochemical, and cytogenetic studies of bone marrow and circulating blood cells in painters exposed to ethylene glycol ethers. Environ. Res. 59(1):250-264.
Doe JE. 1984. Ethylene glycol monoethyl ether and ethylene glycol monoethyl ether acetate teratology studies. Environ. Health Perspect. 57:33-41.

HSDB. 1996. Hazardous Substances Data Bank. National Library of Medicine, Bethesda, Maryland (CD-ROM Version). Denver, CO: Micromedex, Inc. (Edition expires 7/31/96).

HSDB. 1999. Hazardous Substances Data Bank. Available online at http://sis.nlm.nih.gov

Nelson BK, Brightwell WS, and Setzer JV. 1982a. Prenatal interactions between ethanol and the industrial solvent 2-ethoxyethanol in rats: maternal and behavioral teratogenic effects. Neurobehav. Toxicol. Teratol. 4:387-94.

Nelson BK, Brightwell WS, Setzer JV, and O'Donohue TL. 1982b. Prenatal interactions between ethanol and the industrial solvent 2-ethoxyethanol in rats: Neurochemical effects in the offspring. Neurobehav. Toxicol. Teratol. 4:395-401.

Nelson BK, Brightwell WS, Setzer JV, Taylor BJ, Hornung RW, and O'Donohue TL. 1981. Ethoxyethanol behavioural teratology in rats. Neurotoxicology 2:231-249.

Ratcliffe JM, Schrader SM, Clapp DE, Halperin WE, Turner TW, and Hornung R.W. 1989. Semen quality in workers exposed to 2-ethoxyethanol. Br. J. Ind. Med. 46:399-406.

Sparer J, Welch LS, McManus K, and Cullen MR. 1988. Effects of exposure to ethylene glycol ethers on shipyard painters: I. Evaluation of exposure. Am. J. Ind. Med. 14:497-507.

Tinston DJ, Doe JE, Godley MJ, Head LK, Killick M, Litchfield MH, and Wickramaratne GA. 1983a. Ethylene glycol monoethyl ether (EE): teratogenicity study in rats. Imperial Chemical Industries PLC, Report No. CTL/P/761 to Chemical Manufacturers Association.

Tinston DJ, Doe JE, Thomas M, and Wickramaratne GA. 1983b. Ethylene glycol monoethyl ether (EE): inhalation teratogenicity study in rabbits. Imperial Chemical Industries PLC. Report No. CTL/P/776 to Chemical Manufacturers Association.

U.S. EPA (United States Environmental Protection Agency). 1990. Documentation of the reference concentration for chronic inhalation exposure (RfC) for 2-ethoxyethanol. Integrated Risk Information System (IRIS on-line). Washington, DC: US EPA.

Welch LS, Cullen MR. 1988. Effect of exposure to ethylene glycol ethers on shipyard painters: III. Hematologic effects. Am. J. Ind. Med. 14(5):527-236.

Welch LS, Schrader SM, Turner TW, Cullen MR. 1988. Effects of exposure to ethylene glycol ethers on shipyard painters: II. Male reproduction. Am. J. Ind. Med. 14(5):509-526.

CHRONIC TOXICITY SUMMARY

ETHYLENE GLYCOL MONOETHYL ETHER ACETATE

(EGEEA; 1-acetoxy-2-ethoxyethane; 2-ethoxyethanol acetate; 2-ethoxyethyl acetate; acetic acid, 2-ethoxyethyl ester; beta-ethoxyethyl acetate; Cellosolve® acetate; ethoxy acetate; ethyl Cellosolve® acetate; Poly-solv® EE acetate; ethyl glycol acetate; oxitol acetate)

CAS Registry Number: 111-15-9

I. Chronic Toxicity Summary

Inhalation reference exposure level	**300 µg/m^3 (60 ppb)**
Critical effect(s)	Teratogenicity and fetotoxicity in rabbits
Hazard index target(s)	Development

II. Chemical Property Summary (HSDB, 1996)

Description	Colorless liquid
Molecular formula	$C_6H_{12}O_3$
Molecular weight	132.16 g/mol
Boiling point	156°C
Vapor pressure	2 torr @ 20°C
Solubility	Soluble in water (229 g/l at 20°C); sol. in alcohol, ether, acetone; miscible with olive oil, aromatic hydrocarbons
Conversion factor	5.41 µg/m^3 per ppb at 25ºC

III. Major Uses and Sources

Ethylene glycol monoethyl ether acetate (EGEEA) is used in automobile lacquers where it retards “blushing” and evaporation and imparts a high gloss (HSDB, 1996). It is also used as a solvent for nitrocellulose, oils, and resins and as a component of varnish removers and wood stains. EGEEA is also used in the treatment of textiles and leather. The annual specific statewide industrial emissions of EGEEA from facilities reporting under the Air Toxics Hot Spots Act in California based on the most recent inventory were estimated to be 66,851 pounds (CARB, 1999).

IV. Effects of Human Exposure

No studies relating exposure to EGEEA to adverse health effects in humans were located in the literature.

Ten male volunteers were exposed to EGEEA by inhalation. Five were exposed to 14, 28, and 50 mg EGEEA/m^3 and five to 28 mg/m^3 for 4 hours (Groeseneken *et al.*, 1987a). Twenty-two percent of the absorbed dose was eliminated in the urine as ethoxyacetic acid within 42 hours. In another study, male volunteers exposed to EGEEA by inhalation under various conditions were found to eliminate some in the form of ethylene glycol monoethyl ether (EGEE) (Groeseneken *et al.*, 1987b).

V. Effects of Animal Exposure

Pregnant rabbits (24 or 25/group) were exposed to 0, 25, 100, or 400 ppm EGEEA by inhalation for 6 hours/day on gestational days 6-18 (Tinston *et al.*, 1983; reviewed in Doe, 1984). The animals were killed on gestational day 29. Maternal effects (decreased weight gain, decreased food consumption, decreased hemoglobin) were observed in the high-dose group. The number of rabbits with total fetal resorptions was increased in the 400 ppm dose group, accompanied by a decrease in weight in surviving fetuses. A reduction in average fetal weight was also observed at 100 ppm EGEEA, but this effect may relate to the increased litter size among dams in this dose group. Evidence of teratogenicity was observed in the 400 ppm dose group, with increased major malformations of the vertebral column. Both 400 and 100 ppm EGEEA were found to be fetotoxic as indicated by retarded ossification. No statistically significant effects were observed in the 25 ppm dose group, although a single case of a major defect (kidney agenesis) was observed in both the 25 and 400 ppm EGEEA dose groups.

Rats (10/sex/dose) and rabbits (2/sex/dose) were exposed for 4 hours/day, 5 days/week for 10 months to 0 or 200 ppm EGEEA (Truhaut *et al.*, 1979). Observation of body weight gain, hematology, clinical chemistry, and gross pathology revealed no toxic effects among treated animals. Among male rats and rabbits, “discrete lesions of tubular nephritis with clear degeneration of the epithelium with hyaline and granular tubular casts” were observed. Four hour exposure to 2000 ppm EGEEA resulted in transient hemoglobinuria and hematuria in rabbits (2/sex/dose), but not rats (10/sex/dose). No pathological lesions were observed following a 2 week observation period.

Dogs were exposed to 600 ppm EGEEA for 7 hours/day for 120 days (Carpenter *et al.*, 1956; Gingell *et al.*, 1982). Hematological, clinical chemistry, and histopathological examination revealed no adverse effects.

Pregnant rats and rabbits (24/group) were exposed to nominal concentrations of 0, 50, 100, 200 or 300 ppm EGEEA by inhalation during gestational days 6-15 and sacrificed on gestational day 21 (Union Carbide Corporation, 1984). Maternal effects in rats included increased absolute liver weights (all treated groups); increased relative liver weights, and decreased RBC count, hemoglobin, hematocrit, and RBC size (all but low-dose group); decreased food consumption, increased white blood cell count, and decreased platelet count (200 and 300 ppm groups). An increase in the number of non-viable implantations per litter was observed at 300 ppm and

decreased average fetal body weight per litter was observed at 200 and 300 ppm EGEEA. Visceral and skeletal malformations were widely observed at both 200 and 300 ppm EGEEA. Among rabbits, maternal effects included decreased platelets (100, 200, and 300 ppm); decreased weight gain, decreased gravid uterine weight, increased number of dams with non-viable implants, and increased number of non-viable implants per litter (200 and 300 ppm); increased occult blood, increased mean corpuscular volume, decreased corpora lutea/litter and increased early resorptions/litter (300 ppm). Visceral and skeletal malformations were observed in the 100, 200, and 300 ppm EGEEA dose groups.

Pregnant rats were exposed to 0, 130, 390, or 600 ppm EGEEA for 7 hours/day on gestational days 7-15 (Nelson *et al.*, 1984). Dams were sacrificed on day 20. Complete resorption of litters was observed at 600 ppm. Skeletal and cardiovascular defects and decreased fetal weight and fetal resorptions were observed at 390 ppm EGEEA. Reduced fetal weights were also observed at 130 ppm EGEEA.

Ethylene glycol monoethyl ether acetate (0.35 ml = 2.6 mmole/treatment) or water was applied to the shaved skin of pregnant rats four times daily on days 7 to 16 gestation (Hardin *et al.*, 1984). EGEEA treated rats showed reduced body weight (from litter resorption) and significantly fewer live fetuses per litter. Litters from treated dams also showed significantly increased visceral malformations and skeletal variations.

VI. Derivation of Chronic Reference Exposure Level (REL)

Study	Tinston *et al.*, 1983
Study population	Rabbits
Exposure method	Discontinuous inhalation exposure
Critical effects	Fetotoxicity
LOAEL	100 ppm
NOAEL	25 ppm
Exposure continuity	6 hours/day, 7 days/week
Exposure duration	13 days
Average experimental exposure	6.2 ppm for NOAEL group (25 x 6/24)
LOAEL uncertainty factor	1
Subchronic uncertainty factor	1
Interspecies factor	10
Intraspecies factor	10
Cumulative uncertainty factor	100
Inhalation reference exposure level	0.06 ppm (60 ppb, 0.03 mg/m^3, 300 µg/m^3)

A review of the literature on the toxicity of EGEEA indicates that the most sensitive endpoint of toxicity is that seen in experimental animals showing developmental effects from inhalation exposure during pregnancy. There are no adequate data associating exposures in humans with toxic effects for the development of a chronic reference exposure level. Separate studies in animals have demonstrated developmental toxicity. Reduced fetal weights were observed in rats exposed to 130 ppm EGEEA on gestational days 7-15 (Nelson *et al.*, 1984). Skeletal and

cardiovascular defects were observed at the next higher dose of 390 ppm EGEEA, and all litters were resorbed in the high-dose group. Visceral and skeletal defects were observed in all but the low-dose group (50 ppm EGEEA) in the litters of rabbit dams exposed to EGEEA on gestational days 6-15 (Union Carbide Corporation, 1984). Fetotoxicity, as indicated by retarded bone development, was observed in all but the low-dose group (25 ppm EGEEA) in the litters of rabbit dams exposed on gestational days 6-18 (Tinston *et al.*, 1983). The lowest dose levels showing developmental toxicity are those reported by Union Carbide Corporation (1984) and Tinston *et al.* (1983), with 100 ppm EGEEA showing developmental defects in the offspring of exposed dams. Since only the Tinston *et al.* (1983) study also showed an exposure level without effect (a NOAEL), this study has been selected for the development of the chronic REL.

VII. Data Strengths and Limitations for Development of the REL

Strengths of the database for EGEEA include the large number of animal studies available. Limitations include the lack of any human data for exposures longer than 4 hours and the lack of sperm count studies, a critical effect for the related compounds, EGEE and EGME. However, the REL calculated is similar to that for EGEE which is based on testicular degeneration.

VIII. References

CARB. 1999. Air toxics emissions data collected in the Air Toxics Hot Spots Program CEIDARS Database as of January 29, 1999.

Carpenter CP, Pozzani UC, Weil CS, Nair JH, Keck GA, and Smyth HF. 1956. The toxicity of butyl cellosolve solvent. Arch. Ind. Health 14:114-31.

Doe JE. 1984. Ethylene glycol monoethyl ether and ethylene glycol monoethyl ether acetate teratology studies. Environ. Health Perspect. 57:33-41.

Gingell R, Boatman RJ, Bus J, Cawley TJ, Knaak JB, Krasavage WJ, Skoulis NP, Stack CR, and Tyler TR. 1982. Glycol ethers and other selected glycol derivatives. In: Patty's Industrial Hygiene and Toxicology. Fourth ed. Clayton, G.D. and Clayton, F.E. (eds.). New York: John Wiley Sons.

Groeseneken D, Veulemans H, Masschelein R, and Van Vlem E. 1987a. Ethoxyacetic acid: a metabolite of ethylene glycol monoethyl ether acetate in man. Br. J. Ind. Med. 44:488-493.

Groeseneken D, Veulemans H, Masschelein R, and Van Vlem E. 1987b. Pulmonary absorption and elimination of ethylene glycol monoethyl ether acetate in man. Br. J. Ind. Med. 44:309-316.

Hardin BD, Goad PT, and Burg JR. 1984. Developmental toxicity of four glycol ethers applied cutaneously to rats. Environ. Health Perspect. 57:69-74.

HSDB. 1996. Hazardous Substances Data Bank. National Library of Medicine, Bethesda, Maryland (CD-ROM Version). Denver, CO: Micromedex, Inc. (Edition expires 7/31/96).

Nelson BK, Setzer JV, Brightwell WS, Mathinos PR, Kuczuk MH, Weaver TE, and Goad PT. 1984. Comparative inhalation teratogenicity of four glycol ether solvents and an amino derivative in rats. Environ. Health Perspect. 57:261-271.

Tinston DJ, Doe JE, Killick M, and Thomas M. 1983. Ethylene glycol monoethyl ether acetate (EEAc): Inhalation teratogenicity study in rabbits. Report No. CTL/P/840. Conducted by the Imperial Chemical Industries PLC, Central Toxicology Laboratory, Alderley Park, Macclesfield, Cheshire, UK.

Truhaut R, Dutertre-Catella H, Phu-Lich N, and Huyen VN. 1979. Comparative toxicological study of ethylglycol acetate and butylglycol acetate. Toxicol. Appl. Pharmacol. 51:117-27.

Union Carbide Corporation. 1984. Bushy Run Research Center. Teratologic evaluation of Cellosolve acetate in Fischer 344 rats and New Zealand white rabbits following inhalation exposure. EPA Doc. No. FYI-AX-1184-0360 (Fiche No. 0000360-0).

CHRONIC TOXICITY SUMMARY

ETHYLENE GLYCOL MONOMETHYL ETHER

(EGME; 2-methoxyethanol; 1-hydroxy-2-methoxyethane; methyl cellosolve)

CAS Registry Number: 109-86-4

I. Chronic Toxicity Summary

Inhalation reference exposure level	**60 µg/m^3 (20 ppb)**
Critical effect(s)	Testicular toxicity in rabbits
Hazard index target(s)	Reproductive system

II. Physical and Chemical Properties (HSDB, 1995)

Description	Colorless liquid
Molecular formula	$C_3H_8O_2$
Molecular weight	76.09
Density	0.965 g/cm^3 @ 20° C
Boiling point	125°C
Melting point	-85.1°C
Vapor pressure	6.2 torr @ 20°C
Solubility	Miscible with water, alcohol, benzene, ether, acetone
Conversion factor	1 ppm = 3.1 mg/m^3 @ 25°C

III. Major Uses and Sources

Ethylene glycol monomethyl ether (EGME) is used as a solvent for cellulose acetate and resins (HSDB, 1995) as well as a solvent in the semiconductor industry. It is also used in dyeing leather and in the manufacture of photographic film. EGME is used as an anti-freeze in jet fuels. Quick drying varnishes, enamels, nail polishes, and wood stains may also contain EGME. The specific annual statewide industrial emissions of EGME from facilities reporting under the Air Toxics Hot Spots Act in California based on the most recent inventory were estimated to be 7398 pounds (CARB, 1999). (Many industries did not report emissions of specific glycol ethers. Thus there were also emitted 2,922,744 pounds of the general category glycol ethers, which can include EGME.)

IV. Effects of Human Exposure

Human exposures to ethylene glycol monomethyl ether have been associated with hematological and neurological abnormalities. To determine whether employees potentially exposed to ethylene glycol monomethyl ether during manufacturing and packaging had a higher prevalence of anemia, leukopenia, or sterility than an in-plant comparison group, a cross-sectional study was

conducted. Blood samples on 65 of 97 potentially exposed and control white males, and semen samples from a subset of 15 were analyzed. No gross abnormalities or clinically meaningful differences in hematological or fertility indices were noted. Decreased testicular size was reported in workers (who were exposed to an 8-hour TWA concentration of 0.42 ppm EGME or less) but it was not statistically significant (Cook *et al.*, 1982).

Cullen *et al*. (1983) studied possible bone marrow toxicity of workplace substances including dipropylene glycol monomethyl ether, EGME, and various aliphatic, aromatic and halogenated hydrocarbons used for offset and ultraviolet cured multicolor printing. Evaluation of seven co-workers of a printer with aplastic anemia indicated normal peripheral blood, but bone marrow specimens demonstrated clear patterns of injury in three while the others had nonspecific signs of marrow effect. The authors could not assign the changes to known risk factors and concluded that further evaluation of possible bone marrow toxicity resulting from exposure to glycol ethers and ultraviolet curing printing processes was warranted. This was done to some extent in their studies on shipyard painters below.

Welch and Cullen (1988) evaluated shipyard painters exposed to ethylene glycol ethers (EGEE and EGME). Air concentrations at the workplace were estimated based on 102 samples over six shifts in Sparer *et al*. (1988). Time –weighted average (TWA) exposures to EGEE ranged from 0 to 80.5 mg/m3 with a mean of 9.9 mg/m3. TWA exposures to EGME ranged from 0 to 17.7 mg/m3 (mean = 2.6 mg/m3). The authors note that during the time period of measurement, painting activities were unusually low and previous NIOSH analyses indicated considerably higher exposures. Ninety-four painters and 55 controls answered a medical and environmental exposure questionnaire including work history and provided blood, urine, and in some cases semen samples. Mean hemoglobin levels, total cell counts and differential counts did not differ between exposed and control. However, the authors found that the lowest quartile of hemoglobin was mostly painters and the lowest polymorphonuclear leukocyte counts were in painters. Nine painters were considered anemic and five were considered granulocytopenic. The authors note that the absence of a significant difference in the group as a whole and the inability to detect a dose-response pattern in the exposed group makes a strong conclusion unwarranted.

Welch *et al*. (1988) evaluated the semen samples from the workers in the cohort from Welch and Cullen (1988). Sperm concentration, velocity, motility, morphology, morphometry, and viability were measured. Although not statistically significant, the measures of sperm count tended to be lower in the painters with a $p = 0.10$ for density and $p = 0.11$ for count. When nonsmokers were analyzed separately from smokers, the number of oligospermic painters was larger than that in controls at $p = 0.05$. There was no difference between controls and exposed men who were smokers. The authors state that although mean values of sperm count did not differ significantly between controls and exposed groups, biologically important differences were seen when the proportion of men with oligospermia was examined. The proportion of painters with azoospermia was 5% with only 1% expected based on other population surveys. The authors note that to create a dose-response model for an effect of glycol ethers on semen parameters would require description of the exposure of each individual 3 to 6 months prior to sampling. The painters moved frequently from one exposure area to the next, making exposure assessment particularly difficult in this cohort.

Cullen *et al*. (1992) conducted a histopathologic analysis of the bone marrow and circulating blood cells in the workers previously examined in Welch *et al*. (1988). The objects of the study included : 1) to exclude other causes for granulocytopenia and depressed hemoglobin levels noted in some painters exposed to ethylene glycol ethers, 2) to determine if subclinical evidence of hematologic damage is present in healthy coworkers, and 3) to identify host or exogenous factors which may increase the risk of hematologic damage in glycol ether exposed painters. Workers were grouped as follows: Group I consisted of those painters that had anemia or granulocytopenia in the Welch and Cullen (1988) study; Group II consisted of exposed painters with normal hematology; Group III consisted of unexposed controls. A battery of hematologic and biochemical parameters were measured and a questionnaire was completed to determine occupational exposure status, health status and drug and alcohol consumption. All hematologic parameters were normal in all groups. Tests of liver, renal, and thyroid function were normal in all groups. Bone marrow histology showed no differences between groups. One biochemical parameter, pyruvate kinase activity, was lower in Group I than Groups II and III ($p = 0.05$). Depression of red cell pyruvate kinase did not vary by race and was lower in every subject in Group I by more than one standard deviation. Low pyruvate kinase is the most consistent red cell enzyme defect noted in acquired hematologic disorders.

Reversible neurological symptoms (apathy, fatigue, decreased appetite) and macrocytic anemia were observed in a worker following occupational dermal and inhalation exposure to an average concentration of 35 ppm EGME for 1-1.5 years (Cohen, 1984). The worker was also exposed to methyl ethyl ketone and propylene glycol monomethyl ether at concentrations of 1-5 ppm and 4.2-12.8 ppm, respectively.

Hematologic effects were also reported in three women employed in a factory working with glue consisting of 70% acetone and 30% EGME (Larese *et al.*, 1992). The women exhibited abnormally low white blood cell counts, relative lymphocytosis and macrocytosis. These hematological parameters returned to normal following cessation of exposure.

Older case reports support findings of neurological and hematological toxicity following occupational exposure to EGME (Greenburg *et al.*, 1938; Zavon, 1963; Parsons and Parsons, 1938).

V. Effects of Animal Exposure

A concentration dependent decrease in testes weight was observed in male rabbits exposed to 30, 100, or 300 ppm EGME 6 hours per day, 5 days per week for 13 weeks (Miller *et al.*, 1983). Degenerative changes in the germinal epithelium were observed in male rabbits of <u>all</u> exposed groups, but were not statistically significant at 30 ppm. Two of five male rabbits exposed to 300 ppm EGME died during the course of the study. Female rabbits were also exposed; two of five female rabbits exposed to 100 or 300 ppm EGME died during the course of the study. The animals died at different times of different causes and thus the authors were uncertain if the deaths were treatment related. Reduced body weight gain, pancytopenia (abnormal depression of all the cellular elements of the blood), and thymic atrophy were observed in rabbits of both sexes

exposed to 300 ppm EGME. No effects on the reproductive organs of the female rabbits were observed.

In the same study (Miller *et al.*, 1983) male and female rats were exposed to 30, 100, or 300 ppm EGME 6 hours per day, 5 days per week for 13 weeks. Moderate to severe degeneration of the germinal epithelium and seminiferous tubules was observed in male rats exposed to 300 ppm EGME. A significant decrease in body weight was observed in male rats exposed to 300 ppm and in female rats exposed to concentrations of EGME of 100 ppm or greater. Pancytopenia, lymphoid tissue atrophy, and decreased liver weights were observed in animals of both sexes exposed to the highest concentration. Also in the highest exposure group, mean values for total serum protein, albumin and globulins were lower than control values.

Doe *et al*. (1983) designed a two-part study to provide a rapid assessment of the effect of glycol ethers on some aspects of reproduction in the rat. Exposure to EGME was by inhalation at 100 and 300 ppm for 6 hr/day. First, pregnant females were exposed on Days 6 to 17 of gestation. Body weight gain was reduced in both groups. No litters were delivered in the 300-ppm group and only 9/20 rats in the 100-ppm group produced litters; the number, weight, and viability of the pups were reduced, but the pups appeared normal externally. Second, male rats were exposed for 10 days. There was a reduction in testicular weight accompanied by seminiferous tubular atrophy in the 300-ppm group. There were no effects at 100 ppm. Exposure at 300 ppm EGME caused significant reductions in white blood cell count, red blood cell count, hemoglobin concentration, hematocrit, and mean cell hemoglobin.

More recent data point to the immune system as a key endpoint of EGME toxicity. A statistically significant dose-related decrease in thymus weight was observed both in male rats administered drinking water containing 2000 and 6000 ppm EGME (161 or 486 mg/kg/day) and in female rats administered drinking water containing 1600 and 4800 ppm EGME (200 or 531 mg/kg/day) for 21 days (Exon *et al.*, 1991). Histopathological examination revealed thymic atrophy and loss of demarcation between the cortex and medulla. Decreased spleen cell numbers were observed in female rats at both dose levels and male rats at the high dose level. Male rats in the high dose group exhibited a statistically significant decrease in body weight gain. Testicular effects were also observed in exposed male rats.

Pregnant mice were exposed to 100, 150, or 200 mg/kg/day EGME on days 10-17 of gestation (Holladay *et al.*, 1994). Thymic atrophy and inhibition of fetal thymocyte maturation were observed in EGME-treated offspring examined on day 18 of gestation. Also, the ability of the EGME-treated fetal mouse liver cells to repopulate the spleen of irradiated mice was significantly impaired as compared to that of control fetal mouse liver cells.

VI. Derivation of Reference Exposure Level

Study	Miller *et al.*, 1983; U.S. EPA, 1995
Study population	Rats and rabbits
Exposure method	Inhalation (0, 30, 100, or 300 ppm)
Critical effects	Decreased testes weight and degenerative changes in the testicular germinal epithelium.
LOAEL	100 ppm
NOAEL	30 ppm
Exposure continuity	6 hours/day, 5 days/week
Average experimental exposure	5.4 ppm for NOAEL group
Human equivalent concentration	5.4 ppm for NOAEL group (gas with systemic effects, based on RGDR = 1.0 using default assumption that lambda (a) = lambda (h))
Exposure duration	13 weeks
LOAEL uncertainty factor	1
Subchronic uncertainty factor	10
Interspecies uncertainty factor	3
Intraspecies uncertainty factor	10
Cumulative uncertainty factors	300
Inhalation reference exposure level	0.02 ppm (20 ppb; 0.06 mg/m^3; 60µg/m^3)

The REL is based on the same study on which U.S. EPA based its RfC. However, OEHHA declined to use a modifying factor because the criteria for use of such factors are not well described by U.S. EPA. However, since rabbits were the more sensitive species and live 6 years (312 weeks), a 13 week study in rabbits merits a subchronic UF of 10.

A comparison with the proposed REL for EGME of 20 ppb (60 µg/m^3) can be made using the occupational study of Welch *et al*. (1988) of the semen of shipyard painters exposed to both EGEE and EGME. The men supplied demographic characteristics, medical conditions, personal habits, and reproductive history; underwent a physical examination; and provided a semen sample. The painters were exposed to EGEE at a TWA concentration of 0 to 80.5 mg/m^3 (mean = 9.9 mg/m^3, and to EGME at a TWA concentration of 0 to 17.7 mg/m^3 (mean = 2.6 mg/m^3). The painters had an increased prevalence of oligospermia and azoospermia and an increased odds ratio for a lower sperm count per ejaculate compared to shipyard employees who were not painters. (The results were controlled for smoking.) Adding the mean exposure levels together results in a total glycol ether concentration (EGME + EGEE) of 12.5 mg/m^3, equivalent to a continuous exposure of 4.5 mg/m^3. Division by a UF of 10 for a LOAEL and by another of 10 for human intraspecies variability results in a REL of 40 µg/m^3 (10 ppb), similar to the REL based on rabbits. Since exposure was primarily to EGEE with co-exposure to EGME, and exposure assessment ws difficult to quantify, this study was not deemed suitable for developing a REL. Nonetheless, the REL developed using this study is close in value to the proposed REL of 20 ppb.

VII. Data Strengths and Limitations for Development of the REL

The strengths of the inhalation REL for EGME include the availability of subchronic inhalation exposure data from a well-conducted study with histopathological analysis and the observation of a NOAEL. In addition, there are a number of human studies showing similar toxicological endpoints to those demonstrated in animal studies. Major areas of uncertainty are the lack of adequate human exposure data, and the lack of chronic inhalation exposure studies.

VIII. References

CARB. 1999. Air toxics emissions data collected in the Air Toxics Hot Spots Program CEIDARS Database as of January 29, 1999.

Cohen R. 1984. Reversible subacute ethylene glycol monomethyl ether toxicity associated with microfilm production: A case report. Am. J. Ind. Med. 6(6):441-446. [cited in U.S. EPA, 1995].

Cook RR, Bodner KM, Kolesar RC, Uhlman CS, Van Peenen PFD, Dickson GS, and Flanagan K. 1982. A cross-sectional study of ethylene glycol monomethyl ether process employees. Arch. Environ. Health 37(6):346-351.

Cullen MR, Rado T, Waldron JA, Sparer J, Welch LS. 1983. Bone marrow injury in lithographers exposed to glycol ethers and organic solvents used in multicolor offset and ultraviolet curing printng processes. Arch Environ Health 38(6):347-354.

Cullen MR, Solomon LR, Pace PE, Buckley P, Duffy TP, McPhedran P, Kelsey KT, Redlich CA. 1992. Morphologic, biochemical, and cytogenetic studies of bone marrow and circulating blood cells in painters exposed to ethylene glycol ethers. Environ. Res. 59(1):250-264.

Doe JE, Samuels DM, Tinston DJ, and de Silva Wickramaratne GA. 1983. Comparative aspects of the reproductive toxicology by inhalation in rats of ethylene glycol monomethyl ether and propylene glycol monomethyl ether. Toxicol. Appl. Pharmacol. 15;69(1):43-47.

Exon JH, Mather GG, Bussiere JL, Olson DP, and Talcott PA. 1991. Effects of subchronic exposure of rats to 2-methoxyethanol or 2-butoxyethanol: thymic atrophy and immunotoxicity. Fundam. Appl. Toxicol. 16(4):830-840.

Greenburg L, Mayers MR, Goldwater JL, Burke WJ, and Moskovitz S. 1938. Health hazards in the manufacture of “fused collars.” I. Exposure to ethylene glycol monomethyl ether. J. Ind. Hyg. Toxicol. 20(2):134-147. [cited in U.S. EPA, 1995].

HSDB 1995. Hazardous Substances Data Bank. National Library of Medicine, Bethesda, Maryland (TOMES® CD-ROM Version). Denver, CO: Micromedex, Inc. (Edition expires 4/30/95).

Holladay SD, Comment CE, Kwon J, Luster MI. 1994. Fetal hematopoietic alterations after maternal exposure to ethylene glycol monomethyl ether: Prolymphoid cell targeting. Toxicol. Appl. Pharmacol. 129:53-60.

Larese F, Fiorito A, De Zotti R. 1992. The possible hematologic effects of glycol monomethyl ether in a frame factory. Br. J. Ind. Med. 49:131-133.

Miller RR, Ayres JA, Young JT, and McKenna MJ. 1983. Ethylene glycol monomethyl ether. I. Subchronic vapor inhalation study with rats and rabbits. Fundam. Appl. Toxicol. 3:49-54.

Parsons CE, and Parsons MEM. 1938. Toxic encephalopathy and “granulopenic anemia” due to volatile solvents in industry: Report of two cases. J. Ind. Hyg. Toxicol. 20(2):124-133. [cited in U.S. EPA, 1995].

Sparer J, Welch LS, McManus K, and Cullen MR. 1988. Effects of exposure to ethyelen glycol ethers on shipyard painters: I. Evaluation of exposure. Am J Ind Med 14:497-507.

U.S. EPA. 1995. U.S. Environmental Protection Agency. Integrated Risk Information System (IRIS). Office of Health and Environmental Assessment, Environmental Criteria Assessment Office, Cincinnati, OH.

Welch LS, Cullen MR. 1988. Effect of exposure to ethylene glycol ethers on shipyard painters: III. Hematologic effects.Am. J. Ind. Med. 14(5):527-236.

Welch LS, Schrader SM, Turner TW, Cullen MR. 1988. Effects of exposure to ethylene glycol ethers on shipyard painters: II. Male reproduction. Am. J. Ind. Med. 14(5):509-526.

Zavon MR. 1963. Methyl cellosolve intoxication. Am. Ind. Hyg. Assoc. J. 24:36-41.

CHRONIC TOXICITY SUMMARY

ETHYLENE GLYCOL MONOMETHYL ETHER ACETATE

(EGMEA; 2-methoxyethanol acetate; 2-methoxyethylester acetic acid; methyl glycol acetate; methyl Cellosolve® acetate)

CAS Registry Number: 110-49-6

I. Chronic Toxicity Summary

Inhalation reference exposure level	**90 µg/m^3 (20 ppb)**
Critical effect(s)	Reproductive (testicular) toxicity in rabbits (EGME)
Hazard index target(s)	Reproductive system

II. Chemical Property Summary (HSDB, 1995)

Description	Colorless liquid
Molecular formula	$C_5H_{10}O_3$
Molecular weight	118.3 g/mol
Boiling point	144-145°C
Vapor pressure	2 torr @ 20°C
Solubility	Miscible with water, organic solvents, oils
Conversion factor	4.83 µg/m^3 per ppb at 25ºC

III. Major Uses and Sources

Ethylene glycol monomethyl ether acetate (EGMEA) is used as a solvent for nitrocellulose, cellulose acetate, and various other gums, resins, waxes, and oils (HSDB, 1995). It is also used in the semiconductor industry and in textile printing, photographic films, lacquers, and silk-screening inks. The annual specific statewide industrial emissions of EGMEA from facilities reporting under the Air Toxics Hot Spots Act in California based on the most recent inventory were estimated to be 3,060 pounds (CARB, 1999).

IV. Effects of Human Exposure

Developmental defects have been described in the offspring of a mother who was occupationally exposed to EGMEA during pregnancy (Bolt and Golka, 1990). The mother was exposed during pregnancy by skin absorption and inhalation for approximately 1-4 hours/day to 1-2 liters of EGMEA. Her first child was born with congenital hypospadia, chordee, micropenis, and

scrotum bifida and her second child (3 years later) was born with chordee, cryptorchidism, penile hypospadia and scrotum bifida. Both children had normal karyotypes. No estimates of exposure were made.

A single case report described allergic dermatitis which may have developed from contact with EGMEA (Jordan and Dahl, 1971). A 58-year-old woman developed dermatitis on the nose possibly from contact with EGMEA on her eyeglasses. Ethylene glycol monoethyl ether acetate (EGEEA) was also present.

V. Effects of Animal Exposure

Cats, rabbits, guinea pigs, and mice were repeatedly exposed by inhalation for 8 hours daily to 500 and 1000 ppm EGMEA (Gross, 1943; as described by Gingell *et al.*, 1982). This exposure regimen was fatal to cats at 500 ppm EGMEA. Death occurred after the animals showed slight narcosis. Similarly, exposure to 1000 ppm EGMEA produced deaths among rabbits, guinea pigs, and mice within a few days. Kidney toxicity was observed in animals in both dose groups. Repeated 4- and 6-hour exposure of cats to 200 ppm EGMEA resulted in decreased “blood pigments” and red blood cell counts.

The toxic effects of EGMEA were examined in male mice treated by gastric intubation 5 days/week for 5 weeks with 0, 62.5, 125, 250, 500, 1000, or 2000 mg EGMEA/kg/day (Nagano *et al.*, 1984). Dose-related testicular atrophy was observed at doses above 250 mg EGMEA/kg/day. Decreased white blood cell counts were observed in all EGMEA-exposed groups.

EGMEA was readily converted in vitro to ethylene glycol monomethyl ether (EGME) by the nasal mucosal carboxylesterases of mice and rabbits (Stott and McKenna, 1985). The enzyme activity in the nasal mucosa was equal to that of the liver and greater than that of the kidney and lung.

A concentration dependent decrease in testes weight was observed in male rabbits exposed to 30, 100, or 300 ppm ethylene glycol monomethyl ether (EGME) 6 hours/day, 5 days/week for 13 weeks (Miller *et al.*, 1983). Degenerative changes in the germinal epithelium were observed in male rabbits of all exposed groups, but the changes were not statistically significant at 30 ppm. Two of five male rabbits exposed to 300 ppm EGME died during the course of the study. Female rabbits were also exposed; two of five female rabbits exposed to 100 or 300 ppm EGME died during the course of the study. Reduced body weight gain, pancytopenia (abnormal depression of all the cellular elements of the blood), and thymic atrophy were observed in rabbits of both sexes exposed to 300 ppm EGME. No effects on the reproductive organs of the female rabbits were observed.

In the same study male and female rats were exposed to 30, 100, or 300 ppm EGME 6 hrs/day, 5 days/week for 13 weeks. Moderate to severe degeneration of the germinal epithelium and seminiferous tubules was observed in male rats exposed to 300 ppm EGME. A significant decrease in body weight was observed in male rats exposed to 300 ppm and in female rats

exposed to concentrations of EGME of 100 ppm or greater. Pancytopenia, lymphoid tissue atrophy, and decreased liver weights were observed in animals of both sexes exposed to the highest concentration. Also in the highest exposure group, mean values for total serum protein, albumin and globulins were lower than control values.

VI. Derivation of Chronic Reference Exposure Level (REL)

Study	Miller *et al.*, 1983 (see below)
Study population	Rabbits
Exposure method	Discontinuous inhalation exposure (0, 30, 100, or 300 ppm EGME)
Critical effects	Testicular effects
LOAEL	100 ppm EGME
NOAEL	30 ppm EGME
Exposure continuity	6 hr/day, 5 days/week
Exposure duration	13 weeks
Average experimental exposure	5.4 ppm EGME for NOAEL group (30 x 6/24 x 5/7)
Human equivalent concentration	5.4 ppm EGME for NOAEL group (gas with systemic effects, based on RGDR = 1.0 using default assumption that lambda (a) = lambda (h))
LOAEL uncertainty factor	1
Subchronic uncertainty factor	10
Interspecies factor	3
Intraspecies factor	10
Cumulative uncertainty factor	300
Inhalation reference exposure level	0.02 ppm (20 ppb, 0.06 mg/m^3, 60 µg/m^3) EGME 90 µg/m^3 EGMEA (20 ppb) (60 x MW_{EGMEA} / MW_{EGME})

Data relating specific EGMEA exposure levels to toxicity in humans are not available for the development of a chronic REL. Data from experimental animals indicate that EGMEA is toxic to the hematopoietic and reproductive systems (Gross, 1943; Nagano *et al.*, 1984), however good, quantitative data relating chronic exposure to toxicity are lacking. Because of evidence that EGMEA is readily converted to EGME by several organ systems (Stott and McKenna, 1985) and since the scant data on EGMEA toxicity in animals indicate that the spectrum of toxicity of the two compounds is similar, the chronic REL was derived based upon the assumption of equimolar toxicity of EGMEA and EGME.

VII. Data Strengths and Limitations for Development of the REL

The strengths of the inhalation REL for EGMEA include the availability of subchronic inhalation exposure data from a well-conducted study of EGME as well as a number of supportive human studies on EGME showing the same toxicological endpoint, and the observation of a NOAEL.

Major areas of uncertainty are the assumption that EGMEA toxicity is comparable to that of EGME, the lack of adequate human exposure data, and the lack of chronic inhalation exposure studies.

VII. References

Bolt HM, and Golka K. 1990. Maternal exposure to ethylene glycol monomethyl ether acetate and hypospadia in offspring: a case report. Br. J. Ind. Med. 47:352-353.

CARB. 1999. Air toxics emissions data collected in the Air Toxics Hot Spots Program CEIDARS Database as of January 29, 1999.

Gingell R, Boatman RJ, Bus JS, Cawley TJ, Knaak JB, Krasavage WJ, Skoulis NP, Stack CR, and Tyler TR. 1994. Glycol ethers and other selected glycol derivatives. In: Patty's Industrial Hygiene and Toxicology. Fourth ed. Vol. II, Part B. Clayton GD, and Clayton FE. (eds.). New York: John Wiley Sons. p. 2761.

Gross E. 1943. In: Toxicology and Hygiene of Industrial Solvents. Lehmann KB, and Flury F. (eds). Baltimore: Williams and Wilkins. p. 290.

HSDB. 1995. Hazardous Substances Data Bank. National Library of Medicine, Bethesda, Maryland (TOMES® CD-ROM Version). Denver, CO: Micromedex, Inc. (Edition expires 7/31/96).

Jordan WP, and Dahl MV. 1971. Contact dermatitis to a plastic solvent in eyeglasses. Cross-sensitivity to ethyl acetate. Arch. Dermatol. 104:524-528.

Miller RR, Ayres JA, Young JT, and McKenna MJ. 1983. Ethylene glycol monomethyl ether. I. Subchronic vapor inhalation study with rats and rabbits. Fundam. Appl. Toxicol. 3:49-54.

Nagano K, Nakayama E, Oobayashi H, Nishizawa T, Okuda H, and Yamazaki K. 1984. Experimental studies on toxicity of ethylene glycol alkyl ethers in Japan. Environ Health Perspect. 57:75-84.

Stott WT, and McKenna MJ. 1985. Hydrolysis of several glycol ether acetates and acrylate esters by nasal mucosal carboxylesterase *in vitro*. Fundam. Appl. Toxicol. 5:399-404.

CHRONIC TOXICITY SUMMARY

ETHYLENE OXIDE

(oxirane, dimethylene oxide, epoxyethane)

CAS Registry Number: 75-21-8

I. Chronic Toxicity Summary

Inhalation reference exposure level	**30 μg/m^3** (18 ppb)
Critical effect(s)	Neurotoxicity in rats
Hazard index target(s)	Nervous system

II. Physical and Chemical Properties (HSDB, 1995; CRC, 1994)

Description	Colorless gas
Molecular formula	C_2H_4O
Molecular weight	44.06 g/mol
Density	1.80 g/L @ 25ºC
Boiling point	10.6ºC
Melting point	–111.6ºC
Vapor pressure	1095 torr @ 20ºC
Conversion factor	1 ppm = 1.80 mg/m^3

III. Major Uses or Sources

The majority of all ethylene oxide (EtO) produced is used as a chemical intermediate in the production of various compounds including ethylene glycol, glycol ethers, and non-ionic surfactants (ATSDR, 1990). EtO is also used as a fumigant for food and cosmetics, and in hospital sterilization of surgical equipment and heat sensitive materials such as plastics. The annual statewide industrial emissions from facilities reporting under the Air Toxics Hot Spots Act in California based on the most recent inventory were estimated to be 43,972 pounds of ethylene oxide (CARB, 2000).

IV. Effects of Human Exposure

Ten hospital sterilizer workers were matched with controls and examined for physical and neuropsychological health (Estrin *et al.*, 1990). The workers had operated sterilizers using 12% EtO and 88% Freon for an average of 5 years (range 0.5-10 years). Regular monitoring of workroom air had not been done. Measurements at the time of the study indicated concentrations of 15 ppm EtO or less. However, a second measurement showed an air concentration of 250 ppm EtO. A significantly greater percent of exposed workers exhibited a

bilateral reflex reduction in the ankle compared to controls. Nerve conduction tests did not identify significant differences between control and exposed workers, but a highly significant reduction ($p = 0.009$) in finger tapping speed was observed in exposed workers. The exposed group also performed more poorly on tests of spatial and visual abilities, and on tests of visual motor function. The results extended previous work by the same group (Estrin *et al*., 1987).

Cognitive impairment and personality dysfunction were observed more frequently in hospital workers chronically exposed to EtO, compared to a control group (Klees *et al.*, 1990). A group of 22 hospital workers, who had been exposed to an 8-hour TWA of 4.7 ppm EtO for a mean of 6.13 years (range 1-11 years), were matched with 24 control subjects. Neuropsychological function in the workers was classified as either normal or impaired on the basis of the questionnaires and of neuropsychological tests by 2 clinical psychologists (who were unaware of exposure status). (If the classification of the two clinicians did not agree, the subject was classified as "disagreement." Disagreement occurred in 7/23 (30%) of the controls and 10/22 (45%) of the exposed.) Exposed subjects were significantly more frequently classified as impaired (5/12) compared to controls (1/16) ($\chi^2 = 6.0861$; $p<0.05$). The Klees *et al*. (1990) study cites several earlier case reports of EtO neurotoxicity.

Recent studies have identified hemoglobin adducts, sister chromatid exchanges, and other hematological effects as indicators of ethylene oxide exposure (Ribeiro *et al.*, 1994; Sarto *et al.*, 1991). However, a recent study of 68 female workers from 9 hospitals in the U.S. and one in Mexico not only reports biological indicators of ethylene oxide exposure, but also provides a complete blood count with differential (Schulte *et al.*, 1995). The workers were classified as low- or high-exposure based on a mean 8-hour time weighted average of 0.08 or 0.17 ppm EtO. The mean length of employment for workers from U.S. hospitals was 5.5 and 10 years for low- and high-exposure workers, respectively. The mean length of employment in low- and high-exposure workers from the hospital in Mexico was 5.9 and 4.2 years, respectively. In workers from U.S. hospitals only, statistically significant decreases in hematocrit and hemoglobin were observed in high-exposure workers compared to low-exposure workers. Also, a statistically significant increase in lymphocytes and a significant decrease in neutrophils were observed in high-exposure workers compared to controls. In the workers from the hospital in Mexico, a significant relationship of EtO exposure and elevated neutrophil count was observed using regression.

At least 2 epidemiological reports indicate a possible association of EtO exposure and spontaneous abortion. Hemminki *et al.* (1982) analyzed spontaneous abortions in Finnish hospital sterilizing staff using data from a postal questionnaire and from a hospital discharge register. The study included all sterilizing staff employed in Finnish hospitals in 1980; the controls were nursing auxiliaries. When the women were involved in sterilizing procedures during their pregnancies, the frequency of spontaneous abortion was 16.7% versus 5.6% for the non-exposed pregnancies. The independent analysis of spontaneous abortions using the hospital discharge register confirmed the findings. Thus two analyses suggested that EtO exposure may carry a risk of spontaneous abortion among sterilizing staff.

More recently Rowland *et al*. (1996) sent questionnaires to 7,000 dental assistants (ages 18-39 years) registered in California in 1987. Of these, 4,856 responded (69%). They analyzed 1,320

women whose most recent pregnancy was conceived while working full-time. Thirty-two reported exposure to EtO; unexposed dental assistants comprised the comparison group. Among exposed women, the age-adjusted relative risk (RR) of spontaneous abortion was 2.5 [95% (CI) = 1.0-6.3]. The RR for pre-term birth was 2.7 (95% CI = 0.8-8.8) and the RR for post-term birth was 2.1 (95% CI = 0.7-5.9). The RR of any of these adverse outcomes among exposed women was estimated to be 2.5 (95% CI = 1.0-6.1). These results also indicate a possible relationship of EtO and spontaneous abortion.

V. Effects of Animal Exposure

A 2 year inhalation bioassay exposed groups of 80 male rats to 0, 50, or 100 ppm EtO 7 hours per day, 5 days per week for 104 weeks (Lynch *et al.*, 1984). Mean body weights were significantly lower and mortality was significantly higher in both exposure groups. Inflammatory lesions of the lung, nasal cavity, trachea, and inner ear were observed more frequently in EtO exposed rats. Skeletal muscle myopathy, consisting of atrophy and degeneration of skeletal muscle fibers, was observed more frequently in rats exposed to 100 ppm EtO compared to controls. Neoplastic changes were also observed in EtO exposed rats.

Mice (30 per sex) were exposed to 0, 10, 50, 100, or 250 ppm EtO for 6 hours per day, 5 days per week, for 10 weeks (males) or 11 weeks (females) (Snellings *et al.*, 1984). Neuromuscular screening was conducted, and samples of urine and blood were collected. A significantly greater percent of exposed mice exhibited abnormal posture during gait and reduced locomotor activity. A dose-response was observed for these effects, with significant changes at 50 ppm and greater. An abnormal righting reflex was observed in a significantly greater percent of mice exposed to 100 ppm and above. Reduced or absent toe and tail pinch reflexes were observed in a significantly greater percent of mice exposed to 250 ppm EtO. Hematological changes observed in mice exposed to 250 ppm include slight, yet significant, decreases in red blood cell count, packed cell volume, and hemoglobin concentration. Absolute and relative spleen weights were significantly decreased in female mice exposed to 100 and 250 ppm and in male mice exposed to 250 ppm EtO. A significant increase in relative liver weight was observed in female mice exposed to 250 ppm EtO. Male mice exhibited a significant decrease in body weight at 10, 50, and 250 ppm and a significant decrease in absolute testes weights at 50, 100, or 250 ppm EtO. This study indicates a subchronic NOAEL for neurological effects of 10 ppm EtO.

In a study of the testicular effects of EtO, male rats were exposed to 500 ppm EtO 6 hours per day, 3 days per week for 2, 4, 6, or 13 weeks (Kaido *et al.*, 1992). An awkward gait was observed in rats after 6-9 weeks of exposure. Although no significant changes in body weight were observed, a statistically significant dose-related decrease in testes weight was observed at 4, 6, and 13 weeks of exposure. Progressive degeneration and loss of germ cells were also observed during the 13 week exposure. While severe loss of germ cells and marked morphological changes in remaining germ cells were observed at 6 weeks of exposure, some intact spermatids were observed at 13 weeks of exposure. This suggests that recovery of spermatogenesis occurred.

Saillenfait *et al*. (1996) studied the developmental toxicity of EtO in pregnant Sprague-Dawley rats using inhalation exposure during gestation days 6 to 15. Two protocols were used: (1)

exposure for 0.5 hr once a day to 0, 400, 800, or 1200 ppm EtO; or (2) exposure for 0.5 hr three times a day to 0, 200, or 400 ppm EtO or to 0, 800, or 1200 ppm EtO. The second protocol caused fetal toxicity as indicated by reduced fetal weight at 800 ppm (the LOAEL for this endpoint) and at 1200 ppm, and overt maternal toxicity manifested as reduced body weight gain at 1200 ppm. No embryolethality or teratogenicity occurred in either exposure protocol.

VI. Derivation of Chronic Reference Exposure Level (REL)

Study	Snellings *et al*., 1984
Study population	Male and female B6C3F1 mice
Exposure method	Inhalation chamber exposure to 0, 10, 50, 100, or 250 ppm ethylene oxide
Critical effects	Impaired neurological function
LOAEL	50 ppm
NOAEL	10 ppm
Exposure continuity	6-hours/day, 5 days/week
Exposure duration	10 weeks (males), or 11 weeks (females)
Average experimental exposure	1.79 ppm (10 x 8/24 x 5/7)
Human equivalent concentration	1.79 ppm ((gas with systemic effects, based on RGDR = 1.0 using default assumption that lambda (a) = lambda (h)))
LOAEL uncertainty factor	1
Subchronic uncertainty factor	3
Interspecies uncertainty factor	3
Intraspecies uncertainty factor	10
Cumulative uncertainty factor	100
Inhalation reference exposure level	18 ppb (30 $\mu g/m^3$)

Snellings *et al*. (1984) found a subchronic NOAEL of 10 ppm for neurological effects in mice. A neuromuscular screening test indicated that certain reflex responses and locomotor activities were altered in EtO-exposed animals. Human studies have also indicated neurological impairment in ethylene oxide exposed workers.

VII. Data Strengths and Limitations for Development of the REL

The strengths of the inhalation REL for ethylene oxide include the use of an animal study with both a LOAEL and a NOAEL and the use of an endpoint seen in both animals and humans.

Major areas of uncertainty are the short time-frame of the key study, the lack of an appropriate human study, and the limited number of developmental toxicity studies.

VIII. References

ATSDR. 1990. Agency for Toxic Substances and Disease Registry. Toxicological Profile for Ethylene Oxide. Prepared under contract No. 205-88-0608 for: US Department of Health and Human Services, Public Health Service, ATSDR.

CARB. 2000. California Air Resources Board. California Emissions Inventory Development and Reporting System (CEIDARS). Data from Data Base Year 1998. February 12, 2000.

CRC. 1994. CRC Handbook of Chemistry and Physics, 75th edition. Lide DR, ed. Boca Raton, FL: CRC Press Inc.

Estrin WJ, Bowler RM, Lash A, and Becker CE. 1990. Neurotoxicological evaluation of hospital sterilizer workers exposed to ethylene oxide. Clin. Toxicol. 28(1):1-20.

Estrin WJ, Cavalieri SA, Wald P, Becker CE, Jones JR, and Cone JE. 1987. Evidence of neurologic dysfunction related to long-term ethylene oxide exposure. Arch. Neurol. 44(12):1283-1286.

HSDB. 1995. Hazardous Substances Data Bank. National Library of Medicine, Bethesda, MD (TOMES® CD-ROM Version). Denver, CO: Micromedex Inc. (Edition expires 7/31/95).

Hemminki K, Mutanen P, Saloniemi I, Niemi ML, and Vainio H. 1982. Spontaneous abortions in hospital staff engaged in sterilising instruments with chemical agents. Br. Med. J. (Clin. Res. Ed.) 285(6353):1461-1463.

Kaido M, Mori K, and Koide O. 1992. Testicular damage caused by inhalation of ethylene oxide in rats: Light and electron microscopic studies. Toxicologic Path. 20(1):32-43.

Klees JE, Lash A, Bowler RM, Shore M, and Becker CE. 1990. Neuropsychological “impairment” in a cohort of hospital workers chronically exposed to ethylene oxide. Clin. Toxicol. 28(1):21-28.

Lynch DW, Lewis TR, Moorman WJ, Burg JR, Groth DH, Khan A, Ackerman LJ, and Cockrell BY. 1984. Carcinogenic and toxicologic effects of inhaled ethylene oxide and propylene oxide in F344 rats. Toxicol. Appl. Pharmacol. 76:69-84.

Ribeiro LR, Salvadori, DMF, Rios ACC, Costa SL, Tates AD, Tornquist M, and Natarajan AT. 1994. Biological monitoring of workers occupationally exposed to ethylene oxide. Mutat. Res. 313:81-87.

Rowland AS, Baird DD, Shore DL, Darden B, and Wilcox AJ. 1996. Ethylene oxide exposure may increase the risk of spontaneous abortion, preterm birth, and postterm birth. Epidemiology 1996;7(4):363-368.

Sarto F, Tornquist MA, Tomanin R, Bartolucci GB, Osterman-Golkar SM, and Ehrenberg L. 1991. Studies of biological and chemical monitoring of low-level exposure to ethylene oxide. Scand. J. Work Env. Health 17:60-64.

Saillenfait AM, Gallissot F, Bonnet P, and Protois JC. 1996. Developmental toxicity of inhaled ethylene oxide in rats following short-duration exposure. Fundam. Appl. Toxicol. 34(2):223-227

Schulte PA, Walker JT, Boeniger MF, Tsuchiya Y, and Halperin WE. 1995. Molecular, cytogenetic, and hematologic effects of ethylene oxide on female hospital workers. J. Occup. Environ. Med. 37(3):313-320.

Snellings WM, Weil CS, and Maronpot RR. 1984. A subchronic inhalation study on the toxicologic potential of ethylene oxide in $B6C3F_1$ mice. Toxicol. Appl. Pharmacol. 76:510-518.

CHRONIC TOXICITY SUMMARY

FLUORIDES *including* HYDROGEN FLUORIDE

(hydrofluoric acid (aqueous solution); hydrogen fluoride (as a gas); fluoride salts (particulates or in solution))

CAS Registry Number: 7664-39-3

I. Chronic Toxicity Summary

Inhalation reference exposure level	**14 μg HF/m^3** (17 ppb); **13 μg F/m^3**
Oral reference exposure level	**0.04 mg/kg-day**
Critical effect(s)	Skeletal fluorosis
Hazard index target(s)	Bone and teeth; respiratory system

II. Physical and Chemical Properties of HF (HSDB, 1995; CRC, 1994)

Description	Colorless gas (HF), or as particulates
Molecular formula	HF
Molecular weight	20.0 g/mol
Density	0.83 g/L @ 25°C
Boiling point	19.54°C
Melting point	–83.1°C
Vapor pressure	400 torr @ 2.5°C
Solubility	Soluble in water and alcohol
Conversion factor	1 ppm = 0.83 mg/m^3 @ 25°C

III. Major Uses or Sources

Hydrofluoric acid (HF) is a colorless, fuming liquid with a sharp, penetrating odor (Fairhall, 1949). This acid is used in the glass etching, electronic, microelectronic, and petroleum refining and chemical industries (Bertolini, 1992). These industries use HF in the manufacture of such things as computer chips (an important industry in California), phosphate fertilizer, metal cans, plastics, refrigerant chemicals (fluorocarbons), inorganic chemicals, soaps and detergents, high-octane gasoline, and aircraft parts (Wohlslagel *et al.*, 1976; Wing *et al.*, 1991). HF is also used in commercial rust removal products. Another high profile use of HF in California has been as a catalyst in petroleum alkylation to make high-octane gasoline. HF is also a product of combustion of any F containing materials; as such, it is produced during structural fires.

Sodium fluoride has been used as a topical and ingested anticaries agent due to its ability to harden tooth enamel during development. The optimal doses are not well established, but have been suggested to be approximately 0.080 mg/kg/day for 7 to 9 month old infants decreasing to 0.034 mg/kg/day at 13 years of age (Shulman *et al*., 1995). A dose of 1.0 mg F ingested per day was reported to reduce dental caries 43%, and to be associated with a greatly increased rate of minor tooth mottling which caused no esthetic damage (Van Nieuwenhuysen and D'Hoore, 1992). Many communities in California routinely add fluoride to the drinking water. The California Department of Health Services has adopted regulations that establish standards for the addition of F (CDHS, 2002). Any public water system using fluoridation must maintain F levels within the range established for its climate. The ranges vary according to average air temperatures, since people in cooler climates typically drink less water per day than people in warmer climates. Thus, in cooler areas, more F is required to provide the same dental benefit. For 2001-2002, F levels in San Francisco municipal water ranged from 0.65 to 1.1 ppm, while in Los Angeles the range was 0.44 to 0.83 ppm (CDHS, 2002).

The annual statewide industrial emissions from facilities reporting under the Air Toxics Hot Spots Act in California based on the most recent inventory were estimated to be 48,221 pounds of fluorides and compounds, and 62,670 pounds of hydrogen fluoride (CARB, 2000).

IV. Effects of Human Exposure

The chronic exposure to fluorides, including HF, and the incidence of minimal osseous changes were studied in the workplace by Derryberry *et al.* (1963). In this study, the 8-hour time-weighted average fluoride exposure was calculated for the employment period of each of 74 male workers (30 Caucasian, 44 African-American). The overall average fluoride exposure in these workers was measured as a time-weighted average of 2.81 mg F/m^3. In comparison, the 17 workers within this group who had evidence of minimally increased bone density had an average fluoride exposure of 3.38 mg F/m^3. The other workers were exposed to an average measured concentration of 2.64 mg F/m^3. In addition, urinary fluoride levels were greater in the 17 individuals with greatest exposure compared to the remaining 57 workers (average = 5.18 mg F/L vs. 4.53 mg F/L). No differences between exposed and unexposed individuals were observed for gastrointestinal, cardiovascular, or hematologic systems, or in a physical exam. A statistically significant ($p < 0.05$) increase in the incidence of acute respiratory disease as determined from past medical histories was observed in fluoride-exposed individuals (19/74 vs. 8/67 in controls); radiographic examination revealed a difference of lesser significance ($p < 0.10$) for pulmonary changes (11/74 vs. 4/67). No pulmonary function tests were reported.

An analysis of these data by OEHHA (see derivation section below) showed a statistically significant relationship between air fluoride and the minimal bone density increases. The raw data from the Derryberry *et al.* (1963) study are shown in Table 1. A Pearson correlation matrix of the variables measured in the Derryberry *et al.* study indicated that bone density was best correlated with mean air fluoride level, and to a lesser extent with the age of the individual. A log-logistic regression using the log air fluoride concentration as the independent variable showed a significant ($p < 0.033$) relationship between increasing air fluoride concentrations and probability of skeletal fluorosis. The parameters for the regression were $\beta_0 = -2.3468$ (std. error

= 0.6462), and β_1 = 1.1736 (std error = 0.5508); the odds ratio for the occurrence of skeletal fluorosis was 3.24. Years of exposure were not correlated with increased bone-density, according to a Pearson Correlation procedure (p = 0.63). Bone density has been shown to decrease with age after the age of 40 among normal, non-fluoride-exposed males (Runge *et al*., 1979). As expected, age was very highly correlated with years exposed (p<0.00001). Therefore including years exposed in the dose-metric likely introduces a confounding variable (see discussion in Section VI.). In addition, Runge *et al*. (1979) found no association between years exposed and mineral content or bone width among 245 aluminum smelter workers exposed to 2.75 or 3.2 mg F/m^3. For these reasons, years exposed were not used as the dose-metric for bone-density in this analysis.

Although a threshold was not readily apparent from the logistic regression model, grouping the 74 individuals by air fluoride exposure level into quintiles of 15 each with one group of 14, allowed for a comparison of group mean responses (Table 2). The 14 employees exposed to a time-weighted average concentration of 1.07 mg F/m^3 did not exhibit bone density changes. An analysis of the grouped responses using a binomial distribution showed a probability of p = 0.008 for obtaining 4/15 increased bone density observations in the 2.34 mg/m^3 group, and a probability of p = 0.047 for obtaining 3/15 positive observations in the 1.89 mg F/m^3 group. The 1.89 mg F/m^3 group was therefore considered a LOAEL for chronic skeletal fluorosis, and the 1.07 mg/m^3 group was considered a NOAEL. The above probabilities assume that a chance occurrence is, at most, 1 in 18 of skeletal fluorosis or other cause leading to an abnormally dense x-ray in the general population. Since osteosclerosis is a rare condition that is associated with several types of hematological malignancies such as myeloid leukemia, the actual incidence of conditions leading to osteosclerosis is far below 1 in 18. This lends strong support to the consideration of 1.89 mg/m^3 as a LOAEL for skeletal fluorosis.

Table 1. Data on worker exposure to fluoride from Derryberry *et al.* (1963)

Obser-vation #	ID	Bone density	Years exposed	Urine max F (mg F/L)	Urine min F (mg F/L)	Mean urinary F (mg F/L)	Age (years)	Air fluoride (mg/m^3)	*OEHHA exposure grouping*
1	119	normal	18.5	43.0	2.8	14.7	58	8.16	5
2	0	normal	8.4	24.7	5.3	9.6	42	3.19	4
3	41	normal	15.8	35.0	2.5	9.1	35	3.29	4
4	147	minimally increased	9.6	17.1	2.1	8.9	60	5.98	5
5	120	normal	16.7	20.5	3.4	8.6	55	3.29	4
6	54	minimally increased	17.0	44.0	4.0	8.6	56	7.73	5
7	148	normal	10.5	14.0	3.7	8.4	41	8.32	5
8	314	minimally increased	14.4	22.7	1.7	8.3	56	3.24	4
9	29	normal	17.0	18.2	2.5	7.7	50	2.60	3
10	14	normal	14.3	19.4	2.1	6.3	46	2.33	3
11	115	normal	15.2	18.5	1.4	6.3	38	2.11	3
12	10	minimally increased	10.3	22.0	2.3	6.1	38	2.72	4
13	4	minimally increased	7.1	7.7	2.0	5.7	54	3.22	4
14	51	normal	14.9	42.0	0.8	5.6	46	3.18	4
15	94	normal	16.2	15.4	3.3	5.5	56	5.12	5
16	217	normal	7.1	7.1	2.6	5.3	42	2.54	3
17	281	minimally increased	7.8	8.6	1.1	5.2	36	3.79	4
18	114	normal	10.4	13.2	2.8	5.2	38	7.66	5
19	7	normal	7.8	9.1	2.2	5.1	43	2.91	4
20	308	normal	11.9	6.7	3.5	5.1	44	1.89	2
21	301	minimally increased	15.2	9.5	2.5	5	36	2.56	3
22	72	normal	25.9	13.7	2.1	4.9	55	5.55	5
23	241	minimally increased	17.0	10.0	1.9	4.9	46	4.48	5
24	345	normal	10.5	7.1	2.0	4.9	47	1.49	1
25	26	normal	16.4	12.2	0.5	4.7	39	2.41	3
26	231	minimally increased	16.3	8.2	2.8	4.6	62	1.88	2
27	2	normal	24.7	8.9	2.1	4.6	46	3.53	4
28	295	normal	14.5	10.7	0.9	4.6	44	2.07	3
29	1	normal	8.9	5.9	2.4	4.5	30	1.92	2
30	203	minimally increased	18.2	6.8	1.6	4.4	43	2.66	3
31	63	normal	16.2	7.4	2.0	4.3	55	3.90	5
32	5	normal	4.5	11.5	1.9	4.3	43	1.12	1
33	460	normal	12.5	6.1	1.6	4.3	60	2.13	3

Obser-vation #	ID	Bone density	Years exposed	Urine max F (mg F/L)	Urine min F (mg F/L)	Mean urinary F (mg F/L)	Age (years)	Air fluoride (mg F/m^3)	*OEHHA exposure grouping*
34	249	minimally increased	15.0	8.0	1.8	4.3	39	2.95	4
35	3	normal	7.6	14.5	2.1	4.3	31	3.90	5
36	322	normal	9.3	6.3	2.0	4.3	35	4.23	5
37	8	minimally increased	24.8	5.9	3.0	4.2	55	2.50	3
38	3	normal	15.2	12.2	2.1	4.2	42	1.14	1
39	309	normal	12.1	5.5	2.4	4.1	42	1.94	2
40	36	normal	9.1	13.2	0.8	4.1	33	1.94	2
41	45	normal	11.3	14.0	2.2	4.1	33	3.84	4
42	70	normal	17.9	8.0	1.0	3.9	44	4.00	5
43	250	minimally increased	9.8	6.7	1.5	3.9	35	1.78	2
44	38	normal	16.9	5.9	1.0	3.9	35	2.10	3
45	200	minimally increased	14.0	7.0	2.8	3.8	66	3.92	5
46	183	normal	9.8	4.9	2.2	3.7	48	1.67	2
47	32	normal	12.5	6.6	0.9	3.7	47	2.21	3
48	25	normal	13.6	5.5	1.5	3.7	44	1.86	2
49	21	normal	13.9	9.1	0.4	3.7	50	1.98	2
50	304	normal	13.4	5.0	2.1	3.7	36	2.62	3
51	132	normal	10.9	5.1	2.4	3.6	39	1.81	2
52	6	minimally increased	8.4	4.8	0.9	3.6	35	3.85	5
53	244	normal	16.6	7.1	1.4	3.6	62	2.87	4
54	30	normal	14.0	14.0	0.9	3.6	43	1.56	1
55	88	minimally increased	15.5	4.9	1.7	3.5	66	2.06	2
56	227	normal	16.6	5.7	1.0	3.5	41	1.18	1
57	271	normal	17.7	4.1	3.0	3.4	60	1.82	2
58	19	normal	13.9	10.0	1.8	3.4	41	1.32	1
59	190	normal	9.3	7.7	1.9	3.3	36	1.95	2
60	258	normal	17.8	5.6	1.6	3.2	58	0.87	1
61	278	normal	10.0	7.0	0.3	3.2	34	1.93	2
62	331	normal	12.8	5.6	1.5	3.1	34	1.23	1
63	91	normal	25.3	7.9	0.2	3.1	63	3.49	4
64	342	normal	18.5	6.0	1.3	3	40	2.73	4
65	261	normal	18.1	5.3	0.9	2.9	52	4.41	5
66	291	normal	13.5	4.5	1.5	2.8	34	2.14	3
67	149	normal	11.3	4.5	2.1	2.8	34	0.76	1
68	2	normal	24.7	4.5	1.5	2.7	51	1.15	1
69	4	normal	16.8	5.7	1.2	2.7	56	0.71	1
70	109	normal	8.3	5.1	0.8	2.7	36	1.89	2
71	242	normal	18.1	4.1	1.2	2.5	49	1.26	1

Obser-vation #	ID	Bone density	Years exposed	Urine max F (mg F/L)	Urine min F (mg F/L)	Mean urinary F (mg F/L)	Age (years)	Air fluoride (mg F/m^3)	*OEHHA exposure grouping*
72	179	normal	18.9	3.9	1.0	2.4	46	0.50	1
73	325	minimally increased	11.8	5.0	0.5	2.2	40	2.10	3
74	159	normal	18.9	5.0	0.7	2.1	45	0.67	1

Table 2. Grouped mean exposure

Exposure group	Mean age ± SD	Mean air level mg F/m^3 ± SD	Number of responses	Probability of difference from group 1*
1	45.0 ± 7.0	1.07 ± 0.32	0/14**	Not Applicable
2	43.9 ± 11.2	1.89 ± 0.09	3/15***	0.047
3	43.0 ± 7.6	2.34 ± 0.23	4/15	0.008
4	45.9 ± 9.8	3.22 ± 0.35	5/15	0.001
5	48.5 ± 10.7	5.41 ± 1.72	5/15	0.001

* Probability of obtaining result assuming a chance occurrence of abnormally dense x-ray of, at most, 1 in 18 individuals, using a binomial distribution (Systat for Windows v.5.05, 1994).
** NOAEL
*** LOAEL ($p < 0.05$)

Largent *et al.* (1951) found a significant increase in bone density in the lower thoracic spine, with calcification extending into the lateral ligaments of 3 workers exposed for 17, 14, and 10 years to HF (concentrations not estimated).

A group of 74 men, who were occupationally exposed to unspecified concentrations of HF for an average of 2.7 years, reported occasions of upper respiratory irritation (Evans, 1940). Repeated chest X-rays over a 5-year period did not reveal any visible evidence of lung changes. The death rate of these workers from pneumonia and other pulmonary infections was the same as that of unexposed plant employees.

There are various reports of asthma and related respiratory effects in pot room workers in the primary aluminum smelting industry. Exposure to fluoride (among other materials such as sulfur trioxide and polycyclic aromatic hydrocarbons) was measured as a possible index of exposures related to this condition (Seixas *et al*., 2000). However multiple exposures to respiratory irritants and other compounds which may affect immune response appear to be common in this work environment making it difficult to quantitatively relate the respiratory symptoms to inhaled HF or fluorides.

Workers in a warehouse containing HF retorts experienced transitory hyperemia of the skin on their face and hands (Dale and McCauley, 1948). Twenty four of the 40 workers had definite changes in the thickness and number of trabeculae in the upper and lower jaw.

Examinations of 107 pot room workers in two aluminum plants with airborne fluorides revealed 22 subjects with limited motion of the dorsolumbar spine, compared with none in a control group of 108 workers with no history of exposure to fluorides (Kaltreider *et al.*, 1972). In one plant, 76 of 79 workers had increased bone density as measured by roentgenogram, with diagnosis of slight to moderate fluorosis. Moderate and marked fluorosis was observed after 15 years employment. The 8-hour time-weighted average fluoride content in these workplaces was 2.4 to 6.0 mg/m^3. Balazova (1971) measured significant fluoride uptake and distribution in children living near an aluminum smelter but reported no incidence of fluorosis.

No studies regarding the chronic irritant or respiratory effects of pure HF exposure in humans were available.

Fluoride ion produced by various fluorocarbons has been associated with toxicity to human kidney collecting duct cells leading to sodium and water disturbances (Cittanova *et al*., 1996).

Oral supplementation of greater than 0.1 mg F/kg body weight daily has been associated with enamel fluorosis in young children (Forsman, 1977).

The Agency for Toxic Substances and Disease Registry (ATSDR, 2001) recently reviewed fluorides since they are found at hazardous waste sites which are candidates for remediation. The focus of this document was on oral exposure studies as that is the main concern for waste site remediation.

V. Effects of Chronic Exposures to Animals

Stokinger (1949) studied the subchronic effects of HF inhalation in several animal species. Animals (dogs, rabbits, rats, guinea pigs, and mice; 1 to 6 per group) were exposed to 0, 7.2 mg/m^3, or 25.1 mg/m^3 6 hours/day, 6 days/week, for 30 days. Mortality, body weight, blood coagulation mechanisms, and gross pathology were measured. Exposure to 25.1 mg/m^3 HF for 30 days resulted in degenerative testicular changes and ulceration of the scrotum in all 4 dogs and hemorrhage and edema in the lungs of 3 dogs. Pulmonary hemorrhage was also seen in 20 of 30 rats, and 4 of 10 rabbits. Renal cortical degeneration was observed in 27 of 30 rats. All of the rats and mice at the 25.1 mg/m^3 concentration died. No mortality was observed in the other species tested. Blood fibrinogen levels were significantly increased in dogs, rats, and rabbits exposed to 25.1 mg/m^3. Exposure to 7.2 mg/m^3 HF resulted in pulmonary hemorrhage in 1 out of 5 dogs. No other significant effects were observed at the lower concentration.

Shusheela and Kumar (1991) administered male rabbits 10 mg NaF/kg-bw per day orally for 18 months (7 rabbits) or 29 months (3 rabbits), then studied the testis, epididymis, and vas deferens microscopically. After 29 months of F administration, the spermatogenic cells in the seminiferous tubules had degenerated and lacked spermatozoa. After both 18 and 29 months, cilia were lost from the epithelial cells lining the ductuli efferentes of the caput epididymidis. Stereocilia on the epithelial cells lining the vas deferens were also lost. In some regions of epithelia, the cell boundaries were not clear, and even appeared to be peeled off. Mucus droplets were abundant in the vas deferens of controls, but none were present in F treated rabbits.

Spermatogenesis ceased sometime between 18 and 29 months. The authors concluded that ingestion of a high concentration of F has adverse effects (including infertility) on the male rabbit reproductive system.

Ghosh *et al*. (2002) investigated the effects of NaF on steroidogenic and gametogenic activities in rat testes. Male Wistar rats were given 20 mg/kg/day NaF by gavage for 29 days. F treatment resulted in significantly lower relative wet weight of the testis, prostate, and seminal vesicle, decreased testicular delta(5),3beta-hydroxysteroid dehydrogenase (HSD) and 17beta-HSD activities, and significant lowering in plasma levels of testosterone. Epididymal sperm count was decreased significantly in F-treated rabbits and there were fewer mature luminal spermatozoa. Indicators of oxidative stress due to F included increased conjugated dienes in the testis, epididymis, and epididymal sperm pellet, and decreases of peroxidase and catalase in the sperm pellet. Thus F, at a dose encountered in drinking water in contaminated areas (at least of India), exerts an adverse effect on the male rat reproductive system. These effects on rats and rabbits (and dogs; see above) may be relevant to anecdotal reports of reproductive system malfunction in human chronic fluorosis.

Parameter	Control (n=6)	NaF (n=6)	p value
Body weight, final (g)	127.00±3.75	122.00±5.10	
Testis, relative weight (%)	1.522±0.034	1.923±0.081	< 0.05
Prostate, relative weight	0.297±0.043	0.148±0.014	< 0.05
Seminal vesicles, rel. weight	0.448±0.025	0.174±0.027	< 0.05
Testicular delta(5),3beta HSD	~28[a]	~24[a]	< 0.05[b]
Testicular 17betaHSD	~29[a]	~24[a]	< 0.05[b]
Plasma testosterone (ng/ml)	~2[a]	~1[a]	< 0.05[b]
Epididymal sperm count (10^6/ml)	7.02±0.17	3.70±0.57	< 0.05

[a] approximate values based on reading Figures 2 and 3 of paper; [b] p values of authors

Long *et al*. (2002) used ligand binding and Western blotting to study neuronal nicotinic acetylcholine receptors (nAChRs) in the brains of male and female Wistar rats ingesting 0.5 ppm (controls), 30 ppm, or 100 ppm F in their drinking water for 7 months. (All received 4 ppm F in their diet.) The brains of rats exposed to 100 ppm had significantly less binding sites for [^{3}H]epibatidine, an analgesic agonist, but no change occurred at 30 ppm. Binding sites for [^{125}I]alpha-bungarotoxin, a competitive antagonist, were significantly decreased in the brains of rats exposed to both levels. The brain levels of the nAChR alpha4 subunit protein was significantly lowered by exposure to 100 ppm F. Alpha7 subunit protein was significantly decreased by both levels of F. No significant changes were seen in levels of the beta2 subunit protein. These nicotinic receptors have roles in learning and memory. Some of the effects were also seen in rat PC cells cultured for 48 h in up to 50 ppm F (Chen *et al*., 2003). The results may help to explain anecdotal reports of nervous system symptoms in human chronic fluorosis (Waldbott, 1978).

NTP (1990) exposed F344/N rats and B6C3F1 mice of both sexes for two years to 0, 25, 100, and 175 ppm sodium fluoride (NaF) in their drinking water. NaF caused a dose dependent whitish discoloration of the teeth in both rats and mice. Male rats had an increased incidence of

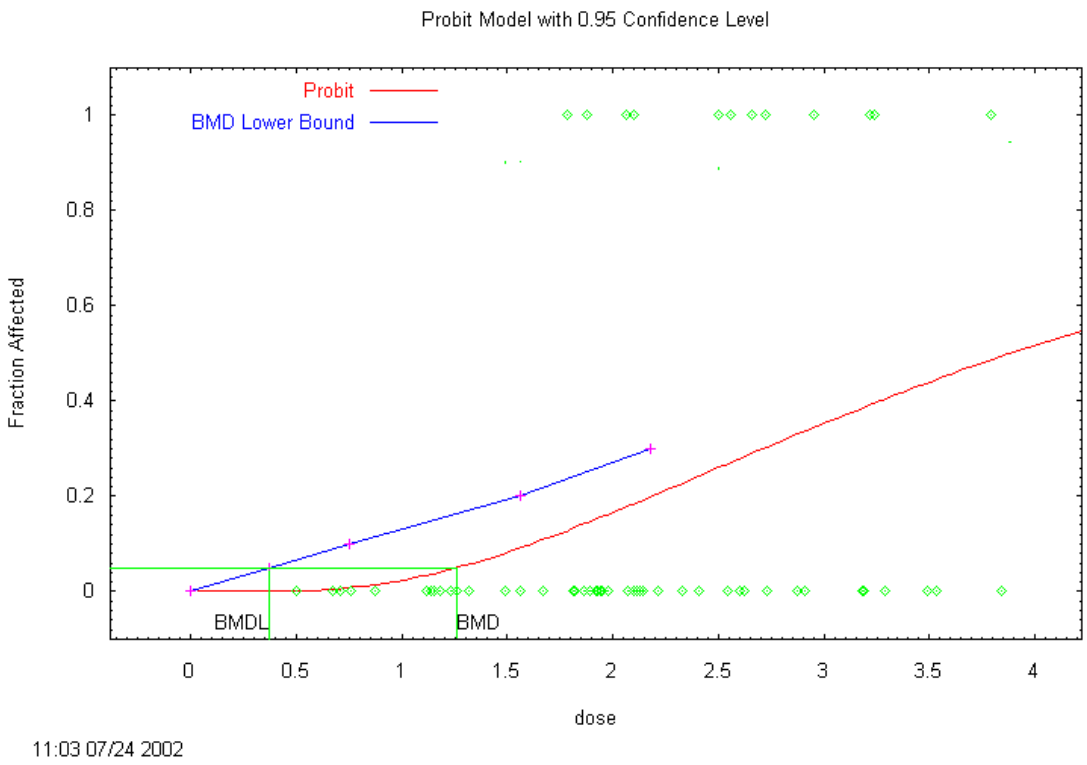
tooth deformities and attrition. NaF increased the dysplasia of dentine in both rats and mice. At the highest dose (175 ppm), osteosclerosis of long bones was increased in female rats. There was also equivocal evidence of carcinogenic activity of NaF in male rats based on four osteosarcomas in dosed animals (Bucher *et al*., 1991). Other organ systems showed no dose-dependent effects.

VI. Derivation of Chronic Reference Exposure Level (REL)

Study	Derryberry *et al.* (1963)
Study population	74 fertilizer plant workers (67 unexposed control subjects)
Exposure method	Occupational
Critical effects	Increased bone density (skeletal fluorosis)
LOAEL	1.89 mg F/m^3 (1.98 mg HF/m^3)
NOAEL	1.07 mg F/m^3 (1.13 mg HF/m^3)
BMC_{05}	0.37 mg F/m^3 (0.39 mg HF/m^3)
Exposure continuity	8 hours/day, 5 days/week
Exposure duration	14.1 years (range = 4.5 to 25.9 years)
Average exposure concentration	0.14 mg HF/m^3 (0.39 x 10/20 x 5/7) or 0.13 mg F/m^3 (0.37 x 10/20 x 5/7)
Human equivalent concentration	0.14 mg HF/m^3 or 0.13 mg F/m^3
LOAEL uncertainty factor	1
Subchronic uncertainty factor	1
Interspecies uncertainty factor	1
Intraspecies uncertainty factor	10
Cumulative uncertainty factor	10
Inhalation reference exposure level for F or HF	0.013 mg F/m^3 (13 μg /m^3; 0.016 ppm; 16 ppb) or 0.014 mg HF/m^3 (14 μg /m^3; 0.017 ppm; 17 ppb)

OEHHA's analysis of the data in Derryberry *et al*. (1963) indicates a LOAEL of 1.89 mg/m^3, and a NOAEL of 1.07 mg/m^3. A benchmark concentration (BMC_{05}) of 0.37 mg/m^3 was derived by fitting the probit model to the log dose in the U.S. EPA's BMDS (version 1.3) software, for the individual mean air exposure data and incidence data in Table 1 above. Individuals in the highest dose group (group 5 in Table 2) were not included in the model, since none of the models fit this range of exposures well. Several other models produced reasonable fits to the data, but the probit model with log-transformed dose was selected since it produced a good fit not only by statistical criteria ($p = 0.71$) but also, as determined by inspection, it fit the low dose curve shape better than other models. This model also has the advantage of biological plausibility, in that, since lower doses of fluoride have a beneficial or nutritional effect, a threshold type of response for adverse effects is clearly expected. A graphical representation of the fit is shown in Figure 1. Adjusting for exposure continuity and utilizing an intraspecies uncertainty factor of 10 (UF_H) results in a REL for F of 13 μg/m^3.

Figure 1.



Changes in bone density in association with fluoride exposure have been observed in several studies, and appear to be the most sensitive health effect for chronic exposure. The minimally increased bone density in the Derryberry study was significantly ($p < 0.04$, Fisher's Exact Test) associated with "other osseous changes," which reportedly included disc lesions, arthritis, and calcified ligaments. An increase in pulmonary changes in the workers with high bone density was marginally significant ($p < 0.06$) and included emphysema, fibrosis, and healed tuberculous lesions. Although dental fluorosis is a sensitive endpoint in many fluoride studies, the dental examinations of exposed workers in this study showed healthier teeth than in controls. The increased bone density observed was considered as indicating that adverse effects had occurred, based on the adverse effects associated with the increased density in the study, and on other research showing that increased bone density caused by fluoride exposure (75 mg sodium fluoride per day for four years) also leads to decreased bone strength and increased fragility (Riggs *et al.*, 1990). Symptoms of abdominal pain, backache, restricted joint movement, and respiratory symptoms have been associated with airborne fluoride exposures and bone density increases in industrial settings (Zhiliang *et al.*, 1987).

The absorption of particulate and gaseous fluorides is reported to be similar (Collings *et al.*, 1951). Therefore, it would be expected that the effects on bone density would be similar regardless of the form of fluoride.

As noted in the study description, Derryberry *et al.* (1963) did not find a good correlation between years of exposure to fluoride and bone density change. OEHHA reexamined the original individual data and confirmed that the presence of bone density changes showed a better correlation with mean air fluoride concentration than with years of exposure, or with the product of the individual values of mean air fluoride concentration and years of exposure. However, the product of exposure concentration and time did show a consistent pattern of cumulative incidence suggesting a dose-response relationship for this parameter. An attempt to derive a benchmark value by fitting the probit model to the log of (exposure duration*concentration) and response (presence or absence of bone density change) did not result in an acceptable fit, so a $BMDL_{05}$ could not be reported. However a maximum likelihood estimate of the benchmark (BMD_{05}) was found to be 6.04 (mg F*years/m^3), with exclusion of the three highest values that appeared to be outliers to the main distribution. If this value is divided by the mean exposure duration for the data set of 14.1 years, a benchmark exposure concentration of 0.43 mg F/m^3 is obtained. While this value is evidently less reliable than that obtained by fitting the mean exposure concentration, it is consistent with it, suggesting that, although other confounding factors related to age or duration prevent the demonstration of a relationship between the exposure/time integral and response in this data set, such a relationship probably does exist, as would be expected.

VII. Data Strengths and Limitations for Development of the REL

The major strengths of the key study for fluoride are the observation of health effects in a large group of workers exposed over many years, the availability of individual exposure estimates for each worker, and the identification of a NOAEL. The primary uncertainty in the study is the lack of a comprehensive health effects examination. Another source for concern is the potentially greater susceptibility of children to the effects of inhaled fluorides, considering the rapid bone growth in early years.

Derivation of Chronic Oral REL

In addition to being inhaled, airborne fluoride salts in particulate form can settle onto crops and soil and enter the body by ingestion. Thus an oral chronic reference exposure level (REL) for fluoride is also required in order to conduct a health risk assessment under the Air Toxics Hot Spots Act. California has developed a Public Health Goal (PHG) of 1 ppm (1,000 ppb) fluoride in drinking water (OEHHA, 1997). This level is intended to be an approximate year-round average. Thus it has properties similar to a chronic oral REL. (The PHG assumed that drinking water was the only source of fluoride since it was based on comparing communities with and without added fluoridation.)

Study	Dean, 1942; U.S. Public Health Service, 1991; National Research Council, 1993
Study population	Inhabitants of several U.S. cities
Exposure method	Drinking water
Critical effects	Dental fluorosis
LOAEL	2 ppm
NOAEL	1 ppm = 0.04 mg/kg-day*
Exposure continuity	Continuous
Exposure duration	Long-term
Average experimental exposure	1 ppm = 0.04 mg/kg-day
LOAEL uncertainty factor	1
Subchronic uncertainty factor	1
Interspecies uncertainty factor	1
Intraspecies uncertainty factor	1 (studies included children)
Cumulative uncertainty factor	1
Oral reference exposure level	0.04 mg/kg-day

* based on the assumption that an 18 kg child drinks 720 ml of water per day (OEHHA, 2000).

The PHG is based on a no-observed adverse-effect-level (NOAEL) of 1 mg/L for dental fluorosis in children (equivalent to 720 μg/day from drinking water for an 18 kg child drinking 40 ml/kg body weight/day of water). Moderate to severe dental fluorosis is rare when the drinking water fluoride level is near 1 mg/L, but begins to become significant at concentrations close to 2 mg/L. Since the study involved long term exposure to humans including children, a sensitive population, the cumulative uncertainty factor was 1. If one were to do a route-to-route extrapolation from this oral REL using the specific parameters for an 18 kg child breathing 4.2 m^3/day, an equivalent inhalation REL would be about 170 μg/m^3. Thus, the inhalation REL of 13 μg/m^3 based on the adult occupational data is likely to be protective of children.

VIII. Potential for Differential Impacts on Children's Health

The critical effect for inhalation exposures is skeletal fluorosis. Since infants' and children's skeletons are developing, they may be more sensitive to this effect. This applies with particular importance to the teeth, and it is established that excessive exposure to fluoride during the period of tooth development in infancy and childhood causes dental fluorosis (Dean, 1942; U.S. Public Health Service, 1991; NRC, 1993). The oral REL and the California PHG for fluoride in drinking water are based on dental fluorosis. Although the inhalation chronic REL proposed is based on a study in adults, the inhalation chronic REL (see section VI) is lower than that implied by the oral REL and PHG. Since the oral REL and PHG are based on exposures throughout life, including the pre-natal period, infancy, and childhood, it is reasonable to conclude that the proposed inhalation REL is generally protective of infants and children, barring some unknown difference in toxicity between the two routes of exposure. The ratio of the intake at the PHG level in drinking water is closer to the effect level than the default intraspecies uncertainty factor of 10; this is to be expected since children are a sensitive subpopulation for the dental fluorosis effect.

Extensive interindividual variation in total fluoride intake (930.7 ± 391.5 μg/day) was recently documented for a small group (n = 11) of healthy German children ages 3 to 6 years (Haftenberger *et al*., 2001). Similar interindividual variation has also been reported for slightly younger children in Connersville (n = 14) and Indianapolis, Indiana (n = 29) and in San Juan, Puerto Rico (n = 11) (Rojas-Sanchez *et al*., 1999). Consideration should therefore be given to populations with exceptionally high fluoride intake due to locally elevated concentrations in drinking water, since some of these populations are already close to adverse effect levels of fluoride intake, and certain individuals in California experience dental fluorosis. For these individuals, even exposure to fluorides at the oral and/or inhalation RELs, which are acceptable in isolation, might be deleterious. The table below compares the data of Haftenberger *et al*. (2001) with recent estimates of F intake ranges in California (OEHHA, 1997).

Fluoride Intake (mg/day)

F in drinking water (mg/L)	F from drinking water	F from food	F from toothpaste	F from mouthwash	F from a supplement	Total F
Children (OEHHA)						
<0.3	0.1 - 0.3	0.1 – 0.5	0.2 – 1.2	0.1 - 0.5	0.5	1.0 – 3.0
0.7 - 1.2	0.7 - 1.2		0.2 – 1.2	0.1 - 0.5	0	1.1 – 4.6
Haftenberger						
0.25	(see food)	0.20±0.12	0.27±0.18	No data	0 - 1.0	0.93±0.39
Adults (OEHHA)						
<0.3	0.2 – 0.6	0.3 – 1.0	0.02 – 0.15	0.2 – 1.0	0	0.7 – 2.8
0.7 - 1.2	1.4 – 2.4	0.3 – 3.4	0.02 – 0.15	0.2 – 1.0	0	1.9 – 7.0

IX. References

ATSDR. 2001. Agency for Toxic Substances and Disease Registry. Toxicological Profile for Fluorides. Draft for Public Comment. Atlanta: ATSDR.

Balazova G. 1971. Long-term effect of fluoride emission upon children. Fluoride 4(2):85-88.

Benke G, Abramson M, Sim M. 1998. Exposures in the alumina and primary aluminum industry: an historical review. Ann. Occup. Hyg. 42(3):173-189.

Bertolini JC. 1992. Hydrofluoric acid: A review of toxicity. J. Emerg. Med. 10:163-168.

Bucher JR, Hejtmancik MR, Toft JD 2nd, Persing RL, Eustis SL, Haseman JK. 1991. Results and conclusions of the National Toxicology Program's rodent carcinogenicity studies with sodium fluoride. Int. J. Cancer 48(5):733-737.

CARB. 2000. California Air Resources Board. California Emissions Inventory Development and Reporting System (CEIDARS). Data from Data Base Year 1998. February 12, 2000.

CDHS. 2002. California Department of Health Services. Public Water System Fluoridation. Available on-line at www.dhs.cahwnet.gov/ps/ddwem/Fluoridation/Fluoridation.htm

CRC. 1994. CRC Handbook of Chemistry and Physics, 75th edition. Lide DR, ed. Boca Raton, FL: CRC Press Inc.

Chen J, Shan KR, Long YG, Wang YN, Nordberg A, Guan ZZ. 2003. Selective decreases of nicotinic acetylcholine receptors in PC12 cells exposed to fluoride. Toxicology 183(1-3):235-242.

Cittanova ML, Lelongt B, Verpont MC, Geniteau-Legendre M, Wahbe F, Prie D, Coriat P, and Ronco PM. 1996. Fluoride ion toxicity in human kidney collecting duct cells. Anesthesiology 84(2):428-435.

Collings GH, Fleming RBL, and May R. 1951. Absorption and excretion of inhaled fluorides. A.M.A. Arch. Ind. Hyg. 4:585-590.

Dale PP, and McCauley HB. 1948. Dental conditions in workers chronically exposed to dilute and anhydrous hydrofluoric acid. J. Am. Dent. Assoc. 37:131-140.

Dean, H. 1942. The investigation of physiological effects by the epidemiological method. In: Fluoride and Dental Health, F. R. Moulton, ed. Washington, DC: American Association for Advancement of Science, p. 23.

Derryberry OM, Bartholomew MD, and Fleming RBL. 1963. Fluoride exposure and worker health - The health status of workers in a fertilizer manufacturing plant in relation to fluoride exposure. Arch. Environ. Health 6:503-514.

Evans EE. 1940. An X-ray study of effects of industrial gases upon the human lung. Radiology 34:411-424.

Fairhall L. 1949. Industrial Toxicology. Baltimore, MD: Williams and Wilkins. [as cited in Smyth HF. 1956. Improved communication - hygienic standards for daily inhalation. Am. Ind. Hyg. Assoc. 129-185.]

Forsman B. 1977. Early supply of fluoride and enamel fluorosis. Scand. J. Dent. Res. 85(1):22-30.

Ghosh D, Das Sarkar S, Maiti R, Jana D, Das UB. 2002. Testicular toxicity in sodium fluoride treated rats: association with oxidative stress. Reprod. Toxicol. 16(4):385-90.

Haftenberger M, Viergutz G, Neumeister V, Hetzer G. 2001. Total fluoride intake and urinary excretion in German children aged 3-6 years. Caries Res. 35(6):451-7

HSDB. 1995. Hazardous Substances Data Bank. TOMES® Denver, CO: Micromedex, Inc.

Hodge HC. 1950. The concentration of fluorides in drinking water to give the point of minimum caries with maximum safety. J. Am. Dent. Assoc. 40:436.

Kaltreider NL, Elder MJ, Cralley LV, and Colwell MO. 1972. Health survey of aluminum workers with special reference to fluoride exposure. J. Occup. Med. 14:531-541.

Largent EJ, Bovard PG, and Heyroth FF. 1951. Roentgenographic changes and urinary fluoride excretion among workmen engaged in the manufacture of inorganic fluorides. Am. J. Roentgenol. Radium Ther. Nucl. Med. 65:42-48.

Long YG, Wang YN, Chen J, Jiang SF, Nordberg A, Guan ZZ. 2002. Chronic fluoride toxicity decreases the number of nicotinic acetylcholine receptors in rat brain. Neurotoxicol. Teratol. 24(6):751-7.

NRC 1993. National Research Council. Health Effects of Ingested Fluoride. Washington, DC: National Academy Press.

NTP). 1990. National Toxicology Program. NTP Technical Report on the Toxicology and Carcinogenesis Studies of Sodium Fluoride (CAS No. 7681-49-4) in F344/N Rats and B6C3F1 Mice (Drinking Water Studies). NTP TR 393. Research Triangle Park, NC: National Toxicology Program.

OEHHA. 1997. Office of Environmental Health Hazard Assessment. Public Health Goal for Fluoride in Drinking Water. Available online at http://www.oehha.ca.gov

OEHHA. 2000. Office of Environmental Health Hazard Assessment. Air Toxics Hot Spots Program Risk Assessment Guidelines. Part IV. Exposure Assessment and Stochastic Technical Support Document. Available online at http://www.oehha.ca.gov

Riggs BL, Hodgson WM, O'Fallon WM, Chao EY, Wahner HW, Muhs JM *et al.* 1990. Effect of fluoride treatment on the fracture rate of postmenopausal women with osteoporosis. New Engl. J. Med. 322:802-809.

Rojas-Sanchez F, Kelly SA, Drake KM, Eckert GJ, Stookey GK, Dunipace AJ. 1999. Fluoride intake from foods, beverages and dentifrice by young children in communities with negligibly and optimally fluoridated water: a pilot study. Community Dent. Oral Epidemiol. 27(4):288-297.

Runge H, Franke J, Geryk B, Hein G, Fengler F, Paul H, Bismarck M, and Schmidt CW. 1979. Bone mineral analysis in persons with long-time fluoride exposure. Fluoride 12(1):18-27.

Seixas NS, Cohen M, Zevenbergen B, Cotey M, Carter S, Kaufman J 2000. Urinary fluoride as an exposure index in aluminum smelting. Amer. Ind. Hyg. Assoc. J. 61(1):89-94.

Shulman JD, Lalumandier JA, and Grabenstein JD. 1995. The average daily dose of fluoride: a model based on fluid consumption. Pediatr. Dent. 17(1):13-18.

Stokinger HE. 1949. Toxicity following inhalation of fluorine and hydrogen fluoride. In: Pharmacology and Toxicology of Uranium Compounds. Voegtlin C, and Hodge HC. (eds) New York: McGraw-Hill Co., Inc. pp. 1021-1057.

Susheela AK, Kumar A. 1991. A study of the effect of high concentrations of fluoride on the reproductive organs of male rabbits, using light and scanning electron microscopy. J. Reprod. Fertil. 92(2):353-60.

U.S. EPA. 2001. United States Environmental Protection Agency. Fluorine (soluble fluoride). IRIS database. Available online at http://www.epa.gov/iris/subst/index.html.

U.S. Public Health Service. 1991. Review of fluoride, benefits and risks, report of the ad hoc subcommittee on fluoride of the committee to coordinate environmental health and related programs. February 1991.

Van Nieuwenhuysen JP, D'Hoore W. 1992. [Dental caries, fluoride tablets and enamel opacities]. Arch. Fr. Pediatr. 49(7):617-621.

Waldbott GL. Fluoridation: The Great Dilemma. Lawrence, KS: Coronado Press, 1978.

Wing JS, Brender JD, Sanderson LM, Perrotta DM, and Beauchamp RA. 1991. Acute health effects in a community after a release of hydrofluoric acid. Arch. Environ. Health 46(3):155-160.

Wohlslagel J, DiPasquale LC, and Vernot EH. 1976. Toxicity of solid rocket motor exhaust: effects of HCl, HF, and alumina on rodents. J. Combustion Toxicol. 3:61-70.

Zhiliang Y, Yihua L, Liansheng Z, and Zhengping Z. 1987. Industrial fluoride pollution in the metallurgical industry in China. Fluoride 20(3):118-125.

CHRONIC TOXICITY SUMMARY

GLUTARALDEHYDE

(1,5-pentanedial; 1,5-pentanedione; glutaric dialdehyde; Aldesen; Cidex; Sonacide)

CAS Registry Number: 111-30-8

I. Chronic Toxicity Summary

Inhalation reference exposure level	**0.08 µg/m^3** (0.02 ppb)
Critical effect(s)	Squamous metaplasia of the respiratory epithelium in the nose of male and female mice
Hazard index target(s)	Respiratory system

II. Chemical Property Summary (HSDB, 1996; CRC, 1994; Chemfinder, 2000)

Description	Colorless liquid/oil
Molecular formula	$C_5H_8O_2$
Molecular weight	100.12 g/mol
Boiling point	188°C (decomposes) (CRC, 1994)
Melting point	–6°C (Chemfinder, 2000)
Solubility	Soluble in water, alcohol, benzene
Conversion factor	4.1 µg/m^3 per ppb at 25ºC

III. Major Uses and Sources

Glutaraldehyde is a chemical frequently used as a disinfectant and sterilizing agent against bacteria and viruses (2% solution), an embalming fluid and tissue fixative, a component of leather tanning solutions, and an intermediate in the production of certain sealants, resins, dyes, and electrical products (HSDB, 1996). For commercial purposes, solutions of 99%, 50%, and 20% are available. Glutaraldehyde is also an atmospheric reaction product of cyclohexene. The annual statewide industrial emissions from facilities reporting under the Air Toxics Hot Spots Act in California based on the most recent inventory were estimated to be 29,603 pounds of glutaraldehyde (CARB, 2000).

IV. Effects of Human Exposure

Evidence of the toxicity of glutaraldehyde to humans is limited to reports of occupational exposure from its use as a disinfectant and sterilizing agent. Frequently observed effects from exposure include skin sensitivity resulting in dermatitis, and irritation of the eyes and nose with accompanying rhinitis (Jordan *et al.*, 1972; Corrado *et al.*, 1986; Hansen, 1983; Wiggins *et al.*,

1989). Occupational asthma has also been reported among workers repeatedly exposed to glutaraldehyde, particularly respiratory technologists who use glutaraldehyde as a sterilizing agent for endoscopes (Chan-Yeung *et al.*, 1993; Stenton *et al.*, 1994; Gannon *et al.*, 1995). Quantitation of the exposure levels that led to glutaraldehyde sensitization was not available from the studies.

V. Effects of Animal Exposure

The histopathology of the respiratory tract in rats and mice exposed to glutaraldehyde by inhalation was examined (Gross *et al.*, 1994). F344 rats and B6C3F1 mice (20 animals of each sex and of each species at each exposure level for a total of 480 rodents) were continuously exposed to glutaraldehyde in recirculating exposure chambers at concentrations of 0, 62.5, 125, 250, 500, or 1000 ppb glutaraldehyde for one day, 4 days, 6 weeks, or 13 weeks. At termination, respiratory tract tissue as well as duodenum and any gross lesions were collected and formalin fixed. Animals were treated with tritiated thymidine two hours before termination to evaluate cell replication in certain respiratory tract tissues. Respiratory tract tissue sections were made as follows: transverse sections of the nose and trachea, frontal section of the carina, and longitudinal section of the lung. Ten male and 10 female mice exposed to 1000 ppb and one female mouse exposed to 500 ppb group died during the course of the study. Two male and 3 female rats exposed to 1000 ppb died during the course of the study. Histopathological examination of animals surviving to the end of the study entailed scoring the severity of the finding from “no response” to “very severe” response on a 0 to 5 scale. Unit length labeling index, the indicator of cell proliferation, was evaluated by autoradiography at two sites: the nasal vestibule and the dorsal atrioturbinate.

Lesions in animals treated with glutaraldehyde appeared primarily in the anterior third of the nose. Lesions were apparently more increased in mice compared to rats due to some level of “background” non-suppurative lesions in the rats. Mice were considered devoid of background lesions. In the 13-week study, female mice were the most sensitive, with lesions averaging a score of 2 (mild and clear, but of limited extent and/or severity). The lesions were characterized as neutrophilic infiltration primarily in the squamous epithelium of the vestibule, with thickening of the epithelium leading to loss of the characteristic surface grooves. Both cell size and number were reported to be increased. Lesions were generally found to increase in nature and severity with increased time and level of exposure. Obstruction of the nasal vestibule was thought to account for the mortality of animals in the higher dose groups. In female mice at 13 weeks, all glutaraldehyde dose groups showed the accumulation of eosinophilic proteinaceous deposits in the respiratory epithelium of the maxilloturbinate margin. Examination of unit length labeling indices as a measure of growth showed significant increases in all treated groups of female mice. No evidence of exposure related lesions was found in the respiratory tract in the trachea, carina, bronchi, or lungs.

Mean Subjective Pathology Scores for Nasal Lesions in Female Mice at 13 Weeks

	Glutaraldehyde	*Intraepithelial neutrophils*	*Subepithelial neutrophils*	*Squamous metaplasia*
	0 ppb	0	0.4	0
	62.5 ppb	2.0	2.0	0
	125 ppb	2.4	2.8	0
	250 ppb	3.2	3.2	0
	500 ppb	2.8	2.8	0.5
	1000 ppb*	--	--	--

*Animals exposed to 1000 ppb died early in the experiment.

Greenspan *et al.* (1985) exposed male and female F-344 rats to 0, 0.3, 1.1 and 3.1 ppm glutaraldehyde and 0, 0.2, 0.63, and 2.1 ppm glutaraldehyde, respectively, in a 9-day study, and both sexes to 0, 21, 49, and 194 ppb glutaraldehyde in a 14 week study. Animal numbers were not specified. Exposures were conducted for 6 hours per day, 5 days per week. In the 9-day study, observations in the high and intermediate dose level groups included reduced body weight gain, inflammation of the nasal and olfactory mucosa, and sensory irritation. In the two highest doses of the 14-week study, statistically significant differences in body weight gain were observed as well as perinasal wetness. No histopathological indication of inflammation in olfactory or nasal mucosa was observed.

Mice were exposed to 0, 0.3, 1.0, and 2.6 ppm glutaraldehyde vapors for 6 hours/day for 4, 9, or 14 days (Zissu *et al.*, 1994). These mice were killed immediately after the exposure period. Other groups exposed to 1.0 ppm for 14 days were killed after recovery periods of 1, 2, and 4 weeks. After 4 days of exposure to the lowest dose, mice showed lesions in the respiratory epithelium of the septum, and the naso- and maxilloturbinates. After exposure to 1.0 ppm glutaraldehyde, lesions were still judged as severe after 2 weeks of recovery.

A study comparing the effects of intra-nasally instilled glutaraldehyde and formaldehyde on rat nasal epithelium found inflammation, epithelial degeneration, respiratory epithelial hypertrophy, and squamous metaplasia in treated animals (St. Clair *et al.*, 1990). Acute inhalation exposure to formaldehyde produced identical lesions. Ten-fold higher concentrations of instilled formaldehyde were required to produce the same effect as instilled glutaraldehyde.

In a chronic study, NTP (1998, 1999) exposed groups of 50 male and 50 female F344/N rats to 0, 250, 500, or 750 ppb glutaraldehyde vapor by inhalation for 6 h/day, 5 days/week, for 104 weeks. Survival of 500 and 750 ppb female rats was less than that of the chamber controls. Mean body weights of all exposed groups of male rats and 500 and 750 ppb female rats were generally less than those of the chamber controls. Increased incidences of nonneoplastic nasal lesions occurred primarily within the anterior section of the nose in 500 and 750 ppb rats and to a lesser extent in 250 ppb rats. The more significant lesions included hyperplasia and inflammation of the squamous and respiratory epithelia and squamous metaplasia of the respiratory epithelium. Thus 250 ppb (1000 $\mu g/m^3$) is a chronic LOAEL for rats.

In the same study NTP (1998, 1999) exposed groups of 50 male and 50 female B6C3F1 mice to 0, 62.5, 125, or 250 ppb glutaraldehyde vapor by inhalation for 6 h/day, 5 days/week, for 104

weeks. Survival of exposed mice was similar to that of the chamber controls. Mean body weights of female mice exposed to 250 ppb were generally less than those of the controls. The incidence of inflammation of the nose was marginally increased in 250 ppb females. Incidences of squamous metaplasia of the respiratory epithelium were increased in 250 ppb males and females and 125 ppb females. Incidences of hyaline degeneration of the respiratory epithelium were increased in all exposed groups of females. Thus 62.5 ppb was a chronic LOAEL for female mice.

Incidence of Nasal Lesions in Female Mice exposed for 104 weeks

	Glutaraldehyde	*Inflammation*	*Respiratory epithelium hyaline degeneration*	*Respiratory epithelium squamous metaplasia*
	0 ppb	6/50	16/50	7/50
	62.5 ppb	7/49	35/49	11/49
	125 ppb	13/50	32/50	16/50
	250 ppb	14/50	30/50	21/50

VI. Derivation of Chronic Reference Exposure Level (REL)

Study	NTP 1998, 1999
Study population	Male and female F344 rats and B6C3F1 mice (50/sex/group)
Exposure method	Continuous inhalation exposure (0, 62.5, 125, and 250 ppb in mice; 0, 250, 500, or 750 ppb in rats)
Critical effects	Respiratory epithelium squamous metaplasia
LOAEL	62.5 ppb (female mice)
NOAEL	Not observed
BMC_{05}	20.5 ppb
Exposure continuity	6 hr/day, 5 days/week
Exposure duration	104 weeks
Equivalent continuous exposure	3.7 ppb (20.5 x 6/24 x 5/7)
Human equivalent concentration	0.62 ppb (gas with extrathoracic respiratory effects, RGDR = 0.17, BW = 28 g, MV = 0.032 L/min, SA = 3 cm^2)
LOAEL uncertainty factor	not needed in BMC approach
Subchronic uncertainty factor	1
Interspecies uncertainty factor	3
Intraspecies uncertainty factor	10
Cumulative uncertainty factor	30
Inhalation reference exposure level	0.02 ppb (0.08 $\mu g/m^3$)

Several studies indicate that the upper respiratory tract is a target for the toxicity of glutaraldehyde from inhalation exposure. Reports of toxicity to humans show that exposure can

lead to occupational asthma as well as cause irritation of the eyes and nose with accompanying rhinitis. Likewise, animals exposed to glutaraldehyde by the inhalation route show evidence of respiratory irritation with the induction of lesions of the anterior nasal cavities upon long-term exposure (Gross *et al.*, 1994; Greenspan *et al.*, 1985; NTP, 1998, 1999). The NTP (1998, 1999) study yielded a chronic LOAEL for female mice of 62.5 ppb. Gross *et al.* (1994) showed neutrophilic infiltration in the olfactory epithelium in the lowest dose exposure group. (Female mice exposed to 62.5 ppb also showed subepithelial neutrophilic infiltration.) This level was taken to be the subchronic LOAEL. This effect on the nasal epithelium was demonstrated to be both concentration- and exposure duration-dependent.

A benchmark concentration was determined using EPA's version 1.20 BMC software and the dose-response data on respiratory epithelium squamous metaplasia in female mice. The quantal-linear model gave an MLE_{05} of 31.24 ppb, a BMC_{05} of 20.51 ppb, and a p value of 0.9471. With the benchmark approach no LOAEL UF is needed. The study was a lifetime study so the subchronic UF is 1. An interspecies UF of 3 rather than 10 was used since an RGDR adjustment had been made. The default intraspecies UF of 10 was used so that the total UF was 30. The resulting chronic REL for glutaraldehyde is 0.02 ppb (0.08 $\mu g/m^3$).

For comparison with the proposed REL, the study of Gross *et al*. (1994) used 62.5 ppb continuous exposure. Multiplying by the RGDR of 0.17 and dividing by a cumulative uncertainty factor of 300 (3 for a LOAEL, 3 for subchronic, 3 for interspecies, and 10 for intraspecies) results in a REL of 0.035 ppb (0.1 $\mu g/m^3$).

VII. Data Strengths and Limitations for Development of the REL

The major strength of the inhalation REL for glutaraldehyde is the availability of controlled exposure inhalation studies in multiple species at multiple exposure concentrations and with adequate histopathogical analysis. Major areas of uncertainty are the lack of human data, the lack of reproductive and developmental toxicity studies, the lack of dermal sensitization studies, and the lack of observation of a NOAEL.

VIII. References

CARB. 2000. California Air Resources Board. California Emissions Inventory Development and Reporting System (CEIDARS). Data from Data Base Year 1998. February 12, 2000.

Chan-Yeung M, McMurren T, Catonio-Begley F, and Lam S. 1993. Occupational asthma in a technologist exposed to glutaraldehyde. J. Allergy Clin. Immunol. 91:974-978.

Chemfinder, 2000. Available online at http://www.chemfinder.com

CRC. 1994. CRC Handbook of Chemistry and Physics, 75th edition. Lide DR, ed. Boca Raton, FL: CRC Press Inc.

Corrado OJ, Osman J, and Davies RJ. 1986. Asthma and rhinitis after exposure to glutaraldehyde. Hum. Toxicol. 5:325-328.

Gannon PF, Bright P, Campbell M, O'Hickey SP, and Burge PS. 1995. Occupational asthma to glutaraldehyde and formaldehyde in endoscopy and x-ray departments. Thorax 50:156-159.

Greenspan BJ, Ballantyne B, Fowler EH, and Snellings WM. 1985. Subchronic inhalation toxicity of glutaraldehyde. Toxicologist 5:29 (abstract).

Gross EA, Mellick PW, Kari FW, Miller FJ, and Morgan KT. 1994. Histopathology and cell replication responses in the respiratory tract of rats and mice exposed by inhalation to glutaraldehyde for up to 13 weeks. Fundam. Appl. Toxicol. 23:348-362.

Hansen KS. 1983. Glutaraldehyde occupational dermatitis. Contact Dermatitis 9:81-82.

HSDB. 1996. Hazardous Substances Data Bank. National Library of Medicine, Bethesda, MD (TOMES® CD-ROM Version). Denver, CO: Micromedex, Inc. (Edition expires 7/31/96).

Jordan WP, Dahl MV, and Albert HL. 1972. Contact dermatitis from glutaraldehyde. Arch. Dermatol. 105:94-95.

National Toxicology Program (NTP). 1998. Toxicology and Carcinogenesis Studies of Glutaraldehyde (CAS NO. 111-30-8) in F344/N Rats and B6C3F1 Mice (Inhalation Studies). TR-490. Board Draft.

National Toxicology Program (NTP). 1999. Toxicology and Carcinogenesis Studies of Glutaraldehyde (CAS NO. 111-30-8) in F344/N Rats and B6C3F1 Mice (Inhalation Studies). TR-490. September 1999. NIH Publication No. 99-3980. Available online at http://ntp-server.niehs.nih.gov/htdocs/LT-studies/tr490.html

St. Clair MBG, Gross EA, and Morgan KT. 1990. Pathology and cell proliferation induced by intra-nasal instillation of aldehydes in the rat: comparison of glutaraldehyde and formaldehyde. Toxicol. Pathol. 18:353-361.

Stenton SC, Beach JR, Dennis JH, Keaney NP, and Hendrick DJ. 1994. Glutaraldehyde, asthma and work -- a cautionary tale. Occup. Med. 44:95-98.

Wiggins P, McCurdy SA, and Zeidenberg W. 1989. Epistaxis due to glutaraldehyde exposure. J. Occup. Med. 31:854-856.

Zissu D, Gagnaire F, and Bonnet P. 1994. Nasal and pulmonary toxicity of glutaraldehyde in mice. Toxicol. Lett. 71:53-62.

CHRONIC TOXICITY SUMMARY

n-HEXANE

(normal hexane)

CAS Registry Number: 110-54-3

I. Chronic Toxicity Summary

Inhalation reference exposure level	**7000 μg/m^3 (2000 ppb)**
Critical effect(s)	Neurotoxicity; electrophysiological alterations in humans
Hazard index target(s)	Nervous system

II. Physical and Chemical Properties (HSDB, 1999)

Description	Colorless liquid, gas
Molecular formula	C_6H_{14}
Molecular weight	86.10
Density	0.660 g/cm^3 @ 20° C
Boiling point	68.95°C
Melting point	–95.3°C
Vapor pressure	150 torr @ 25° C
Solubility	Insoluble in water; soluble in most organic solvents; very soluble in alcohol
Conversion factor	1 ppm = 3.52 mg/m^3 @ 25° C

III. Major Uses or Sources

n-Hexane is used in the extraction of vegetable oil from seeds such as safflower, soybean, cotton, and flax (HSDB, 1995). It is also used as a alcohol denaturant and as a paint diluent. The textile, furniture and leather industries use n-hexane as a cleaning agent. Many petroleum and gasoline products contain n-hexane. The annual statewide industrial emissions from facilities reporting under the Air Toxics Hot Spots Act in California based on the most recent inventory were estimated to be 999,225 pounds of hexane (CARB, 1999).

IV. Effects of Human Exposure

In an offset printing factory with 56 workers, symptomatic peripheral neuropathy was noted in 20 of 56 (36%) workers, while another 26 (46%) had evidence of subclinical neuropathy (Chang *et al*., 1993). Reduced sensory action potentials; reduced motor action potentials; decreased motor nerve conduction velocity; and increased distal latency were found in most workers. Giant axonal swellings with accumulation of 10 nm neurofilaments, myelin sheath attenuation, and widening of nodal gaps were noted upon sural nerve biopsy of a severe case. Optic neuropathy and CNS impairment were not usually found. Personal air samples had 80 to 210 ppm hexane (mean = 132 ppm), 20 to 680 ppm isopropanol (mean = 235 ppm), and 20 to 84 ppm (mean = 50 ppm) toluene. The workers worked 12 hours per day for 6 days per week. The mean duration of employment was 2.6 years, with a range of 1 month to 30 years.

An epidemiologic study was performed on workers employed in a factory producing tungsten carbide alloys and exposed for an average of 6.2 years to solvent vapors consisting of an 8-hour time weighted average of 58 ppm (±41 ppm) n-hexane and 39 ppm (±30 ppm) acetone (Sanagi *et al.*, 1980). Neurological examinations performed on both control and exposed workers examined cranial nerves, motor and sensory nerves, reflexes, coordination and gait. Neurophysiological and nerve stimulation studies were also performed. While no overt neurological abnormalities were noted, the mean motor nerve conduction velocity and residual latency of the exposed group were significantly decreased as compared to unexposed workers. The effects observed are consistent with other reports of n-hexane-induced peripheral neuropathy. The study reports a LOAEL of 58 ppm n-hexane.

Polyneuropathy with subsequent development of muscular atrophy and paresthesia in the distal extremities was observed in workers exposed to between 500 and 1000 ppm n-hexane in a pharmaceutical plant (Yamada, 1967).

A group of 15 industrial workers exposed to n-hexane in vegetable oil extracting and adhesive bandage manufacturing processes was examined for signs of neurotoxicity and ophthalmological changes (Raitta *et al*., 1978; Seppalainen *et al*., 1979). The workers (11 males and 4 females) had been exposed to hexane for 5 to 21 years (mean of 12 years). Ten healthy workers served as controls. Exposures were found to be variable; concentrations as high as 3000 ppm were found on some occasions, although exposure concentrations were usually well below 500 ppm. The authors concluded that the high short-term exposures, occurring occasionally for 1 to 2 hours at a time, could have been major factors in the effects observed. Visual evoked potentials (VEPs) were generally reduced among the exposed subjects and latencies tended to be increased (Seppalainen *et al*., 1979). Visual acuity, visual fields, intraocular pressure, and biomicroscopical findings were normal. Macular changes were noted in 11 and impaired color discrimination was found in 12 of the 15 subjects, largely in the blue-yellow spectrum (Raitta *et al*., 1978).

Fifteen (25%) of 59 press proofing workers had polyneuropathy (Wang *et al*., 1986). All of the patients with polyneuropathy were regularly exposed to n-hexane, and there was a significant association between n-hexane concentration and prevalence of polyneuropathy. The ambient concentration of n-hexane of 190 ppm was found in one factory in which all six workers

developed polyneuropathy. Workers exposed to less than 100 ppm n-hexane who frequently worked overtime demonstrated significant decreases in motor nerve conduction velocities in median, ulnar, and peroneal nerves. Twelve of 13 workers who regularly slept in the factory had polyneuropathy compared to three (7%) of 46 employees who did not sleep in the factory.

Ninety-three of 1662 Japanese workers were found to have polyneuropathy (Yamamura, 1969; Sobue *et al*., 1978). All of the workers developing polyneuropathy were employed in pasting with rubber cement containing 70% or more hexane and small amounts of toluene. The worksites were poorly ventilated and concentrations in workrooms were measured at between 500 and 2500 ppm hexane. One patient developed numbness and weakness of the legs after 6 months of exposure to hexane-based solvents. This patient was hospitalized for over a year until the muscle weakness and atophy improved enough to discharge the patient.

Urinary 2,5-hexanedione concentrations were significantly higher in 35 male workers exposed to n-hexane than in an unexposed group (Karakaya *et al*., 1996). Significant decreases in serum IgG, IgM and IgA levels were also found, and a significant correlation was noted between urinary 2,5-hexanedione concentrations and serum Ig level of the exposed group.

An association between n-hexane and parkinsonism has been proposed based on two case reports (Pezzoli *et al*., 1989; 1995). Regional striatal abnormalities of the nigrostriatal dopaminergic system and of glucose metabolism, observed with positron emission tomography studies, were considered distinct from those seen in idiopathic Parkinson's disease.

Co-exposure to acetone increased the urinary concentrations of free and total 2,5-hexanedione (2,5-HD) in a study of 87 hexane-exposed workers (Cardona et al., 1996). Increased urinary 2,5-HD is noted also with coexposure to hexane and methyl ethyl ketone (Ichihara et al., 1998).

V. Effects of Animal Exposure

Groups of 12 Sprague-Dawley (SD) rats inhaled n-hexane (0, 6, 26, or 129 ppm) for 6 hours/day, 5 days/week for 26 weeks (Bio/dynamics, 1978). A second experiment from the same report involved inhalation exposures of SD rats for 26 weeks to 0, 5, 27, or 126 ppm hexane for 21 hours/day, 7 days/week. There were no consistent dose-related differences between exposed and control animals, although small numbers of animals were involved and examinations were limited to physical observation, body weight, hematological parameters, clinical chemistry, and necropsy of spontaneous deaths. The highest concentration (126 ppm for 21 hours/day, 7 days/week) was a NOAEL and represents a time-weighted average exposure of 110.2 ppm over the duration of the experiment.

F-344 rats and B6C3F1 mice (50/sex/concentration/species) inhaled commercial hexane solvent (0, 900, 3000, or 9000 ppm) for 6 h/day, 5 days/week over 2 years (Daughtrey *et al*., 1999). No significant differences in mortality were noted between hexane-exposed and control groups. Small statistically significant reductions in body weight gain were noted in male and female rats inhaling 3000 ppm or more and in female mice inhaling 9000 ppm. Epithelial cell hyperplasia was increased in the nasoturbinates and larynx of exposed rats.

Fischer 344 rats (5/sex/dose) inhaled >99.5% pure n-hexane (0, 3000, 6500, or 10,000 ppm) for 6 hours/day, 5 days/week over 13 weeks (Cavender *et al*., 1984). No statistically significant differences were notes in food consumption, ophthalmologic examination, neurological function, or hematological or serum chemistry parameters in either males or females. Female body weights and clinical observations were unaltered by hexane treatment. The mean body weight gain of male rats in the 10,000-ppm group was significantly decreased compared with controls at 4 weeks of exposure and thereafter. Axonopathy was noted in the tibial nerve of four of five male rats exposed to 10,000 ppm and in one of five male rats exposed to 6500 ppm. Axonopathy in the medulla was noted in one male rat exposed to 10,000-ppm. Males inhaling 10,000 ppm had slightly but significantly lower brain weights. No other adverse histopathological effects were reported. This study identifies a NOAEL for neurotoxicity of 3000 ppm, with an average experimental exposure of 540 ppm.

B6C3F$_1$ mice were exposed to 500, 1000, 4000, or 10,000 ppm n-hexane 6 hours per day, 5 days per week for 13 weeks or to 1000 ppm n-hexane for 22 hours per day, 5 days per week for 13 weeks (Dunnick *et al.*, 1989). Mild inflammatory, erosive and regenerative lesions in the olfactory and respiratory epithelium were observed in the nasal cavity of mice exposed to 1000 ppm n-hexane and higher. "Minimal lesions" were noted in those mice exposed to 500 or 1000 ppm n-hexane. Paranodal axonal swelling in the tibial nerve was observed in 6/8 mice exposed to 1000 ppm for 22 hours per day and in 6/8 mice exposed to 10,000 ppm for 6 hours per day. No such swelling was noted in neurohistological examination of the control animals; neurohistological examination was not performed in those animals exposed to 500 and 1000 ppm for 6 hours per day. A NOAEL for histological lesions of the nasal turbinates of 500 ppm n-hexane was identified. Because neurohistological examinations were not performed in animals exposed to 500 or 1000 ppm (the NOAEL and LOAEL, respectively), the interpretation of the results from this study are seriously limited.

Male SM-A strain mice (10/group) were exposed continuously to 0, 100, 250, 500, 1000, or 2000 ppm commercial grade hexane (65 to 70% n-hexane with the remainder being other hexane isomers) for 6 days/week for 1 year (Miyagaki, 1967). Electromyography, strength-duration curves, electrical reaction time, and flexor/extensor chronaxy ratio, gait posture and muscular atrophy were studied. Increased complexity of NMU (neuromuscular unit) voltages during electromyographic analysis was noted in 0/6 controls, 1/6 in the 100 ppm group, 3/6 in the 250 ppm group, 5/6 in the 500 ppm group, 3/3 in the 1000 ppm group, and 4/4 in the 2000 ppm group. A dose-related increase in incidence and severity of reduced interference voltages from muscles was noted in mice exposed to 250 ppm or more, but not in controls (0/6 examined) or in the 100 ppm group (0/6). Dose-related abnormal posture and muscle atrophy were noted at 250 ppm or more. This study identifies a NOAEL of 100 ppm for neurotoxicity (68 ppm when adjusted for 67.5% n-hexane).

Rats inhaling 400-600 ppm n-hexane developed peripheral neuropathy after forty-five days of exposure (Schaumburg and Spencer, 1976). Giant axonal swellings and fiber degeneration were observed in the central and peripheral nervous systems. The changes were most notable in tibial nerves and in the cerebellum, medulla and spinal cord.

A dose-dependent decrease in motor nerve conduction velocity and body weight gain was observed in rats exposed to 500, 1200, or 3000 ppm n-hexane for 12 hours per day, 7 days per week for 16 weeks (Huang *et al.*, 1989). The neurotoxicity was significant in the two highest exposure groups; peripheral nerve degeneration, characterized by paranodal swellings and demyelination and remyelination in the myelinated nerve fibers, was observed and was more advanced in the highest exposure group.

Available studies indicate that the neurotoxicity of n-hexane is potentiated by concurrent exposure to methyl ethyl ketone (Altenkirch *et al.*, 1982).

Acetone has also been shown to potentiate the neurotoxicity of hexane and 2,5-HD. Male rabbits administered acetone and 2,5-HD intravenously had decreased body clearance of 2,5-HD (Lagefoged and Perbellini, 1986). Male rats were treated for 6 weeks with 0.5% w/v 2,5-hexanedione alone or in combination with 0.50% w/v acetone in the drinking water (Ladefoged et al., 1994). Acetone potentiated effects on open field ambulation, or rearing and on the rotarod test. Giant axonal swelling was greater in acetone administered animals. During a dose-free 10-week recovery period, the acetone-supplemented group had less improvement in neurological parameters. Male Wistar rats were administered 0.5% w/v 2,5-hexanedione alone or in combination with 0.50% w/v acetone in the drinking water for 7 weeks (Lam et al., 1991). Effects on radial arm maze behavior, a "brain-swelling" reaction, and synaptosomal functions were noted with 2,5-HD and exacerbated with acetone coexposure. In another study of male rats using the same doses for 6 weeks, testis weight, testis tubuli diameter and fertility were reduced with 2,5-HD exposure and potentiated with acetone coexposure (Larsen et al., 1991).

Pregnant rats were exposed to 200, 1000, or 5000 ppm n-hexane 20 hours per day on days 9-19 of gestation (Mast *et al.*, 1987). A statistically significant decrease in fetal body weight compared to controls was observed in male offspring following maternal exposure to 1000 and 5000 ppm n-hexane. Maternal toxicity, indicated by decreased body weight gain, was observed in all exposure groups.

Pregnants rats were exposed to hexane (0, 93.4, or 408.7 ppm) on days 6 through 15 of gestation (Litton Bionetics, 1979). There were no adverse effects noted in dams, and no hexane-induced teratogenicity, changes in sex ratio, embryotoxicity, or impaired fetal growth or development.

Male New Zealand rabbits exposed to 3000 ppm n-hexane for 8 hours per day, 5 days per week for 24 weeks developed exposure-related lesions of the respiratory tract with the terminal bronchioles exhibiting the most characteristic damage (Lungarella *et al.*, 1984). These changes were noted even after a 120-day recovery period. Clinical signs of ocular and upper respiratory tract irritation and respiratory difficulties (such as gasping, lung rales, mouth breathing) were observed throughout the study in exposed rabbits.

VI. Derivation of Chronic Reference Exposure Level

Three studies, an experimental study with mice (Miyagaki, 1967) and two occupational studies (Sanagi et al., 1980; Chang et al., 1993), were considered by OEHHA to be most informative and relevant to the derivation of a chronic REL. This was because these studies (1) evaluated the most sensitive endpoint (peripheral neuropathy) and (2) involved exposures over a significant fraction of a lifetime. While significant limitations may be noted for each of these studies individually, viewed collectively they provide a consistent view of the chronic inhalation toxicity of hexane and yield a stronger basis for deriving a chronic inhalation REL.

While the animal study has the disadvantage of introducing the uncertainty of interspecies differences, the limitations of the human studies were considered to be more significant. Specifically, both human studies were considered likely to overestimate effects of inhalation exposures to hexane.

The Sanagi study, which U.S. EPA used as the basis of its RfC, may overestimate hexane effect because of a confounding coexposure to acetone, which is known to potentiate hexane neuropathy. The minimum effective acetone inhalation concentration for potentiating hexane neuropathy is unclear, as studies (Ladefoged et al., 1994; Lam et al., 1991; Larsen et al., 1991) have used orally administered acetone. The minimum effective acetone inhalation dose for potentiation of carbon tetrachloride hepatotoxicity in male Sprague-Dawley rats was 2500 ppm over 4 hours (Charbonneau et al., 1986). A dose of 0.5% acetone in human drinking water is comparable, assuming equal absorption, to an inhalation concentration of approximately 1400 ppm (0.5% w/v x 2 L/day ÷ 2 m^3/day = 5 g/m^3; 5 g/m^3 x 1000 mg/g ÷ 3.52 mg/m^3 per ppm = 1400 ppm). As the acetone potentiating effects were all noted at higher exposures than are being considered in occupational studies and are at much higher concentrations than the REL itself, the significance of these findings is uncertain.

In the Chang study, the workers were probably intermittently exposed to higher inhalation exposures than were estimated from ambient air sampling, and significant dermal exposures were also likely. Furthermore, coexposure to high levels of isopropanol and toluene, may have confounded the results, although CNS effects were not noted and these substances are not known to induce or potentiate peripheral neuropathy.

As shown in Table 1, the human studies by Sanagi *et al*. (1980) and Chang *et al*. (1993) yield 7 to 10-fold lower RELs than the Miyagaki study. In view of the likely overprediction of hexane risks from these studies, due to co-exposure to other materials which may potentiate the effects of hexane, these calculations may be viewed as generally supporting the 7000 µg/m^3 REL.

Key study	Miyagaki (1967)
Study population	Male mice
Exposure method	Discontinuous inhalation
Critical effects	Peripheral neuropathy (electromyographic alterations; dose-related abnormal posture and muscle atrophy)
LOAEL	250 ppm
NOAEL	100 ppm
Exposure continuity	24 hours/day, 6 days/week
Exposure duration	1 year
Average experimental exposure	57.9 ppm for LOAEL group (100 ppm * 0.675 * 6/7)
Human equivalent concentration	57.9 ppm (gas with systemic effects, based on default RGDR = 1 for lambda (a) = lambda (h))
LOAEL uncertainty factor	1
Subchronic uncertainty factor	1
Interspecies uncertainty factor	3
Intraspecies uncertainty factor	10
Cumulative uncertainty factor	30
Inhalation reference exposure level	2 ppm (2000 ppb; 7 mg/m^3; 7000 µg/m^3)

Table 1: Reference Exposure Levels (RELs) from Selected Human Studies

Study	Duration	Effect	LOAEL (ppm)	LOAEL (ppm) (TWA)	NOAEL (ppm)	NOAEL (ppm) (TWA)	total UF	REL (ppb)	REL (µg/m^3)
Sanagi *et al.*, 1980	6.2 years	decreased motor nerve conduction velocity; increased residual latency	58	20.7	Not observed		100[a]	200	700
Chang *et al.*, 1993	mean 2.6 years: range 1 month to 12 years	Symptomatic peripheral neuropathy; decreased motor nerve conduction velocity; increased residual latency; axonal swelling of sural nerve	mean 132: range 80 - 210	83	*Not observed*		300[b]	300	1000

[a] LOAEL uncertainty factor, 10; Intraspecies uncertainty factor, 10
[b] LOAEL uncertainty factor, 10; Subchronic uncertainty factor, 3; Intraspecies uncertainty factor, 10

The hexane exposure estimate was reduced for the Miyagaki data as the solvent used contained 67.5% n-hexane.

The average occupational exposure for the Chang study involving an unusual 72-hour work week was calculated by assuming that 12 hours of occupational exposures at an inhalation rate of 20 L/min was followed by 4 hours of light work at 20 L/min and 8 hours of rest at 7.5 L/min. Using these assumptions an estimated 63% of daily inhaled air occurred at the workplace.

The Chang study found that the severity of effects was not correlated with the length of exposure, suggesting that (1) susceptibility may differ markedly between individuals and/or (2) shorter exceedances of the time-weighted average concentration might be significant. Thus the subchronic uncertainty factor was reduced to 3-fold.

VII. Data Strengths and Limitations for Development of the REL

There is a substantial database on the health effects of n-hexane in both humans and animals from which to derive a chronic reference exposure level. Some relevant studies are summarized in the table below.

Study	***Species***	***Exposure concentration***	***Exposure regimen***	***TWA from NOAEL[a]***	***TWA from LOAEL[a]***
Sanagi *et al*. (1980)	Humans	58 ppm (mean)	10 m^3/d, 5 d/wk, 6.2 yr (mean)	None	20.7 ppm
Chang *et al*. (1993)	Humans	130 ppm (mean)	12 hr/d, 6 d/wk, 2.6 yr (mean)	None	83 ppm
Miyagaki (1967)	Male mice	0, 100, 250, 500, 1000, 2000 ppm	Continuous, 6 d/wk, 1 yr	57.9 ppm	121 ppm
Daughtrey *et al*. (1999)	F344 rats	0, 900, 3000, 9000 ppm	6 hr/d, 5 d/wk, 2 yr	None	161 ppm
Daughtrey *et al*. (1999)	B6C3F1 mice	0, 900, 3000, 9000 ppm	6 hr/d, 5 d/wk, 2 yr	None	161 ppm
Dunnick *et al*. (1989)	B6C3F1 mice	0, 500, 1000, 4000, 10,000 ppm	6 hr/d, 5 d/wk, 13 wk	89 ppm	179 ppm
Huang *et al*. (1989)	Wistar rats	0, 500, 1200, 3000 ppm	12 hr/d, 7 d/wk, 16 wk	None	250 ppm
Bio/dynamics (1978)	SD rats	0, 5, 27, 126 ppm	21 hr/d, 7 d/wk, 26 weeks	110 ppm	None
Cavender *et al*. (1984)	F344 rats	0, 3000, 6500, 10,000 ppm	6 hr/d, 5 d/wk, 13 wk	540 ppm	1160 ppm

[a] The experimental exposure was extrapolated to an equivalent (time-weighted average or TWA) continuous exposure.

The major strengths of the REL for hexane include (1) the primary use of an animal study (Miyagaki, 1967) with controlled, nearly continuous chronic hexane exposures not confounded by coexposure to other solvents, which observed both a NOAEL and LOAEL; and (2) the results obtained from two different human studies (Sanagi, 1980; Chang et al., 1993) which were viewed as being generally consistent with the animal study based REL.
There is uncertainty about interspecies as well as intraindividual differences in susceptibility to n-hexane peripheral neuropathy. In one study, controlled TWA exposures of 540 ppm (Cavender

et al., 1984) were not found to cause neuropathy in rats. Also human studies (especially that of Chang *et al*., 1993) have shown that some individuals develop peripheral neuropathy within months, whereas others remain symptom-free despite years of employment at the same occupation at the same workplace.

OEHHA staff also estimated RELs from two other animal studies for comparison. In Bio/Dynamics (1978), 126 ppm for 21 hours/day, 7 days/week for 26 months was a NOAEL and represents a time-weighted average exposure of 110.2 ppm. Using an RGDR of 1 and a cumulative 30-fold uncertainty factor (3 for interspecies differences not accounted for by the RGDR method and 10-fold for intraspecies differences), a REL of 4 ppm (10,000 $\mu g/m^3$) was derived. Cavender *et al*. (1984) identified a NOAEL for neurotoxicity of 3000 ppm, with an average experimental exposure of 540 ppm. A REL based on this study, using an RGDR of 1 and a 100-fold uncertainty factor (3 for subchronic (13 weeks) to chronic, 3 for interspecies, and 10 for intraspecies) would be 5.4 ppm (19,000 $\mu g/m^3$).

VIII. References

Altenkirch H, Wagner HM, Stoltenburg G, and Spencer PS. 1982. Nervous system responses of rats to subchronic inhalation of N-hexane and N-hexane + methyl-ethyl-ketone mixtures. J. Neurol. Sci. 57:209-219.
Bio Dynamics Inc. 1978. 26 Week Inhalation Toxicity Study of n-Hexane in the Rat. US EPA; Document #FYI-AX-1081-0137.

Cardona A, Marhuenda D, Prieto MJ, Marti J, Periago JF, Sanchez JM. 1996. Behaviour of urinary 2,5-hexanedione in occupational co-exposure to n-hexane and acetone. Int Arch Occup Environ Health 68(2):88-93.

Cavender FL, Casey HW, Salem H, Graham DG, Swenberg JA, Gralla EJ. 1984. A 13-week vapor inhalation study of n-hexane in rats with emphasis on neurotoxic effects. Fundam. Appl. Toxicol. 4(2 Pt 1):191-201.

Chang CM, Yu CW, Fong KY, Leung SY, Tsin TW, Yu YL, Cheung TF, Chan SY. 1993. N-hexane neuropathy in offset printers. J. Neurol. Neurosurg. Psychiatry 56(5):538-542.

Charbonneau M, Brodeur J, du Souich P, Plaa GL. 1986. Correlation between acetone-potentiated CCl_4-induced liver injury and blood concentrations after inhalation or oral administration. Toxicol. Appl. Pharmacol. 30;84(2):286-294.

Daughtrey W, Newton P, Rhoden R, Kirwin C, Haddock L, Duffy J, Keenan T, Richter W, Nicolich M. 1999. Chronic inhalation carcinogenicity study of commercial hexane solvent in F-344 rats and B6C3F1 mice. Toxicol. Sci. 48(1):21-29.

Dunnick JK, Graham DG, Yang RS, Haber SB, and Brown HR. 1989. Thirteen-week toxicity study of n-hexane in B6C3F1 mice after inhalation exposure. Toxicology 57(2):163-172.

HSDB. 1999. Hazardous Substances Data Bank. National Library of Medicine, Bethesda, Maryland. WWW database (http://sis.nlm.nih.gov/sis1/).

Huang J, Kato K, Shibate E, Sugimura K, Hisanaga N, Ono Y, Takeuchi Y. 1989. Effects of chronic n-hexane exposure on nervous system-specific and muscle-specific proteins. Arch. Toxicol. 63(5):381-385.

Ichihara G, Saito I, Kamijima M, Yu X, Shibata E, Toida M, Takeuchi Y. 1998. Urinary 2,5-hexanedione increases with potentiation of neurotoxicity in chronic coexposure to n-hexane and methyl ethyl ketone. Int. Arch. Occup. Environ. Health 71(2):100-104.

Karakaya A, Yucesoy B, Burgaz S, Sabir HU, Karakaya AE. 1996. Some immunological parameters in workers occupationally exposed to n-hexane. Hum. Exp. Toxicol. 15(1):56-8.

Ladefoged O, Perbellini L. 1986. Acetone-induced changes in the toxicokinetics of 2,5-hexanedione in rabbits. Scand. J. Work Environ. Health 12(6):627-629.

Ladefoged O, Roswall K, Larsen JJ. 1994. Acetone potentiation and influence on the reversibility of 2,5- hexanedione-induced neurotoxicity studied with behavioural and morphometric methods in rats. Pharmacol. Toxicol. 74(4-5):294-299.

Lam HR, Larsen JJ, Ladefoged O, Moller A, Strange P, Arlien-Soborg P. 1991. Effects of 2,5-hexanedione alone and in combination with acetone on radial arm maze behavior, the "brain-swelling" reaction and synaptosomal functions. Neurotoxicol. Teratol. 13(4):407-412.

Larsen JJ, Lykkegaard M, Ladefoged O. 1991. Infertility in rats induced by 2,5-hexanedione in combination with acetone. Pharmacol. Toxicol. 69(1):43-46.

Litton Bionetics Inc. 1979. Teratology Study in Rats. n-Hexane. US EPA; Document #FYI-AX-0183-0231.

Lungarella G, Barni-Comparini I, and Fonzi L. 1984. Pulmonary changes induced in rabbits by long-term exposure to n-hexane. Arch. Toxicol. 55(4):224-228.
Mast TJ, Decker JR, Clark ML, *et al.* 1987. Inhalation developmental toxicology studies: teratology study of n-hexane in rats: Final report. Iss. PNL-6453; Order No. DE88006812. p.208.

Miyagaki H. 1967. Electrophysiological studies on the peripheral neurotoxicity of n-hexane. Jap. J. Ind. Health 9(12-23): 660-671
Paulson GW, and Waylonis GW. 1976. Polyneuropathy due to n-hexane. Arch. Int. Med. 136:880-882. [cited in U.S. EPA, 1995].

Pezzoli G, Barbieri S, Ferrante C, Zecchinelli A, Foa V. 1989. Parkinsonism due to n-hexane exposure. Lancet 2(8667):874.

Pezzoli G, Antonini A, Barbieri S, Canesi M, Perbellini L, Zecchinelli A, Mariani CB, Bonetti A, Leenders KL. 1995. n-Hexane-induced parkinsonism: pathogenetic hypotheses. Mov. Disord. 10(3):279-82.

Raitta C, Seppalainen AN, Huuskonen MS. 1978. N-hexane maculopathy in industrial workers. Albrecht Von Graefes Arch. Klin. Exp. Ophthalmol. 209(2):99-110. Sanagi S, Seki Y, Sugimoto K, and Hirata M. 1980. Peripheral nervous system functions of workers exposed to n-hexane at a low level. Int. Arch. Occup. Environ. Health 47:69-79.

Schaumburg HH, Spencer PS. 1976. Degeneration in central and peripheral nervous systems produced by pure n-hexane: an experimental study. Brain 99(2):183-192.Seppalainen AM, Raitta C, and Huuskonen MS. 1979. n-Hexane-induced changes in visual evoked potentials and electroretinograms of industrial workers. Electroencephal. Clin. Neurophysiol. 47:492-498.

Sobue I, Iida M, Yamamura Y, and Takayanagui T. 1978. N-Hexane polyneuropathy. Int. J. Neurol. 11(4):317-330.
U.S. EPA. 1999. U. S. Environmental Protection Agency. Integrated Risk Information System (IRIS). Cincinnati, OH: Office of Health and Environmental Assessment, Environmental Criteria Assessment Office.

Wang J-D, Chang Y-C, Kao K-P, Huang C-C, Lin C-C, and Yeh W-Y. 1986. An outbreak of n-hexane induced polyneuropathy among press proofing workers in Taipei. Am. J. Ind. Med. 10(2):111-118.

Yamada S. 1967. Intoxication polyneuritis in the workers exposed to n-hexane. Jap. J. Ind. Health 9:651-659 [cited in U.S. EPA, 1994].

Yamamura Y. 1969. n-Hexane polyneuropathy. Folia Psychiatr. Neurol. Jap. 23(1):45-57.

CHRONIC TOXICITY SUMMARY

HYDRAZINE

(diamine; diamide; nitrogen hydride; levoxine)

CAS Registry Number: 302-01-2

I. Chronic Toxicity Summary

Inhalation reference exposure level	**0.2 µg/m^3** (0.1 ppb)
Critical effect(s)	Amyloidosis of the liver and thyroid in hamsters
Hazard index target(s)	Alimentary system; endocrine system

II. Chemical Property Summary (HSDB, 1995; CRC, 1994)

Description	Colorless, oily liquid or white crystals
Molecular formula	N_2H_4
Molecular weight	32.05 g/mol
Boiling point	113.5°C (Merck, 1983; CRC, 1994)
Melting point	2.0°C
Vapor pressure	14.4 torr @ 25°C
Solubility	Miscible with water, methyl-, ethyl-, isobutyl alcohols; slightly miscible with hydrocarbons; insoluble in chloroform, ether
Conversion factor	1.31 µg/m^3 per ppb at 25ºC

III. Major Uses and Sources

Hydrazine is a highly reactive base and reducing agent. Its primary uses are as a high-energy rocket propellant, as a reactant in military fuel cells, in nickel plating, in the polymerization of urethane, for removal of halogens from wastewater, as an oxygen scavenger in boiler feedwater to inhibit corrosion, and in photographic development (Von Burg and Stout, 1991). Hydrazine was historically used experimentally as a therapeutic agent in the treatment of tuberculosis, sickle cell anemia, and non-specific chronic illnesses (Von Burg and Stout, 1991; Gold, 1987). The annual statewide industrial emissions from facilities reporting under the Air Toxics Hot Spots Act in California based on the most recent inventory were estimated to be 1664 pounds of hydrazine (CARB, 2000).

IV. Effects of Human Exposure

One person was occupationally exposed to hydrazine at unknown levels once per week for a period of 6 months (Sotaniemi *et al.*, 1971). The worker showed symptoms of conjunctivitis, tremors, and lethargy for 1-2 days following each exposure. Vomiting, fever, and diarrhea developed on the last day of exposure and progressed to abdominal pain and incoherence. The previously healthy 59-year old individual died three weeks after the last exposure. Evidence of tracheitis, bronchitis, heart muscle degeneration, and liver and kidney damage was found at autopsy. A single case report can not prove a cause and effect relationship between hydrazine exposures and the noted symptoms and death, but the repeated association between exposures and symptoms is highly suspicious. Liver toxicity is also associated with acute exposure to hydrazine.

The only epidemiological studies of human hydrazine exposures found involve workers in a hydrazine manufacturing plant (Wald *et al*., 1984; Wald, 1985; Morris *et al*., 1995). Workers were exposed to various durations of at least 6 months between 1945 and 1972 and have been followed through 1992. The studies are based on a review of medical records. Only 78 of 427 workers were believed to have had more than incidental exposure to hydrazine. Only cumulative mortality was reviewed. Health effects reported during or after hydrazine exposure were not examined. No increase in mortality was noted for lung cancer, other cancers, or causes other than cancer. However, these small studies have little power to detect increased mortality, and age of death was not examined. The authors reported that relative risks up to 3.5 could have gone undetected.

Dermal sensitization has also been reported from repeated contact with hydrazine (Van Ketal, 1964; Von Keilig and Speer, 1983; Wrangsjo and Martensson, 1986).

V. Effects of Animal Exposure

An inhalation study of the toxicity and carcinogenicity of hydrazine was conducted in cats, mice, hamsters, and dogs (Vernot *et al.*, 1985). Various animal groups were exposed 6 hours/day, 5 days/weeks for one year to concentrations of 0.05, 0.25, 1.0, and 5.0 ppm anhydrous hydrazine base. Exposed and controls groups were made up of the following animals: 100 Fischer 344 rats/sex at 0.05, 0.25, 1.0, and 5.0 ppm hydrazine plus 150 rats/sex as controls; 400 female C57BL/6 mice at 0.05, 0.25, and 1.0 ppm hydrazine plus 800 female mice as controls; 200 male Golden Syrian hamsters at 0.25, 1.0, and 5.0 ppm hydrazine plus 200 male hamsters as controls; 4 beagle dogs/sex at 0.25 and 1.0 ppm hydrazine plus 4 dogs/sex as controls. Animals were observed post-exposure for the following periods: 18 months for rats, 15 months for mice, 12 months for hamsters, and 38 months for dogs. Animals were observed hourly during the exposure period and daily in the post-exposure period.

No non-cancer toxic effects were observed in mice or dogs, with the exception of a single dog, exposed to 1.0 ppm hydrazine, which showed cyclic elevations in serum glutamic-pyruvic transaminase levels and, upon necropsy at 36 months post-exposure, showed liver effects described as “clusters of swollen hepatocytes that had highly vacuolated cytoplasm.” Of the

other species examined, hamsters showed toxicity at the lowest dose levels, particularly amyloidosis in various organs including liver, spleen, kidney, thyroid, and adrenal glands. An increased incidence of amyloidosis was seen at the lowest exposure level (0.25 ppm hydrazine) in the liver and thyroid (67/160 exposed vs. 42/180 control for the liver and 20/117 exposed vs. 9/155 control in the thyroid; $p \leq 0.01$ by Fisher's exact test). This effect was found to be dose related. The incidence of hemosiderosis of the liver was also significantly increased in all exposed groups. Significantly increased incidences of toxic effects observed in the 1.0 and 5.0 ppm hydrazine groups include amyloidosis of the spleen, kidney glomerulus, and adrenals glands, and lymphadenitis of the lymph nodes. Significantly increased toxic effects observed only in the highest dose group include amyloidosis of the kidney interstitium and thyroid, and senile atrophy of the testis. The authors note these effects appear to reflect accelerated changes commonly associated with aging in hamsters.

Incidence of Nonneoplastic Lesions in Male Hamsters (from Table 3 of Vernot *et al*.)

Lesion	*Control*	*0.25 ppm*	*1.0 ppm*	*5.0 ppm*
Liver				
Amyloidosis	42/180 (23)*	67/160 (42)[a]	68/148 (46)[a]	79/159 (50)[a]
Hemosiderosis	42/180 (23)	63/160 (39)[a]	77/148 (52)[a]	94/159 (59)[a]
Bile duct hyperplasia	14/180 (8)	31/160 (19)[a]	28/148 (19)[a]	44/159 (28)[a]
Biliary cyst	45/180 (25)	45/160 (28)	42/148 (28)	55/159 (35)[b]
Thyroid				
Amyloidosis	9/155 (6)	20/117 (17)[a]	11/127 (9)	22/137 (16)[a]
Adrenal				
Amyloidosis	38/177 (22)	49/199 (32)[b]	52/141 (37)[a]	76/153 (50)[a]

* Incidence of lesion (% of animals with lesion)
[a] Incidence significantly greater than control, $p \leq 0.01$
[b] Incidence significantly greater than control, $0.01 < p \leq 0.05$

In the hydrazine exposed rats, effects were observed in the respiratory tract of exposed animals. Specifically, squamous metaplasia of the larynx, trachea, and nasal epithelium (males only) was observed in the highest dose group (5.0 ppm hydrazine). Inflammation was also observed in the larynx and trachea of rats exposed to 5.0 ppm hydrazine. Increased incidence of focal cellular change of the liver was observed in female mice at 1.0 and 5.0 ppm hydrazine. Other effects with increased incidence only in the high dose group include hyperplastic lymph nodes in females, endometriosis, and inflammation of the uterine tube.

The toxic effects from inhalation of hydrazine over a six month period from both intermittent and continuous exposure scenarios were examined (Haun and Kinkead, 1973). Groups of 8 male beagle dogs, 4 female rhesus monkeys, 50 male Sprague-Dawley rats, and 40 female ICR rats per dose group were continuously exposed to 0.2 or 1.0 ppm hydrazine or intermittently (6 hours/day, 5 days/week) to 1.0 or 5.0 ppm hydrazine. A control group consisted of equal numbers of animals. The experimental design was such that each intermittent exposure group had a time-weighted-average matching continuous exposure group. Dose-related body weight reductions were observed in all treated groups as well as evidence of hepatic degeneration, fatty

deposition in the liver, central nervous system depression and lethargy, eye irritation, and anemia.

Toxic effects from the exposure of rats, mice, and dogs to airborne hydrazine at levels of 0, 4.6, or 14 ppm intermittently for 6 months were reported (Comstock *et al.*, 1954). Observed adverse effects included anorexia, irregular breathing, vomiting, fatigue, and emphysema in dogs; pulmonary congestion and emphysema in rats and mice; and lung and liver damage in rats.

Lymphoid bronchial hyperplasia was observed in guinea pigs exposed to 2-6 ppm hydrazine for 5 days/week for 19-47 days (Weatherby and Yard, 1955).

VI. Derivation of Chronic Reference Exposure Level (REL)

Study	Vernot *et al.*, 1985
Study population	Hamster
Exposure method	Inhalation of 0, 0.25, 1, and 5 ppm
Critical effects	Amyloidosis and hemosiderosis of the liver; thyroid amyloidosis
LOAEL	0.25 ppm
NOAEL	Not observed
Exposure continuity	6 hour/day, 5 days/week
Exposure duration	1 year
Average experimental exposure	0.045 ppm for LOAEL group (0.25 x 6/24 x 5/7)
Human equivalent concentration	0.045 ppm for LOAEL group (gas with systemic effects, based on RGDR = 1.0 using default assumption that lambda (a) = lambda (h))
LOAEL uncertainty factor	10 (low incidence above controls but serious adverse effects)
Subchronic uncertainty factor	1
Interspecies uncertainty factor	3
Intraspecies uncertainty factor	10
Cumulative uncertainty factor	300
Inhalation reference exposure level	0.0001 ppm (0.1 ppb, 0.0002 mg/m^3, 0.2 µg/m^3)

Vernot *et al.*(1985) present a thorough examination of chronic health effects from inhalation exposure to hydrazine. This study was chosen for the development of the chronic reference exposure level because (1) it was conducted with an adequate number of animals, (2) the critical/sensitive adverse effect (degenerative change in the liver in hamsters) showed a dose-response relationship, and (3) the findings of this study support data found in studies by other groups.

This study shows a dose-related increase in the incidence of amyloidosis and hemosiderosis in hamsters intermittently exposed by inhalation to levels of hydrazine greater than 0.25 ppm. Other effects noted at 0.25 ppm included weight depression during exposure, mineralization of the kidney, and amyloidosis of the thyroid. Haun and Kinkead (1973) have also noted lesions of the

liver in dogs, monkeys, and mice exposed continuously to 0.2 ppm hydrazine for 6 months by inhalation. Comstock *et al.* (1954) observed liver damage in groups of rats exposed to hydrazine vapors. The single case report of hydrazine inhalation toxicity in humans showed necrosis and degeneration of the liver (Sotaniemi *et al.*, 1971).

VII. Data Strengths and Limitations for Development of the REL

The strengths of the inhalation REL for hydrazine include the availability of chronic inhalation exposure data from a well-conducted study with histopathological analysis. Major areas of uncertainty are the lack of adequate human exposure data, the lack of reproductive and developmental toxicity studies, and the lack of observation of a NOAEL in the key study.

VIII. References

CARB. 2000. California Air Resources Board. California Emissions Inventory Development and Reporting System (CEIDARS). Data from Data Base Year 1998. February 12, 2000.

CRC. 1994. CRC Handbook of Chemistry and Physics, 75th edition. Lide DR, ed. Boca Raton, FL: CRC Press Inc.

Comstock CC, Lawson LH, Greene EA, and Oberst FW. 1954. Inhalation toxicity of hydrazine vapor. Arch. Ind. Hyg. Occup. Med. 10:476-490.

Gold J. 1987. Hydrazine sulfate: a current perspective. Nutr. Cancer 9:59-66.

Haun CC, and Kinkead ER. 1973. Chronic inhalation toxicity of hydrazine. Proceedings of the 4th Annual Conference on Environmental Toxicology. Wright-Patterson Air Force Base, OH. AMRL-TR-73-125:351-363.

HSDB. 1995. Hazardous Substances Data Bank. National Library of Medicine, Bethesda, MD (CD-ROM Version). Denver, CO: Micromedex, Inc. (Edition expires 7/31/96).

Merck. 1983.The Merck Index. 10th ed. Windholz M. (ed.) Rahway, NJ: Merck & Co.

Morris J, Densem JW, Wald NJ, Doll R. 1995. Occupational exposure to hydrazine and subsequent risk of cancer. Occup. Environ. Med. 52(1):43-45.

Sotaniemi E, Hivonen J, Isomaki H, Takkunen J, and Kaila J. 1971. Hydrazine toxicity in the human. Report of a fatal case. Ann. Clin. Res. 3:30-33.

Van Ketal WG. 1964. Contact dermatitis from a hydrazine derivative in a stain remover. Cross-sensitization to apresoline and isoniazid. Acta Derm. Venereol. (Stockh.) 44:49-53.

Vernot EH, MacEwen JD, Bruner RH, Haun CC, Kinkead ER, Prentice DE, Hall A, Schmidt RE, Eason RL, Hubbard GB, and Young JT. 1985. Long-term inhalation toxicity of hydrazine. Fundam. Appl. Toxicol. 5:1050-1064.

Von Burg R, and Stout T. 1991. Toxicology Update: Hydrazine. J. Appl. Toxicol. 11:447-450.

Von Keilig, W. and Speer U 1983. Occupational allergic contact dermatitis from hydrazine. Dermatosen in Beruf und Umwelt 31:25-27.

Wald N, Boreham J, Doll R, and Bonsall J. 1984. Occupational exposure to hydrazine and subsequent risk of cancer. Br. J. Ind. Med. 41(1):31-34.

Wald N. 1985. Hydrazine: epidemiological evidence. IARC Scientific Publication 65:75-80.

Weatherby JH, and Yard AS. 1955. Observations on the subacute toxicity of hydrazine. Arch. Ind. Health 11:413.

Wrangsjo K, and Martensson A. 1986. Hydrazine contact dermatitis from gold plating. Contact Dermatitis, 15:244-245.

CHRONIC TOXICITY SUMMARY

HYDROGEN CHLORIDE

(Hydrochloric acid; anhydrous hydrogen chloride; muriatic acid)

CAS Registry Number: 7647-01-0

I. Chronic Reference Exposure Level

Inhalation reference exposure level	**9 µg/m^3 (6 ppb)**
Critical effect(s)	Hyperplasia of nasal mucosa, larynx, and trachea in rats
Hazard index target(s)	Respiratory system

II. Physical and Chemical Properties (HSDB, 1999)

Description	Colorless gas
Molecular formula	HCl
Molecular weight	36.46
Density	1.49 g/L @ 25° C
Boiling point	-84.9° C (HCl gas)
Melting point	-114.8° C (HCl gas)
Solubility	Soluble in water, alcohol, benzene, ether; insoluble in hydrocarbons
Conversion factor	1 ppm = 1.49 mg/m^3 at 25°C

III. Major Uses or Sources

Hydrogen chloride (HCl) is used in the manufacture of vinyl chloride, fertilizers, dyes, artificial silk, and pigments for paints. It is also used in electroplating, soap refining, and leather tanning. Other consumers of HCl include the photographic, textile and rubber industries (HSDB, 1999).

Hydrogen chloride is produced in large quantities during combustion of most materials and especially materials with a high chlorine content. Thus, HCl is a major product formed during the thermal decomposition of polyvinyl chloride, a commonly used plastic polymer (Burleigh-Flayer *et al.*, 1985). It is also released in large quantities during the test firing of some rocket and missile engines (Wohlslagel *et al.*, 1976). Since HCl is extremely hygroscopic, it generally exists as an aerosol in the ambient atmosphere. The annual statewide industrial emissions from facilities reporting under the Air Toxics Hot Spots Act in California based on the most recent inventory were estimated to be 2,570,888 pounds of HCl (CARB, 1999b).

IV. Effects of Human Exposure

Few reports are available on the effects of chronic HCl exposure on humans. Bleeding of the nose and gums and ulceration of the mucous membranes was observed following repeated occupational exposure to HCl mist at high but unquantified concentrations (Stokinger, 1981).

In another report, workers exposed to various mineral acids, including HCl, exhibited etching and erosion of the front teeth (Ten Bruggen Cate, 1968). Dental erosion was noted in 176 of 555 (32%) workers examined between 1962 and 1964, and progressive erosion was reported in 66 of 324 (20%) workers examined repeatedly. Rates of active erosion were highest (50%) in the most highly-exposed category (battery formation workers), intermediate (23%) in an intermediate-exposure category (picklers), and low (7%) in a low-exposure category (other processes). Grade 1 erosion (enamel loss) was noted in workers exposed for greater than 3 months; grade 2 erosion (loss of enamel and dentine) was noted after 2.5 to 5 years exposure; and grade 3 (loss of enamel and dentine with exposure of secondary dentine) was noted after six or more years of exposure.

V. Effects of Animal Exposure

Male Sprague-Dawley rats were exposed to 10 ppm HCl for 6 hours per day, 5 days per week over their lifetime (Sellakumar *et al.*, 1985). No differences in body weights or survival were observed between 99 exposed and 99 control animals. Increased incidences of hyperplasia of the nasal mucosa (62/99 vs. 51/99), larynx (22/99 vs. 2/99), and trachea (26/99 vs. 2/99) were observed in exposed rats compared to air-exposed controls.

A 90-day inhalation study using B6C3F1 mice and Sprague-Dawley and Fisher 344 rats exposed the animals (groups of 31 males and 31 females for each species and strain) to 10, 20, or 50 ppm HCl for 6 hours per day, 5 days per week over 90 days (Toxigenics, 1984). Several animals died during the study, though the deaths were not considered to be exposure related. A slight but significant decrease in body weight gain was reported in male and female mice and in male Fischer 344 rats in the high-exposure groups. No effect were noted in hematology, clinical chemistry, or urinalysis. Minimal or mild rhinitis was observed in both strains of rats Concentration- and time-related lesions were noted in the anterior portion of the nasal cavity of exposed rats. Cheilitis, eosinophilic globules in the nasal epithelium and accumulation of macrophages in the peripheral tissues were observed in mice of all exposed groups. This study thus observed a LOAEL for both mice and rats of 10 ppm. The U.S. EPA considered this study supportive of the portal-of-entry effects observed at 10 ppm in the lifetime rat study (USEPA, 1999). Female rats (8-15/group) exposed to 302 ppm HCl for 1 hour either 12 days prior to mating or on day 9 of gestation exhibited severe dyspnea and cyanosis; the exposure was lethal to one-third of the exposed animals (Pavlova, 1976). Fetal mortality was significantly higher in rats exposed during pregnancy. Organ functional abnormalities observed in offspring exposed at 2-3 months of age were reported to be similar to those observed in the exposed dams.

Female rats were exposed to 302 ppm HCl for 1 hour prior to mating (GEOMET Technologies, 1981). Exposure killed 20 to 30% of the rats. In rats surviving 6 days after exposure, a decrease in blood oxygen saturation was reported, as were kidney, liver, and spleen effects. Estrus cycles

were also altered. In rats mated 12-16 days postexposure and killed on day 21 of pregnancy, a decrease in fetal weight, an increase in relative fetal lung weights, and reduced numbers of live fetuses were observed.

Derivation of Chronic Reference Exposure Level

Study	Sellakumar *et al.*, 1985
Study population	Sprague-Dawley rats (100 males)
Exposure method	Discontinuous whole-body inhalation (0 or 10 ppm)
Critical effects	Hyperplasia of the nasal mucosa, larynx and trachea
LOAEL	10 ppm
NOAEL	Not identified
Exposure continuity	6 hours per day, 5 days per week
Average experimental exposure	1.8 ppm for LOAEL group
Human equivalent concentration	0.57 ppm (gas with extrathoracic respiratory effects, RGDR =0.32, based on rat MV_a = 0.33 L/min, MV_h = 13.8 L/min, $SA_a(ET)$ = 15 cm^2); Sa_h = 200 cm^3) (U.S. EPA, 1994)
Exposure duration	Lifetime
LOAEL uncertainty factor	3 (<30% incidence; mild effect)
Subchronic uncertainty factor	1
Interspecies uncertainty factor	3
Intraspecies uncertainty factor	10
Cumulative uncertainty factor	100
Reference Concentration (RfC)	0.006 ppm (6 ppb; 0.009 mg/m^3; 9 $\mu g/m^3$)

Both extrathoracic and tracheobronchial effects have been associated with exposures to hydrogen chloride. The REL was based on extrathoracic effects as humans are predicted to be relatively more susceptible to the effects of hydrogen chloride in that region. An intermediate LOAEL factor was used as the effects were both mild and occurring at a low incidence at the dose tested.

VII. Data Strengths and Limitations for Development of the REL

The USEPA based its RfC of 7 $\mu g/m^3$ on the same study. U.S. EPA evaluated this RfC as a having a low level of confidence because of (1) the use of only one dose; (2) limited toxicity evaluation; (3) the lack of reproductive toxicity data; and (4) the lack of chronic exposure studies (U.S. EPA, 1994). OEHHA agrees with this assessment. The database for chronic exposure to this common chemical is limited.

VIII. References

Burleigh-Flayer H, Wong KL, and Alarie Y. 1985. Evaluation of the pulmonary effects of HCl using CO_2 challenges in guinea pigs. Fundam. Appl. Toxicol. 5:978-985.

CARB. 1999b. Air toxics emissions data collected in the Air Toxics Hot Spots Program CEIDARS Database as of January 29, 1999.

GEOMET Technologies, Inc. 1981. Hydrogen chloride: Report 4, Occupational Hazard Assessment. U.S. Department of Health and Human Services, NIOSH, Cincinnati, OH. NTIS PB83-105296.

HSDB. 1999. Hazardous Substance Data Bank. National Library of Medicine, Bethesda, Maryland. WWW database (http://sis.nlm.nih.gov/sis1/).

Pavlova TE. 1976. Disturbance of development of the progeny of rats exposed to hydrogen chloride. Bull. Exp. Biol. Med. 82(7):1078-1081.

Sellakumar AR, Snyder CA, Solomon JJ, and Albert RE. 1985. Carcinogenicity of formaldehyde and hydrogen chloride in rats. Toxicol. Appl. Pharmacol. 81:401-406.

Stokinger HE. 1981. Hydrogen chloride, HCl. In: Clayton GD, and Clayton FE, eds. Patty's Industrial Hygiene and Toxicology. 3rd rev. ed. Volume 2B, Toxicology. New York: Wiley Interscience. pp.2959-2961.

Ten Bruggen Cate H.J. 1968. Dental erosion in industry. Br. J. Ind. Med. 25:249-266
Toxigenics Inc. 1984. 90-Day inhalation study of hydrogen chloride gas in B6C3F1 mice, Sprague-Dawley rats and Fischer-344 rats. Study conducted for CIIT, Research Triangle Park, NC. CIIT Docket No. 20915.

U.S.EPA.1999. United States Environmental Protection Agency. Integrated Risk Information System (IRIS). (CD-ROM Version) Washington, D.C: US Environmental Protection Agency.

U.S. EPA. 1990. United States Environmental Protection Agency. Interim Methods for Development of Inhalation Reference Concentrations. Environmental Criteria and Assessment Office, Office of Health and Environmental Assessment, Office of Research and Development, U.S. EPA, Research Triangle Park, NC. Review Draft.

Wohlslagel J, Di Pasquale LC, and Vernot EH. 1976. Toxicity of solid rocket motor exhaust of HCl, HF, and alumina on rodents. J. Combustion Toxicol. 3:61-70.

CHRONIC TOXICITY SUMMARY

HYDROGEN CYANIDE

(Formonitrile; hydrocyanic acid; prussic acid)

CAS Registry Number: 74-90-8

I. Chronic Toxicity Summary

Inhalation reference exposure level	**9 µg/m^3 (8 ppb)**
Critical effect(s)	CNS effects, thyroid enlargement, and hematological disorders in workers
Hazard index target(s)	Nervous system; endocrine system; cardiovascular system

II. Physical and Chemical Properties (HSDB, 1999)

Description	Colorless liquid/gas
Molecular formula	HCN
Molecular weight	27.03
Boiling point	25.6 ^{o}C
Melting point	-13.4 ^{o}C
Vapor pressure	630 torr @ 20°C
Solubility	Miscible in water, alcohol; slightly soluble in ether
Conversion factor	1 ppm = 1.10 mg/m^3 @ 25 ^{o}C

III. Major Uses or Sources

Hydrogen cyanide is used in a variety of syntheses including the production of adiponitrile (for nylon), methyl methacrylate, sodium cyanide, cyanuric chloride, chelating agents, pharmaceuticals, and other specialty chemicals. Manufacturing activities releasing hydrogen cyanide include electroplating, metal mining, metallurgy and metal cleaning processes. Additionally, hydrogen cyanide has some insecticide and fungicide applications (ATSDR, 1993). Fires involving some nitrogen-containing polymers, often found in fibers used in fabrics, upholstery covers, and padding, also produce hydrogen cyanide (Tsuchiya and Sumi, 1977).

Another common source of hydrogen cyanide is cigarette smoke. Levels in inhaled mainstream cigarette smoke range from 10 to 400 µg per cigarette (U.S. brands); 0.6% to 27% (w/w) of these mainstream levels are found in secondary or sidestream smoke (Fiskel *et al.*, 1981). The annual statewide industrial emissions from facilities reporting under the Air Toxics Hot Spots Act in

California based on the most recent inventory were estimated to be 188,665 pounds of hydrogen cyanide (CARB, 1999b).

IV. Effects of Human Exposure

Occupational epidemiological studies investigating hydrogen cyanide exposure are complicated by the mixed chemical environments created by synthetic and metallurgic processes. However, several reports indicate that chronic low exposure to hydrogen cyanide can cause neurological, respiratory, cardiovascular, and thyroid effects (Blanc *et al.*, 1985; Chandra *et al.*, 1980; El Ghawabi *et al.*, 1975). Although these studies have limitations, especially with incomplete exposure data, they also indicate that long-term exposure to inhaled cyanide produces CNS and thyroid effects.

El Ghawabi *et al.* (1975) studied 36 male electroplating workers in three Egyptian factories exposed to plating bath containing 3% copper cyanide, 3% sodium cyanide, and 1% sodium carbonate. Breathing zone cyanide concentrations ranged from 4.2 to 12.4 ppm (4.6 to 13.7 mg/m^3), with means from 6.4 to 10.4 ppm (7.1 to 11.5 mg/m^3), in the three factories at the time of this cross-sectional study. The men were exposed for a duration of 5 to 10 years, except for one man with 15 years exposure. Twenty non-exposed male volunteers were used as controls. None of the subjects, controls or workers, currently smoked cigarettes. Complete medical histories were taken, and medical exams were performed. Urinary levels of thiocyanate (a metabolite of cyanide) were utilized as a biological index of exposure. Thyroid function was measured as the uptake of radiolabeled iodine, since thiocyanate may block the uptake of iodine by the thyroid leading to iodine-deficiency goiters. Frequently reported symptoms in the exposed workers included headache, weakness, and altered sense of taste or smell. Lacrimation, abdominal colic, and lower stomach pain, salivation, and nervous instability occurred less frequently. Increased blood hemoglobin and lymphocyte counts were present in the exposed workers. Additionally, punctate basophilia were found in 78% (28/36) of the exposed subjects. Twenty of the thirty six exposed workers had thyroid enlargements, although there was no correlation between the duration of exposure with either the incidence or the degree of enlargement. Thyroid function test indicated significant differences in uptake between controls and exposed individuals after 4 and 24 hours. Urinary excretion of thiocyanates correlated with the breathing zone concentrations of cyanides. Symptoms persisted in 50% of the dyspneic workers in a 10-month nonexposure follow up period. This study reported a LOAEL of 6.4 ppm (7.1 mg/m^3) for the CNS symptoms and thyroid effects.

Another retrospective study (Blanc *et al.*, 1985) examined 36 former silver-reclaiming workers with long-term exposure to hydrogen cyanide fumes. The authors found significant trends between the incidence of self-reported CNS symptoms during active employment (headache, dizziness, nausea, and bitter almond taste), the symptoms reported post-exposure, and a qualitative index of exposure retroactively defined by the investigators as low-, moderate-, or high-exposure through work histories. Some symptoms persisted for 7 months or more after exposure. None of the workers had palpable thyroid gland abnormalities, but clinical tests revealed decreases in vitamin B12 absorption and folate levels and statistically significant

increases in thyroid-stimulating hormone levels, which in combination with the CNS effects, suggest long-term adverse effects associated with cyanide exposure.

Due to the systemic nature of the lesions produced by cyanide, orally ingested cyanide will likely result in injuries similar to that seen by inhalation exposure. Cassava root, a dietary staple in many tropical regions, contains cyanogenic glycosides such as linamarin which release cyanide (CN^-) when metabolized endogenously (Sharma, 1993; Kamalu, 1995). Consumption of insufficiently processed cassava roots over a period of time in combination with a protein deficient diet has been implicated in neurotoxic effects. One such neuropathy known as konzo results in nerve cell degeneration leading to a permanent but non-progressive spastic weakness of the legs and degeneration of corresponding corticospinal pathways (Tylleskar *et al*., 1992; Tor-Agbidye *et al*., 1999). The development of this sydrome is hypothesized to depend on (a) the amount and duration of exposure to dietary cyanide, and (b) the ability of the body to detoxify cyanide, a function that may vary with nutritional status. The endogenous conversion of cyanide to cyanate (OCN^-) is thought to be a contributor to the neurotoxic symptoms, but other substances found in cassava flour have been implicated (Obidoa and Obasi, 1991; Tor-Agbidye *et al*., 1999; Kamalu, 1995). Tylleskar et al. (1992) determined daily cassava flour consumption at above 0.5 kg per adult in a konzo-affected, albeit malnourished, African population. Thus, the potential daily cyanide exposure was estimated to be 0.5-1 mmol (13-26 mg), which correlated well with urinary concentrations of the metabolite, thiocyanate. A similar daily cyanide intake via cassava ingestion was estimated at 15-31.5 mg (approximately 0.2-0.45 mg/kg) following a major outbreak of konzo in Mozambique (Casadei et al.,1984; Cliff et al., 1984).

Other effects associated with cassava consumption include pancreatic diabetes, vitamin B_{12} deficiency and decreased iodine uptake (Sharma, 1993; Jansz and Uluwaduge, 1997). Cretinism in children, associated with a deficiency of dietary iodine, is worsened by eating cassava (Miller, 1974). Excess thiocyanate due to cyanide metabolism results in a depressed uptake of iodine by the thyroid gland that may lead to symptoms of iodine deficiency, including goiter. A comparison of three villages in Ethiopia observed increased total goiter rate with increasing rate of cassava consumption (Abuye *et al*., 1998). Goiter was also more prevalent in females and in individuals under 20 years of age. In one village, the incidence of goiter increased following the introduction of cassava, indicating that cassava exacerbated pre-existing iodine deficiency. Urinary iodine levels of school children revealed marginal dietary consumption of iodine, but were within the normal range. However, low T4 and high TSH levels indicated insufficient iodine uptake by the thyroid gland due to cassava consumption.

V. Effects of Animal Exposures

There is little animal data for chronic inhalation exposure to hydrogen cyanide; only two subchronic studies were noted by U.S. EPA, one in rabbits (Hugod, 1979, 1981) and the other in dogs (Valade, 1952). Continuous exposure of rabbits to 0.5 ppm HCN (0.55 mg/m^3) for either 1 or 4 weeks produced no microscopically detectable morphological changes of the lungs, pulmonary arteries, coronary arteries or aorta. This study observed a subacute inhalation NOAEL for HCN in rabbits of 0.5 ppm (Hugod, 1979, 1981). Four dogs exposed to 50 mg/m^3 (45 ppm) hydrogen cyanide in a series of 30-minute inhalation periods conducted at 2-day intervals

demonstrated extensive CNS toxicity, including dyspnea and vomiting, with vascular and cellular CNS lesions identified post-mortem (Valade, 1952).

Male Sprague-Dawley rats were administered potassium cyanide (0, 40, 80, or 160 mg KCN/kg bw-day) in the drinking water for 13 weeks (Leuschner et al., 1991). At the highest dose, blood cyanide concentrations were between 16 and 26 mmol CN^-/ml blood and thiocyanate ranged between 341 and 877 mmmol SCN^-/ml plasma. The high dose group exposure was reduced to 140 mg/kg-day after 12 weeks because of decreased body weight gain, reduced drinking water consumption, and mortality in this group.

Male New Zealand white rabbits (6 per group) were administered potassium cyanide in the diet over a 40 week experiment (Okolie and Osagie, 1999). The average cyanide intake was 36.5 mg/day. Based on the growth data presented in the report, cyanide intake was estimated at approximately 20 mg/kg-day. The cyanide exposed group had higher feed consumption with reduced weight gain, and focal necrosis was noted in the liver and kidney.

Male weanling rats (strain not identified, 10 animals per group) were administered potassium cyanide (1500 ppm) in the diet for 11.5 months (Philbrick et al., 1979). There were no deaths or overt signs of toxicity. There was a reduction in body weight gain in the exposed group. Myelin degeneration was noted in the spinal cord white matter of cyanide exposed animals.

Kamalu (1993) fed groups of dogs (6/group; strain not specified) either a control diet containing rice as the carbohydrate source, a diet with cassava as a carbohydrate source, or a control diet containing NaCN, for 14 weeks. Both the cassava and NaCN diets were adjusted to release 10.8 mg HCN/kg cooked food. Growth was depressed only in the dogs fed rice + NaCN. Plasma thiocyanate was significantly lower in dogs fed cassava compared to dogs fed rice + NaCN. These effects indicate that all the intact cyanogenic glycosides absorbed from cassava, primarily linamarin, was not hydrolyzed to HCN. However, evidence of liver inflammation and hemorrhage were observed only in the cassava fed dogs. Kidney, adrenal, myocardial, and testicular lesions were noted in both treated groups but were considered more severe in the cassava fed dogs. It was concluded that the lesions observed in the cassava fed dogs were not entirely due to cyanide.

No information was found regarding developmental and reproductive effects in humans for any route of hydrogen cyanide exposure. No animal studies utilizing dermal exposure have been reported for either hydrogen cyanide or cyanide salts. Dietary studies of the high cyanogenic glycoside cassava diet have shown adverse effects, increased runting and decreased ossification in hamsters (Frakes *et al.*, 1986), but not in rats fed cassava alone, or supplemented with potassium cyanide (Tewe and Maner, 1981). Hamsters with gestational cassava exposure did not display reproductive effects (Frakes *et al.*, 1986).

VI. Derivation of Chronic Reference Exposure Level

Study	El Ghawabi *et al.* (1975); U.S. EPA (1994)
Study population	36 male electroplating workers
Exposure method	Discontinuous occupational inhalation exposures
Critical effects	CNS effects, thyroid enlargement, and hematological disorders
LOAEL	7.1 mg/m^3
NOAEL	Not observed
Exposure continuity	8 hr/day (10 m^3/day/20 m^3/day), 5 days/week
Average occupational exposure	2.5 mg/m^3 for LOAEL group
Human equivalent concentration	2.5 mg/m^3 for LOAEL group
Exposure duration	5 to 10 years (except one man for 15 years)
LOAEL uncertainty factor	10
Subchronic uncertainty factor	3
Interspecies uncertainty factor	1
Intraspecies uncertainty factor	10
Cumulative uncertainty factor	300
Inhalation reference exposure level	0.008 ppm (8 ppb, 0.009 mg/m^3, 9 $\mu g/m^3$)

The USEPA based its RfC of 3 $\mu g/m^3$ on the same study but included a Modifying Factor (MF) of 3 for lack of chronic and multigenerational reproduction studies. The criteria for use of modifying factors are not well specified by U.S. EPA. Such modifying factors were not used by OEHHA. OEHHA used a 3-fold subchronic uncertainty factor because most workers were exposed for less than ten years (78%) and many were exposed for less than 5 years (39%)..

An alternative analysis was conducted using data from an animal ingestion study reporting effects at low cyanide concentrations:

Study	Jackson (1988)
Study population	Miniature swine
Exposure method	Daily oral administration of aqueous potassium cyanide
Critical effects	Behavioral effects; decreased blood T_3 and T_4
LOAEL	0.4 mg/kg-day
NOAEL	Not observed
Exposure continuity	Apparently 7 days per week
Average exposure	0.4 mg/kg-day (1.4 mg/m^3 for LOAEL group assuming 20 m^3/day inhalation by a 70 kg person)
Human equivalent concentration	Not derived due to lack of species-specific data
Exposure duration	24 weeks
LOAEL uncertainty factor	3 (minimal effects at lowest dose)
Subchronic uncertainty factor	10 (based on assumed 27 year lifespan)
Interspecies uncertainty factor	10
Intraspecies uncertainty factor	10
Cumulative uncertainty factor	3,000
Inhalation reference exposure level	0.0005 mg/m^3 (0.5 µg/m^3; 0.0004 ppm; 0.4 ppb)

This study reported neurobehavioural and thyroid effects at cyanide exposure levels (equivalent to 1.4 to 4.2 mg/m^3) similar to that reported by El Ghawabi (2.5 mg/m^3). However, as greater uncertainty factors are required for use of the animal study, a lower REL was derived. Use of a cross-route extrapolation also introduces uncertainty. Therefore the REL derived from the human data is more appropriate.

VII. Data Strengths and Limitations for Development of the REL

The major strength of the RfC for hydrogen cyanide is the use of human health effects data. The major uncertainties are the lack of a NOAEL observation in the key study, the difficulty in estimating exposures, and the discontinuous and variable nature of the exposures.

VIII. References

Abuye C, Kelbessa U, and Wolde-Gebriel S. 1998. Health effects of cassava consumption in South Ethiopia. East African Med. J. 75(3):166-170.

ATSDR. 1993. Agency for Toxic Substances and Disease Registry. Toxicological Profile for Cyanide. U.S. Dept. of Health and Human Services, Public Health Service. TP-92/09.

Ansell M, and Lewis FAS. 1970. A review of cyanide concentrations found in human organs: A survey of literature concerning cyanide metabolism, ‘normal’, non-fatal and fatal body cyanide levels. J. Forensic Med. 17:148-155.

Blanc P, Hogan M, Mallin K, Hryhorczuk D, Hessl S, and Bernard B. 1985. Cyanide intoxication among silver-reclaiming workers. JAMA 253:367-371.

CARB. 1999. Air toxics emissions data collected in the Air Toxics Hot Spots Program CEIDARS Database as of January 29, 1999.

Casadei E, Jansen P, Rodrigues A, Molin A, and Rosling H. 1984. Mantakassa: An epidemic of spastic paraparesis associated with chronic cyanide intoxication in a cassava staple area of Mozambique. 2. Nutritional factors and hydrocyanic acid content of cassava products. Bull. World Health Org. 62(3): 485-492.

Chandra H, Gupta BN, Bhargave SH, Clerk SH, and Mahendra PN. 1980. Chronic cyanide exposure - a biochemical and industrial hygiene study. J. Anal. Toxicol. 4:161-165.

Cliff J, Martelli A, Mondlane E, Molin A, and Rosling H. 1984. Mantakassa: An epidemic of spastic paraparesis associated with chronic cyanide intoxication in a cassava staple area of Mozambique. 1. Epidemiology and clinical and laboratory findings in patients. Bull. World Health Org. 62 (3): 477-484.

El Ghawabi SH, Gaafar MA, El-Saharti AA, Ahmed SH, Malash KK, and Fares R. 1975. Chronic cyanide exposure: a clinical, radioisotope, and laboratory study. Br. J. Ind. Med. 32:215-219.

Fiskel J, Cooper C, and Eschenroeder A. 1981. Exposure and risk assessment for cyanide. EPA/440/4-85/008. NTIS PB85-220572.

Frakes RA, Sharma RP, Willhite CC, and Gomez G. 1986. Effect of cyanogenic glycosides and protein content in cassava diets on hamster prenatal development. Fundam. Appl. Toxicol. 7:191-198.

HSDB. 1999. Hazardous Substances Data Bank. National Library of Medicine, Bethesda, Maryland. WWW database (http://sis.nlm.nih.gov/sis1/).

Hugod C. 1979. Effect of exposure to 0.5 ppm hydrogen cyanide singly or combined with 200 ppm carbon monoxide and/or 5 ppm nitric oxide on coronary arteries, aorta, pulmonary artery, and lungs in the rabbit. Int. Arch. Occup. Environ. Health 44:13-23.

Hugod C. 1981. Myocardial morphology in rabbits exposed to various gas-phase constituents of tobacco smoke. Artherosclerosis 40:181-190.

Jackson LC. 1988. Behavioral effects of chronic sublethal dietary cyanide in an animal model: implications for humans consuming cassava (Manihot esculenta). Hum. Biol. 60(4):597-614.

Jansz ER and Uluwaduge DI. 1997. Biochemical aspects of cassava (Manihot esculenta Crantz) with special emphasis on cyanogenic glucosides – a review. J. Natn. Sci. Coun. Sri Lanka 25(1):1-24.

Kamalu BP. 1993. Pathological changes in growing dogs fed on a balanced cassava (Manihot esculenta Crantz) diet. Br. J. Nutr. 69(3):921-34.

Kamalu BP. 1995. The adverse effects of long-term cassava (*Manihot esculenta* Crantz) consumption. Int. J. Food Sci. Nutr. 46(1):65-93.

Leuschner J, Winkler A, Leuschner F. 1991. Toxicokinetic aspects of chronic cyanide exposure in the rat. Toxicol. Lett. 57(2):195-201.

Miller RW. 1974. Susceptibility of the fetus and child to chemical pollutants. Science 184:812-814.

Obidoa O and Obasi SC. 1991. Coumarin compounds in cassava diets: 2. Health implications of scopoletin in gari. Plant Foods Hum. Nutr. 41(3):283-289.

Okolie NP, Osagie AU. 1999. Liver and kidney lesions and associated enzyme changes induced in rabbits by chronic cyanide exposure. Food Chem. Toxicol. 37(7):745-750.

Philbrick DJ, Hopkins JB, Hill DC, Alexander JC, Thomson RG. 1979. Effects of prolonged cyanide and thiocyanate feeding in rats. J. Toxicol. Environ. Health 5(4):579-592.
Sharma RP. 1993. Cyanide containing foods and potential for fetal malformations. In: Dietary Factors and Birth Defects (RP Sharma, ed.), Pacific Division, AAAS, San Francisco, CA.

Tewe OO, and Maner JH. 1981. Long-term and carry-over effect of dietary inorganic cyanide (KCN) in the life cycle performance and metabolism of rats. Toxicol. Appl. Pharmacol. 58:1-7.

Tor-Agbidye J, Palmer VS, Lasarev MR, Craig AM, Blythe LL, Sabri MI, Spencer PS. 1999. Bioactivation of cyanide to cyanate in sulfur amino acid deficiency: Relevance to neurological disease in humans subsisting on cassava. Toxicol. Sci. 50(2):228-235.

Tsuchiya Y, and Sumi, K. 1977. Thermal decomposition products of polyacrylonitrile. J. Appl. Polym. Sci. 21:975-980.

Tylleskar T, Banea M, Bikangi N, Cooke RD, Poulter NH, and Rosling H. 1992. Cassava cyanogens and konzo, an upper motoneuron disease found in Africa. Lancet (N Am Ed). 339(8787):208-211.

U.S.EPA. 1999. U.S. Environmental Protection Agency. Integrated Risk Information System (IRIS) Database. Reference concentration (RfC) for Hydrogen Cyanide.

Valade MP. 1952. [Central nervous system lesions in chronic experimental poisoning with gaseous hydrocyanic acid.] Bull. Acad. Nat. Med. (Paris) 136:280-285.

CHRONIC TOXICITY SUMMARY

HYDROGEN SULFIDE

(hydrogen sulphide; dihydrogen sulfide; dihydrogen monosulfide; sulfur hydride; sulfureted hydrogen; hydrosulfuric acid)
CAS registry number: 7783-06-4

I. Chronic Toxicity Summary

Inhalation reference exposure level	**10 μg/m^3 (8 ppb)**
Critical effect(s)	Nasal histological changes in B6C3F1 mice
Hazard index target(s)	Respiratory system

II. Physical and Chemical Properties (HSDB, 1999)

Description	Colorless gas
Molecular formula	H_2S
Molecular weight	34.08
Density	1.4 g/L @ 25° C (air = 1) (AIHA, 1991)
Boiling point	–60.7° C (CRC, 1994)
Melting point	–85.5ºC (CRC, 1994)
Vapor pressure	15,600 Torr @ 25ºC
Solubility	Soluble in water, hydrocarbon solvents, ether, and ethanol
Odor threshold	8.1 ppb (11 μg/m^3) (Amoore and Hautala, 1983)
Odor description	Resembles rotten eggs
Conversion factor	1 ppm = 1.4 mg/m^3 @ 25° C

III. Major Uses or Sources

Hydrogen sulfide (H_2S) is used as a reagent and an intermediate in the preparation of other reduced sulfur compounds (HSDB, 1999). It is also a by-product of desulfurization processes in the oil and gas industries and rayon production, sewage treatment, and leather tanning (Ammann, 1986). The annual statewide industrial emissions from point sources at facilities reporting under the Air Toxics Hot Spots Act in California based on the most recent inventory were estimated to be 5,688,172 pounds of hydrogen sulfide (CARB, 1999).

IV. Effects of Human Exposure

Although numerous case studies of acutely toxic effects of H_2S exist, there is inadequate occupational or epidemiological information for specific chronic effects in humans exposed to H_2S.

Bhambhani and Singh (1991) showed that 16 healthy subjects exposed for short durations to 5 ppm (7 mg/m^3) H_2S under conditions of moderate exercise exhibited impaired lactate and oxygen uptake in the blood. Bhambhani and Singh (1985) reported that exposure of 42 individuals to 2.5 to 5 ppm (3.5 to 7 mg/m^3) H_2S caused coughing and throat irritation after 15 minutes.

In another study, ten asthmatic volunteer subjects were exposed to 2 ppm H_2S for 30 minutes and pulmonary function was tested (Jappinen *et al.*, 1990). All subjects reported detecting "very unpleasant" odor but "rapidly became accustomed to it." Three subjects reported headache following exposure. No significant changes in mean FVC or FEV_1 were reported. Although individual values for specific airway resistance (SR_{aw}) were not reported, the difference following exposure ranged from –5.95% to +137.78%. The decrease in specific airway conductance, SG_{aw}, ranged from -57.7% to +28.9%. The increase in mean SR_{aw} and decrease in mean SG_{aw} were not statistically significant.

Kilburn and Warshaw (1995) investigated whether people exposed to sulfide gases, including H_2S, as a result of working at or living downwind from the processing of "sour" crude oil demonstrated persistent neurobehavioral dysfunction. They studied thirteen former workers and 22 neighbors (of a California coastal oil refinery) who complained of headaches, nausea, vomiting, depression, personality changes, nosebleeds, and breathing difficulties. Their neurobehavioral functions and a profile of mood states were compared to 32 controls (matched for age and educational level). The exposed subjects' mean values were statistically significantly different (abnormal) compared to controls for several tests (two-choice reaction time; balance (as speed of sway); color discrimination; digit symbol; trail-making A and B; immediate recall of a story). Their profile of mood states scores were much higher than those of controls. Visual recall was significantly impaired in neighbors, but not in the former workers. The authors concluded that neurophysiological abnormalities were associated with exposure to reduced sulfur gases, including H_2S from crude oil desulfurization.

Xu *et al*. (1998) conducted a retrospective epidemiological study in a large petrochemical complex in Beijing, China in order to assess the possible association between petrochemical exposure and spontaneous abortion. The facility consisted of 17 major production plants divided into separate workshops, which allow for the assessment of exposure to specific chemicals. Married women (n = 2853), who were 20-44 years of age, had never smoked, and who reported at least one pregnancy during employment at the plant, participated in the study. According to their employment record, about 57% of these workers reported occupational exposure to petrochemicals during the first trimester of their pregnancy. There was a significantly increased risk of spontaneous abortion for women working in all of the production plants with frequent exposure to petrochemicals compared with those working in nonchemical plants. Also, when a comparison was made between exposed and non-exposed groups within each plant, exposure to

petrochemicals was consistently associated with an increased risk of spontaneous abortion (overall odds ratio (OR) = 2.7 (95% confidence interval (CI) = 1.8 to 3.9) after adjusting for potential confounders). When the analysis was performed with the exposure information obtained from interview responses for (self reported) exposures, the estimated OR for spontaneous abortions was 2.9 (95% CI = 2.0 to 4.0). When the analysis was repeated by excluding those 452 women who provided inconsistent reports between recalled exposure and work history, a comparable risk of spontaneous abortion (OR 2.9; 95% CI = 2.0 to 4.4) was found. In analyses for exposure to specific chemicals, an increased risk of spontaneous abortion was found with exposure to most chemicals. There were 106 women (3.7% of the study population) exposed only to hydrogen sulfide, and the results for hydrogen sulphide (OR 2.3; 95% CI = 1.2 to 4.4) were significant. No hydrogen sulfide exposure concentration was reported.

Four workers were exposed for several minutes to concentrations of hydrogen sulfide sufficient to cause unconciousness. Four other workers were exposed chronically to H_2S and developed lacrimation, eye irritation, nausea, vomiting, headache, sore throat, and skin irritation but retained conciousness as the result of a 150-minute release. Both groups were subjected to olfactory testing 2 to 3 years later (Hirsch and Zavala, 1999). Six of eight workers showed deficits in odor detection and identification, with the workers who had experienced unconciousness most severely affected in the followup tests.

Three patients exposed acutely to unknown concentrations of hydrogen sulfide developed persistent cognitive impairment (Wasch *et al*,. 1989). While standard neurological and physical examinations were unremarkable, all three subjects had prolonged P-300 latencies and persistent neurological and neurobehavioral deficits.

V. Effects of Animal Exposure

Rats (Fischer and Sprague-Dawley, 15 per group) were exposed to 0, 10.1, 30.5, or 80 ppm (0, 14.1, 42.7, or 112 mg/m^3, respectively) H_2S for 6 hours/day, 5 days/week for 90 days (CIIT, 1983a,b). Measurements of neurological and hematological function revealed no abnormalities due to H_2S exposure. A histological examination of the nasal turbinates also revealed no significant exposure-related changes. A significant decrease in body weight was observed in both strains of rats exposed to 80 ppm (112 mg/m^3).

In a companion study, the Chemical Industry Institute of Toxicology conducted a 90-day inhalation study in mice (10 or 12 mice per group) exposed to 0, 10.1, 30.5, or 80 ppm (0, 14.1, 42.7, or 112 mg/m^3, respectively) H_2S for 6 hours/day, 5 days/week (CIIT, 1983c). Neurological function was measured by tests for posture, gait, facial muscle tone, and reflexes. Ophthalmological and hematological examinations were also performed, and a detailed necropsy was included at the end of the experiment. The only exposure-related histological lesion was inflammation of the nasal mucosa of the anterior segment of the noses of mice exposed to 80 ppm (112 mg/m^3) H_2S. Weight loss was also observed in the mice exposed to 80 ppm. Neurological and hematological tests revealed no abnormalities. The 30.5 ppm (42.5 mg/m^3) level was considered the NOAEL for histological changes in the nasal mucosa. (Adjustments were made by U. S. EPA to this value to calculate an RfC of 0.9 μg/m^3.)

Fischer F344 rats inhaled 0, 1, 10, or 100 ppm hydrogen sulfide for 8 hours/day for 5 weeks (Hulbert *et al*, 1989). No effects were noted on baseline measurements of airway resistance, dynamic compliance, tidal volume, minute volume, or heart rate. Two findings were noted more frequently in exposed rats: (1) proliferation of ciliated cells in the tracheal and bronchiolar epithelium, and (2) lymphocyte infiltration of the bronchial submucosa. Some exposed animals responded similarly to controls to aerosol methacholine challenge, whereas a subgroup of exposed rats were hyperreactive to concentrations as low as 1 ppm.

Male rats were exposed to 0, 10, 200, or 400 ppm H_2S for 4 hours (Lopez *et al.*, 1987). Samples of bronchoalveolar and nasal lavage fluid contained increased inflammatory cells, protein, and lactate dehydrogenase in rats treated with 400 ppm. Lopez and associates later showed that exposure to 83 ppm (116 mg/m^3) for 4 hours resulted in mild perivascular edema (Lopez *et al.*, 1988).

A study by Saillenfait *et al.* (1989) investigated the developmental toxicity of H_2S in rats. Rats were exposed 6 hours/day on days 6 through 20 of gestation to 100 ppm hydrogen sulfide. No maternal toxicity or developmental defects were observed..

Hayden *et al*. (1990) exposed gravid Sprague-Dawley rat dams continuously to 0, 20, 50, and 75 ppm H_2S from day 6 of gestation until day 21 postpartum. The animals demonstrated normal reproductive parameters until parturition when delivery time was extended in a dose dependent manner (with a maximum increase of 42% at 75 ppm). Pups which were exposed in utero and neonatally to day 21 postpartum developed with a subtle decrease in time of ear detachment and hair development and with no other observed change in growth and development through day 21 postpartum.

VI. Derivation of Chronic REL

Study	CIIT, 1983c
Study population	B6C3F1 mice (10-12 per group)
Exposure method	Discontinuous inhalation
Critical effects	Histopathological inflammatory changes in the nasal mucosa
LOAEL	80 ppm (112 mg/m^3)
NOAEL	30.5 ppm (42.5 mg/m^3)
Exposure continuity	6 hours/day, 5 days/week
Exposure duration	90 days
Average experimental exposure	5.4 ppm for NOAEL group (30.5 x 6/24 x 5/7)
Human equivalent concentration	0.85 ppm (gas with extrathoracic respiratory effects, RGDR = 0.16, based on mouse MV_a = 0.033 L/min; MV_h = 13.8 L/min; $SA_a(ET)$ = 3.0 cm^2; $Sa_h(ET)$ = 200 cm^3) (U.S. EPA, 1994)
LOAEL uncertainty factor	1
Subchronic uncertainty factor	3
Interspecies uncertainty factor	3
Intraspecies uncertainty factor	10
Cumulative uncertainty factor	100
Inhalation reference exposure level	8 ppb (10 μg/m^3)

The adverse effects reported in chronic animal studies occur at higher concentrations than effects seen in acute human exposures. For example, human irritation was reported at concentrations of 2.5-5 ppm for 15 minutes (Bhambhani and Singh, 1985), yet no effects on laboratory animals were observed at concentrations up to 80 ppm for 90 days. This suggests either that humans are more sensitive to H_2S, or that the measurements in laboratory animals are too crude to detect subtle measures of irritation. However, the uncertainty factor and HEC attempt to account for these interspecies differences.

VII. Data Strengths and Limitations for Development of the REL

Hydrogen sulfide is the leading chemical agent causing human fatalities following inhalation exposures. Although lower concentration acute exposures have been quantitatively studied with human volunteers, the dose-response relationship for human toxicity due to hydrogen sulfide exposure is not known. Thus, a major area of uncertainty is the lack of adequate long-term human exposure data. Subchronic (but not chronic) studies have been conducted with several animal species and strains, and these studies offer an adequate basis for quantitative risk assessment.

The strengths of the inhalation REL include the availability of controlled exposure inhalation studies in multiple species at multiple exposure concentrations, adequate histopathogical analysis, and the observation of a NOAEL.

Hydrogen sulfide has a strong unpleasant odor. The threshold for detection of this odor is low, but shows wide variation among individuals. A level of 7 $\mu g/m^3$, based on a 30 minute averaging time, was estimated by a Task Force of the International Programme on Chemical Safety (IPCS) (1981) to not produce odor nuisance in most situations. On the other hand, the current California Ambient Air Quality standard for hydrogen sulfide, based on a 1 hour averaging time, is 42 $\mu g/m^3$ (30 ppb).

Amoore (1985) analyzed a large number of reports from the scientific literature and found that reported thresholds for detection were log-normally distributed, with a geometric mean of 10 $\mu g/m^3$ (8 ppb). Detection thresholds for individuals were reported to be log-normally distributed in the general population, with a geometric standard deviation of 4.0, *i.e.* 68% of the general population would be expected to have a detection threshold for hydrogen sulfide between 2.5 and 40 $\mu g/m^3$ (2 and 32 ppb). Sources of variation included age, sex, medical conditions, and smoking. Training and alertness of the subject in performing the test also affected the results.

Amoore (1985) drew attention to the difference between a detection threshold under laboratory conditions, and the levels at which an odor could be recognized, or at which it was perceived as annoying. Analysis of various laboratory and sociological studies suggested that a level at which an odor could be recognized was typically a factor of three greater than the threshold for detection, while the level at which it was perceived as annoying was typically a factor of five greater than the threshold. Annoyance was characterized both in terms of esthetic or behavioral responses, and by physiological responses such as nausea and headache. He therefore predicted that, although at 10 $\mu g/m^3$ (the proposed REL) 50% of the general population would be able to detect the odor of hydrogen sulfide under controlled conditions, only 5% would find it annoying at this level. At 50 $\mu g/m^3$, 50% would find the odor annoying.

On this basis, the proposed REL of 10 $\mu g/m^3$ (8 ppb) is likely to be detectable by many people under ideal laboratory conditions, but it is unlikely to be recognized or found annoying by more than a few. It is therefore expected to provide reasonable protection from odor annoyance in practice. However, this consideration cannot be entirely dismissed due to the wide inter-individual variation in sensitivity to odors. Amoore (1985) also points out that many industrial operations generating hydrogen sulfide also generate organic thiol compounds with similar, but even more potent odors (e.g., methyl mercaptan, butyl mercaptan). Such compounds may in fact have detection thresholds as much as a hundred-fold lower than hydrogen sulfide, so even minute quantities have a powerful impact on odor perception. Because of the concurrent emission of these contaminants, the incidence of odor complaints near hydrogen sulfide emitting sites correlated poorly with the levels of hydrogen sulfide measured in the affected areas.

VIII. References

AIHA. 1991. American Industrial Hygiene Association. Emergency Response Planning Guideline for Hydrogen Sulfide. Set 6. Akron, HI: AIHA.

Ammann HM. 1986. A new look at physiologic respiratory response to H_2S poisoning. J. Haz. Mat. 13 :369-374.

Amoore JE (1985). The perception of hydrogen sulfide odor in relation to setting an ambient standard. Olfacto-Labs, Berkeley, CA: prepared for the California Air Resources board.

Amoore JE, and Hautala E. 1983. Odor as an aid to chemical safety: Odor thresholds compared with threshold limit values and volatilities for 214 chemicals in air and water dilution. J. Appl. Toxicol. 3(6):272-290.

Bhambhani Y, and Singh M. 1985. Effects of hydrogen sulphide on selected metabolic and cardio-respiratory variables during rest and exercise. Report submitted to Alberta Worker's Health and Safety and Compensation. June, 1985.

Bhambhani Y, and Singh M. 1991. Physiological effects of hydrogen sulfide inhalation during exercise in healthy men. J. Appl. Physiol. 71:1872-1877.

CARB. 1999. Air toxics emissions data collected in the Air Toxics Hot Spots Program CEIDARS Database as of January 29, 1999.

CIIT. 1983a. Chemical Industry Institute of Toxicology. 90-Day vapor inhalation toxicity study of hydrogen sulfide in Fischer-344 rats. U.S. EPA, Office of Toxic Substances Public Files. Fiche number 0000255-0. Document number FYI-OTS-0883-0255.

CIIT. 1983b. Chemical Industry Institute of Toxicology. 90-Day vapor inhalation toxicity study of hydrogen sulfide in Sprague-Dawley rats. U.S. EPA, Office of Toxic Substances Public Files. Fiche number 0000255-0. Document number FYI-OTS-0883-0255.

CIIT. 1983c. Chemical Industry Institute of Toxicology. 90-Day vapor inhalation toxicity study of hydrogen sulfide in B6C3F1 mice. U.S. EPA, Office of Toxic Substances Public Files. Fiche number 0000255-0. Document number FYI-OTS-0883-0255.

CRC. 1994. CRC Handbook of Chemistry and Physics, 75th edition. Lide DR, ed. Boca Raton, FL: CRC Press Inc.

Hannah RS, Hayden LJ, and Roth SH. 1989. Hydrogen sulfide exposure alters the amino acid content in developing rat CNS. Neurosci. Lett. 99(3):323-327.

Hannah RS, and Roth SH. 1991. Chronic exposure to low concentrations of hydrogen sulfide produces abnormal growth in developing cerebral Purkinje cells. Neurosci. Lett. 122(2):225-228.

Hayden LJ, Goeden H, and Roth SH. 1990. Growth and development in the rat during subchronic exposure to low levels of hydrogen sulfide. Toxicol. Ind. Health 6(3-4):389-401.Hirsch AR, Zavala G. 1999. Long-term effects on the olfactory system of exposure to hydrogen sulphide. Occup. Environ. Med. 56(4):284-287.

HSDB. 1999. Hazardous Substances Data Bank. U.S. National Library of Medicine, Bethesda, MD 20894. (http://sis.nlm.nih.gov/sis1/)

Hulbert, W.C., M.G. Prior, P. Pieroni and Z. Florence. 1989. Hyperresponsiveness in rats after 5 weeks exposure to hydrogen sulfide. Clin. Invest. Med. 12(4): B89.

International Programme on Chemical Safety. 1981. Environmental Health Criteria 19. Hydrogen Sulfide. Geneva: World Health Organization. p. 41.

Jappinen P, Vilkka V, Marttila O, Haahtela T. 1990. Exposure to hydrogen sulphide and respiratory function. Br. J. Ind. Med. 47(12):824-828.

Kilburn KH, Warshaw RH. 1995. Hydrogen sulfide and reduced-sulfur gases adversely affect neurophysiological functions. Toxicol. Ind. Health 11:185-197.

Lopez A, Prior M, Yong S, Albassam M, and Lillie L. 1987. Biochemical and cytological alterations in the respiratory tract of rats exposed for 4 hours to hydrogen sulfide. Fundam. Appl. Toxicol. 9:753-762.

Lopez A, Prior M, Lillie L, Gulayets C, and Atwal O. 1988. Histologic and ultrastructural alterations in lungs of rats exposed to sublethal to lethal concentrations of hydrogen sulfide. Vet. Pathol. 25:376-384.

Saillenfait A, Bonnet P, and DeCeaurriz J. 1989. Effects of inhalation exposure to carbon disulfide and its combination with hydrogen sulfide on embryonal and fetal development in rats. Toxicol. Lett. 48:57-66.

U.S.EPA. 1999. U.S. Environmental Protection Agency. Integrated Risk Information System (IRIS) database. Reference concentration (RfC) for hydrogen sulfide.

Wasch HH, Estrin WJ, Yip P, Bowler R, Cone JE. 1989. Prolongation of the P-300 latency associated with hydrogen sulfide exposure. Arch. Neurol. 46(8):902-904.

Xu X, Cho SI, Sammel M, You L, Cui S, Huang Y, *et al*. Association of petrochemical exposure with spontaneous abortion. Occup. Environ. Med. 1998;55(1):31-36.

CHRONIC TOXICITY SUMMARY

ISOPHORONE

(1,1,3-trimethyl-3-cyclohexene-5-one; 3,5,5-trimethyl-2-cyclohexen-1-one; isoforon; isoacetophorone)

CAS Registry Number: 78-59-1

I. Chronic Toxicity Summary

Inhalation reference exposure level	**2,000 μg/m^3** (400 ppb)
Critical effect(s)	Developmental effects (reduced crown-rump length of female rat fetuses); hepatocytomegaly and coagulative necrosis of the liver in mice
Hazard index target(s)	Development; liver

II. Chemical Property Summary (HSDB, 1995; CRC, 1994; CARB, 1997)

Description	Water-clear liquid with a peppermint-like odor
Molecular formula	$C_9H_{14}O$
Molecular weight	138.21 g/mol
Boiling point	215.2°C
Melting point	–8.1°C
Vapor pressure	0.44 torr at 25°C
Solubility	Slightly soluble in water (12,000 mg/L water at 25°C); miscible in organic solvents.
Conversion factor	5.65 μg/m^3 per ppb at 25°C

III. Major Uses and Sources

Isophorone is used extensively as a solvent in some printing inks, paints, lacquers, adhesives, vinyl resins, copolymers, coatings, finishes, and pesticides, in addition to being used as a chemical intermediate (HSDB, 1995). Since this compound has many different applications, release to the environment may originate from a wide variety of industrial sources including iron and steel manufacturers, manufacturers of photographic equipment and supplies, automobile tire plants, and printing operations. Coal-fired power plants may also emit isophorone to the air. Although it is mostly a man-made compound, isophorone has been found to occur naturally in cranberries (ATSDR, 1989). Occupational exposure may occur by inhalation or dermal contact. The annual statewide industrial emissions from facilities reporting under the Air Toxics Hot Spots Act in California based on the most recent inventory were estimated to be 2809 pounds of isophorone (CARB, 2000).

IV. Effects of Human Exposures

No information is available concerning long-term exposure or pharmacokinetics of isophorone in humans. In occupational monitoring studies, the time-weighted average concentration in breathing zones and workplace air of a screening plant ranged from 8.3-23 ppm and from 3.5-14.5 ppm, respectively (Samimi, 1982). Up to 25.7 ppm was detected in air of a silk screening printing plant in Pittsburgh, PA (Kominsky, 1983). The concentration in breathing zone samples from a decal manufacturing plant in Ridgefield, NJ was 0.7-14 ppm (Lee and Frederick, 1982). It was suspected that the reported eye and nose irritation of workers at the silk screening plant and at the decal manufacturing plant was the result of acute and subacute exposure to isophorone vapors.

Workers exposed to 5-8 ppm (28-45 mg/m^3) of isophorone for one month complained of fatigue and malaise (NIOSH, 1978). When concentrations were reduced to 1-4 ppm, no adverse effects were reported. Acute exposure studies in humans (up to 400 ppm for 1 to 4 minutes) resulted in eye, nose and throat irritation, nausea, headache, and dizziness or faintness (Union Carbide, 1963). Inhalation exposure for 15 minutes to 10 ppm isophorone produced only mild effects in human subjects while 25 ppm produced irritation to eyes, nose, and throat (Silverman *et al.*, 1946).

V. Effects of Animal Exposures

Few reports have been published regarding the pharmacokinetics of isophorone in experimental animals. Isophorone was widely distributed in the major organs of the rat following 4 hour inhalation exposure to 400 ppm (ATSDR, 1989). Oral gavage of 4000 mg/kg body wt to rats and a rabbit also resulted in wide distribution of the chemical. The highest blood levels of isophorone were reached by 30 min in rabbits following oral gavage and had decreased dramatically by 21 hours, indicating rapid absorption and elimination of the chemical. Preliminary results of a pharmacokinetic study indicate that rats treated orally with ^{14}C-isophorone excreted 93% of the radiolabel in the urine, expired air, and feces in 24 hours (ATSDR, 1989). The highest levels of ^{14}C-isophorone were found in the liver, kidney, preputial gland, testes, brain, and lungs. Several metabolites were identified in the urine of orally dosed rats and rabbits, including 3-carboxy-5,5-dimethyl-2-cyclohexene-1-one, 3,5,5-trimethylcyclohexanol, and some glucuronide conjugates (Dutertre-Catella *et al.*, 1978). A portion of the chemical was excreted unchanged in expired air.

In an early inhalation study, 10 Wistar rats/group and 10 guinea pigs/group, all of mixed sex, were exposed to 0, 25, 50, 100, 200, or 500 ppm isophorone 8 hr/day, 5 days/week for 6 weeks (Smyth *et al.*, 1942). Increased mortality and reduced body weights were observed at 100 ppm and up in both species. However, eye and nose irritation was noted only at the highest dose. Minor changes in blood chemistry and histopathological changes in the kidney and lungs were noted in treated animals. However, later investigations determined that the isophorone used in this study was contaminated with appreciable amounts of compounds (Rowe and Wolf, 1963).

Therefore, some of the adverse effects (i.e., the lung lesions) may have been due to the contaminants. The accuracy of the concentration data in the 1942 study is also questionable.

No treatment-related histopathological lesions were found in lungs, livers, or kidneys of male and female rats exposed intermittently (6 hr/day, 5 days/week) to 37 ppm isophorone for 4 weeks compared to controls (Hazleton Labs, 1968; summarized by ATSDR, 1989). Histological examination was limited to 30% of the control and treated rats. Body weight gain, mean absolute liver weights, and mean liver-to-body weight ratios of treated rats were significantly reduced compared to controls. Slight variations in hematological findings were noted in treated rats (increased lymphocytes and hemoglobin content; decreased neutrophils) but were not considered different from controls.

Rats (10/sex) were exposed to 500 ppm isophorone 6 hr/day, 5 days/week for up to 6 months (Dutertre-Catella, 1976; summarized by ATSDR, 1989). Irritation of eyes and nasal mucosa was observed. One female and three males in the treatment group died during the study, which was considered to be a treatment-related effect. But no exposure-related histopathological lung or liver lesions were observed compared to controls. Dutertre-Catella (1976) also exposed rats and rabbits (number per group per sex not stated) to 250 ppm isophorone 6 hr/day, 5 days/week for 18 months (Dutertre-Catella, 1976). Irritation of eyes and nasal mucosa was observed in both species, but no deaths occurred in the treatment groups. Histopathological examination of the lungs and kidneys, urinalysis, and hematological analysis revealed no exposure-related changes in either species. However, cytoplasmic microvacuolization of hepatocytes was observed in both species (ATSDR, 1989).

In a 90-day feeding study, 20 CFE albino rats/group/sex were given isophorone in their diet at concentrations of 0, 750, 1500, or 3000 ppm. Four beagle dogs/group/sex received isophorone in gelatin capsules at concentrations of 0, 35, 75, or 150 mg/kg body wt-day (AME, 1972a,b). High dose rats exhibited slightly reduced weight gain compared to controls (8-10%) for most of the study. Average weight gain among the exposure groups of beagle dogs remained essentially unchanged during the entire study. Urinalysis, hematology, and clinical chemistry indices found no treatment-related effects in the animals at either the interim or final toxicological examinations. Gross pathology and a limited histopathological examination observed no treatment-related effects in either species. Data on isophorone purity and possible loss of isophorone from rat diet due to vaporization were not presented.

In the most comprehensive isophorone toxicity study to date, 50 F344/N rats/group/sex and 50 B6C3F1 mice/group/sex were administered 0, 250 or 500 mg isophorone/kg body wt 5 days/week by oral gavage (in corn oil) for 103 weeks (Bucher *et al.*, 1986; NTP, 1986). Clinical signs of toxicity were not seen during the length of the study. However, several deaths in male and female rats at the high dose occurred early in the study. A steep decline in survival rate of high dose male rats occurred after week 90. Male and female rats and female mice in the high dose group exhibited only a slight decrease in body weight (<10%) compared to controls. A 13-week range finding study for the 2-year study did not find compound-related lesions in the kidney (or any other organs) of rats and mice exposed up to 1000 mg/kg body wt-day. However, pathological examination of rats exposed to isophorone for 2 years revealed non-neoplastic lesions in the kidney. Increased mineralization of the collecting ducts in isophorone-exposed

male (but not female) rats was observed. This lesion was characterized by basophilic aggregates of mineral most often found in the medullary collecting ducts and occurred coincidentally with lesions of chronic nephropathy. Nephropathy was observed in almost half the female controls and nearly all the male controls. Isophorone exposure appeared to increase both the severity of nephropathy in low dose male rats and the incidence of nephropathy in dosed female rats, but the effects were not pronounced. However, the isophorone potentiation of nephropathy in rats may be due to 'male rat-specific nephropathy' and may not have any relevance to human exposure (Strasser *et al.* 1988). Other adverse effects in kidneys of isophorone-treated male rats include tubular cell hyperplasia (in a dose-related manner) and epithelial hyperplasia of the renal pelvis. In mice, an increased incidence of chronic focal inflammation was observed in the kidneys of males, but was not considered treatment-related. A dose-dependent increase in fatty metamorphosis occurred in the adrenal cortex of male rats, but the biological significance of this change is unknown. All isophorone-exposed male mice had an increased incidence of hepatocytomegaly and coagulative necrosis of the liver. However, treatment-related liver lesions were not observed in female mice. Increased incidence of hyperkeratosis of the forestomach was observed in dosed male and high dose female mice, but was probably not a relevant treatment-related effect.

Published studies on possible reproductive effects of isophorone are lacking. An unpublished inhalation study conducted by a commercial laboratory (Bio/dynamics, 1984b) studied possible teratogenicity due to isophorone in rats or mice at inhaled doses up to 115 ppm. Groups of 22 female rats and 22 female mice were exposed to 0, 25, 50, or 115 ppm isophorone (6 hr/day) on gestational days 6-15. Maternal toxicity in rats included dose-dependent alopecia and cervical/anogenital staining. Low body weights (7-8%) were occasionally observed in the 115 ppm group. In mice, maternal toxicity was confined to slightly decreased weight (7-8%) on one day in the 115 ppm group. No significant differences were found in uterine implantations, fetal toxicity, and external and internal malformations among the animals. However, a slight, but significant, growth retardation in the form of decreased crown-rump length was present among the high dose fetal rats. Also, a slight, but insignificant, increase in extra ribs and/or rudimentary ribs was seen in rat and mouse fetuses at the highest dose. In a pilot study for this developmental toxicity investigation (12 females/species), exencephaly was observed in 1 rat and 1 mouse undergoing late reabsorption and in 2 live rat fetuses from dams exposed to 150 ppm isophorone on gestational days 6-15 (Bio/dynamics, 1984a). Exencephaly was not observed at any dose level in the primary study.

Dutertre-Catella (1976) did not find adverse reproductive or developmental effects in rats exposed to 500 ppm isophorone (6 hr/day, 5 days/week) for 3 months before mating and throughout gestation (females only) as well. The pups were not examined for internal malformations so the study was incomplete for determination of developmental effects.

VI. Derivation of Chronic Reference Exposure Level (REL)

Study	Bio/dynamics 1984a,b
Study population	22 female mice/group, 22 female rats/group
Exposure method	Discontinuous whole body inhalation exposure during gestation (0, 25, 50, or 115 ppm)
Critical effects	Developmental effects (reduced crown-rump length of female rat fetuses); teratogenicity (exencephaly in fetal rats and mice) in range finding study at 150 ppm
LOAEL	115 ppm for reduced crown-rump length of female rat fetuses
NOAEL	50 ppm
Exposure continuity	6 hr/day during gestation
Exposure duration	Days 6-15 of gestation
Average experimental exposure	12.5 ppm (50 x 6/24)
Human equivalent concentration	12.5 ppm (gas with systemic effects, based on RGDR = 1.0 using default assumption that lambda (a) = lambda (h))
LOAEL uncertainty factor	1
Subchronic uncertainty factor	1
Interspecies uncertainty factor	3
Intraspecies uncertainty factor	10
Cumulative uncertainty factor	30
Inhalation reference exposure level	0.4 ppm (400 ppb, 2 mg/m^3, 2,000 μg/m^3)

The inhalation study by Bio/dynamics (1984a,b) presents data that indicate exposure during gestation may be the most sensitive indicator of non-neoplastic toxicity by isophorone. Exposure of pregnant rats to 115 ppm isophorone during gestation resulted in significant growth retardation of female rat fetuses (reduced crown-rump length). Exposure to 50 ppm isophorone, the NOAEL, produced no developmental effects. The authors had removed the two shortest female fetuses prior to statistical analysis. The result was that there was no significant difference in fetal growth retardation; therefore, this adverse effect is not significant. However, this selective culling before the statistical analysis is not scientifically appropriate in this case. In addition, the authors did not perform some of the scheduled fetal examinations. Otherwise, the growth retardation might have had even greater statistical significance. The pilot study (Bio/dynamics, 1984a) observed exencephaly in a few mouse and rat fetuses at 150 ppm. Exencephaly was not considered significant by the authors because it was not present in any fetuses of the primary study (Bio/dynamics, 1984b). However, exencephaly is included as a critical effect in this summary because it is considered a serious teratogenic effect that was present at a dose (150 ppm) only slightly higher than the LOAEL of the primary study (115 ppm). Alopecia of adult female rats was observed in many of the exposed animals. However, this effect may be considered more of an acute dermal irritation than a chronic effect. In addition, cervical and anogenital staining seen in many exposed rats is not considered a chronic ‘adverse’ effect.

For comparison with the proposed REL of 0.4 ppm, the inhalation LOAEL of 250 ppm for mild liver effects (Dutertre-Catella, 1976) in rats and rabbits intermittently exposed to isophorone for 18 months was used to estimate a REL. Use of a time adjustment (6/24 x 5/7), an RGDR of 1, and a total UF of 100 (LOAEL to NOAEL = 3, interspecies = 3, and intraspecies = 10), also resulted in an estimated REL of 0.4 ppm. These results indicate that the REL will also protect against adverse liver effects.

While the toxicological significance of this liver effect observed by Dutertre-Catella (1976) is unknown, the NTP (1986) study observed an increased incidence of hepatocytomegaly and coagulative necrosis of the liver in treated male mice, but not in female mice and rats, orally gavaged with isophorone. Using 250 mg/kg-day as a LOAEL for mice and dividing by a total UF of 1000 (10 each for LOAEL to NOAEL, 10 for interspecies, and 10 for intraspecies) results in an oral REL of 0.25 mg/kg-day. Multiplying the oral REL by 3,500 $\mu g/m^3$ per mg/kg-day for route-to-route extrapolation results in a chronic inhalation REL estimate of 900 $\mu g/m^3$ (0.16 ppm), which is in good agreement with the REL developed from Dutertre-Catella (1976) and Biodynamics (1984a,b).

VII. Data Strengths and Limitations for Development of the REL

The strength of the database for isophorone is the consistent lack of relevant severe histopathological effects in the chronic inhalation study (Dutertre-Catella, 1976) and in the oral gavage study (NTP, 1986). Weaknesses of the database for isophorone include the lack of human exposure data, the lack of comprehensive long-term inhalation studies, and the lack of published peer-reviewed reproductive/developmental studies. The lack of human data may be due to isophorone's rather low potency for causing chronic, non-neoplastic, adverse effects. Inhalation of isophorone is a relevant route of exposure under occupational settings, but is most likely a minor route of exposure for the general population. Due to the insufficient characterization of the kidney and liver lesions in the oral gavage NTP study (Bucher *et al*, 1986; NTP, 1986) and the inhalation study (Dutertre-Catella, 1976), a comprehensive chronic study in rodent and non-rodent species would enhance the database for isophorone.

VIII. Potential for Differential Impacts on Children's Health

Since the REL is based on a developmental study, it is expected to be adequately protective of infants and children. However, there is no direct evidence in the literature to quantify a differential effect of isophorone in children relative to adults. Isophorone occurs in cranberries and thus presumably in cranberry juice, which is often mixed with other fruit juices. Children tend to consume more fruit juice. However, isophorone as a Hot Spot emission is unlikely to be a multimedia chemical, and there is no evidence to suggest that normal dietary levels of isophorone are associated with adverse health effects.

IX. References

AME. 1972a. Affiliated Medical Enterprises, Inc. 90-Day subchronic toxicity of isophorone in the rat (final report). Unpublished study performed by Affiliated Medical Enterprises, Inc. Princeton, NJ for Rohm and Haas Co. Philadelphia, PA. OTS 8d submission Doc. ID. 87812179, Microfiche No. 205975.

AME. 1972b. Affiliated Medical Enterprises, Inc. 90-Day subchronic toxicity of isophorone in the dog (final report). Unpublished study performed by Affiliated Medical Enterprises, Inc. Princeton, NJ for Rohm and Haas Co. Philadelphia, PA. OTS 8d submission Doc. ID. 87812178, Microfiche No. 205975.

ATSDR. 1989. Agency for Toxic Substances and Disease Registry. Toxicological profile for isophorone. U.S. Public Health Service. Atlanta, GA: ATSDR. PB90-180225.

Bio/dynamics. 1984a. Inhalation teratology probe study in rats and mice. Project No. 323771. Unpublished study performed by Bio/dynamics Inc. East Millstone, NJ. OTS Section 4 submission Doc. ID 40-8455042. Microfiche No. OTS0507219, pp. 1-33.

Bio/dynamics. 1984b. Inhalation teratology study in rats and mice. Final Report 3223772. Unpublished study performed by Bio/dynamics Inc. East Millstone, NJ for Exxon Biomedical Science, East Millstone NJ. OTS Section 4 submission Doc. ID 40-855049. Microfiche No. OTS 0507224, pp. 1-107.

Bucher JR, Huff J, and Kluwe WM. 1986. Toxicological and carcinogenesis studies of isophorone in F344 rats and B6C3F1 mice. Toxicology 39:207-219.

CARB. 1997. California Air Resources Board. Toxic Air Contaminant Identification List Summaries.

CARB. 2000. California Air Resources Board. California Emissions Inventory Development and Reporting System. (CEIDARS). Data from Data Base Year 1998. February 12, 2000.

CRC. 1994. CRC Handbook of Chemistry and Physics, 75th edition. Lide DR, ed. Boca Raton, FL: CRC Press Inc.

Dutertre-Catella H. 1976. Contribution to the analytical toxicological and bio-chemical study of isophorone (in French). Thesis for doctorate in pharmacology, Universite Rene Descartes, Paris. [Cited in Joint Assessment of Commodity Chemicals, No. 110, Isophorone, ECETOC, Brussels, 1989.]

Dutertre-Catella H, Nguyen PL, Dang Quoc Q, and Truhaut R. 1978. Metabolic transformations of the 3,5,5-2-cyclohexene-1-one trimethyl (isophorone). Toxicol. Eur. Res. 1(4):209-216.

Hazleton Labs. 1968. Assessment and comparison of subacute inhalation toxicities of three ketones. Final Report. Prepared by Hazleton Laboratories, Inc. Falls Church, VA for Exxon Chem Amers. Houston, TX. OTS 8d submission Doc ID. 878210935, Microfiche No. 206267.

HSDB. 1995. Hazardous Substances Data Bank. National Library of Medicine, Bethesda, MD (TOMES® CD-ROM Version). Denver, CO: Micromedex, Inc. (Edition expires 11/31/95).

Kominsky JR. 1983. Health hazard determination report no. HE 78-107-563. Pittsburgh, PA: Swinston Company.

Lee SA, and Frederick L. 1982. NIOSH health hazard evaluation report no. HHE80-103-827; NTIS PB82-189226.

NIOSH. 1978. National Institute for Occupational Safety and Health. Occupational exposure to ketones: criteria for a recommended standard. U.S. Department of Health, Education, and Welfare. DHEW (NIOSH) Publication No. 78-173.

NTP. 1986. National Toxicology Program. Toxicology and carcinogenesis studies of isophorone in F/344 rats and $B6C3F_1$ mice. NTP TR 291. NIH Publication No. 86-2547.

Rowe VK, and Wolf MA. 1963. Ketones. In: Industrial Hygiene and Toxicology, Second ed. Patty FA (ed.) New York: Interscience Publishers. p. 1764.

Samimi B. 1982. Exposure to isophorone and other organic solvents in a screen printing plant. Am. Ind. Hyg. Assoc. J. 43(1):43-48.

Silverman L, Schulte HF, and First MW. 1946. Further studies on sensory response to certain industrial solvent vapors. J. Ind. Hyg. Toxicol. 28(6):262-266.

Strasser J Jr, Charbonneau M, Borgoff SJ, Turner MJ, and Swenberg JA. 1988. Renal protein droplet formation in male Fischer 344 rats after isophorone (IPH) treatment. Toxicologist 8(1):136 (abstract).

Smyth HF Jr, Seaton J, and Fischer L. 1942. Response of guinea pigs and rats to repeated inhalation of vapors of mesityl oxide and isophorone. J. Ind. Hyg. Toxicol. 24(3):46-50.

Union Carbide Corporation. 1963. Toxicology Studies - Isophorone Summary Data Sheet. Industrial Medical Toxicology Dept. New York: Union Carbide.

CHRONIC TOXICITY SUMMARY

ISOPROPANOL

(2-propanol; dimethylcarbinol; isopropyl alcohol)

CAS Registry Number: 67-63-0

I. Chronic Toxicity Summary

Inhalation reference exposure level	**7,000 μg/m^3 (3000 ppb)**
Critical effect(s)	Kidney lesions in mice and rats; fetal growth retardation and developmental anomalies in rats
Hazard index target(s)	Kidney; development

II. Chemical Property Summary (HSDB, 1995)

Description	Colorless liquid at room temperature (25°C) with a pleasant odor. Slightly bitter taste.
Molecular formula	C_3H_8O
Molecular Weight	60.09
Boiling point	82.5°C
Vapor Pressure	44.0 torr at 25°C
Solubility	Miscible in water and most organic solvents; insoluble in salt solutions.
Conversion factor	1 ppb = 2.45 μg/m^3 at 25°C

III. Major Uses and Sources

Isopropanol is used as a solvent and in making many commercial products (HSDB, 1995). The annual production volume of isopropanol has been in excess of one billion pounds since 1956; it was ranked 50th among chemicals produced in the U.S. in 1994 (C&EN, 1995). Rubbing alcohol is a solution of 70% isopropanol in water. Specific uses and sources include: a component of antifreeze; a solvent for gums, shellac, essential oils, creosote and resins; extraction of alkaloids; component of quick drying oils and inks; component of denaturing alcohol; antiseptic for hand lotions; rubefacient; component of household products (after-shave lotions, cosmetics, etc.); the manufacture of acetone; deicing agent for liquid fuels; dehydrating agent and synthetic flavoring adjuvant. Isopropanol can enter the environment as emissions from its manufacture and use as a solvent. It naturally occurs as a plant volatile and is released during the microbial degradation of animal wastes. Human exposure will be both in occupational atmospheres and from use of consumer products containing isopropanol as a volatile solvent. An odor threshold has been estimated as 22 ppm (Amoore and Hautala, 1983), which is 7-fold higher than the chronic REL.

The annual statewide industrial emissions from facilities reporting under the Air Toxics Hot Spots Act in California based on the most recent inventory were estimated to be 525,826 pounds of isopropanol (CARB, 1999b).

IV. Effects of Human Exposures

Currently, there are no adequate chronic exposure data for isopropanol in humans. While isopropanol is not considered a dermal irritant, it is a defatting agent and can cause dermatitis with prolonged exposure to skin (IARC, 1977). A subacute study of daily oral intake of isopropanol (2.6 or 6.4 mg/kg body weight) by groups of 8 men for 6 weeks had no effect on blood cells, serum or urine and produced no subjective symptoms (Wills *et al.*, 1969). A pharmacokinetic study of men occupationally exposed to isopropyl alcohol revealed that uptake occurs readily via the inhalation route; acetone is the major metabolite (Brugnone *et al.*, 1983). Acetone was eliminated mainly by the lung but was also eliminated in the urine.

V. Effects of Animal Exposures

In metabolism studies with rats and mice, up to 92% of the administered dose (via i.v. or inhalation) of isopropanol was exhaled as acetone, CO_2 and the unmetabolized alcohol (Slauter *et al.*, 1994). Approximately 3-8% of the administered dose was excreted in urine as isopropanol, acetone, and a metabolite tentatively identified as isopropyl glucuronic acid. Isopropanol is readily absorbed from the GI tract and persists in the circulation longer than ethyl alcohol. Alcohol dehydrogenase oxidizes most isopropanol to acetone. Acetone may be further metabolized to acetate, formate, and finally CO_2. In another metabolism study, the amount of acetone in the blood stream was found to be directly related to the air concentration of isopropanol (Laham *et al.*, 1980). This finding indicated that the acetone metabolite could be used as a biochemical indicator of isopropanol exposure.

Subchronic studies by Guseinov and Abasov (1982) and Baikov *et al.* (1974) reported changes in certain hematologic and clinical chemistry parameters, as well as increases in some organ weights. But the Environmental Protection Agency deemed these studies insufficient to reasonably predict subchronic toxicity of isopropanol (Burleigh-Flayer *et al.*, 1994). Three different routes of exposure have been used by researchers for isopropanol toxicity studies: inhalation, oral gavage and presence in drinking water. The following subchronic and chronic studies exposed experimental animals to isopropanol by the inhalation route:

Toxicological and neurobehavioral endpoints were investigated in rats and mice following 13-week inhalation exposure (6 hr/day, 5 days/week) to 0, 100, 500, 1500 or 5000 ppm isopropanol (Burleigh-Flayer *et al.*, 1994). In rats, clinical signs observed following exposures included swollen periocular tissue (females) at the highest dose and perinasal encrustation (males) at 500 ppm and above. Narcosis was observed in a few animals of both species during exposure to 5000 ppm and possibly 1500 ppm as well. However, the animals became tolerant to the narcotic effects of isopropanol after week 2. No neurobehavioral changes were observed in any parameters of the functional observational battery. However, increased motor activity was noted

at week 9 of exposure in female rats of the 5000 ppm group. After an initial drop in body weight gain in the first week of exposure at the high dose (5000 ppm), rats in the 1500 and 5000 ppm groups had significant increases in body weight gain and/or body weight throughout most of the exposure period. But only the 5000 ppm group had greater than 10% body weight gain compared to controls. Increases in body weight and body weight gain greater than 10% were also noted in female mice in the 5000 ppm group. Consistent clinical pathology changes included an increase in mean corpuscular volume (rats; female mice) and mean corpuscular hemoglobin (male rats; female mice) at the 5000 ppm exposure level. Other changes noted include a slight anemia in rats at week 6 only and a slight dehydration in female mice at the end of the study. Relative liver weight in rats was elevated no more than 8% in the 5000 ppm groups. However, a 10 and 21% increase in relative liver weight was observed in female mice at 1500 and 5000 ppm, respectively. No gross lesions were observed in any organs. The only microscopic change observed was hyaline droplets within kidneys of all male rats. This change was not clearly concentration related, although it was most pronounced in the 5000 ppm group.

In a follow-up inhalation study spanning the lifetime of rats and mice, Burleigh-Flayer *et al*. (1997) exposed four groups of animals, each consisting of 75 CD-1 mice/sex and 75 Fischer 344 rats/sex, to 0, 500, 2500, or 5000 ppm isopropanol vapor. Of these, 55 mice/sex/group and 65 rats/sex/group were exposed 6 hr/day, 5 days/week for at least 78 weeks (mice) or 104 weeks (rats). Transient signs of narcosis were observed at the higher doses. Increased mortality and a decreased mean survival time (577 days versus 631 days for controls) were noted for male rats in the 5000 ppm group. Increases in body weight and/or body weight gain were observed for both sexes of mice and rats from the 2500 and 5000 ppm groups throughout the study. Concentration-related increases in absolute and relative liver weight were observed for male and female mice. In addition, increased absolute and/or relative liver and kidney weight were observed for male and/or female rats from the 2500- and 5000-ppm groups. Urinalysis and changes in urine chemistry, indicative of impaired kidney function (i.e. decreased osmolality and increased total protein, volume, and glucose), were noted for male rats in the 2500 ppm group and for male and female rats in the 5000 ppm group. At necropsy, the most significant noncancer lesions in rats were observed in the kidney, and were associated with an exacerbation of spontaneous chronic renal disease. The kidney lesions noted with increased severity and/or frequency included mineralization, tubular dilation, glomerulosclerosis, interstitial nephritis, interstitial fibrosis, hydronephrosis, and transitional cell hyperplasia. The authors considered chronic renal disease to be the main cause of death for male and female rats exposed to 5000 ppm and to account for much of the mortality observed for male rats exposed to 2500 ppm. Unlike the subchronic study, anemia was not observed in rats in the chronic study. In mice, an increased incidence of seminal vesicle enlargement was observed grossly in males in the 2500 and 5000 ppm groups. Microscopically, the lesions in mice included an increased incidence of ectasia (dilation) of the seminal vesicles for male mice in the 2500 and 5000 ppm groups, minimal renal tubular proteinosis for male and female mice from all isopropanol groups, and renal tubular dilation for female mice in the 5000-ppm group. The seminal vesicle effects did not have any associated inflammatory or degenerative changes. The enlargement may have been the result of either increased secretion or decreased evacuation of the secretory product by these glands. Microscopic evaluation of the livers of rats and mice revealed no exposure-related lesions.
In a 13-week behavioral/neurotoxicity study by the same investigators, the reproducibility and reversibility of increased motor activity in isopropanol-exposed female Fischer 344 rats was

investigated (Burleigh-Flayer *et al.* 1998). Rats were exposed to 0 or 5000 ppm isopropanol for 6 hr/day, 5 days/week. Increased motor activity was characterized as the summation of ambulation, rearing and fine movements and was first observed 4 weeks following exposure to 5000 ppm isopropanol. Reversibility of this effect was observed 2 days following cessation of exposure in a subgroup of rats exposed to isopropanol for only 9 weeks. In the subgroup exposed for 13 weeks, reversal of the increased motor activity did not occur until 2 weeks following cessation of exposure. However, complete reversibility of the time versus activity profile, or habituation curve, was not noted until 42 days following exposure to isopropanol for 13 weeks.. Other effects included a significant increase in body weight and an increased incidence of swollen periocular tissue in isopropanol-exposed animals.

In a study conducted to investigate neurochemical and behavioural effects, 20 male Wistar rats/group were exposed to 0 or 300 ppm isopropanol 6 hr/day, 5 days/week for up to 21 weeks (Savolainen *et al.*, 1979). Enzyme activity of superoxide dismutase and azoreductase in cerebellar homogenate was decreased at week 20-21. Acid protease activity in glial cells was increased up to week 10. Open-field tests indicated sporadic changes in urination (10th week) and defecation (15th week). Isopropanol also appeared to depress caffeine stimulation activity at 15 weeks.

In a subchronic neurotoxicity study by Teramoto *et al.* (1993), motor and sensory nerve conduction velocity increased significantly following a 20-week exposure (8 hr/day, 5 days/week) of Jcl-Wistar rats to 8000 ppm isopropanol. Low dose (1000 ppm) exposure had no effect on conduction velocity. Conduction velocities returned to normal following the end of exposure. The sex of the rats in this study was not specified.

A developmental study in rats exposed pregnant dams (15/group) to 0, 3500, 7000 or 10,000 ppm isopropanol 7 hr/day on gestation days 1-19 (Nelson *et al.*, 1988). At the two highest exposure levels, feed intake (weeks 1 and 2 of exposure) and maternal body-weight gain were reduced. Narcosis was evident only at the 10,000 ppm level.Increased fetal resorptions and reduced fetal weights (59% of controls) occurred at the highest exposure level. Fetal weights were also significantly reduced (85% of controls) at 7000 ppm. A slight reduction in fetal weight (96% of controls) occurred at 3500 ppm but was significant in the sense that a dose-dependent relationship in fetal weight reduction was present across all exposed groups. Skeletal malformations (primarily rudimentary cervical ribs) were seen only in the presence of maternal toxicity at the two highest exposure levels. No detectable teratogenic effects were observed in the 3500 ppm group. The authors noted that the developmental effects at 3500 ppm were considered very slight, indicating that this exposure level is close to the LOAEL for isopropanol.

The following studies administered isopropyl alcohol to experimental animals by oral gavage:

In a developmental study, pregnant (VAF)CD(SD) rats (25/group) were gavaged with either 0, 400, 800 or 1200 mg/kg body wt-day of isopropanol daily on gestational days 6 through 15 (Tyl *et al.*, 1994). In the same study, pregnant New Zealand white rabbits (15/group) were dosed orally with either 0, 120, 240 or 480 mg/kg body wt-day of isopropanol daily during gestational days 6 through 18. In rats, fetal body weight exhibited a linear downward trend with increasing dose and was significantly lower at the highest dose compared to controls. However, the fetal

body weight differences at each dose level was less than 10% of controls. Maternal weight gain during gestation was significantly reduced at the highest dose level. In rabbits, maternal weight gain and food consumption was reduced during gestation at 480 mg/kg body wt-day. Four rabbits died after dosing at this level. No differences were observed in reproduction indices or in fetal development. No teratogenic effects were seen in either species.

In another developmental study performed to investigate neurotoxicity in rat pups, 64 time-mated Sprague-Dawley rats/group were administered 0, 200, 700 or 1200 mg/kg body wt-day isopropanol by oral gavage from gestational day 6 through postnatal day 21 (Bates *et al.*, 1994). One high-dose dam died on postnatal day 15, but there were no other clinical observations of effects on maternal weight, food consumption or gestation length. All fetal developmental indices were unaffected at the dose levels used. Developmental neurotoxicity, in the form of motor activity, auditory startle and active avoidance tests, was not found at any dose of isopropanol.

In a multi-generation study carried out to investigate potential reproductive and developmental effects of isopropanol, Sprague-Dawley rats were administered 0, 100, 500 or 1000 mg/kg body wt-day of isopropanol by oral gavage (Bevan *et al*., 1995). P1 and P2 rats were dosed daily for 10 weeks prior to mating, throughout the mating, and during the gestation and lactation period for the F1 and F2 litters, respectively. In adult rats, centrilobular hepatocyte hypertrophy and increased relative liver weight (>10%) was observed in P2 males at 1000 mg/kg. A general increase in absolute and relative liver and kidney weights was observed (less than 10% in most cases) in treated animals in both P1 and P2 generations. However, with the exception of hepatocellular hypertrophy in P2 males, no histopathological effects relevant to human risk were present. Reproductive effects due to isopropanol were not seen at any dose level. Statistically significant reduction of body weights (5-12%) in F1 and F2 offspring and increased mortality (14%) in F1 offspring were observed at the 1000 mg/kg dose level.

The following toxicology studies administered isopropanol to experimental animals in drinking water:

In a study designed to investigate neurotoxicological effects, 22 male SPF rats/group were administered isopropanol in drinking water at concentrations of 0, 1, 2, 3, or 5% (*w*/*v*) for 12 weeks (Pilegaard and Ladefoged, 1993). Average daily intake of isopropanol was 0, 870, 1280, 1680 and 2520 mg/kg body wt, respectively. Water intake and body weights were consistently lower at the two highest doses. Relative weights of liver, kidney and adrenals were increased in a dose-dependent manner. However, histopathology revealed no treatment-related changes in organs other than the male rat-specific kidney lesions. Evidence of astrogliosis, in the form of increased glial fibrillary acidic protein in dorsal hippocampus, was not found in exposed rats.

In a 1-generation study, 10 Wistar-derived rats/sex/group were exposed to 0, 0.5, 1.25, 2.0, and 2.5% isopropanol in water for up to 18 weeks (USEPA/OTS, 1986). The doses are equivalent to 0, 325, 711, 1002, and 1176 mg/kg body wt-day, respectively, for males; to 0, 517, 1131, 1330, and 1335 mg/kg body wt-day, respectively, for females during the pre-mating phase; and to 0, 1167, 2561, 2825, and 2722 mg/kg body wt-day, respectively, in females during the post-partum phase. Exposure periods were: 70 days pre-mating, plus 15 days during mating, plus 42 days for

males; 21 days pre-mating plus 15 days during mating, plus 21 days gestation, plus 21 days rearing in females; and 21 days for the F_1 generation. At the highest two levels, body weights of males during the first two weeks were reduced and the body weights of females during the post-partum period were reduced. Water consumption and food ingestion were generally lower at the top three dose levels. The authors concluded that these effects were related to the unpalatability of drinking water containing isopropanol and not due to a toxic effect of the alcohol itself. Anemia was present in post-partum females. Red cell numbers were reduced in a dose-related manner at doses of 1.25% isopropanol or higher. Hematocrit was lower at the two highest doses while hemoglobin was lower at the highest dose. In males, mean cell volume was reduced at 1.25% isopropanol or higher. Absolute and relative liver and kidney weights were higher in most exposure groups at 2.0% or higher in both sexes, but no relevant pathology was seen. Absolute liver weight of females was also higher in the 1.25% group. Fetal weight gain was depressed in a dose-related fashion in the 1.25% and higher groups. Mean pup weights and pup survival were lower than controls at the two highest doses. Fewer pups were born per animal in the 2.5% exposure group. A teratogenic examination was not performed on the pups.

In a similar exposure study investigating the potential teratogenic effects of isopropanol, 20 pregnant Wistar-derived rats/group were exposed to 0, 0.5, 1.25 or 2.5% of the alcohol in drinking water (equivalent to 0, 596, 1242 and 1605 mg/kg body wt-day) during gestational days 6 to 16 (USEPA/OTS, 1992a,b). Water and feed consumption were reduced at the two highest doses while maternal body weight was significantly reduced at the highest dose. Fetal body weights were decreased in the two highest dose groups. Minor abnormalities and variants (reduced ossification of the skeleton) were present in fetuses of exposed groups in a dose-related manner. However, the authors concluded that the reduced fetal weights are probably a consequence of maternal growth retardation during the critical period of organogenesis. Similarly, the fetal abnormalities are probably due to small fetal size, related to slightly retarded development. Therefore, the study found no indication of teratogenesis.

A multi-generation study performed in ‘white’ rats also observed reduced body weights in F1 offspring (Lehman *et al.*, 1945). Body weights of F2 offspring were the same as controls. The adult rats had imbibed an average of 1.9 ml/kg (1470 mg/kg body wt) of isopropanol per day in drinking water 80 days prior to mating. No other developmental or reproductive effects were seen. In the same study, several dogs were given 4% isopropanol in drinking water for approximately 7 months. Histopathology at the end of exposure revealed a decrease in the number of nephrons with hydropic changes and necrosis of some of the tubular epithelium. Some capillary hemorrhages were also noted in the brains of two of the dogs. Average daily dose of isopropanol imbibed by the dogs could not be determined from data provided in the report.

VI. Derivation of Chronic Reference Exposure Level (REL)

Study	Burleigh-Flayer *et al.* (1997)
Study population	Rats and mice
Exposure method	Discontinuous whole-body inhalation (0, 504, 2,509 or 5,037 ppm)
Critical effects	Kidney lesions in mice and rats
LOAEL	2,509 ppm
NOAEL	504 ppm
Exposure continuity	6 hours/day, 5 days/week
Exposure duration	78 weeks in mice; 104 weeks in rats
Average experimental exposure	90 ppm for NOAEL group (500 x 6/24 x 5/7)
Human equivalent concentration	90 ppm for NOAEL group (gas with systemic effects, based on RGDR = 1.0 using default assumption that lambda (a) = lambda (h))
LOAEL uncertainty factor	1
Subchronic uncertainty factor	1
Interspecies uncertainty factor	3
Intraspecies factor	10
Cumulative uncertainty factor	30
Inhalation reference exposure level	3 ppm (3000 ppb, 7 mg/m^3, 7000 μg/m^3)

The Burleigh-Flayer et al. (1997) study was selected because it was a chronic study, was recent, and was published in a respected, peer-reviewed journal. While numerous subchronic studies have been performed, this was the only study that conducted lifetime animal exposures. In addition, the chronic kidney effects observed in rats and mice were not seen in the subchronic studies, indicating that chronic exposure is necessary for development of these lesions.

The lesions observed in the kidneys of male rats in some of the studies described above is typical of a male rat-specific chronic renal disease and is not considered to be relevant to human risk assessment (Phillips and Cockrell, 1984; Beyer, 1992). However, the exacerbation of chronic renal disease in male and female rats, and the slight kidney damage observed in mice of both sexes following chronic isopropanol exposure indicates that the kidney is a sensitive indicator for nonneoplastic effects (Burleigh-Flayer *et al*., 1997). Suggestive evidence also exists for kidney damage in dogs following subchronic exposure to isopropanol in drinking water (Lehman *et al*., 1945).

Some isopropanol exposure studies noted increased liver and kidney weights in exposed animals but no observable relevant pathology. With particular relevance to the liver, this weight change may be considered to be more of a metabolic response rather than a toxic effect of the alcohol. The changes noted in the neurochemical and behavioural study by Savolainen *et al.* (1979) may have also been more of a metabolic response to the increased load of isopropanol. It is also possible that these changes reflected the development of tolerance. The changes in behavior

were small and unconvincing. This study would have benefited from additional dose levels to analyze for dose-response trends.

Other possible sensitive indicators of isopropanol toxicity include blood chemistry changes and reduced fetal body weights. However, the blood chemistry findings were conflicting among the various studies that investigated this endpoint. Reduced fetal weights at doses below maternal body weight reductions were minor (<10% compared to controls), but consistent, suggesting that reduced fetal weights are a manifestation of isopropanol developmental toxicity.

A comparative REL was calculated from the only reproduction/developmental study that utilized inhalation as the route of exposure (Nelson *et al*., 1988). Exposure of pregnant rats to isopropanol during gestation caused dose-dependent reduction in fetal body weights across all treatment groups, resulting in a LOAEL of 3500 ppm (average measured concentration = 3510 ppm). A NOAEL was not observed for this effect. Skeletal malformations probably related to reduced fetal weight was observed at 7000 ppm and 10,000 ppm. The average exposure duration at the LOAEL for this study is 1024 ppm (7hr/24hr x 3510 ppm). Use of an RGDR of 1 and a cumulative uncertainty factor of 100 (3 for LOAEL to NOAEL, 3 for interspecies, and 10 for intraspecies) resulted in a REL of 10 ppm (25 mg/m^3). Since the endpoint is a function of exposure only during gestation, no subchronic to chronic UF was used. This developmental REL is within an order of magnitude of the chronic REL for kidney lesions, and therefore, is also considered to be a critical effect.

The oral dose developmental studies by Tyl *et al*. (1994), Bevan *et al*. (1995), USEPA/OTS (1986), and USEPA/OTS (1992 a, b) provide supportive evidence that reduced fetal weights is a sensitive developmental endpoint. The USEPA/OTS (1992a,b) study provides supportive evidence for skeletal malformations in exposed rat fetuses.

VII. Data Strengths and Limitations for Development of the REL

Strengths of the database for isopropanol include availability of a well-conducted chronic study in two species, similar toxicological endpoints among different studies, and pharmacokinetic similarities between humans and experimental animals. Isopropanol is metabolized through a similar pathway to acetone and CO_2.

Weaknesses of the database for isopropanol include a lack of literature regarding chronic toxicity endpoints in humans. The deficiency of chronic toxicity cases in humans may be related to the relatively low chronic toxicity of isopropanol. Another weakness is that, while most developmental studies observed maternal and fetal effects, only one study was performed via the inhalation route.

VIII. References

Amoore JE, and Hautala E. 1983. Odor as an aid to chemical safety: Odor thresholds compared with threshold limit values and volatiles for 2114 industrial chemicals in air and water dilution. J. Appl. Toxicol. 3(6):272-290.

Baikov BK, Gorlova OE, Gusev MI, Novikov Yu V, Yudina TV, and Sergeev AN. 1974. Hygenic standardization of the daily average maximum admissible concentrations of propyl and isopropyl alcohols in the atmosphere. Gigiena I Sanitariia, 4:6-13.

Bates HK, McKee RH, Bieler GS, Gardiner TH, Gill MW, Strother DE, and Masten LW. 1994. Developmental neurotoxicity evaluation of orally administered isopropanol in rats. Fundam. Appl. Toxicol. 22:152-158.

Bevan C, Tyler T, Gardiner T, Kapp R, Andrews L, Beyer B. 1995. Two-generation reproduction toxicity study with isopropanol in rats. J. Appl. Toxicol. 15:117-223.

Brugnone F, Perbellini L, Apostoli P, Bellomi M, and Caretta D. 1983. Isopropanol exposure: environmental and biological monitoring in a printing works. Br. J. Ind. Med., 40:160-168.

Burleigh-Flayer H, Gill M, Hurley J, Bevan C, Gardiner T, Kapp R, Tyler T, Wright G. 1998. Motor activity effects in female Fischer 344 rats exposed to isopropanol for 90 days. J. Appl. Toxicol. 18:373-381.

Burleigh-Flayer H, Garman R, Neptun D, Bevan C, Gardiner T, Kapp R, Tyler T, Wright G. 1997. Isopropanol vapor inhalation oncogenicity study in Fischer 344 rats and CD-1 mice. Fundam. Appl. Toxicol. 36(2):95-111.

Burleigh-Flayer HD, Gill MW, Strother DE, Masten LW, McKee RH, Tyler TR, Gardiner T. 1994. Isopropanol 13-week vapor inhalation study in rats and mice with neurotoxicity evaluation in rats. Fundam. Appl. Toxicol. 23:421-428.

CARB. 1999. Air toxics emissions data collected in the Air Toxics Hot Spots Program CEIDARS Database as of January 29, 1999.

C&EN. 1995. Chemical & Engineering News. Production by the U.S. chemical industry. M. Heylin, ed., American Chemical Society, Washington D. C., June 26:38-44.

Guseinov VG, and Abasov DM. 1982. Toxicological characteristics of isopropyl alcohol after chronic exposure under experimental conditions. Azerb. Med. Zh. 58:53-57.

HSDB. 1995. Hazardous Substances Data Bank. National Library of Medicine, Bethesda, MD (TOMES® CD-ROM version). Denver, CO: Micromedex, Inc. (Edition expires 11/31/95).

IARC. 1997. IARC monographs on the evaluation of the carcinogenic risk of chemicals to man. Vol. 15. Lyon: IARC. pp. 223-243.

Laham S, Potvin M, Schrader K, and Marino I. 1980. Studies on inhalation toxicity of 2-propanol. Drug Chem. Toxicol. 3(4):343-360.

Lehman AJ, Schwerma H, and Rickards E. 1945. Isopropyl alcohol acquired tolerance in dogs, rate of disappearance from the blood stream in various species, and effects on successive generation of rats. J. Pharmacol. Exper. Therap. 85:61-69.

Nelson BK, Brightwell WS, MacKenzie-Taylor DR, Khan A, Burg JR, Weigel WW, and Goad PT. 1988. Teratogenicity of *n*-propanol and isopropanol administered at high inhalation concentrations to rats. Food Chem. Toxicol. 26(3):247-254.

Phillips RD, and Cockrell B. 1984. Effect of certain light hydrocarbons on kidney function and structure in male rats. Adv. Mod. Environ. Toxicol, 7:89-106.

Pilegaard K, and Ladefoged O. 1993. Toxic effects in rats of twelve weeks' dosing of 2-propanol, and neurotoxicity measured by densitometric measurements of glial fibrillary acidic protein in the dorsal hippocampus. *in vivo* 7:325-330.

Savolainen H, Pekari K, and Helojoki H. 1979. Neurochemical and behavioural effects of extended exposure to isopropanol vapour with simultaneous ethanol intake. Chem.-biol. Interact. 28:237-248.

Slauter R, Coleman D, Gaudette N, McKee R, Masten L, Gardiner T, Strother D, Tyler R, and Jeffcoat A. 1994. Disposition and pharmacokinetics of isopropanol in F-344 rats and B6C3F1 mice. Fundam. Appl. Toxicol. 23:407-420.

Teramoto K, Wakitani F, Horiguchi S, Jo T, Yamamoto T, Mitsutake H, and Nakseko H. 1993. Comparison of the neurotoxicity of several chemicals estimated by the peripheral nerve conduction velocity in rats. Environ. Res. 62:148-154.

Tyl RW, Masten LW, Marr MC, Myers CB, Slauter RW, Gardiner TH, Strother DE, McKee RH, and Tyler TR. 1994. Developmental toxicity evaluation of isopropanol by gavage in rats and rabbits. Fundam. Appl. Toxicol, 22:139-151.

United States Environmental Protection Agency/Office of Toxic Substances. 1986. A pilot one-generation study with isopropyl alcohol in rats (final report) with attachments and cover letter dated 013092. EPA/OTS Doc. #86-920000728. Prepared by British Industrial Biological Research Association. Submitted by Chemical Manufacturers Association. Fiche No. NTIS/OTS0535607.

United States Environmental Protection Agency/Office of Toxic Substances. 1992a. A teratology study with isopropyl alcohol in rats. Letter submitting one enclosed teratology study and one enclosed supplementary study on isopropanol. EPA/OTS Doc. #86-920000727. Prepared by

British Industrial Biological Research Association. Submitted by Chemical Manufacturers Association. Fiche No. NTIS/OTS0535606.

United States Environmental Protection Agency/Office of Toxic Substances. 1992b. Investigations supplementary to studies with isopropyl alcohol. Letter submitting one enclosed teratology study and one enclosed supplementary study on isopropanol. EPA/OTS Doc. #86-920000727. Prepared by British Industrial Biological Research Association. Submitted by Chemical Manufacturers Association. Fiche No. NTIS/OTS0535604.

Wills JH, Jameson EM, and Coulston F. 1969. Effects on man of daily ingestion of small doses of isopropyl alcohol. Toxicol. Appl. Pharmacol. 15:560-565.

CHRONIC TOXICITY SUMMARY

MALEIC ANHYDRIDE

(2,5-furandione; cis-butenedioic anhydride; toxilic anhydride; maleic andride)

CAS Registry Number: 108-31-6

I. Chronic Toxicity Summary

Inhalation reference exposure level	**0.7 µg/m^3** (2.5 ppb**)**
Critical effect(s)	Neutrophilic infiltration of the nasal epithelium; irritation of the respiratory system in rats, hamsters and monkeys
Hazard index target(s)	Respiratory system

II. Chemical Property Summary (HSDB, 1995)

Description	Colorless or white solid
Molecular formula	$C_4H_2O_3$
Molecular weight	98.06 g/mol
Boiling point	202°C
Melting point	52.8°C
Vapor pressure	0.1 torr @ 25°C (AIHA, 1970)
Solubility	Soluble in water, ether, acetate, chloroform, dioxane; @ 25°C, 227 g/100 g acetone, 112 g/100 g ethyl acetate, 52.5 g/100 g chloroform, 50 g/100 g benzene, 23.4 g/100 g toluene, 19.4 g/100 g o-xylene, 0.6 g/100 g CCl_4, 0.25 g/100 g ligroin
Conversion factor	4.0 µg/m^3 per ppb at 25ºC

III. Major Uses and Sources

Maleic anhydride is used as a chemical intermediate in the synthesis of fumaric and tartaric acid, certain agricultural chemicals, resins in numerous products, dye intermediates, and pharmaceuticals (HSDB, 1995). It is also used as a co-monomer for unsaturated polyester resins, an ingredient in bonding agents used to manufacture plywood, a corrosion inhibitor, and a preservative in oils and fats. The annual statewide industrial emissions from facilities reporting under the Air Toxics Hot Spots Act in California based on the most recent inventory were estimated to be 7366 pounds of maleic anhydride (CARB, 2000).

IV. Effects of Human Exposure

In many occupational situations workers are exposed to mixtures of acid anhydrides, including maleic anhydride, phthalic anhydride, and trimellitic anhydride. For example, Barker *et al*. (1998) studied a cohort of 506 workers exposed to these anhydrides. In one factory, workers were exposed only to trimellitic anhydride, which has the lowest acceptable occupational exposure limit (40 $\mu g/m^3$) of the three anhydrides. In that factory there was an increased prevalence of sensitization to acid anhydride and work related respiratory symptoms with increasing full shift exposure even extending down to levels below the current occupational standard. However, none of the workplaces had exposure only to maleic anhydride and a dose-response relationship was not seen with mixed exposures.

The following reports involve exposure only to maleic anhydride.

There are several case reports describing asthmatic responses possibly resulting from exposure to maleic anhydride. An individual showed an acute asthmatic reaction after exposure to dust containing maleic anhydride (Lee *et al.*, 1991). Workplace concentrations of maleic anhydride were 0.83 mg/m^3 in the inspirable particulate mass and 0.17 mg/m^3 in the respirable particulate mass. Bronchial provocation testing was performed with phthalic anhydride, lactose, and maleic anhydride. Exposure of this individual to maleic anhydride (by bronchial provocation testing) at 0.83 mg/m^3 and 0.09 mg/m^3 in inspirable and respirable particulate mass, respectively, showed a response of cough, rhinitis, and tearing within two minutes. Within 30 minutes, rales developed in both lungs and peak flow rate decreased 55%.

An individual occupationally exposed to maleic anhydride developed wheezing and dyspnea upon exposure (Gannon *et al.*, 1992). After a period without exposure, two re-exposures both resulted in episodes of severe hemolytic anemia. There was no evidence of pulmonary hemorrhage. Radioallergosorbent testing showed specific IgE antibodies against human serum albumin conjugates with maleic anhydride, phthalic anhydride, and trimellitic anhydride, but not with tetrachlorphthalic anhydride. A critique of the Gannon *et al.* (1992) study by Jackson and Jones (1993) questions the relationship of maleic anhydride exposure to the onset of the anemia, since there were extended periods of exposure to maleic anhydride before symptoms appeared.

Another case report described occupational asthma due to exposure to maleic anhydride (Guerin *et al.*, 1980).

Humans exposed to maleic anhydride showed respiratory tract and eye irritation at concentrations of 0.25 to 0.38 ppm (1 to 1.6 mg/m^3) maleic anhydride (Grigor'eva, 1964). No irritation was reported at 0.22 ppm maleic anhydride.

V. Effects of Animal Exposure

Short *et al.* (1988) chronically exposed CD rats (15/sex/group), Engle hamsters (15/sex/group), and rhesus monkeys (3/sex/group) to maleic anhydride by inhalation. Four groups of each species were exposed to concentrations of 0, 1.1, 3.3, or 9.8 mg/m^3 maleic anhydride for 6

hours/day, 5 days/week, for 6 months in stainless steel and glass inhalation chambers. Solid maleic anhydride was heated to 53°C to generate vapors, which were then mixed with a stream of nitrogen. Chamber target levels were monitored by gas chromatography as total maleic (maleic anhydride plus maleic acid). No exposure-related increase in mortality occurred. Of the species examined, only rats showed significant changes in body weight during the course of the experiment, with reductions among males in the high-dose groups after exposure day 40 and a transient weight reduction from days 78-127 in the mid-dose group. All species exposed to any level of maleic anhydride showed signs of irritation of the nose and eyes, with nasal discharge, dyspnea, and sneezing reported frequently. No exposure-related eye abnormalities were reported. The severity of symptoms was reported to increase with increased dose. No dose-related effects were observed in hematological parameters, clinical chemistry, or urinalysis. No effects on pulmonary function in monkeys were observed. Dose-related increases in the incidence of hyperplastic change in the nasal epithelium occurred in rats in all exposed groups, and in hamsters in the mid- and high-dose groups. Neutrophilic infiltration of the epithelium of the nasal tissue was observed in all species examined at all exposure levels. All changes in the nasal tissues were judged to be reversible. The only other significant histopathological observation was slight hemosiderin pigmentation in the spleens of female rats in the high-dose group.

Incidence of epithelial hyperplasia of the nasal mucosa in animals from Short *et al*. (1988)

Maleic anhydride (mg/m^3)	0	0	1.1	1.1	3.3	3.3	9.8	9.8
Pathology grade	Trace	Mild	Trace	Mild	Trace	Mild	Trace	Mild
Rat								
Male	0/15	0/15	2/15	6/15	1/15	14/15	0/15	12/15
Female	0/15	0/15	6/15	5/15	4/15	10/15	0/15	14/15
Combined		0/30		11/30		24/30		26/30
Hamster								
Male	0/15	0/15	0/15	0/15	0/15	5/15	0/15	8/15
Female	0/15	0/15	0/15	0/15	4/15	4/15	1/15	4/15
Combined		0/30		0/30		9/30		12/30
Monkey								
Male	0/3	0/3	0/3	0/3	0/3	0/3	0/3	0/3
Female	0/3	0/3	0/3	0/3	0/3	0/3	0/3	0/3
Combined		0/6		0/6		0/6		0/6

The teratogenicity and multigeneration reproductive toxicity of maleic anhydride were also investigated (Short *et al.*, 1986). To evaluate teratogenicity, pregnant CD rats were treated orally with maleic anhydride in corn oil at concentrations of 0, 30, 90, or 140 mg/kg-day from gestational days 6-15. Animals were necropsied on gestational day 20. No statistically significant dose-related effects were observed in maternal weight gain, implantation, fetal viability, post-implantation loss, fetal weight, or malformations. Groups of 10 male rats and 20 female rats/group (F_0 animals) were orally treated with 0, 20, 55, or 150 mg/kg-day maleic anhydride in corn oil to study multigeneration reproductive toxicity. Animals within the same dose group were bred together after 80 days of treatment to produce two F_1 generation animals (F_{1a} and F_{1b}) and animals from the F_1 generation were interbred to produce two F_2 generation animals (F_{2a} and F_{2b}). A significant increase in mortality was observed among both F_0 and F_1

generation animals in the high-dose group. Total body weight was significantly reduced in animals in the high-dose group at Week 11 of exposure for the F_0 generation males and females and at Week 30 of exposure in the F_1 generation males. No consistent pattern of dose- or treatment-related effect on fertility, litter size, or pup survival was observed. Examination of F_0 animals showed necrosis of the renal cortex in the high-dose group (60% of males and 15% of females). Absolute kidney weights were significantly increased in F_1 females in the low- and mid-dose groups, although there was no histological correlate. No changes in organ weight or histology were observed in the F_2 generation animals.

VI. Derivation of Chronic Reference Exposure Level (REL)

Study	Short *et al.*, 1988
Study population	Rats (15/sex/group), hamsters (15/sex/group), monkeys (3/sex/group)
Exposure method	Discontinuous inhalation exposure (0, 1.1, 3.3, or 9.8 mg/m^3)
Critical effects	Neutrophilic infiltration of the nasal epithelium; epithelial hyperplasia; respiratory irritation
LOAEL	1.1 mg/m^3
NOAEL	Not observed in rats
BMC_{05}	0.12 mg/m^3 for mild epithelial hyperplasia in rats (males and females combined)
Exposure continuity	6 hr/day, 5 days/week
Exposure duration	6 months
Average experimental exposure	21 μg/m^3 for the BMC_{05} (0.12 x 6/24 x 5/7 x 1000)
Human equivalent concentration	21 μg/m^3 for the BMC_{05} (Due to the lack of aerosol particle size data for the critical study, a human equivalent concentration could not be developed using recommended methods of inhalation dosimetry.)
LOAEL uncertainty factor	not needed in benchmark approach
Subchronic uncertainty factor	1
Interspecies uncertainty factor	3 (see below)
Intraspecies uncertainty factor	10
Cumulative uncertainty factor	30
Inhalation reference exposure level	0.7 μg/m^3 0.2 ppb)

Short *et al.* (1988) examined the toxicity of maleic anhydride to rats, hamsters, and monkeys by the inhalation route of exposure. Dose- and exposure related effects, although mild and reversible, were observed at all exposure levels. Specifically, exposure to maleic anhydride vapors resulted in hyperplastic change in the nasal epithelium of rats and hamsters (obligate nose breathers). Neutrophilic infiltration of the nasal epithelium was observed in all three species at all levels of exposure. All species also showed signs of irritation at all exposure levels. The observation that acute maleic anhydride is a strong respiratory irritant to humans (ACGIH, 1992)

suggests that this is a valid endpoint of toxicity to humans as well. Human exposure at levels as low as ~1 mg/m^3 appears to trigger acute asthmatic reactions in sensitive individuals (Lee *et al.*, 1991). The histological changes observed by Short *et al*. occurring as a result of inhalation exposure to a known strong irritant such as maleic anhydride are considered to be the adverse effect of repetitive acute exposures, rather than a chronic response, in the development of the REL.

The chronic REL was developed using the benchmark approach. The gamma model in the U.S. EPA's BMDS software yielded a BMC_{05} of 0.12 mg/m3 for mild epithelial hyperplasia in male and female rats combined. Because of the similarities among species and the inclusion of monkeys in the study, an interspecies uncertainty factor of 3, rather than 10, was used. Although there is no evidence of a toxic response similar to the development of asthma in animals, the 1.1 mg/m^3 LOAEL from the animal studies of Short *et al.* (1988) results in a REL of 0.7 μg/m^3 which should protect asthmatics from maleic and other anhydrides.

VII. Data Strengths and Limitations for Development of the REL

The major strengths of the REL for maleic anhydride are the availability of multiple-species, multiple-dose subchronic inhalation studies, and the observation of a mild effect LOAEL. The major uncertainties are the lack of human data and the lack of a NOAEL observation.

VIII. Potential for Differential Impacts on Children's Health

Minimal teratogenic and reproductive adverse effects were seen at the lowest oral dose of maleic anhydride (20 mg/kg-day), given to rats during gestation (Short et al., 1986). This dose is equivalent to a person inhaling 70 mg/m^3. Thus the chronic REL of 0.7 μg/m^3 should protect children. Maleic anhydride is a respiratory irritant and an inducer of asthma. Exacerbation of asthma has a more severe impact on children than on adults. However, there is no direct evidence in the literature to quantify a differential effect of maleic anhydride in children.

IX. References

ACGIH. 1992. American Conference of Governmental Industrial Hygienists, Inc. Documentation of the threshold limit values and biological exposure indices. Sixth ed. Cincinnati, OH: ACGIH.

AIHA. 1970. American Industrial Hygiene Association. Maleic anhydride. Am. Ind. Hyg. Assoc. J. 31:391-394.

Barker RD, van Tongeren MJ, Harris JM, Gardiner K, Venables KM and Newman Taylor AJ. 1998. Risk factors for sensitisation and respiratory symptoms among workers exposed to acid anhydrides: a cohort study. Occup. Environ. Med. 55(10):684-691.

CARB. 2000. California Air Resources Board. California Emissions Inventory Development and Reporting System (CEIDARS). Data from Data Base Year 1998. February 12, 2000.

Gannon PFG, Burge PS, Hewlett C, and Tee RD. 1992. Haemolytic anaemia in a case of occupational asthma due to maleic anhydride. Br. J. Ind. Med. 49:142-143.

Grigor'eva, K.V. 1964. Pollution of atmospheric air with maleic anhydride (Abstract in Chem. Abstr. (1966) 65:14319b). Gig. Sanit. 29:7-11.

Guerin JC, Deschamps O, Guillot TL, Chavallion JM, and Kalb JC. 1980. A propos d'un cas d'asthme a l'anhydride maleique [A case of asthma due to maleic anhydride]. Poumon et le Coeur 36:393-395.

HSDB. 1995. Hazardous Substances Data Bank. National Library of Medicine, Bethesda, MD (TOMES® CD-ROM Version). Denver, CO: Micromedex, Inc. (Edition expires 7/31/95).

Jackson J, and Jones AH. 1993. Correspondence: Haemolytic anaemia in a case of occupational asthma due to maleic anhydride. (with reply by P. Gannon). Br. J. Ind. Med. 50:191-192.

Lee HS, Wang YT, Cheong TH, Tan KT, Chee BE, and Narendran K. 1991. Occupational asthma due to maleic anhydride. Br. J. Ind. Med. 48:283-285.

Short RD, Johannsen FR, Levinskas GJ, Rodwell DE, and Schardein JL. 1986. Teratology and multigeneration reproduction studies with maleic anhydride in rats. Fundam. Appl. Toxicol. 7:359-366.

Short RD, Johannsen FR, and Ulrich CE. 1988. A 6-month multispecies inhalation study with maleic anhydride. Fundam. Appl. Toxicol. 10:517-524.

CHRONIC TOXICITY SUMMARY

METHANOL

(methyl alcohol, wood spirit, carbinol, wood alcohol, wood naphtha)

CAS Registry Number: 67-56-1

I. Chronic Toxicity Exposure Level

Inhalation reference exposure level	**4,000 μg/m^3 (3,000 ppb)**
Critical effect(s)	Increased incidence of abnormal cervical ribs, cleft palate, and exencephaly in mice
Hazard index target(s)	Teratogenicity

II. Chemical Property Summary (HSDB, 1999; CRC, 1994)

Description	Colorless liquid
Molecular formula	CH_3OH
Molecular weight	32.04 g/mol
Boiling point	64.6°C
Melting point	–97.6°C
Vapor pressure	92 torr at 20°C
Solubility	Methanol is miscible with water, ethanol, ether and many other organic solvents.
Conversion factor	1 ppm = 1.31 mg/m^3

III. Major Uses and Sources

Originally distilled from wood, methanol is now manufactured synthetically from carbon oxides and hydrogen. Methanol is used primarily for the manufacture of other chemicals and as a solvent. It is also added to a variety of commercial and consumer products such as windshield washing fluid and de-icing solution, duplicating fluids, solid canned fuels, paint remover, model airplane fuels, embalming fluids, lacquers, and inks. Methanol is also used as an alternative motor fuel (HSDB, 1999). The annual statewide industrial emissions from facilities reporting under the Air Toxics Hot Spots Act in California based on the most recent inventory were estimated to be 3,009,776 pounds of methanol (CARB, 1999b).

IV. Effects of Human Exposure

The majority of the available information on methanol toxicity in humans relates to acute rather than chronic exposure. The toxic effects after repeated or prolonged exposure to methanol are

believed to be qualitatively similar but less severe than those induced by acute exposure (Kavet and Nauss, 1990). These effects include CNS and visual disturbances such as headaches, dizziness, nausea and blurred vision. The role of formate, a metabolite of methanol, in chronic toxicity is unclear.

In one study, symptoms of blurred vision, headaches, dizziness, nausea and skin problems were reported in teachers aides exposed to duplicating fluid containing 99% methanol (Frederick *et al.*, 1984). Individual aides worked as little as 1 hr/day for 1 day a week to 8 hrs/day for 5 days/wk. The workers' total exposure duration was not mentioned. A dose-response relationship was observed between the self-reported amount of time spent at the duplicator and the incidence of symptoms. The concentrations of methanol in the breathing zones near the machines in 12 schools ranged from 485 to 4096 mg/m^3 (365 to 3080 ppm) for a 15 minute sample.

Forty-five percent of duplicating machine operators experienced blurred vision, headache, nausea, dizziness and eye irritation (NIOSH, 1981). Air concentrations of methanol for 25 minutes near the machines averaged 1330 mg/m^3.
Employees working in the proximity of direct process duplicating machines complained of frequent headaches and dizziness (Kingsley and Hirsch, 1954). Air concentrations of methanol ranged from 15 ppm (20 mg/m^3) to 375 ppm (490 mg/m^3).

Thirty young women, who had polished wood pencils with a varnish containing methanol, all experienced headaches, gastric disorders, vertigo, nausea and blurred vision (Tyson, 1912; as cited in NIOSH, 1976).

None of the above studies specified the workers' total duration of exposure.

Ubaydullayev (1968) exposed 3 to 6 subjects to methanol vapor for short durations (40 minutes for some subjects and others for an unspecified amount of time). Electrical brain cortex reflex activity was significantly altered upon exposures to 1.17 mg/m^3 (0.89 ppm) or 1.46 mg/m^3 (1.11 ppm). No effect was observed at 1.01 mg/m^3 (0.77 ppm).

V. Effects of Animal Exposure

With the exception of non-human primates, the signs of methanol toxicity in commonly used laboratory animals are quite different from those signs observed in humans (Gilger and Potts, 1955). The major effect of methanol in non-primates (rodents, dogs, cats, etc) is CNS depression similar to that produced by other alcohols. Metabolic acidosis and ocular toxicity are not observed. The differences in toxicity are attributed to the ability of non-primates to metabolize formate more efficiently than humans and other primates (Tephly, 1991).

Two chronic studies have been conducted with monkeys. In one study, ultrastructural abnormalities of hepatocytes indicating alteration of RNA metabolism were observed in rhesus monkeys given oral doses of 3 to 6 mg/kg methanol for 3 to 20 weeks (Garcia and VanZandt, 1969). In a study aimed at examining ocular effects, cynomolgous monkeys were exposed by

inhalation to methanol concentrations ranging from 680 mg/m^3 (520 ppm) to 6650 mg/m^3 (5010 ppm) for 6 hours per day, 5 days per week for 4 weeks (Andrews *et al.*, 1987). No deaths occurred and no treatment-related effects were found upon histopathologic examination. However, Andrews *et al.* did not examine possible neurologic or reproductive effects which have been observed in other species at lower concentrations (see Sections IV and V).
Exposure to a mixture of methanol and other solvents has been associated with central nervous system birth defects in humans (Holmberg, 1979). However, because of mixed or inadequate exposure data, methanol is not considered a known human teratogen.

In two separate studies in male rats, inhalation exposure to methanol ranging from 260 to 13,000 mg/m^3 for 6 to 8 hours per day for either 1 day or 1, 2, 4 or 6 weeks resulted in a significant reduction in testosterone levels (Cameron *et al.*, 1984; Cameron *et al.*, 1985).

Ubaydullayev (1968) exposed rats (15 per group) to 0, 0.57, or 5.31 mg/m^3 methanol continuously for 90 days. Chronaxy ratios of flexor and extensor muscles were measured in addition to hematologic parameters and acetyl cholinesterase activity. No changes were apparent in the 0.57 mg/m^3 group. Effects observed in the 5.31 mg/m^3 group included decreased blood albumin content beginning 7 weeks after exposure, slightly decreased acetylcholinesterase activity, decreased coproporphyrin levels in the urine after 7 weeks, and changes in muscle chronaxy. (Chronaxy is the minimum time an electric current must flow at a voltage twice the rheobase to cause a muscle to contract. The rheobase is the minimal electric current necessary to produce stimulation (Dorland, 1981).
Pregnant rats were exposed by inhalation to methanol at concentrations ranging from 5000 to 20,000 ppm for 7 hours per day on days 1-19 gestation, and days 7-15 for the highest dose group (Nelson *et al.*, 1985). A dose-related decrease in fetal weight, an increase in extra or rudimentary cervical ribs, and urinary or cardiovascular defects were observed. Exencephaly and encephalocoele were observed in the 20,000 ppm dose group. The no-observed-adverse-effect level (NOAEL) was 5000 ppm.

Pregnant mice were exposed to methanol vapors at concentrations ranging from 1000 to 15,000 ppm for 7 hours per day on days 6-15 of gestation (Rogers *et al.*, 1993). Increased embryonic and fetal death, including an increase in full-litter resorptions, was observed at 7500 ppm and higher. Significant increases in the incidence of exencephaly and cleft palate were observed at 5000 ppm and higher. A dose-related increase in the number of fetuses per litter with cervical ribs (usually small ossification sites lateral to the seventh cervical vertebra) was observed at 2000 ppm and above. The NOAEL was 1000 ppm.

VI. Derivation of Chronic Reference Exposure Level (REL)

Study	Rogers *et al.* (1993)
Study population	Pregnant mice
Exposure method	Discontinuous inhalation, 7 hours/day on days 6-15 of gestation
Critical effects	Abnormal cervical ribs, exencephaly, cleft palate
LOAEL	5000 ppm
NOAEL	1000 ppm
Benchmark Concentration (BMC_{05})	305 ppm
Exposure continuity	7 hr/day
Exposure duration	10 days
Average experimental exposure	89 ppm at BMC_{05} (305 ppm x 7/24)
Human equivalent concentration	89 ppm atBMC_{05} (gas with systemic effects, based on RGDR = 1.0 using default assumption that lambda (a) = lambda (h))
Subchronic uncertainty factor	1 (see below)
LOAEL uncertainty factor	1
Interspecies uncertainty factor	3
Intraspecies uncertainty factor	10
Cumulative uncertainty factor	30
Inhalation reference exposure level	3 ppm (3,000 ppb, 4 mg/m^3, 4,000 µg/m^3)

A NOAEL of 1000 ppm for developmental malformations was observed in mice exposed for 7 hours/day on days 6 through 15 of gestation (Rogers *et al.*, 1993). Although not a chronic study, the endpoint, teratogenicity, is a function of exposure only during gestation, especially in the case of a non-accumulating compound such as methanol. Therefore, an uncertainty factor to account for differences between subchronic and chronic exposures was not required. The investigators calculated maximum likelihood estimates (MLEs) using a log-logistic model for both 1% and 5% added risks above background. The most sensitive developmental toxicity endpoint was an increase in the incidence of cervical ribs. The MLE_{05} and BMC_{05} for cervical ribs were 824 ppm (1079 mg/m^3) and 305 ppm (400 mg/m^3), respectively.

VII. Data Strengths and Limitations for Development of the REL

The major strengths of the REL for methanol are the observation of a NOAEL and the demonstration of a dose-response relationship. The major uncertainties are the lack of human data for chronic inhalation exposure, the lack of comprehensive, long-term multiple dose studies, and the difficulty in addressing reproductive short-term effects within the chronic REL framework.

VIII. References

Andrews LS, Clary JJ, Terrill JB, and Bolte HF. 1987. Subchronic inhalation toxicity of methanol. J. Toxicol. Environ. Health 20:117-124.

CARB. 1999. Air toxics emissions data collected in the Air Toxics Hot Spots Program CEIDARS Database as of January 29, 1999.

Cameron AM, Nilsen OG, Haug E, and Eik-Nes KB. 1984. Circulating concentrations of testosterone, luteinizing hormone and follicle stimulating hormone in male rats after inhalation of methanol. Arch. Toxicol. Suppl. 7:441-443..

Cameron AM, Zahlsen K, Haug E, Nilsen OG, and Eik-Nes KB. 1985. Circulating steroids in male rats following inhalation of n-alcohols. Arch. Toxicol. Suppl. 8:422-424
Dorland. 1981. Dorland's Illustrated Medical Dictionary. 26th ed. Philadelphia: W.B. Saunders.

Frederick LJ, Schlulte PA, and Apol A. 1984. Investigation and control of occupational hazards associated with the use of spirit duplicators. Am. Ind. Hyg. Assoc. J. 45:51-55.

Garcia JH, and VanZandt JP. 1969. Nucleolar segregation and chronic methanol intoxication. Proc. Electron Microsc. Soc. Am. 27:360 [as cited in Rowe and McCollister, 1978].

Gilger AP, and Potts AM. 1955. Studies on the visual toxicity of methanol: V. The role of acidosis in experimental methanol poisoning. Am. J. Ophthalmol. 39:63-86.

Holmberg PC. 1979. Central nervous system defects in children born to mothers exposed to organic solvents during pregnancy. Lancet 2:177-179.

HSDB. 1999. Hazardous Substances Data Bank. National Library of Medicine, Bethesda, Maryland. WWW database (http://sis.nlm.nih.gov/sis1/).

Kavet R, and Nauss KM. 1990. The toxicity of inhaled methanol vapors. Crit. Rev. Toxicol. 21:21-50.

Kingsley WH, and Hirsch FG. 1954. Toxicologic considerations in direct process spirit duplicating mahcines. Comp. Med. 6:7-8.

Nelson BK, Brightwell WS, MacKenzie DR, Khan A, Burg JR, Weigel WW, and Goad PT. 1985. Teratological assessment of methanol and ethanol at high inhalation levels in rats. Fundam. Appl. Toxicol. 5:727-736.

NIOSH. 1976. National Institute for Occupational Safety and Health. Criteria document for methyl alcohol. Cincinnati, OH.

NIOSH. 1981. National Institute for Occupational Safety and Health. Health Hazard Evaluation Report, HETA 81-177, PB82-194648.178-88 [as cited in Kavet and Nauss, 1990].

Rogers JM, Mole ML, Chernoff N, Barbee BD, Turner CI, Logsdon TR, and Kavlock JV. 1993. The developmental toxicity of inhaled methanol in the CD-1 mouse, with quantitative dose-response modeling for estimation of benchmark doses. Teratology 47:175-188.

Rowe RK, and McCollister SB. 1978. Alcohols. In: Patty's Industrial Hygiene and Toxicology. Clayton GD,and Clayton FE (eds). New York: Wiley. pp. 4527-4541.

Tephly TR. 1991. Minireview. The toxicity of methanol. Life Sci. 48:1031-1041.

Tyson HH. 1912. Amblyopia from inhalation of methyl alcohol. Arch. Ophthalmol. 16:459-471 [as cited in NIOSH, 1976].

Ubaydullayev R. 1968. A study of hygienic properties of methanol as an atmospheric air pollutant (translation by B.S. Levine). USSR Literature on Air Pollution and Related Occupational Diseases - A Survey. 17:39-45.

CHRONIC TOXICITY SUMMARY

METHYL BROMIDE

(bromomethane; monobromomethane)

CAS Registry Number: 74-83-9

I. Chronic Toxicity Summary

Inhalation reference exposure level	**5 μg/m^3 (1 ppb)**
Critical effect(s)	Histological lesions of the olfactory epithelium of the nasal cavity in rats
Hazard index target(s)	Respiratory system; nervous system; development

II. Physical and Chemical Properties (HSDB, 1994)

Description	Colorless gas
Molecular formula	CH_3Br
Molecular weight	94.95 g/mol
Density	3.89 g/L @ 25°C
Boiling point	3.6°C
Vapor pressure	1420 torr @ 20°C
Solubility	Soluble in ethanol, benzene, carbon disulfide, and 1.75% (w/w) in water
Odor threshold	20.6 ppm
Odor description	Sweetish odor
Metabolites	Methanol, bromide, 5-methylcysteine
Conversion factor	1 ppm = 3.89 mg/m^3 @ 25° C

III. Major Uses and Sources

Methyl bromide (MeBr) was used historically as an industrial fire extinguishing agent and was introduced in the U.S. from Europe in the 1920s. Current uses of MeBr include the fumigation of homes and other structures for termites and other pests. Methyl bromide is also used to fumigate soil before planting and fruits and vegetables after harvest. In 1981, 6.3 million pounds of MeBr were reportedly used in California (Alexeeff and Kilgore, 1983). By 1991, its use had grown to 18.7 million pounds in the state (Cal/EPA, 1993). The annual statewide industrial emissions from facilities reporting under the Air Toxics Hot Spots Act in California, based on the most recent inventory, were estimated to be 75,575 pounds of methyl bromide (CARB, 1999). This does not include emissions of methyl bromide during its use as a pesticide.

IV. Effects of Human Exposure

Workers (n = 32) exposed to MeBr during fumigation of soil or structures were compared to a referent group of 29 workers not exposed to MeBr, but exposed to other fumigants (Anger *et al.*, 1986). Exposures to MeBr were not quantified. It was found that workers exposed to MeBr had a higher rate of neurological symptoms and performed less well on several behavioral tests. Several confounding factors were present in this study, including lack of adjustments for age, alcohol consumption, prescription medication, illegal drugs, education, or ethnic group between the exposed and the referent groups.

V. Effects of Animal Exposure

The first experimental animal study on repeated MeBr exposures was carried out and reported by Irish and associates (1940). In this study, rats (135 per group), rabbits (104 per group), or female rhesus monkeys (13 per group) were exposed to 0, 17, 33, 66, 100, or 220 ppm (0, 66, 128, 256, 388, or 853 mg/m^3) 7-8 hours/day, 5 days/week for 6 months or until the majority of the animals exhibited severe signs of toxicity. Mortality was seen in rats, guinea pigs, and monkeys at 100 ppm. Rabbits began to die at 33 ppm. Severe effects, including paralysis, were seen after exposure to 66 ppm in rabbits and monkeys. None of the species exhibited adverse effects after exposure to 17 ppm.

Kato and associates (1986) observed focal lesions in the brain and heart in rats (10-12 per group) after inhalation of 150 ppm (585 mg/m^3) MeBr 4 hours/day, 5 days/week for 11 weeks. In another experiment, rats were exposed to 0, 200, 300, or 400 ppm (0, 777, 1160, or 1550 mg/m^3) MeBr 4 hours/day, 5 days/week for 6 weeks. In this experiment, rats exposed to any concentration of MeBr exhibited coronary lesions, and exposures of 300 ppm or greater resulted in neurological dysfunction, including ataxia and paralysis. Testicular atrophy was noted in 6 of the 8 animals exposed to 400 ppm.

Anger *et al.* (1981) determined that rabbits are more sensitive than rats to neurotoxicity of MeBr. In this study, rats or rabbits were exposed to 0 or 65 ppm (0 or 254 mg/m^3) MeBr for 7.5 hours/day, 4 days/week, for 4 weeks. Nerve conduction velocity and eyeblink reflex were impaired in the rabbits but not rats exposed to 65 ppm MeBr. Similarly, rats did not exhibit neurological signs after exposure to 55 ppm (215 mg/m^3) MeBr for 36 weeks. Rabbits exposed to 26.6 ppm (104 mg/m^3) did not display any neurological effects after 8 months exposure (Russo *et al.*, 1984).

In the studies of Reuzel and associates (1987, 1991), groups of 50 male and 60 female Wistar rats were exposed to 0, 3, 30, or 90 ppm methyl bromide (98.8%) for 6 hours per day, 5 days per week. Three groups of animals (10/sex/exposure level) were killed for observations at 14, 53, and 105 weeks of exposure. Body weight, hematology, clinical chemistry, and urinalyses were examined throughout the experiment in addition to histopathology and organ weights at time of necropsy. Exposures of males and females to 90 ppm resulted in reduced body weight. Exposure to 90 ppm also resulted in significant lesions in the heart in the form of cartilaginous metaplasia and thrombus in the males, and myocardial degeneration and thrombus in the

females. Exposure of males to 30 or 90 ppm resulted in a decrease in relative kidney weight. Histological changes in the nose, heart, esophagus, and forestomach were the principal effects of methyl bromide toxicity. At the lowest concentration (3 ppm), very slight degenerative changes in the nasal epithelium, and olfactory basal cell hyperplasia were noted in both sexes at 29 months. Based on this study, a LOAEL of 3 ppm (11.7 mg/m^3) was determined.

The National Toxicology Program (NTP) conducted a 13-week and a chronic study on the toxicology and carcinogenesis of methyl bromide in rats and mice (NTP, 1990). In the 13-week study, 18 rats/sex/group were exposed to 0, 30, 60, or 120 ppm (0, 117, 233, or 466 mg/m^3) MeBr 6 hours/day, 5 days/week. The mice were exposed to 0, 10, 20, 40, 80, or 120 ppm (0, 39, 78, 155, 311, or 466 mg/m^3) 6 hours/day, 5 days/week. Hematological parameters and selected organ weights were measured in both species, in addition to histopathological changes. Pseudocholinesterase activity and neurobehavioral tests were conducted in the mice. Serious effects, including 58% body weight loss, 17% mortality and severe curling and crossing of the hindlimbs were observed in mice exposed to 120 ppm MeBr. Exposure of males to 40 ppm or higher resulted in significant effects on several hematological parameters, including decreased mean cell hemoglobin and increased red blood cell count. The only exposure-related histological effect was olfactory epithelial dysplasia and cysts in the rats of both sexes exposed to 120 ppm.

A 6-week study in rats and mice (5 animals/sex/group) exposed to 0 or 160 ppm (0 or 624 mg/m^3) showed high mortality rates, loss in body weight and histological changes in multiple organ systems including brain, kidney, nasal cavity, heart, adrenal gland, liver, and testes (NTP, 1990).

An exposure of mice (86 animals/group) to 0, 10, 33, or 100 ppm (0, 38.8, 128, or 388 mg/m^3) MeBr for 6 hours/day, 5 days/week, for 103 weeks was also conducted by NTP (1990). In this study, high mortality rates in both males and females in the 100 ppm group resulted in a discontinuation of exposure after 20 weeks. A low incidence of sternal dysplasia and a significant decrease in locomotor activity were noted in the 10 ppm group.

A 5-day exposure of rats (10 animals/group) to 0, 90, 175, 250, or 325 ppm (0, 350, 680, 971, or 1260 mg/m^3) resulted in lesions in the nasal olfactory sensory cells, the cerebellum and adrenal gland beginning at 175 ppm (Hurtt *et al.*, 1987). Hurtt and Working (1988) later observed severe histological damage to the nasal epithelium following a single exposure to 90 or 200 ppm (351 or 780 mg/m^3) MeBr. Olfactory function, measured by the ability to locate buried food, was impaired at the 200 ppm exposure. In this study, reduced testosterone and testicular glutathione levels were observed in the male rats exposed to 200 ppm, but no effects on spermatogenesis, sperm quality, or testes histopathology were noted.

Sikov *et al.* (1981) examined the teratogenic potential of MeBr in rats and rabbits exposed to 0, 20, or 70 ppm (0, 78, or 272 mg/m^3) 7 hours/day, 5 days/week for 3 weeks during days 1-19 (rats) or 1-24 (rabbits) of gestation. No maternal or fetal effects were observed in the rats, however, severe maternal neurotoxic effects were observed in the rabbits that resulted in 24/25 deaths. In this study, no significant maternal or fetal effects were observed at a concentration of 20 ppm.

Another developmental toxicity study was conducted in rabbits by Breslin *et al.* (1990). In this study, rabbits were exposed to 0, 20, 40, or 80 ppm (0, 78, 156, or 312 mg/m^3) MeBr for 6 hours/day on gestation days 6-19. Maternal toxicity was observed at 80 ppm and included reduced body weight gain and signs of neurotoxicity. In addition to the maternal effects observed, a significant increase in incidence of gall bladder agenesis and fused sternebrae were observed in the offspring exposed to 80 ppm. No adverse effects were observed at 40 ppm or lower concentrations.

A 2-generation reproduction and developmental toxicity study on MeBr in rats was conducted by American Biogenics Corporation (1986). Groups of rats (25/sex/concentration) were exposed to 0, 3, 30, or 90 ppm (0, 12, 117, or 350 mg/m^3) MeBr 6 hours/day, 5 days/week during premating, gestation, and lactation through 2 generations. Significant decreases in body weight during the pre-mating period and at the end of the study were observed in the males exposed to 90 ppm. Although some adult organ weights were affected in the 90-ppm group, there was no evidence of histopathology in these organs. Neonatal body weights were decreased by exposure to 30 ppm. There was a decreased cerebral cortex width in the 90 ppm F_1 group, reduced brain weight in 30 ppm F_1 females, and reduced fertility in the 30 and 90 ppm F_{2b} groups.

VI. Derivation of Chronic Reference Exposure Level

Study	Reuzel *et al.*, 1987; 1991
Study population	Male and female Wistar rats (50 and 60 per group, respectively)
Exposure method	Discontinuous inhalation exposures (0, 3, 30, or 90 ppm) over 29 months
Critical effects	Basal cell hyperplasia of the olfactory epithelium of the nasal cavity
LOAEL	3 ppm
NOAEL	Not observed
Exposure continuity	6 hr/day, 5 days/week
Exposure duration	29 months
Average experimental exposure	0.54 ppm for the LOAEL group
Human equivalent concentration	0.12 ppm for the LOAEL group (gas with extrathoracic respiratory effects, RGDR = 0.23, based on MV = 0.03 m^3/min, SA = 11.6 cm^2)
LOAEL uncertainty factor	3 (20% extra risk of a mild effect)
Subchronic uncertainty factor	1
Interspecies uncertainty factor	3
Intraspecies uncertainty factor	10
Cumulative uncertainty factor	100
Inhalation reference exposure level	0.001 ppm (1 ppb, 0.005 mg/m^3, 5 μg/m^3)

The chronic REL for methyl bromide is also the U.S EPA RfC.

VII. Data Strengths and Limitations for Development of the REL

The major strengths of the REL for methyl bromide are the use of a comprehensive, long-term, multiple dose study with large sample sizes, and the availability of supporting data including long-term studies in other species and reproductive and developmental studies. The major uncertainties are the lack of human data and the lack of a NOAEL observation for the critical effect.

The California Department of Pesticide Regulation used a different approach that adjusts for respiration rate differences between humans and animals and which uses 10-fold uncertainty factors for interspecies differences, for intraspecies variability, and for a LOAEL to NOAEL extrapolation. Applying these factors to the same 3 ppm LOAEL results in a level for children and adults of 1 and 2 ppb (4 and 8 $\mu g/m^3$), respectively.

VIII. References

Alexeeff GV, and Kilgore WW. 1983. Methyl bromide. In: Residue Reviews. Vol. 88. New York: Springer-Verlag. pp. 102-153.

American Biogenics Corporation. 1986. Two-generation reproduction study via inhalation in albino rats using methyl bromide. Final Report. American Biogenics Corporation Study 450-1525, OTS 0515364 sponsored by the Methyl Bromide Panel.

Anger WK, Moody L, Burg J, Brightwell WS, Taylor BJ, Russo JM, *et al.* 1986. Neurobehavioral evaluation of soil and structural fumigators using methyl bromide and sulfuryl fluoride. Neurotoxicology. 7(3):137-156.

Anger WK, Setzer JV, Russo JM, Brightwell WS, Wait RG, and Johnson BL. 1981. Neurobehavioral effects of methyl bromide inhalation exposures. Scand. J. Work Environ. Health 7(Suppl. 4):40-47.

Breslin WJ, Zablotny CL, Brabley GJ, and Lomax LG. 1990. Methyl bromide inhalation teratology study in New Zealand white rabbits. Toxicology Research Laboratory, Health and Environmental Sciences, The Dow Chemical Company, Midland, MI. Study No. K-000681-033. OTS Number 8EHQ-1189-0844 S.

CARB. 1999. Air toxics emissions data collected in the Air Toxics Hot Spots Program CEIDARS Database as of January 29, 1999.

Cal/EPA. May, 1993. California Environmental Protection Agency. Letter to Carol Browner, Administrator, U.S.EPA, on methyl bromide uses in California.

HSDB. 1999. Hazardous Substance Data Bank. National Library of Medicine, Bethesda, Maryland. WWW database (http://sis.nlm.nih.gov/sis1/).

Hurtt ME, Morgan KT, and Working PK. 1987. Histopathology of acute toxic responses in selected tissues from rats exposed by inhalation to methyl bromide. Fundam. Appl. Toxicol. 9:352-365.

Hurtt ME, and Working PK. 1988. Evaluation of spermatogenesis and sperm quality in the rat following acute inhalation exposure to methyl bromide. Fundam. Appl. Toxicol. 10(3):490-498.

Irish DD, Adams EM, Spencer HC, and Rowe VK. 1940. The response attending exposure of laboratory animals to vapors of methyl bromide. J. Ind. Hyg. Toxicol. 22(6):218-230.

Kato N, Morinobu S, and Ishizu S. 1986. Subacute inhalation experiment for methyl bromide in rats. Ind. Health. 24:87-103.

NTP. 1990. National Toxicology Program. Toxicology and carcinogenesis studies of methyl bromide (CAS No. 74-83-9) in B6C3F1 mice (inhalation studies). NTP TR 385, NIH Publication No. 91-2840.

Reuzel PGJ, Kuper CF, Dreef-van der Meulen HC, and Hollanders VMH. 1987. Chronic (29-month) inhalation toxicity and carcinogenicity study of methyl bromide in rats. Report No. V86.469/221044. Netherlands Organization for Applied Scientific Research, Division for Nutrition and Food Research, TNO. EPA/OTS Document No. 86-8700001202.

Reuzel PGJ, Dreef-van der Meulen HC, Hollanders VMH, Kuper CF, Feron VJ, and Van der Heijden CA. 1991. Chronic inhalation toxicity and carcinogenicity study of methyl bromide in Wistar rats. Food Chem. Toxicol. 29(1):31-39.

Russo JM, Anger WK, Setzer JV, and Brightwell WS. 1984. Neurobehavioral assessment of chronic low-level methyl bromide exposure in the rabbit. J. Toxicol. Environ. Health. 14:247-255.

Sikov MR, Cannon WC, Carr DB, Miller RA, Montgomery LR, and Phelps DW. 1981. Teratologic assessment of butylene oxide, styrene oxide and methyl bromide. Battelle Pacific Northwest Laboratory, Richland, WA, for the National Institute for Occupational Safety and Health, Cincinnati, OH.

U.S.EPA. 1999. Environmental Protection Agency. Integrated Risk Information System (IRIS) database. Reference concentration (RfC) for methyl bromide.

CHRONIC TOXICITY SUMMARY

METHYL CHLOROFORM

(1,1,1-trichloroethane, methyltrichloromethane)

CAS Registry Number: 71-55-6

I. Chronic Toxicity Summary

Inhalation reference exposure level	**1,000 µg/m^3 (200 ppb)**
Critical effect(s)	Astrogliosis in the sensorimotor cortex (brain) of gerbils
Hazard index target(s)	Nervous system

II. Chemical Property Summary (HSDB, 1999)

Description	Colorless liquid
Molecular formula	$C_2H_3Cl_3$
Molecular weight	133.42 g/mol
Density	1.3376 g/cm^3 @ 20° C
Boiling point	74.1° C
Melting point	-30.4° C
Vapor pressure	127 torr @ 25° C
Solubility	Soluble in acetone, benzene, methanol, carbon tetrachloride
Conversion factor	5.47 µg/m^3 per ppb at 25ºC

III. Major Uses and Sources

Methyl chloroform is used as a solvent for adhesives and for metal degreasing (ACGIH, 1992). It is also used in the manufacture of vinylidene chloride and in textile processing and dry cleaning. The annual statewide industrial emissions from facilities reporting under the Air Toxics Hot Spots Act in California, based on the most recent inventory, were estimated to be 25,316,458 pounds of methyl chloroform (CARB, 1999a). Statewide monitored median and mean concentrations of methyl chloroform have been generally declining; decreasing from 0.8 or 1.71 ppb in 1990 to 0.12 or 0.30 ppb in 1996 (CARB, 1999b).

IV. Effects of Human Exposure

A 44-year old woman was diagnosed with peripheral neuropathy following 18 months of occupational exposure to methyl chloroform in a solvent bath (House *et al.*, 1994). There was no identified exposure to agents known to cause peripheral neuropathy, such as n-hexane or

trichloroethylene. The worker reported that she wore protective gloves and a respirator, both of which frequently leaked. Seven months following removal from exposure, the worker showed improved nerve conduction.

Other case reports have identified the nervous system as a target of methyl chloroform toxicity in similar exposure scenarios. Three workers developed distal sensory neuropathy after working with methyl chloroform in a degreasing operation with repeated dermal exposure (Liss, 1988; Howse *et al.*, 1989). Changes were observed in nerve conduction in the upper extremities accompanied by both axonopathy and myelonopathy.

Twenty-eight workers with chronic exposure to high (but unquantified) concentrations of 1,1,1-trichloroethane had significant deficits in memory, intermediate memory, rhythm, and speed based on the Luria-Nebraska Neuropsychological Battery (Kelafant *et al*., 1994). Deficits in vestibular, somatosensory, and ocular components of balance were noted.

A 13-year-old male died after intentional inhalation of 1,1,1-trichloroethane (Winek *et al*., 1997). Autopsy findings included tissue congestion of lung, liver and kidney.

Cardiac arrhythmia resulting from heightened cardiac sensitivity to epinephrine has been reported in several case reports of high acute inhalation exposures to methyl chloroform (ATSDR, 1990). There are case reports of arrhythmias persisting for two weeks or more after cessation of exposure to methyl chloroform (McLeod *et al.*, 1987).

An epidemiological study of workers chronically exposed to low levels of methyl chloroform (<250 ppm) found no changes in blood pressure, heart rate, or electrocardiogram (Kramer *et al.*, 1978). This study consisted of 151 workers who had been exposed for more than one year. No neurophysiological testing was done.

Another study of 22 female workers exposed to methyl chloroform (plus 7 unexposed control workers) at concentrations ranging from 110-345 ppm in air for a mean of 6.7 years failed to identify neurotoxicity resulting from methyl chloroform exposure (Maroni *et al.*, 1977). The examination included evaluation for neurologic symptoms, changes in nerve conduction, and psychomotor tests.

Liver disease was observed in a worker exposed to methyl chloroform in a clothing factory screen printing room (Cohen and Frank, 1994). The worker was exposed for a total of 4 years before occupational exposure was identified as the cause of the liver disease. The worker sprayed an adhesive (containing 65% methyl chloroform, 25% propane and dimethyl ether, and 10% inert ingredients) during which the worker reported often feeling dizzy or intoxicated. Three months following removal of the worker from exposure, liver function tests, although still abnormal, were significantly improved. Other case reports support these findings (Hodgson *et al.*, 1989; Halevy *et al.*, 1980).

Six male volunteers were exposed to 35 and 350 ppm methyl chloroform for 6-hours on two separate occasions (Nolan *et al.*, 1984). Absorption was determined to be 25% of the inhaled dose. Of the absorbed dose, 91% was excreted unchanged in the expired air. Although the odor

was perceptible for the duration of the exposure, no subjective symptoms were reported by the volunteers.

V. Effects of Animal Exposure

Gerbils (4/sex/dose plus 24 sex-matched control animals) were continuously exposed to 70, 210, or 1000 ppm methyl chloroform for 3 months (Rosengren *et al.*, 1985). A 4-month (solvent-free) recovery period following exposure was included to evaluate "lasting or permanent changes." Body weights were not changed significantly as a result of exposure. Brain weights in the animals in the 1000 ppm dose group were significantly decreased. Fibrillary astrocytes are formed in the brain in response to injury. Brain injury in methyl chloroform exposed gerbils was evaluated by detection of glial fibrillary acidic (GFA) protein, the main protein subunit of astroglial filaments. Increased levels of GFA protein were detected in the sensorimotor cerebral cortex of animals exposed to 210 or 1000 ppm methyl chloroform.

A later study in gerbils examined the effects of a 3-month continuous exposure to 70 ppm methyl chloroform followed by a 4-month recovery period (Karlsson *et al.*, 1987). DNA content was significantly decreased in three areas of the brain: posterior cerebellar hemisphere, anterior cerebellar vermis, and hippocampus. The authors contended that depressions in DNA content reflect decreased cell density.

No evidence of peripheral neuropathy or other neurotoxicity was detected in rats exposed to 200, 620, or 2000 ppm methyl chloroform 6 hours per day, 5 days per week for 13 weeks (Mattson *et al.*, 1993). The study included a functional observational test battery and measured visual, somatosensory, auditory and caudal nerve-evoked potentials. Histopathology of the brain, spinal cord, peripheral nerves and limb muscles was also examined at the end of the 13-week exposure.

Forty percent of all mice continuously exposed to 1000 ppm methyl chloroform for 14 weeks exhibited evidence of hepatocellular necrosis (McNutt *et al.*, 1975). A statistically significant increase in liver weight per body mass was observed throughout the study. Electron microscopy revealed accumulation of triglyceride droplets in the centrilobular hepatocytes following one week of exposure to 1000 ppm methyl chloroform. After 4 weeks of exposure, cytoplasmic alterations in centrilobular hepatocytes included a loss of polyribosomes and increased smooth endoplasmic reticulum. Similar changes observed occasionally in hepatocytes from mice exposed to 250 ppm were not as dramatic.

Mild hepatocellular changes were observed in rats exposed to 1500 ppm methyl chloroform 6 hours per day, 5 days per week for 6, 12, and 18 months (Quast *et al.*, 1988). At 24 months, these slight effects were no longer discernible due to confounding geriatric changes. No hepatocellular changes or other adverse effects were observed in rats exposed to 150 or 500 ppm methyl chloroform for up to 24 months.

The developmental toxicity of inhaled methyl chloroform was studied in CD-1 mice. Mice were exposed on gestation days 12 through 17 to either 2000 ppm methyl chloroform for 17 hours per day or 8000 ppm methyl chloroform for 1 hour three times per day (Jones *et al*., 1996). There

were no effects on pregnancy outcome, but exposed pups has reduced weight gain, had poorer results on motor coordination tests and showed delays in negative geotaxis (orienting towards the top of a sloped screen).

VI. Derivation of Chronic Reference Exposure Level (REL)

Study	Rosengren *et al.* (1985)
Study population	Mongolian gerbils (4/sex/dose)
Exposure method	Whole-body inhalation exposure
Critical effects	Astrogliosis in the sensorimotor cerebral cortex
LOAEL	210 ppm
NOAEL	70 ppm
Exposure continuity	Continuous
Average experimental exposure	70 ppm for NOAEL group
Human equivalent concentration	Not derived (species-specific data for gerbils unavailable to validate assumption of RGDR=1)
Exposure duration	3 months
LOAEL uncertainty factor	1
Subchronic uncertainty factor	3
Interspecies uncertainty factor	10
Intraspecies uncertainty factor	10
Cumulative uncertainty factor	300
Inhalation reference exposure level	0.2 ppm (200 ppb; 1 mg/m^3; 1,000 µg/m^3)

VII. Data Strengths and Limitations for Development of the REL

Case reports indicate that the nervous system and the liver are targets of the toxicity of methyl chloroform (House *et al.*, 1994; Liss, 1988; Howse *et al.*, 1989; Cohen and Frank, 1994). The largest of the epidemiological studies (Kramer *et al.*, 1978; Maroni *et al.*, 1977), however, did not identify adverse effects as a result of chronic methyl chloroform exposure. The Kramer *et al.* (1978) study limited its evaluation to changes in blood pressure, heart rate, or electrocardiogram and exposure levels were only characterized as less than 250 ppm. Maroni *et al.* (1977) conducted their study among 22 women exposed occupationally to methyl chloroform levels as low as 110 ppm. Although the subjects were evaluated specifically for signs of neurotoxicity, the small sample size limits conclusions that can be drawn from their failure to identify adverse effects in this population. If no effects are associated with the exposures in the 2 studies (Kramer *et al.*, 1978; Maroni *et al.*, 1977), the REL predicted would be approximately 3 ppm.

Data from animal studies generally support the findings of the case reports from human exposures. Both neurotoxicity and hepatotoxicity have been identified among animals exposed by inhalation to methyl chloroform. The adverse effect observed at the lowest level in these studies was the development of astrogliosis in the brains of gerbils exposed for 3 months to 210 ppm methyl chloroform (Rosengren *et al.*, 1985). A no-observed-adverse-effect-level (NOAEL)

in this study was 70 ppm methyl chloroform. A subsequent study identified a more subtle change in the brains of gerbils exposed similarly to 70 ppm methyl chloroform, with slightly decreased DNA content found in several discrete brain regions of exposed animals. However, the relationship between tissue DNA content and cell density as an indication of adverse effect in the brain was considered too tenuous for the development of a guidance value for chronic exposure to methyl chloroform.

The major strengths of the REL for methyl chloroform are the observation of the NOAEL and the continuous subchronic exposure regimen. The major uncertainties are the lack of human exposure data, the lack of dose-response information, and the lack of comprehensive multi-organ effects data.

VIII. References

ACGIH. 1992. American Conference of Governmental Industrial Hygienists, Inc. Documentation of the Threshold Limit Values and Biological Exposure Indices. Sixth edition. Cincinnati, OH.

ATSDR. 1990. Agency for Toxic Substances and Disease Registry. US Public Health Service. Toxicological Profile for 1,1,1-Trichloroethane. Prepared by Syracuse Research Corporation under contract to Clement Associates, Inc. under contract no. 205-88-0608.

CARB (California Air Resources Board). 1999a. Air toxics emissions data collected in the Air Toxics Hot Spots Program CEIDARS Database as of January 29, 1999.

CARB. 1999b. 1990-1996 Statewide Methyl Chloroform Summary, ppb. WWW document (http://www.arb.ca.gov/aqd/tcea/tastate.htm).

Cohen C, and Frank AL. 1994. Liver disease following occupational exposure to 1,1,1-trichloroethane: a case report. Am. J. Ind. Med. 26:237-241.Halevy J, Pitlik S, Rosenfeld J, and Eitan B-D. 1980. 1,1,1-Trichloroethane intoxication: a case report with transient liver and renal damage. Review of the literature. Clin. Toxicol. 16:467-72.

Hodgson MJ, Heyl AE, and Van Thiel DH. 1989. Liver disease associated with exposure to 1,1,1-trichloroethane. Arch. Intern. Med. 149:1793-1798.

House RA, Liss GM, and Wills MC. 1994. Peripheral sensory neuropathy associated with 1,1,1-trichloroethane. Arch. Environ. Health 49:196-199.

Howse DC, Shanks GL, and Nag S. 1989. Peripheral neuropathy following prolonged exposure to methyl chloroform. Neurology, 39:242. (Abstract)

HSDB. 1999. Hazardous Substance Data Bank. National Library of Medicine, Bethesda, Maryland. WWW database (http://sis.nlm.nih.gov/sis1/).

Jones HE, Kunko PM, Robinson SE, Balster RL. 1996. Developmental consequences of intermittent and continuous prenatal exposure to 1,1,1-trichloroethane in mice. Pharmacol Biochem Behav 55(4):635-46.

Karlsson J-E, Rosengren LE, Kjellstrand P, and Haglid KG. 1987. Effects of low-dose inhalation of three chlorinated aliphatic organic solvents on deoxyribonucleic acid in gerbil brain. Scand. J. Work Environ. Health 13:453-458.

Kelafant GA, Berg RA, Schleenbaker R. 1994. Toxic encephalopathy due to 1,1,1-trichloroethane exposure. Am J Ind Med 25(3):439-46.

Kramer CG, Ott MG, Fulkerson JE, Hicks N, and Imbus HR. 1978. Health of workers exposed to 1,1,1-trichloroethane: a matched pair study. Arch. Environ. Health 33:331-342.

Liss G. 1988. Peripheral neuropathy in two workers exposed to 1,1,1-trichloroethane. JAMA 260(15):2217.

Maroni M, Bulgheroni C, Cassitto G, Merluzzi F, Gilioli R, and Foa V. 1977. A clinical, neurophysiological and behavioral study of female workers exposed to 1,1,1-trichloroethane. Scand. J. Work Environ. Health 3:16-22.

Mattson JL, Albee RR, Lomax LG, Beekman MJ, and Spencer PJ. 1993. Neurotoxicologic examination of rats exposed to 1,1,1-trichlorethane vapor for 13 weeks. Neurotoxicol. Teratol. 15:313-326.

McLeod AA, Marjot R, and Monaghan MJ. 1987. Chronic cardiac toxicity after inhalation of 1,1,1-trichloroethane. Br. Med. J. 294:727-729.

McNutt NS, Amster RL, McConnell EE, and Morris F. 1975. Hepatic lesions in mice after continuous inhalation exposure to 1,1,1-trichloroethane. Lab. Invest. 32:642-654.

Nolan RJ, Freshour NL, Rick DL, McCarty LP, and Saunders JH. 1984. Kinetics and metabolism of inhaled methyl chloroform (1,1,1-trichloroethane) in human volunteers. Fundam. Appl. Toxicol. 4:654-662.

Quast JF, Calhoun LL, and Frauson LE. 1988. 1,1,1-Trichloroethane formulation: a chronic inhalation toxicity and oncogenicity study in Fischer 344 rats and B6C3F1 mice. Fundam. Appl. Toxicol. 11:611-625.

Rosengren LE, Kjellstrand AA, and Haglid KG. 1985. Astrogliosis in the cerebral cortex of gerbils after long-term exposure to 1,1,1-trichloroethane. Scand. J. Work Environ. Health 11:447-455.

Winek CL, Wahba WW, Huston R, Rozin L. 1997. Fatal inhalation of 1,1,1-trichloroethane.. Forensic Sci Int 87(2):161-165.

CHRONIC TOXICITY SUMMARY

METHYL ISOCYANATE

(MIC, CH_3-N=C=O)

CAS Registry Number: 624-83-9

I. Chronic Toxicity Summary

Inhalation reference exposure level	**1 µg/m^3** (0.5 ppb)
Critical effects(s)	Decreased weight gain and lung pathology at cessation of exposure in rats
Hazard index target(s)	Respiratory system; reproductive system

II. Chemical Property Summary (HSDB, 1995; CRC, 1994)

Description	Colorless liquid
Molecular formula	C_2H_3NO
Molecular weight	57.06 g/mol
Boiling point	39.5^{o}C
Melting point	–45°C
Vapor pressure	348 torr @ 20°C, 600 torr @ 30°C (Varma and Guest, 1993)
Solubility	10 percent in water @ 15°C
Conversion factor	2.3 µg/m^3 per ppb at 25°C

III. Major Uses and Sources (Dave, 1985; U.S. EPA, 1986; HSDB, 1995)

Methylisocyanate (MIC) is prepared industrially by reacting methylamine with phosgene, oxidizing monomethylformamide at high temperatures ($\geq$ 550°C), or heating metal methylisocyanates. Because of its high reactivity, MIC is used as an intermediate in organic synthesis, most notably in the production of carbamate based pesticides. Tobacco smoke from some brands of cigarettes also contains MIC (about 4 µg per cigarette). Workers exposed to the MIC 8-hour threshold limit value of 0.02 ppm (46 µg/m^3) are exposed to approximately 460 µg MIC in a workday. Based on the most recent inventory, the annual statewide industrial emissions from facilities reporting under the Air Toxics Hot Spots Act in California were negligible (CARB, 2000). This does not include estimates of emissions of breakdown products from the use of metam sodium in agricultural applications. Use of metam sodium averaged 15,400,000 pounds/year from 1995 to 1999.

IV. Effects of Human Exposure

Although occupational exposures to MIC have been documented (Varma, 1986), few known exposures to the general public have occurred. A major exposure occurred in Bhopal, India in December 1984. Because of the sudden, short-term release (30-45 minutes), no measurements occurred, but the air concentration was estimated as 13 ppm (Dave, 1985) to 100 ppm (Varma, 1986).

The chemical identity of the ultimate toxicant has not been unequivocally determined and may consist of more than one chemical species. Although the chemistry of MIC suggests that hydrolysis to methylamine and dimethylurea is rapid, such hydrolysis in moist air is probably slow, and the reaction with photochemically produced hydroxyl radical is also slow (chemical $T_{1/2}$ about 3 months) (U.S. EPA, 1986). Brown *et al.* (1987) have shown that the alkylisocyanates (e.g., MIC) are relatively resistant (compared to the arylisocyanates) to hydrolysis in water. Hence, despite the high water reactivity of MIC, this compound could possibly persist in the environment for many days after an initial release.

Within 5 days of the initial exposure to MIC at Bhopal, more than 2,000 deaths occurred (Dave, 1985), while 4,000 more deaths were documented during the following decade (Lepkowski, 1994). The initial symptoms among the population living near the MIC plant were irritation and difficulty in breathing (Varma, 1986). Blindness occurred in more than 10,000 exposed persons but later resolved in most cases (Andersson *et al.*, 1990). The acute damage that led to death was mainly to the respiratory system, most likely pulmonary edema, bronchospasm, and electrolyte imbalance (Varma, 1986). However extrapulmonary damage, including tissue anoxia, gastrointestinal symptoms, and muscular weakness, were also observed (Dave, 1985). Within a year of the exposure, survivors continued to exhibit damage to the lung and eyes. Fibrosis of the lungs was seen in 30 percent of this group (Dave, 1985).

Reproductive toxicity was observed among women exposed to MIC in Bhopal. Varma (1987) reported 43 percent unsuccessful pregnancies among 865 women who were pregnant at the time of the MIC release. Among the live births, 14 percent of the infants died within 30 days, whereas a death rate of only 3 percent for the same interval was recorded 2 years prior to the release. Bhandari *et al.* (1990) reported increased spontaneous abortions and neonatal deaths among exposed women who were pregnant at the time of exposure compared to a control group in another city. In the latter study, stillbirths and congenital malformations were similar in the exposed and non-exposed groups.

Non-reproductive, non-pulmonary responses were evident in a group of exposed Bhopal residents, 3-years following exposure to the MIC vapors. Loss of vision and loss of visual acuity were more prominent among exposed residents than among unexposed people, and the losses appeared to be dose-dependent (Andersson *et al.*, 1990). In this study, the surrogate for dose was extent of early deaths in a housing cluster. Similarly, cataracts were reported more often among the exposed than among the unexposed group.

The lesions associated with lung damage may be expressed as pulmonary edema for immediate effects (Varma, 1986), and lesions associated with the bronchoalveolar area for long-term effects

(Dave, 1985, Varma, 1986). Vijayan *et al.* (1995) studied cellular components of bronchoalveolar lavage (BAL) and pulmonary function in Bhopal patients 1.3, 2.7, and 5.1 years after exposure to MIC. All had lived within 3-miles of the factory and all experienced acute respiratory and ophthalmic symptoms on the day of exposure. All were experiencing continued respiratory symptoms. Among the exposed people, decrements in forced vital capacity and forced expiratory volume (at 1-minute) were observed. In general, the decrements ranged from 12 - 21 percent of predicted values, whereas the control group exhibited decrements of 2 - 4 percent of the expected values. Analysis of the BAL revealed increases in total cells (all exposed groups), increased absolute numbers of macrophages (all exposure groups), decreased percentage of lymphocytes (2.7 and 5.1 year groups), and increased numbers and percentage of neutrophils (5.1 year group). These cell types are involved, through the secretion of various factors, in inflammatory and immunologic processes in the lung (Reiser and Last, 1986). The Vijayan *et al.* (1995) study thus suggests long term damage to lung parenchyma among people who survived the initial acute effects of MIC exposure.

In summary, humans exposed acutely by inhalation to MIC may experience long-term (as well as immediate) damage to pulmonary and extrapulmonary systems. The lung is probably the critical target organ for long-term effects from acute exposure, although adverse effects on other organs (e.g., eye, reproductive, and gastrointestinal) also exist. The late responses to the acute exposure suggest an immunological component, which could involve several systems including lung, eye, liver, and kidney. The chemical identity of the ultimate toxicant is unknown and may be more than one compound.

Avashia *et al*. (1996) assessed pulmonary effects from long-term, low-level MIC for more than 400 workers at a large chemical facility. Serial pulmonary function data, cigarette smoking histories, and industrial-hygiene measurements were available. Jobs were classified according to level of MIC exposure as none, low, moderate or high. Where work records were incomplete, exposures were based on the ratings of supervisors and coworkers. The frequency of pulmonary impairment was evaluated for the assumed four levels of exposure. No specific or consistent pulmonary impairment was evident. Unfortunately the report gave no quantitative classification of low, moderate or high MIC levels.

V. Effects of Animal Exposure

Experimental animal studies have been designed to address the experiences of the victims of the Bhopal disaster, in which the exposure has been described as acute because of the short duration (30-45 min). No studies were found that described exposure duration greater than 10 days. However, a chronic component to MIC exposure may exist as a result of slower rates of hydrolysis in air (compared to water), the presence of carbamylated hemoglobin in MIC-exposed people, and the change from edematous to inflammatory and/or fibrotic lesions with time. Further, a glutathione-dependent reversible MIC transport system has been suggested in experimental animals (see below).

MIC is absorbed through the respiratory tract and distributed to non-respiratory organs in experimental animals. In an acute (30 min) inhalation exposure to a dose of ^{14}C-MIC (labeled in

the isocyanate moiety) equivalent to one-LC_{50} (23 mg/L), rats accumulated protein-bound radioactivity (including carbamylated proteins) in brain, liver, kidney, and lung, but not in blood (Bhattacharya *et al.*, 1988). Ferguson *et al.* (1988) exposed guinea pigs by inhalation to 0.47 ppm ^{14}C-MIC (methyl group) for 6-hours. At the end of exposure, the label was found in arterial and venous blood, bile, and urine. At 2.7 days post-exposure, the label decreased to 2-7 percent. MIC was retained in the nasal-laryngeal area of the guinea pigs.

MIC, like reactive isocyanates in general, can react with biological molecules containing amino, alcohol, or sulfhydryl groups, as well as with water. While hydrolysis in an aqueous environment, such as the lung, is theoretically possible, measurements show that alkyl isocyanates are relatively resistant (compared to arylisocyanates) to such hydrolysis (Brown *et al.* 1987). The absence of a role for MIC hydrolytic products, methylamine (MA) or dimethylurea (DMU), is also suggested by the work of Jeevaratnam and Sriramachari (1994) and Sriramachari *et al*. (1994). Inhalation (30 min) or subcutaneous exposure of rats to either hydrolytic product at levels equivalent to the LC_{50} or LD_{50} did not result in death. Similarly, neither methylamine nor dimethylurea duplicated the acute effects of respiratory necrosis and congestion. However, exposure to these hydrolytic products did lead to interstitial pneumonitis, an observation that suggests MA and/or DMU could lead to subsequent inflammatory responses if sufficient amounts are present.

A role for methylamine in reproductive/developmental toxicity was investigated by Guest and Varma (1991). In a mouse study, pregnant dams were exposed to varying doses (intraperitoneal) of methylamine (as well as the di- and trimethyl compounds). Reproductive toxicity was not observed for methylamines. However, in cultured embryo experiments, decrements in crown-rump length, yolk-sac diameter, head length, and embryo survival were observed. The concentrations were high (>0.75 mM) and the interpretation of the biological activity of methylamine in terms of inhalation exposure is difficult.

MIC is a carbamylating intermediate; this is the basis for its use in the manufacture of carbamate based pesticides. In the same way, MIC should react with the appropriate functional groups of proteins, peptides, and nucleic acids. However, i*n vitro* studies with cholinesterases show that such a reaction is not efficient (Brown *et al.*, 1987), an observation which may be explained by the presence of protonated amino groups at physiological pH (Baillie and Slatter, 1991).

A transport system for MIC via reduced glutathione (GSH) has been suggested by the discovery of the MIC-adduct, S-(N-methylcarbamoyl)glutathione (SMG), in the bile and the MIC-adduct of N-acetylcysteine (mercapturic acid, AMCC) in the urine of rats exposed to MIC by non-inhalation routes (Pearson *et al.*, 1990; Slatter *et al.*, 1991). The reaction of MIC with GSH and with cysteine is reversible, and can provide a source of free MIC in the tissues (Baille and Slatter, 1991). Similar studies in experimental animals exposed to MIC by inhalation have not been reported. However, humans exposed by inhalation to N,N-dimethylformamide (H-C-(=O)-$N(CH_3)_2$) excrete AMCC in urine (Mraz and Nohova, 1992). Hence a reversible MIC-transport system in animals, including humans, is possible, and the presence of high levels of GSH in human lavage fluid (Cantin *et al.*, 1987) would permit the initiation of this mechanism.

The toxicity of the adduct SMG was tested in mouse embryo culture (Guest *et al*., 1992). Mouse embryos, at day 8 of gestation in vivo, were removed from their dams and cultured in the presence (and absence) of SMG. Dose-dependent (0.25 - 2 mM) decrements were observed for yolk sac diameter, crown-rump length, somite number, and protein content. Delayed DNA synthesis in the embryos and in yolk-sacs occurred in the presence of 0.25 mM SMG. Similar to the results obtained with methylamine, the SMG concentrations were high and the exposures were not by inhalation. However, the data show that a MIC metabolite, SMG, has toxic properties. In the presence of GSH (1 or 3 mM), the extent of the SMG-dependent toxicities was decreased. Such data demonstrate the reversibility of the binding between MIC and GSH.

Three inhalation studies were identified in which experimental animals were exposed to more than one dose of MIC. Among these studies, two used exposure durations for more than one day (Dodd and Fowler, 1986; Mitsumori *et al.*, 1987). Rats and mice were exposed by inhalation to 0, 1.1, and 2.8 (female) or 3.0 (male) ppm MIC for 6 hr/day for a total of 4 days, and then followed during a 91-day post-exposure interval (Mitsumori *et al.*, 1987). Among the rats, post-exposure deaths occurred by 49 days (male) and 14 days (female) at the high dose. Among the mice, only 1 male mouse died at 16 days post-exposure. Reduced weight gain was observed among the female and male rats in the high dose group, prior to death, although the absolute weights were not different from the unexposed rats one day before the end of exposure. Among the mice, a slowed weight gain was observed at 3- and 6-days post exposure (male) and 1 day post exposure (female) at the high dose, but normal weight gain returned by 1 week following cessation of exposure. At 7 days post-exposure, microscopic changes were observed in the respiratory system among the high dose rats of both sexes. Between 8- and 27 days post-exposure, increased lesions in the respiratory tract and also in liver, thymus, spleen, heart, and brain were observed at the high dose. Similar lesions were not observed in rats exposed to 1.1 ppm MIC and followed to the 8-27 day post-exposure. Among survivors, the incidence of lesions decreased to control values by 91 days. Among the mice, treatment related changes in the respiratory tract were observed at the high dose at 7 days post-exposure. Between 28 and 91 days, the lesions associated with the upper respiratory tract disappeared, whereas those associated with the major bronchi remained, although somewhat attenuated. These data suggest that the rat is more sensitive than the mouse to the effects of MIC. A LOAEL of 2.9 ppm is indicated, based on post-exposure decreased weight gain and respiratory tract changes in rats.

Dodd and Fowler (1986) exposed rats to 0, 0.15, 0.6, and 3.1 ppm MIC for two 4-day sessions at 6-hours/day and examined the animals within 1-day following exposure. The 2-cycle exposure included a 2-day recess from exposure. No deaths occurred at any MIC concentration during the exposure. Lesser weight gain occurred for rats in the 3.1 ppm groups, whereas weight among the rats in the 0.15 and 0.6 ppm MIC groups was indistinguishable from the air-exposed control animals. On exposure days 3 and 8, mean food consumption values in the high dose group were below those for the non-exposed group. At the time of termination, male rats exposed to 3.1 ppm MIC exhibited a 38 percent increase in hemoglobin concentration and a 26 percent decrease ($p<0.001$) in oxygen saturation, compared to the unexposed rats ($p<0.001$). Such changes were not observed for the female rats exposed to 3.1 ppm or for rats of either sex exposed to 0.15 or 0.6 ppm MIC. Absolute lung weights increased ($p<0.001$) in both sexes after exposure to 3.1 ppm, compared to the control rats. Decreases in liver, kidney and testes absolute weights were observed in this exposure group, but the authors interpreted these data as a reflection of the body

weight losses. No weight changes were observed in rats exposed to 0.15 or 0.60 ppm MIC. Gross and microscopic lesions were observed in rats (female and male) exposed to 3.1 ppm, but not in rats exposed to 0, 0.15, or 0.6 ppm MIC. The microscopic lesions occurred in the respiratory tract and consisted of inflammation, epithelial necrosis, squamous metaplasia, and epithelial hyperplasia. These lesions extended into the bronchioles. These data suggest a NOAEL of 0.6 ppm MIC, based on weight gain loss, absolute lung weight, and lung histopathology in rats, immediately following cessation of exposure.

Post-exposure changes in lung pathology also occurred in the rats surviving 3.1 ppm in the Dodd and Fowler (1986) study. The early lesions associated with inflammation, epithelial necrosis, squamous metaplasia, and epithelial hyperplasia extending to the bronchioles either decreased in severity or receded toward the upper respiratory tract by 85-days post-exposure. In males, the intraluminal and submucosal fibroplasia changed in appearance during this interval, due in part to the maturation of fibrous tissue. Mucous plugs were also seen in the terminal bronchioles and alveoli in some rats. The importance of this observation is the progressive character of MIC induced lung disease. Such progression may be difficult to follow at lower doses, if the times involved are of insufficient duration.

Sethi *et al.* (1989) exposed rats by inhalation to 0, 0.21, 0.26, and 0.35 ppm MIC for 6 days at 0.5 hr/day. Statistical evaluation was not presented. No post-exposure deaths were reported, although lethality was recorded for rats exposed to 3.5 and 35 ppm for only 10 minutes. Following the 0.5 hr × 6-day exposure, the weight gain declined in proportion to the exposure dose. At the lowest dose (0.21 ppm) the weight gain was 111 g after 91 days post-exposure, compared to a weight gain of 218 g during the same interval among the non-exposed rats. The absolute weights of the rats at the end of the exposure were not given. According to the narrative, inflammatory lesions of bronchopulmonary tissue were present; their extent increased with dose. A dose-response increase in markers of lung infection was present and suggests that the MIC exposed rats were more prone to infectious agents than were the unexposed animals. Non-specific lesions in liver and kidneys were also observed and appeared to be dose dependent, but the authors suggested that these effects could be a result of the lung infections.

Fetotoxicity was observed in two experimental animal studies (Schwetz *et al.*, 1987; Varma, 1987). Among female mice exposed to 0, 1, or 3 ppm MIC during gestation days 14 - 17 for 6 hr/day, an increased incidence of fetal deaths was observed at 1 ppm (Schwetz *et al.*, 1987). At 3 ppm, the average number of pups/litter decreased relative to the air-exposed controls. The dams were unaffected in terms of survival, body weight, or length of gestation. Non-gestational exposure (6 hr/day, 4 days) did not affect the number of pregnancies or the live litter sizes, suggesting that the fetotoxic effect may be specific to the female reproductive tract rather than a general attribute of systemic toxicity. Similarly, female mice exposed for 3 hours on gestation day 8 to 0, 2, 6, 9 or 15 ppm MIC gave birth to pups with decreased body weights at the lowest dose, although a good dose-response was not observed (Varma, 1987). At 9 or 15 ppm MIC, the surviving dams lost 75 - 80 percent of their fetuses. Maternal mortality and decreased skeletal lengths were also observed at 9 and 15 ppm. A distinction between maternally induced fetotoxicity and a direct effect on fetal health could not be made. Because the inhalation exposure to the dams occurred for only 3 hrs on one day, a chronic LOAEL is not suggested. Exposure of male rats to one dose of 3.2 mg/L for 8 minutes resulted in a 21 percent fertility rate

among the cohabited female rats within the day 8-14 period post-exposure compared to a fertility rate of 40% for controls; however, the rates increased after 15 days post-exposure (Agarwal and Bose, 1992). There was no evidence of fetotoxicity among the dams impregnated by the MIC-exposed male rats. Exposure of male and female mice to 0, 1, or 3 ppm MIC did not result in altered body weights, fertility, or litter size (Schwetz *et al*, 1987). The results suggest that exposures to MIC at doses that are not toxic to adult male or female (pregestational) mice or rats do not result in adverse reproductive outcomes.

VI. Derivation of Chronic Reference Exposure Level (REL)

Study	Dodd and Fowler (1986)
Study populations	F344 rats
Exposure method	Inhalation (0, 0.15, 0.6, or 3.1 ppm)
Critical effects	Decreased weight gain and lung pathology immediately after cessation of exposure
LOAEL	3.1 ppm
NOAEL	0.6 ppm
Exposure continuity	6 hours/day, 8 days/10 day experiment (2-cycles, with one 2-day recess from exposure)
Exposure duration	10 days
Average experimental exposure	0.12 ppm for the NOAEL group (0.6 x 8/10 x 6/24)
Human equivalent factor	0.15 ppm for the NOAEL group (gas with pulmonary respiratory effects, RGDR = 1.23, based on BW = 152 g, MV = 0.12 L/min, SA = 225 cm^2)
LOAEL uncertainty factor	1
Subchronic uncertainty factor	10
Interspecies uncertainty factor	3
Intraspecies uncertainty factor	10
Cumulative uncertainty factor	300
Inhalation reference exposure level	0.5 ppb (1 $\mu g/m^3$)

Although the exposure was for only 10 days, the Dodd and Fowler (1986) study includes the longest exposure duration of the available investigations and also uses some of the lower exposure levels (down to 0.15 ppm). The microscopic findings of the respiratory tract were statistically analyzed, although an observation of the tabulated data at the four doses (0, 0.15, 0.6, and 3.1 ppm) clearly shows a NOAEL of 0.6 ppm. Other endpoints with the same NOAEL were increased hemoglobin and increased absolute lung weights. The symptomatic ramifications of the increased hemoglobin are unknown, although similar increases were reported for humans exposed to MIC in Bhopal (Srivastava *et al.*, 1988). The lung weight gain may be a reflection of the pathological changes seen in the microscopic studies.

Decreased body weight gain was also seen in the experimental 4 day rat inhalation study of Mitsumori *et al.* (1987) (NOAEL = 1.1 ppm), except that the decrease in the latter study did not

occur until 1 and 3 days (female and male, respectively) post-exposure. The apparent discrepancy could be explained, in part, on the basis of the length of exposure, which was twice as long in the Dodd and Fowler (1986) study. However, the weight gain loss in the Dodd and Fowler (1986) study was initiated within one day of the start of exposure.

The MIC chronic REL of 0.5 ppb is based on endpoints observed within 1 day of cessation of exposure. Post-exposure evaluation showed that, at a higher exposure level (3.1 ppm), progressive changes, including death, occurred. Post-exposure observations, however, were not reported at the 0.15 and 0.6 ppm MIC levels. The attribute of delayed MIC inhalation toxicity has also been observed in other experimental animals studies (Dodd and Fowler, 1986, Mitsumori *et al.*, 1987). In the case of the human MIC exposure in Bhopal, India, death did not occur during the immediate 30 - 45 minute exposure, but exhibited a lag phase. A few deaths occurred during the first few hours, the maximum occurred at 2 - 3 days, and by the end of a week about 2500 deaths were documented (Dave, 1985; Varma, 1986; Varma and Guest , 1993), although Varma (1986) suggests that the immediate number may be closer to 5,000. One report suggests that during the intervening decade as many as 6,000 deaths may be attributed to the initial exposure in Bhopal (Lepkowski, 1994). Such information suggests that the presence of an adverse effect at the NOAEL of 0.6 ppm (Dodd and Fowler, 1986) might be possible if the rats were observed during an extended post-exposure interval. Experimental evidence is needed to test this hypothesis.

Only one study was identified in which post-exposure observations were made on experimental animals exposed subchronically by inhalation to multiple doses of MIC. Mitsumori *et al.* (1987) exposed rats to 0, 1.1, and 2.8 (females) or 3.0 (males) ppm MIC for 6 hr/day for 4 days and observed the rats for 91 days. No deaths and no weight gain loss (in contrast to Dodd and Fowler, 1986) were present until the post-exposure period and were mainly observed in animals exposed at the high dose. Using a NOAEL of 1.1 ppm MIC, a chronic REL of 1.1 ppb (2.6 $\mu g/m^3$) was derived. The REL based on the Mitsumori *et al.* (1987) study is similar to the REL based on immediate effects (Dodd and Fowler, 1986), and may indicate that the time of occurrence of exposure related effects may not be as important as the MIC air concentration.

VII. Data Strengths and Limitations for Development of the REL

The strengths of the inhalation REL for MIC include the availability of controlled exposure inhalation studies in multiple species at multiple exposure concentrations and with adequate histopathogical analysis and the observation of a NOAEL. Major areas of uncertainty are the lack of adequate human exposure data and the lack of chronic inhalation exposure studies.

VIII. Potential for Differential Impacts on Children's Health

Since exposures to MIC at levels that are not toxic to adult male or female (pregestational) mice or rats do not result in adverse reproductive outcomes, the chronic REL of 1 $\mu g/m^3$ should adequately protect infants and children. MIC is a respiratory irritant and the developing respiratory system is more sensitive than that of adults. However, there is no direct evidence in the literature to quantify a differential effect of MIC on the respiratory system of infants and children.

IX. References

Agarwal DK, and Bose M. 1992. Inhalation toxicity of methylisocyanate: assessment of germ cell mutagenicity and reproductive effects in rats. Indian J. Exp. Biol. 30:504-508.

Andersson N, Ajwani MK, Mahashabde S, Tiwari MK, Muir MK, Mehra V, Ashiru K, and Mackenzie DC. 1990. Delayed eye and other consequences from exposure to methyl isocyanate: 93% follow up of exposed and unexposed cohorts in Bhopal. Br. J. Ind. Med. 47:553-558.

Avashia B, Battigelli MC, Morgan WK and Reger RB. 1996. Effects of prolonged low exposure to methyl isocyanate. J. Occup. Environ. Med. 38(6):625-630.

Baillie TA, and Slatter JG. 1991. Glutathione: a vehicle for the transport of chemically reactive metabolites i*n vivo*. Acc. Chem. Res. 24:264-270.

Bhandari NR, Syal AK, Kambo I, Nair A, Beohar V, Saxena NC, Dabke AT, Agarwal SS, and Saxena BN. 1990. Pregnancy outcome in women exposed to toxic gas at Bhopal. Indian J. Med. Res [B]. February. pp. 28-33.

Bhattacharya BK, Sharma SK, and Jaiswal DK. 1988. *In vivo b*inding of [1-^{14}C]methylisocyanate to various tissue proteins. Biochem. Pharmacol. 37:2489-2493.

Brown WE, Green AH, Cedel TE, and Cairns J. 1987. Biochemistry of protein-isocyanate interactions: a comparison of the effects of aryl vs. alkyl isocyanates. Environ. Health Perspect. 72:5-11.

CARB. 2000. California Air Resources Board. California Emissions Inventory Development and Reporting System (CEIDARS). Data from Data Base Year 1998. February 12, 2000.

Cantin AM, North SL, Hubbard RC, and Crystal RG. 1987. Normal alveolar epithelial lining fluid contains high levels of glutathione. J. Appl. Physiol. 63:152-157.

CRC. 1994. CRC Handbook of Chemistry and Physics, 75th edition. Lide DR, ed. Boca Raton, FL: CRC Press Inc.

Dave JM. 1985. The Bhopal methyl isocyanate (MIC) incident: an overview. In: Proceedings of an International Symposium, Highly Toxic Chemicals: Detection and Protection Methods. Schiefer, H.B. ed. Saskatoon, Saskatchewan, Canada. pp 1-38.

Dodd DE, and Fowler EH. 1986. Methyl isocyanate subchronic vapor inhalation studies with Fischer 344 rats. Fundam. Appl. Toxicol. 7:502-522.

Ferguson JS, Kennedy AL, Stock MF, Brown WE, and Alarie Y. 1988. Uptake and distribution of ^{14}C during and following exposure to [^{14}C} methyl isocyanate. Toxicol. Appl. Pharmocol. 94:104-117.

Guest I, Baille TA, and Varma DR. 1992. Toxicity of the methyl isocyanate metabolite S-(n-methylcarbamoyl)GSH on mouse embryos in culture. Teratology 46:61-67.

Guest I, and Varma DR. 1991. Developmental toxicity of methylamines in mice. J. Toxicol. Environ. Health. 32:319-330.

HSDB. 1995. Hazardous Substances Data Bank. TOMES® Vol. 25. Denver, CO: Micromedex, Inc. Expires 07/31/95.

Jeevaratnam K, and Sriramachari S. 1994. Comparative toxicity of methyl isocyanate and its hydrolytic derivatives in rats. I. Pulmonary histopathology in the acute phase. Arch. Toxicol. 69:39-44.

Lepkowski W. 1994. Ten years later - Bhopal. Chemical and Engineering News. 19 December 1994.

Mitsumori K, Boorman GA, Gupta BN, and Bucher JR. 1987. Four-day repeated inhalation and recovery study of methyl isocyanate in F344 rats and B6C3F1 mice. Fundam. Appl. Toxicol. 9:480-495.

Mraz J, and Nohova H. 1992. Absorption, metabolism and elimination of N,N-dimethylformamide in humans. Int. Arch. Occup. Environ. Health. 64:85-92.

Pearson PG, Slatter JG, Rashed MS, Han D-H, Grillo MP, and Baillie TA. 1990. S-(N-methylcarbamoyl)glutathione: a reactive S-linked metabolite of methyl isocyanate. Biochem. Biophys. Res. Comm. 166:245-250.

Reiser KM, and Last JA. 1986. Early cellular events in pulmonary fibrosis. Exp. Lung Res. 10:331-355.

Schwetz BA, Adkins B Jr, Harris M, Moorman M, and Sloane R. 1987. Methyl isocyanate: reproductive and developmental toxicology studies in mice. Environ. Health Perspect. 72:149-152.

Sethi N, Dayal R, and Singh RK. 1989. Acute and subacute toxicity study of inhaled methyl isocyanate in Charles Foster rats. Ecotoxicol. Environ. Saf. 18:68-74.

Slatter JG, Rashed MS, Pearson PG, Han D-H, and Baillie TA. 1991. Biotransformation of methyl isocyanate in the rat. Evidence of glutathione conjugation as a major pathway of metabolism and implications for isocyanate-mediated toxicities. Chem. Res. Toxicol. 4:157-161.

Sriramachari S, Rao GJ, Sharma VK, Jadhav RK, Saraf AK, and Chandra H. 1991. GC-NPD and GC-MS analysis of preserved tissue of Bhopal gas disaster: evidence of methyl carbamylation in post-mortem blood. Med. Sci. Law. 31:289-293.

Srivastava RC, Gupta BN, Athar M, Behari JR, Dwivedi RS, Hasan SK, Bharti RS, Singh A, Misra M, and Ray PK. 1988. Effect of exposure to toxic gas on the population of Bhopal: Part III - Assessment of toxic manifestations in humans - hematological and biochemical studies. Ind. J. Exp. Biol. 26:165-172.

U.S. EPA. 1986. United States Environmental Protection Agency. Health and environmental effects profile for methyl isocyanate. EPA/600/22. Environmental Criteria and Assessment Office, Office of Research and Development. Cincinnati OH: U.S. EPA.

Varma DR. 1986. Anatomy of the methyl isocyanate leak on Bhopal. In: Hazard Assessment of Chemicals. Saxena J, ed. New York: Hemisphere Publishing Corp. pp. 233-299.

Varma DR. 1987. Epidemiological and experimental studies on the effects of methyl isocyanate on the course of pregnancy. Environ. Health Perspect. 72:153-157.

Varma DR, and Guest I. 1993. The Bhopal accident and methyl isocyanate toxicity. J. Toxicol. Environ. Health 40:513-529.

Vijayan VK, Sankaran K, Sharma SK, and Misra NP. 1995. Chronic lung inflammation in victims of toxic gas leak at Bhopal. Resp. Med. 89:105-111

CHRONIC TOXICITY SUMMARY

METHYL *t*-BUTYL ETHER

(MTBE; 2-methoxy-2-methylpropane; tert-butyl methyl ether; methyl 1,1dimethyl ether)

CAS Registry Number: 1634-04-4

I. Chronic Toxicity Summary

Inhalation reference exposure level	**8000 μg/m^3 (2000 ppb)**
Critical effect(s)	Nephrotoxicity, prostration, periocular swelling in Fischer 344 rats
Hazard index target(s)	Kidney; eyes; alimentary system

II. Physical and Chemical Properties (HSDB, 1994)

Description	Colorless liquid
Molecular formula	$C_5H_{l2}0$
Molecular weight	88.15 g/mol
Density	0.7405 g/cm^3 @ 20°C
Boiling point	55.2°C @ 760 mm Hg
Vapor pressure	245 torr @ 20°C
Solubility	Soluble in alcohol, ether, and 5% soluble in water
Conversion factor	1 ppm = 3.61 mg/m^3 @ 25° C; 3.67 mg/m^3 @ 20° C

III. Major Uses or Sources

Methyl t-butyl ether (MTBE) is used as a gasoline additive to improve octane ratings and reduce emissions of some pollutants, in industry to improve miscibility of solvents, and in clinical medicine to dissolve cholesterol gall stones (Yoshikawa *et al.*, 1994). The annual statewide industrial emissions from facilities reporting under the Air Toxics Hot Spots Act in California,based on the most recent inventory, were estimated to be 215,182 pounds of MTBE (CARB, 1999).

IV. Effects of Human Exposure

Gasoline (with 10% MTBE) tanker drivers reported significantly higher fatigue at the end of the work week than before the work week, and those with longer exposure to gasoline with MTBE during the work week reported significantly higher fatigue than drivers with shorter exposure (Hakkola *et al*., 1997). 20% of drivers reported symptoms such as headache, dizziness, nausea,

and dyspnoea at the end of work week. No human chronic toxicity or chronic epidemiology information for MTBE without coexposure to gasoline was found.

Ten healthy male volunteers undergoing light physical work were exposed to 5, 25, and 50 ppm MTBE vapor for 2 hours (Nihlen *et al*., 1998). While a solvent smell was noted at these concentrations, there were no consistent concentration-related effects on reported ocular or nasal irritation. The blockage index (a measure of nasal airway resistance) increased significantly after exposure but was not correlated with exposure concentration.

V. Effects of Animal Exposure

Male and female rats (50/sex/group) were exposed by inhalation for 6 hours/day, 5 days/week to mean concentrations of 0, 403, 3023, or 7977 ppm (0, 1453, 10,900, or 28,760 mg/m^3) MTBE for 24 months (Chun *et al.*, 1992). Clinical signs, hematology, body weights and food consumption were monitored. Necropsy included measurements of organ weights and histopathology. Corticosterone levels were measured on 10 animals prior to sacrifice. Serum enzymes were not monitored. The NOAEL for several endpoints, including non-alpha-2μ-globulin induced nephrotoxicity, increased relative liver and kidney weights and prostration in females, and periocular swelling in both sexes was 403 ppm (1453 mg/m^3).

Mice were exposed for 6 hours/day, 5 days/week for 18 months to MTBE concentrations of 0, 402, 3014, or 7973 ppm (0, 111, 835, or 2208 mg/m^3) (Burleigh-Flayer *et al.*, 1992). The mice exposed to the highest concentration (7973 ppm) all exhibited ataxia. Prostration was also noted in 8 of 50 animals in this group. Liver weights were elevated in a concentration-dependent manner in the female mice but this change was not significant at the lowest concentration (402 ppm). Kidney weights were elevated in the female mice exposed to 7973 ppm. At the highest concentration, a significant increase in hepatocellular hypertrophy and adrenal gland weight was detected in the male mice. Spleen weights were increased in the females exposed to the highest concentration.

Moser *et al*. (1998) exposed female B6C3F$_1$ mice to 7924 ppm (2195 mg/m^3) MTBE for 4 months, or 7919 ppm (2194 mg/m^3) MTBE for 8 months; controls received plain air. Body weight increases for control and MTBE-exposed mice, respectively, were 57% and 37% at 4 months and 79% and 45% at 8 months: the reduced weight gain in MTBE-exposed mice was significantly different from the controls at both time points. In MTBE-exposed mice, mean uterine weight was 83% reduced relative to controls at 4 and 8 months. Ovary weight was also reduced in exposed mice, the mean weight being 55% of control at 4 months and 51% of control at 8 months. Pituitary weights were decreased by 44% and 31% at 4 and 8 months, relative to controls. Disturbances of the estrus cycle and histological changes in the reproductive organs were also noted. Although the changes in organ weights and histology were suggestive of an anti-estrogenic effect of MTBE, serum estrogen levels were unaffected. No changes in estrogen receptor (ER) immunoreactivity in reproductive system tissues were observed. Experiments *in vitro* failed to demonstrate any inhibition of estradiol binding to ER by MTBE or its metabolites. No inhibition of ER by MTBE was detected, nor was there any inhibition of the induction of ER by estradiol. The authors concluded that the apparent anti-estrogenic effects of MTBE were not

mediated via the ER, and drew a parallel with the anti-estrogenic effects of dioxins and chlorinated biphenyls.

Tests for histopathology in the respiratory tract, plasma corticosterone levels, motor activity and neurobehavioral endpoints were performed in rats exposed to MTBE at concentrations of 0, 797, 3920, or 8043 ppm (0, 2877, 14151, or 29035 mg/m^3), 6 hours/day, 5 days/week for 13 weeks (Dodd and Kintigh, 1989). Of these endpoints, the most significant finding was an elevation in plasma corticosterone in the high dose group. This finding was consistent with the elevated adrenal weights reported by Burleigh-Flayer *et al.* (1992). A clear dose-response for neurotoxic effects in these rats was not established.Biles *et al.* (1987) reported a NOAEL of 300 ppm (1083 mg/m^3) MTBE for decreased pup viability in rats exposed for 6 hours/day, 5 days/week for a total of 16 weeks. Animals exposed to 1240 ppm (4470 mg/m^3) or 2860 ppm (10,311 mg/m^3) MTBE exhibited slightly decreased pup survival.

Neeper-Bradley (1991) exposed rats to 0, 402, 3019, or 8007 ppm (0, 111, 836, and 2218 mg/m^3) MTBE over 2 generations. Exposures were for 6 hours/day, 5 days/week during the prebreeding period, and for 7 hours/day, 5 days/week during gestation and lactation. Parental effects of MTBE exposure were observed, including ataxia, blepharospasm, lack of startle reflex, and increased relative liver weights (F1 generation only). There were no histological changes in the organs from either parental generation. Reduced body weights were observed in the F1 and F2 pups at the 3019 and 8007 ppm concentrations. Reduced survivability to postnatal day 4 was observed in the 8007 ppm group. No adverse effects were noted at the 403 ppm (111 mg/m^3) concentration.

In a developmental and reproductive toxicity study, Conaway and associates (1985) found no significant increases in maternal or fetal toxicity, nor in pregnancy rates or in any gross toxicologic parameter tested with pregnant rats or mice exposed during gestation to concentrations of MTBE up to 3300 ppm (11,897 mg/m^3).

Maternal toxicity, in the form of hypoactivity and ataxia, was observed in pregnant mice exposed during gestation to 4076 ppm (14,690 mg/m^3) MTBE (Bushy Run Research Center, 1989a). Significant reductions in food intake and body weight gain were observed in dams exposed to 8153 ppm (29,390 mg/m^3). Fetal body weight was significantly reduced in the 4076 ppm group, and there were significant increases in the incidences of skeletal variations and unossified phalanges in the 4076 and 8153 ppm groups. Pregnant rabbits exposed to similar concentrations during gestation showed no significant maternal or fetal toxicity or developmental toxicity up to a concentration of 8021 ppm (28,918 mg/m^3) (Bushy Run Research Center, 1989b).

VI. Derivation of Chronic Reference Exposure Level

Study	Chun *et al.*, 1992; Bird 1997
Study population	Male and female rats (50 per sex/group)
Exposure method	Discontinuous whole-body inhalation exposures (0, 403, 3023, or 7977 ppm)
Critical effects	Nephrotoxicity, increased liver and kidney weight, prostration and periocular swelling
LOAEL	3023 ppm
NOAEL	403 ppm
Exposure continuity	6 hours per day, 5 days per week
Exposure duration	24 months
Average experimental exposure	72 ppm for the NOAEL group
Human equivalent concentration	72 ppm for the NOAEL group (gas with systemic effects, based on RGDR = 1.0 using default assumption that lambda (a) = lambda (h))
LOAEL uncertainty factor	1
Subchronic uncertainty factor	1
Interspecies uncertainty factor	3
Intraspecies uncertainty factor	10
Cumulative uncertainty factor	30
Inhalation reference exposure level	2 ppm (2000 ppb, 8 mg/m^3, 8000 μg/m^3)

The USEPA (1995) based its RfC of 3000 μg/m^3 on the same study but included a Modifying Factor (MF) of 3 for database deficiencies. The criteria for use of modifying factors are not well specified by U.S. EPA. Such modifying factors were not used by OEHHA.

VII. Data Strengths and Limitations for Development of the REL

The major strengths of the REL for MTBE are the use of a comprehensive, long-term multiple dose study with large sample sizes and the observation of a NOAEL. The major uncertainty is the lack of human data.

VIII. References

Biles RW, Schroeder RE, and Holdsworth CE. 1987. Methyl tertiary butyl ether inhalation in rats: A single generation study. Toxicol. Ind. Health 3:519-534.

Bird MG, Burleigh-Flayer HD, Chun JS, Douglas JF, Kneiss JJ, Andrews LS. 1997. Oncogenicity studies of inhaled methyl tertiary-butyl ether (MTBE) in CD-1 mice and F-344 rats. J. Appl. Toxicol. 17 Suppl 1:S45-55.

Burleigh-Flayer HD, Chun JS, and Kintigh WJ. 1992. Methyl tertiary butyl ether: Vapor inhalation oncogenicity study in CD- 1 mice (unpublished material). Prepared for the MTBE Committee by Bushy Run Research Center, Union Carbide Chemicals and Plastics Company, Inc. Docket No. OPTS-42098.

Bushy Run Research Center, Union Carbide Chemicals and Plastics Company, Inc. 1989a. Developmental toxicity study of inhaled methyl tertiary butyl ether in CD- 1 mice (final report). TSCATS/403186. EPA/OTS No. FYI-OTS-0889-0689. [cited in U.S. EPA, 1995].

Bushy Run Research Center, Union Carbide Chemicals and Plastics Company, Inc. 1989b. Developmental toxicity study of inhaled methyl tertiary butyl ether in New Zealand White rabbits (final report). TSCATS/403186. EPA/OTS No. FYI-OTS-0889-0689. [cited in U.S. EPA, 1995].

CARB. 1999. Air toxics emissions data collected in the Air Toxics Hot Spots Program CEIDARS Database as of January 29, 1999.

Chun JS, Burleigh-Flayer HD, and Kintigh WJ. 1992. Methyl tertiary butyl ether: Vapor inhalation oncogenicity study in Fischer 344 rats (unpublished material). Prepared for the MTBE committee by Bushy Run Research Center, Union Carbide Chemicals and Plastics Company, Inc., Docket No. OPTS-42098.

Conaway CC, Schroeder RE, and Snyder NK. 1985. Teratology evaluation of methyl tertiary butyl ether in rats and mice. J. Toxicol. Environ. Health 16(6):797-809.

Dodd DE, and Kintigh WJ. 1989. Methyl tertiary butyl ether (MTBE): Repeated (13 week) vapor inhalation study in rats with neurotoxicity evaluation (unpublished material). Prepared for the MTBE Committee by Bushy Run Research Center, Union Carbide Chemicals and Plastics Company, Inc., TSCATS 403187. EPA/OTS No. FYI-OTS-08890689.

Hakkola M, Honkasalo ML, Pulkkinen P. 1997. Changes in neuropsychological symptoms and moods among tanker drivers exposed to gasoline during a work week. Occup Med (Lond) 47(6):344-8.

HSDB. 1999. Hazardous Substance Data Bank. National Library of Medicine, Bethesda, Maryland. WWW database (http://sis.nlm.nih.gov/sis1/).

Moser GJ, Wolf DC, Sar M, Gaido KW, Janszen D, Goldsworthy TL. 1998. Methyl tertiary butyl ether-induced endocrine alterations in mice are not mediated through the estrogen receptor. Toxicol. Sci. 41(1):77-87.

Neeper-Bradley TL. 1991. Two-generation reproduction study of inhaled methyl tertbutyl ether in CD Sprague-Dawley rats (unpublished material). Bushy Run Research Center, Union Carbide Chemicals and Plastics Company, Inc.

Nihlen A, Walinder R, Lof A, Johanson G. 1998. Experimental exposure to methyl tertiary-butyl ether. II. Acute effects in humans. Toxicol. Appl. Pharmacol. 148(2):281-287.

U.S.EPA. 1999. U.S. Environmental Protection Agency. Integrated Risk Information System (IRIS) database. Reference concentration (RfC) for methyl t-butyl ether (MTBE).

Yoshikawa M, Arashidani K, Katoh T, Kawamoto T, and Kodama Y. 1994. Pulmonary elimination of methyl tertiary butyl ether after intraperitoneal administration in mice. Arch. Toxicol. 68:517-519.

CHRONIC TOXICITY SUMMARY

METHYLENE CHLORIDE

(dichloromethane, methylene dichloride)

CAS Registry Number: 75-09-2

I. Chronic Toxicity Summary

Inhalation reference exposure level	**400 µg/m^3 (100 ppb)**
Critical effect(s)	Carboxyhemoglobin formation above 2% in human workers
Hazard index target(s)	Cardiovascular system; nervous system

II. Physical and Chemical Properties (HSDB, 1999, except as noted)

Description	Colorless liquid
Molecular formula	CH_2Cl_2
Molecular weight	84.93
Density	1.32 g/cm^3 @ 20° C (ACGIH, 1991)
Boiling point	39.75° C
Vapor pressure	400 torr @ 24.1° C
Solubility	Miscible with most organic solvents, slightly soluble in water (ACGIH, 1991)
Conversion factor	1 ppm = 3.47 mg/m^3 @ 25° C

III. Major Uses and Sources

Methylene chloride (MC) is used in paint and varnish remover, in aerosols as a cosolvent or vapor pressure depressant, and in solvent degreasing and metal cleaning. It is also used in plastics processing and in extraction of fats and oils from food products (HSDB, 1999). The annual statewide industrial emissions from facilities reporting under the Air Toxics Hot Spots Act in California, based on the most recent inventory, were estimated to be 3,504,271 pounds of methylene chloride (CARB, 1999a). Both mean and maximum monitored ambient methylene chloride concentrations have decreased slightly between 1990 and 1996 (CARB, 1999b). Median and maximum concentrations were 1.09 and 11 ppb in 1990 and 0.66 and 5.6 ppb in 1996.

IV. Effects of Human Exposure

Effects of a controlled 2-hour inhalation exposure to MC included CNS depression at concentrations of 1000 ppm (3500 mg/m^3) or more and increased blood carboxyhemoglobin

(COHb) content at lower concentrations (500 ppm) due to metabolism of MC to carbon monoxide (Stewart *et al.*, 1972). High levels of COHb can be found in the blood hours after exposure to methylene chloride, due to its partitioning into fat and its slow release into circulation with subsequent metabolism, leading to formation of carbon monoxide (Engstrom and Bjurstrom, 1977). In situations of chronic exposure, carbon monoxide toxicity is also of concern. Barrowcliff (1978) documented the case of an adult male who developed an unsteady gait, a peculiar dysarthria and a loss of memory. The man had worked with 15-50 liters of methylene chloride daily for 3 years in a poorly ventilated room while cleansing road materials. No natural disease could be found to explain his conditions and the effects were attributed to chronic carbon monoxide poisoning.

Twelve women volunteer subjects were exposed to 0, 300, or 800 ppm methylene chloride for 4 hours (Fodor and Winneke, 1971). Neurobehavioral vigilance was measured by auditory discrimination of intensity of certain sound pulses against a background of continuous white noise. A significant interactive effect between methylene chloride concentration and duration of exposure using 2-way ANOVA ($p < 0.01$) was found.

Human erythrocytes enzymatically convert methylene chloride to formaldehyde in cell-culture experiments (Hallier *et al.*, 1994).

A subacute controlled exposure of eleven resting non-smokers to methylene chloride was conducted by DiVincenzo and Kaplan (1981a). The eleven subjects were exposed to 50, 100, 150, or 200 ppm methylene chloride for 7.5 hours on 5 consecutive days. Exposure to all concentrations led to dose-dependent elevation in COHb concentrations in the blood and elevated exhaled CO. The peak blood COHb saturations were 1.9, 3.4, 5.3, and 6.8%, respectively, for the 50, 100, 150, and 200 ppm groups.

Divencenzo and Kaplan (1981a) also measured COHb percentage in the blood of workers occupationally exposed to methylene chloride and a group of workers not exposed to methylene chloride. The 19 workers exposed to methylene chloride had mean blood COHb concentrations of 2.3% in the morning and 3.9% at the end of the work-shift. Ambient concentrations in the workplace were estimated from 57 samples, which ranged from 0 to 250 ppm, with a mean concentration of 40 ppm. Three exposed workers also wore monitors to estimate personal exposures. The time-weighted average exposure for these workers was 33 ppm. Controls (8 subjects) had significantly lower mean blood COHb concentrations of 0.8% in the AM and 1.3% in the PM compared with the exposed workers. The length of employment of the exposed workers was not given.

A companion study by DiVincenzo and Kaplan (1981b) showed that smoking and methylene chloride exposure result in an additive effect on COHb levels compared with levels in non-smokers. Similarly, light, moderate or heavy exercise workloads resulted in higher COHb levels.

Soden *et al.* (1996) showed a dose-response increase in carboxyhemoglobin levels in non-smokers with increasing methylene chloride exposure in workers involved in triacetate fiber production. Carboxyhemoglobin levels ranged from 1.77% to 4% from exposures ranging from 6.5 to 89.7 ppm, respectively. The number of employees in the study was not reported.

Although animal studies have shown COHb-induced cardiovascular effects following MC exposure (Aviado *et al.*, 1977), no data exist on this outcome in humans. However, studies of men with coronary artery disease and exercise-induced angina report a decrease in time to onset of exercise-induced angina following exposure to carbon monoxide (CO) at concentrations sufficient to result in blood COHb levels of about 2% (Kleinman *et al.*, 1989; Allred *et al.*, 1989). A physiologically based pharmacokinetic model of MC and CO estimated that a 1-hour exposure to 340 ppm (1200 mg/m^3) MC at a ventilation rate of 9 liters/min would result in a peak blood COHb level of 2% (Andersen *et al.*, 1991; Reitz, 1994). The California Ambient Air Quality Standard for CO is based on a blood COHb level of 2% (CARB, 1982).

An epidemiological study of 751 male workers in the Eastman Kodak Company exposed to daily 8-hour time-weighted average concentrations of 30-125 ppm methylene chloride for up to 30 years was conducted by Friedlander and associates (1978). A control group of workers in production but not exposed to methylene chloride was used together with New York state cause and age-specific mortality rates. The follow-up period for these workers was 13 years, with 97% success. The studies did not indicate any increase in risk of death from circulatory disease, cancer, or other causes due to methylene chloride exposure.

A study of female pharmaceutical workers in eight different factories exposed to a variety of organic solvents indicated that solvent exposure, and particularly methylene chloride exposure, resulted in an increase in spontaneous abortions (Taskinen *et al.*, 1986). In all, 1795 pregnancies were followed, with 142 spontaneous abortions occurring. The odds ratio for methylene chloride exposure was 1.0 to 5.7 (average = 2.3; $p < 0.06$). There was a significant effect of exposure to 4 or more solvents, compared with age-matched controls ($p < 0.05$). The concentrations of MC were not reported in the study.

The U.S. Occupational Safety and Health Administration reduced its permissible exposure limits (PEL) for MC from 500 ppm to 25 ppm in 1997 (U.S. CFR, 1997).

V. Effects of Animal Exposure

Nitschke *et al.* (1988) found that a 2-year exposure to 0, 50, 200, or 500 ppm MC for 6 hours/day, 5 days/week resulted in significant histopathologic lesions in the livers of rats exposed to 500 ppm. No significant adverse effects were observed at 200 ppm or lower. The predominant hepatocellular lesion was fatty vacuolization of hepatocytes.

Female B6C3F1 mice inhaling 2000 ppm MC for 1 to 26 weeks had 40 to 60% lower cell turnover rates of bronchiolar cells compared with controls (Kanno *et al*., 1993). At this concentration no observable pathological changes were found in the lungs of MC exposed animals.

A continuous exposure of mice (16 per group) to 100 ppm MC for 1, 2, 3, 4 or 10 weeks resulted in signifcant elevation in liver triglycerides beginning at 2 weeks and lasting throughout the 10-week period (Weinstein and Diamond, 1972). Liver/body weight ratios were unaffected at any

time point. After 1 week, small fat droplets were apparent in centrilobular hepatocytes and a decrease in hepatic glycogen was also noted. Necrosis was not observed during the 10-week period, but fat droplet size increased and glycogen depletion persisted.

Male and female Sprague-Dawley rats and Golden Syrian hamsters inhaled methylene chloride (0, 500, 1500, or 3500 ppm) for 6 hr per day, 5 days a week over 2 years (Burek *et al.*, 1984). The groups consisted of 129 rats per sex per concentration, and 107 to 109 hamsters per sex per concentration. Females rats inhaling 3500 ppm had an increased mortality rate while female hamsters inhaling 1500 or 3500 ppm had decreased mortality rates. Slight histopathological findings were noted in livers of rats exposed to 500, 1500, or 3500 ppm MC. Decreased amyloidosis was also found in livers and other organs of hamsters at each of the three MC concentrations. Overall, effects were more potent in rats compared with hamsters, which had fewer spontaneous age-related changes, decreased mortality (at least for females), and evidence of specific target organ toxicity was weak. Carboxyhemoglobin values were elevated in both rats and hamsters exposed to 500 ppm or more of MC, with the percentage increase greater in hamsters than in rats.
Monkeys were observed to be more susceptible subjects for methylene chloride induced COHb than dogs upon 14-week subchronic continuous exposure to 25 or 100 ppm (Haun *et al.*, 1972). At 25 ppm, approximately 1.5% COHb was reached in the 4 monkeys, compared to approximately 0.5% in 16 dogs. Monkeys exposed to 100 ppm MC had COHb levels of approximately 4% compared with 2% in the dogs.

Oral ethanol pretreatment in rats has been shown to suppress the COHb formation characteristic of methylene chloride exposure through inhibition of biotransformation of methylene chloride (Glatzel *et al.*, 1987).

Gerbils (10/sex per group; 60 controls) exposed continuously to MC concentrations of 210, 350, or 700 ppm for a period of 3 months, with a 4-month follow-up period, showed irreversible cellular and biochemical changes in brain (Rosengren *et al.*, 1986). A high mortality rate (19/20) was observed in the 700 ppm group, and this exposure was terminated after 7 weeks. The gerbils exposed to 350 ppm also had a high mortality rate (9/20) and this exposure was terminated after 10 weeks. The gerbils exposed to 210 ppm had no premature mortality and the exposure continued for the full 3 months. Four months after termination of exposure, the animals in the 350 and 210 ppm groups had significantly decreased brain DNA content in the hippocampus. The 350 ppm group exhibited elevated astroglial proteins in the frontal and sensory motor cerebral cortex, consistent with astrogliosis in these regions. In addition, the gerbils exposed to 350 ppm MC had significantly decreased DNA in the cerebellar hemispheres. Complimentary studies by these investigators showed that the formation of carboxyhemoglobin did not increase in gerbils between the 210 and 350 ppm exposures, indicating that the metabolism of MC to CO is saturable at concentrations below those in the study. On the other hand, the neurotoxic brain biochemical alterations were significantly greater in gerbils exposed to 350 ppm as compared with the 210 ppm group, implying that carboxyhemoglobin induced cerebral hypoxia is not the major cause of MC-induced neurotoxicity in the brain.

Rats (50 per sex per group) were exposed to 0, 1000, 2000, or 4000 ppm methylene chloride 6 hours/day, 5 days/week for 102 weeks (NTP, 1986). Both sexes exhibited hemosiderin

pigmentation in the liver in a dose-dependent fashion, beginning with the 1000 ppm concentration. Squamous metaplasia of the nasal cavity was observed in female rats, and thyroid C-cell hyperplasia was observed in males exposed to 2000 ppm or greater. Kidney tubule degeneration (not otherwise specified) was increased at all exposure levels.

Mice (50 per sex per group) exposed to 0, 2000, or 4000 ppm methylene chloride 6 hours/day, 5 days/week for 102 weeks showed increased incidence of liver cytologic degeneration and splenic atrophy at 4000 ppm (males) (NTP, 1986). Male and female mice also had an increased incidence of kidney tubule casts (not otherwise specified) at 2000 ppm or greater, and significant testicular atrophy was observed in males at 4000 ppm. Female mice showed cytologic degeneration in the liver at 2000 ppm or greater, and ovarian atrophy at 2000 ppm or greater.

A six month exposure to 5000 ppm MC of 8 guinea pigs for 7 hours/day, 5 days/week resulted in 3 deaths; 2 showed moderate centrilobular fatty degeneration of the liver and extensive pneumonia at necropsy (Heppel *et al.*, 1944). None of the 14 control animals died. Food consumption and body weight were lower in the exposed guinea pigs, compared with control pigs. One out of 12 rats died at this concentration, and the liver histology in this animal revealed multiple thrombi in renal vessels, associated with marked cortical infarction. By comparison, dogs and rabbits showed no signs of illness, nor were blood pressure or hematological values altered at the 5000 ppm concentration. At 10,000 ppm, 2 of 4 dogs showed moderate centrilobular congestion, narrowing of liver cell cords, and slight to moderate fatty degeneration. One of 2 monkeys revealed disseminated tuberculosis lesions, but no other histological alterations. Four out of 6 guinea pigs had moderate fatty degeneration of the liver at this concentration.

The offspring of rats (10 dams per group) exposed during gestation to 0 or 4500 ppm methylene chloride exhibited altered rates of behavioral habituation to novel environments (Bornschein *et al.*, 1980). This effect was observed beginning at 10 days of age but was still demonstrable in rats 150 days old. The authors concluded that elevated maternal COHb could have been a contributing factor in the developmental impairment.

In a study of the effects of methylene chloride on estrous cycle and serum prolactin, groups of 15 female rats were exposed to 0 or 3500 ppm for 6 hours/day for 15 to 19 consecutive days (Breslin and Landry, 1986). Males (15 per group) were exposed for 5 hours/day for 5 consecutive days. Female rats exhibited decreased body weight and increases in the estrous cycle duration and in serum prolactin. Males did not show any significant effects on serum prolactin from methylene chloride exposure.

Pregnant mice and rats were exposed to 0 or 1250 ppm MC 7 hours/day, on days 6 through 15 of gestation (Schwetz *et al.*, 1975). Significantly elevated absolute liver weights were seen in maternal animals from both species. In addition, significantly increased incidences of delayed ossification of the sternebrae were seen in both species, compared to controls.

Methylene chloride exposure of female rats before or during gestation to 4500 ppm resulted in elevated maternal liver weights and decreased birth weights of the offspring, but no terata or skeletal/soft tissue anomalies (Hardin and Manson, 1980).

A 2-generation reproduction test was conducted by Dow Chemical Company (Nitschke *et al.*, 1985) which showed no significant reproductive or developmental effects in rats exposed to 0, 100, 500, or 1500 ppm MC 6 hours/day, 5 days/week, for 14 weeks. The exposure conditions were identical for the F_0 and F_1 generations.

VI. Derivation of Chronic Reference Exposure Level (REL)

Study	DiVincenzo and Kaplan (1981a)
Study population	19 workers, 8 controls
Exposure method	Occupational inhalation exposure
Critical effects	Significantly elevated carboxyhemoglobin levels (> 2%)
LOAEL	40 ppm (ambient workplace exposures averaged 40 ppm with a range of 0 to 250 ppm); controls = 0 ppm
NOAEL	Not observed
Exposure continuity	8 hours/day, 5 days/week
Exposure duration	Length of employment unspecified
Average occcupational exposure	14 ppm for LOAEL group (40 x 10/20 x 5/7)
LOAEL uncertainty factor	10
Subchronic uncertainty factor	1 (see following text for explanation)
Interspecies uncertainty factor	1
Intraspecies uncertainty factor	10
Cumulative uncertainty factor	100
Inhalation reference exposure level	0.1 ppm (100 pbb; 0.4 mg/m^3; 400 µg/m^3)

Workers were exposed to average measured concentrations of 40 ppm during the workday, and the personal monitors on 3 of the subjects indicated a 8-hour time-weighted average of 33 ppm over a 2-week period. The average COHb levels were 3.9% at the end of the work-shift. Elevated carboxyhemoglobin concentrations of above 2% are considered to aggravate angina in some individuals (CARB, 1982). In effect, 2% COHb can be considered a NOAEL for aggravation of angina. Therefore, the 33 ppm concentration was considered a LOAEL for the formation of greater than 2% COHb. The duration of the employment period was not specified. However, in the DiVincenzo and Kaplan (1981a) study, the levels of COHb did not appear to increase over a period of 5 days in experimental exposures using volunteers, therefore an uncertainty factor for subchronic exposure was not necessary. A number of factors contribute to the uncertainty in determining the degree of sensitivity to methylene chloride, including activity level, metabolic enzyme activity, age, and background COHb status (e.g., from smoking, etc.).

The subchronic study by Haun *et al.* (1972) with monkeys reported a NOAEL of 25 ppm and a LOAEL of 100 ppm for 2% COHb formation following a 14-week exposure. These results are consistent with the LOAEL reported in the DiVincenzo and Kaplan study. However, the human occupational study likely contains less uncertainty, since the toxicokinetics of the effect,

including rate of formation of CO and thus COHb is metabolism-dependent, resulting in considerable potential interspecies differences.

The study in hamsters by Burek *et al.*(1984) showed a LOAEL for elevated carboxyhemoglobin of 500 ppm. A time-weight average exposure and HEC of 89 ppm was calculated. Using a 10-fold LOAEL uncertainty factor, a 3-fold interspecies uncertainty factor for residual uncertainty not accounted for in the HEC calculation, and a 10-fold intraspecies uncertainty factor, a REL of 300 ppb or 1000 µg/m^3 was derived. Thus, the REL derived from the best available animal study is comparable to the 400 µg/m^3 REL derived from the best-available human study.

VII. Data Strengths and Limitations for Development of the REL

The major strength of the key study (DiVincenzo and Kaplan, 1981a) used to derive the REL for methylene chloride is that human health effects were observed. The major uncertainties from the key study itself are the lack of a NOAEL observation, the difficulty in estimating exposures, and the discontinuous and variable nature of the exposures.

The health effects database for methylene chloride includes, in addition to an adequate study of human occupational exposures (DiVincenzo and Kaplan, 1981a), an adequate lifetime inhalation exposure study in 2 species of laboratory animals (Burek *et al*., 1984). The REL values derived from these studies (400 µg/m^3 vs. 1,000 µg/m^3) are comparable. That both the human and animal studies measured the same endpoint and arrived at similar conclusions is a circumstance that is rarely found but one that considerably increases the weight of evidence from which the REL was derived. The two studies complement each other, as the animal study involved controlled, measured exposures over a lifetime but introduces the uncertainty of predicting human health effects from animal observations, and the human study involved poorly characterized human exposures but lacks the uncertainty inherent in interspecies extrapolation.

VIII. References

Allred EN, Bleecker ER, Chaitman BR, Dahms TE, Gottlieb SO, Hackney JD, Hayes D, Pagano M, Selvester RH, Walden SM, and Warren J. 1989. Acute effects of carbon monoxide exposure on individuals with coronary artery disease. Health Effects Research Institute (HEI) Research Report No. 25., Cambridge, MA: HEI.

ACGIH. 1991. American Conference of Governmental Industrial Hygienists. Documentation of Threshold Limit Values and Biological Exposure Indices. 6th edition Cincinnati, OH: ACGIH.

Andersen ME, Clewell HJ III, Gargas ML, MacNaughton MG, Reitz RH, Nolan RJ, and McKenna MJ. 1991. Physiologically based pharmacokinetic modeling with dichloromethane, its metabolite, carbon monoxide, and blood carboxyhemoglobin in rats and humans. Toxicol. Appl. Pharmacol. 108:14-27.

Aviado DM, Zakhari S, Watanabe T. 1977. Methylene chloride. In: Golberg L. (ed.) Non-Fluorinated Propellants and Solvents for Aerosols, Cleveland, OH: CRC Press, pp.19-45.

Barrowcliff DF. 1978. Chronic carbon monoxide poisoning caused by methylene chloride paintstripper. Med. Sci. Law 18(4):238.

Bornschein RL, Hastings L, and Manson JM. 1980. Behavioral toxicity in the offspring of rats following maternal exposure to dichloromethane. Toxicol. Appl. Pharmacol. 52:29-37.

Breslin WJ, and Landry TD. 1986. Methylene chloride: Effects on estrous cycling and serum prolactin in Sprague-Dawley rats. Unpublished study by Dow Chemical U.S.A., Midland, MI 48674.

Burek JD, Nitschke KD, Bell TJ, Wackerle DL, Childs RC, Beyer JE, Dittenber DA, Rampy LW, and McKenna MJ. 1984. Methylene chloride: A two-year inhalation toxicity and oncogenicity study in rats and hamsters. Fundam. Appl. Toxicol. 4:30-47.

CARB (California Air Resources Board). 1982. California Air Resources Board. California Ambient Air Quality Standards for Carbon Monoxide (Sea Level). Technical Support Document. Sacramento, CA: California Air Resources Board, Technical Support Division.

CARB (California Air Resources Board). 1999a. Air toxics emissions data collected in the Air Toxics Hot Spots Program CEIDARS Database as of January 29, 1999.

CARB (California Air Resources Board). 1999b. 1990-1996 Statewide Methylene Chloride Summary, ppb. WWW document (http://www.arb.ca.gov/aqd/dcm/dmstate.htm).

DiVincenzo GD, and Kaplan CJ. 1981a. Uptake, metabolism, and elimination of methylene chloride vapor by humans. Toxicol. Appl. Pharmacol. 59:130-140.

DiVincenzo GD, and Kaplan CJ. 1981b. Effect of exercise or smoking on the uptake, metabolism, and excretion of methylene chloride vapor. Toxicol. Appl. Pharmacol. 59:141-148.

Engstrom J, and Bjurstrom R. 1977. Exposure to methylene chloride: content in subcutaneous adipose tissue. Scand. J. Work Environ. Health 3:215-224.

Fodor GG, and Winneke H. 1971. Nervous system disturbances in men and animals experimentally exposed to industrial solvent vapors. Proc. 2nd Intl. Clean Air Congr. New York: Academic Press. pp. 238-243.

Friedlander BR, Hearne T, and Hall S. 1978. Epidemiologic investigation of employees chronically exposed to methylene chloride. J. Occup. Med. 20:657-666.

Glatzel W, Tietze K, Gutewort R, and Pankow D. 1987. Interaction of dichloromethane and ethanol in rats: Toxicokinetics and nerve conduction velocity. Alcohol. Clin. Exp. Res. 11(5):450-452.

Hallier E, Schroder KR, Asmuth K, Dommermuth A, Aust B, Goerens HW. 1994. Metabolism of dichloromethane (methylene chloride) to formaldehyde in human erythrocytes: influence of polymorphism of glutathione transferase theta (GST T1-1). Arch. Toxicol. 68:423-427.

Hardin BD, and Manson JM. 1980. Absence of dichloromethane teratogenicity with inhalation exposure in rats.Toxicol Appl Pharmacol 1980 52(1):22-28.

Haun CC, Vernot EH, and Darmer KI. 1972. Continuous animal exposure to low levels of dichloromethane. Aerospace Medical Research Laboratories, Wright-Patterson Air Force Base, Dayton, OH. AMRL-TR-72-130.

HSDB. 1999. Hazardous Substance Data Bank. National Library of Medicine, Bethesda, Maryland. WWW database (http://sis.nlm.nih.gov/sis1/).

Heppel LA, Neal PA, Perrin TL, Orr ML, and Porterfield VT. 1944. Toxicology of dichloromethane (methylene chloride). I. Studies on effects of daily inhalation. J. Ind. Hyg. Toxicol. 26(1):8-16.

Kanno J, Foley JF, Kari F, Anderson MW, Maronpot RR. 1993. Effect of methylene chloride inhalation on replicative DNA synthesis in the lungs of female B6C3F1 mice. Environ Health Perspect 101 Suppl 5:271-276.

Kleinman MT, Davidson DM, Vandigraff RB, Caiozzo VJ, and Whittenberger JL. 1989. Effects of short-term exposure to carbon monoxide in subjects with coronary artery disease. Arch. Env. Health 44(6):361-369.

NTP. 1986. National Toxicology Program. Toxicology and carcinogenesis studies of dichloromethane (methylene chloride) in F344/N rats and B6C3F1 mice. NIH Publication 86-2562. Reasearch Triangle Park, NC: U.S. Dept. of Health and Human Services.

Nitschke KD, Eisenbrandt DL, and Lomax LG. 1985. Methylene chloride: Two -generation reproduction study in Fischer 344 rats. Midland, MI: Dow Chemical Company. February 5.

Nitschke KD, Burek JD, Bell TJ, Kociba RJ, Rampy LW, and McKenna MJ. 1988. Methylene chloride: A 2-year inhalation toxicity and oncogenicity study in rats. Fundam. Appl. Toxicol. 11:48-59.

Reitz RH. 1994. Personal communication. August 5, 1994.

Rosengren LE, Kjellstrand P, Aurell A, and Haglid KG. 1986. Irreversible effects of dichloromethane on the brain after long term exposure: a quantitative study of DNA and the glial cell marker proteins S-100 and GFA. Br. J. Ind. Med. 43:291-299.

Schwetz BA, Leong KJ, and Gehring PJ. 1975. The effect of maternally inhaled trichloroethylene, perchloroethylene, methyl chloroform, and methylene chloride on embryonal and fetal development in mice and rats. Toxicol. Appl. Pharmacol. 32(1):84-96

Soden KJ, Marras G, and Amsel J. 1996. Carboxyhemoglobin levels in methylene chloride-exposed employees. J. Occup. Environ. Med. 38(4):367-371.

Stewart RD, Fisher TN, Hosko MJ, Peterson JE, Baretta ED, and Dodd HC. 1972. Experimental human exposure to methylene chloride. Arch. Env. Health 25(5):342-348.

Taskinen H, Lindbohm ML, and Hemminki K. 1986. Spontaneous abortions among women working in the pharmaceutical industry. Br. J. Ind. Med. 43:199-205.

U.S. Code of Federal Regulations (29 CFR). Final Rule for Occupational Exposure to Methylene Chloride. Federal Register 62(7):1494.

Weinstein RS, and Diamond SS. 1972. Hepatotoxicity of dichloromethane (methylene chloride) with continuous inhalation exposure at a low dose level. Proceedings of the 3rd Annual Conference on Environmental Toxicology, 25, 26, and 27 October, 1972. Aerospace Medical Research Laboratory, Aerospace Medical Division, Air Force Systems Command, Wright-Patterson Air Force Base, OH.

CHRONIC TOXICITY SUMMARY

4,4′-METHYLENE DIANILINE

(MDA; 4,4'-diaminodiphenylmethane; 4,4'-diphenylmethanediamine; DAPM; dianilinemethane)

CAS Registry Number: 101-77-9

I. Chronic Toxicity Summary

Inhalation reference exposure level	**20 µg/m^3** (2 ppb)
Critical effect(s)	Ocular toxicity to the retinas of guinea pigs
Hazard index target(s)	Eyes; alimentary system (hepatotoxicity)

II. Chemical Property Summary (HSDB, 1995; CRC, 1994)

Description	Colorless to pale yellow flakes; tan
Molecular formula	$C_{13}H_{14}N_2$
Molecular weight	198.3 g/mol
Boiling point	398-399°C
Melting point	92.5°C
Vapor pressure	1 torr @ 197°C
Solubility	Soluble in alcohol, benzene, ether; 273 g/100 g acetone; 0.1 g/100 g water @ 25°C
Conversion factor	8.1 µg/m^3 per ppb at 25ºC

III. Major Uses and Sources

4,4'-Methylene dianiline (MDA) is synthesized by the reaction of aniline with formaldehyde. MDA's major uses are as a chemical intermediate in the synthesis of certain isocyanates and polyurethane polymers, as a corrosion inhibitor, in the preparation of azo dyes, as a rubber preservative, and in the curing of epoxy resins and neoprene (HSDB, 1995; ACGIH, 1992). The annual statewide industrial emissions from facilities reporting under the Air Toxics Hot Spots Act in California based on the most recent inventory were estimated to be 1133 pounds of MDA (and its dichloride) (CARB, 2000).

IV. Effects of Human Exposure

Several cases of human exposure to MDA have identified the compound as a hepatotoxicant which produces cholestatic jaundice (Kopelman *et al.*, 1966; McGill and Motto, 1974; Williams *et al.*, 1974; Bastian, 1984). Bastian (1984) described cases of acute hepatic illness in four workers exposed from laying floors using an epoxy resin base, which contained MDA as a curing agent. The workers were exposed via fumes and dusts in the air as well from hand

contact with powder and had worked with epoxy resins for periods ranging from one to 12 years. The level of exposure was not quantified. The workers initially reported to the hospital with symptoms of abdominal pain three days after the most recent exposure and all were discharged within four days. Two workers continued to show severe symptoms five days after the onset, with abdominal pain, jaundice, a tender liver, nausea, dyspnea, and muscular pain. Plasma bilirubin, alkaline phosphatase, and aspartate aminotransferase levels were elevated. Some symptoms did not subside until two months after the onset. One worker, after another exposure, experienced nausea, abdominal pain, and muscular pain. A second worker reported further symptoms of headache, tiredness, and decreased libido.

Williams *et al.* (1974) reported symptoms in 6 of approximately 300 workers exposed to MDA by surface coating concrete walls with epoxy resins. Exposure probably occurred by inhalation, ingestion, and skin contact as a result of mixing powder containing MDA. Symptoms of clinical hepatitis in the 6 workers appeared two days to two weeks after beginning work; five of the six had elevated bilirubin levels, and one liver biopsy showed bile stasis. All the workers recovered completely after an unspecified time.

McGill and Motto (1974) described hepatitis among 13 men who, over the course of 6 years, were occupationally exposed to MDA in the blending of epoxy resins used in the manufacture of insulating material. Among the 13 patients showing symptoms, all reported weakness, jaundice, and dark urine; 11 reported abdominal pain, nausea or vomiting, and anorexia; and over half reported fever, chills and/or headache. All the workers recovered within a 10 week period. After the first cases of hepatitis occurred, air sampling showed initial levels of MDA to be 0.1 ppm in the work area. After additional cases of hepatitis occurred, measures were taken to reduce worker exposure, and air levels were reduced to as low as 0.0064 ppm. The authors concluded that percutaneous absorption was the likely major route of exposure in light of the fact that cases occurred in spite of measures taken to reduce air levels and there was evidence that significant hand contact with the compound occurred during the workday. Since the symptoms appeared within one to 18 days after “working intensively” with the compound and exposure routes were not clearly established, quantitation of exposure levels was considered difficult.

The most well-known incident of MDA toxicity to humans resulted from ingestion of bread made with flour contaminated with MDA during transport (Kopelman *et al.*, 1966a). Eighty-four persons showed symptoms of abdominal pain and some degree of jaundice. All patients had elevated serum alkaline phosphatase and glutamic oxaloacetic transaminase levels. Seventeen had serum bilirubin levels over 5 mg/100 ml. Liver biopsy was performed on 8 persons and evaluated in a separate study (Kopelman *et al.*, 1966b). The primary finding was an unusual lesion described during the early course of the disease as portal zone cholangitis and later as centrilobular cholestasis with necrosis. The initial study reported that all but 2 patients had complete recovery within several weeks. However, a two year follow-up study of 14 individuals showed that 10 still had symptoms of some severity 7 to 23 months after initial onset including food intolerance, gastrointestinal disturbances, fatigue, and visual disturbances (Kopelman, 1968).

Human effects other than hepatotoxicity have been described including several cases of contact dermatitis and skin sensitization (LeVine, 1983; Van Joost *et al.*, 1987; de Pablo *et al.*, 1992;

Bruynzeel and van der Wegen-Keijser, 1993). A case report of a man exposed to MDA with potassium carbonate and γ-butyrolactone by accidental ingestion has been described (Roy *et al.*, 1985). In addition to hepatitis and abnormal liver function, which persisted over 18 months, the patient developed a progressively worsening retinopathy described as a "malfunction of the retinal pigment epithelium" accompanied by diminished visual acuity. The patient improved after approximately 3 months, but after examination at 18 months had not completely recovered.

Another report described the development of acute cardiomyopathy in addition to hepatitis in a worker exposed to a large quantity of MDA dust as the result of air filtration malfunction (Brooks *et al.*, 1979). The patient showed an abnormal ECG and an elevated cardiac LDH isoenzyme profile, which returned to normal within one month of onset.

V. Effects of Animal Exposure

The carcinogenicity of MDA was investigated in F344/N rats and $B6C3F_1$ mice (50/sex/dose group) administered in the drinking water at concentrations of 0, 150, and 300 ppm MDA (dihydrochloride) for 103 weeks (Lamb *et al.*, 1986). A 14-day range finding study was also conducted with 5 animal/sex/species/dose group, with exposure levels of 0, 200, 400, 800, 1600, and 3200 ppm MDA. A 13-week subchronic study was conducted with 10 animals/sex/species/dose group and exposure levels of 0, 25 (mice), 50, 100, 200, 400, and 800 (rats) ppm MDA. Using body weight and drinking water values from the study, low and high daily doses in the chronic study were calculated to be 9 and 16 mg/kg-day for male rats, 10 and 19 for female rats, 25 and 57 for male mice, and 19 and 43 for female mice. In the chronic study, survival was reduced among male mice treated with 300 ppm MDA. Final mean body weights were reduced in the 300 ppm dose group of female rats (-9%), male mice (-13%), and female mice (-16%). Among rats, non-cancer effects included follicular cysts and follicular-cell hyperplasia of the thyroid (significantly increased incidence in high-dose females; $p<0.05$ by Fisher's exact test). In the liver, the incidence of fatty and focal cellular change was elevated in low-dose male and female rats and also in high dose male rats. Incidence of unspecified dilatation of the liver was also elevated in high-dose male rats. Increased incidence of kidney mineralization was found in male rats treated with 300 ppm MDA. Among mice, incidence of liver degeneration was elevated in males in both treatment groups and females in the high-dose group ($p<0.01$ by Fisher's exact test). Incidence of kidney nephropathy was increased in male and female mice in both treatment groups and mineralization of the renal papilla was increased in both sexes in the high-dose group ($p<0.01$). From the 13-week study, the authors noted thyroid and bile duct effects in rats at 800 ppm MDA in water and in mice at 400 ppm MDA in water.

Albino and pigmented guinea pigs were exposed to aerosols of methylene dianiline in polyethylene glycol 200 (PEG) in nose-only exposure chambers (Leong *et al.*, 1987). Animals (8 of each strain) were exposed to a time-weighted average aerosol concentration of 0.44 g MDA/m^3 in air for 4 hours/day, 5 days/week for 2 weeks. Eight control animals were neither exposed to aerosol nor placed in the exposure chamber. Two weeks after the exposure period, animals were evaluated for dermal sensitization and irritation by challenge with 0.05 ml of 0, 2, 20, and 200 mg MDA/ml in PEG for up to 24 hours. No evidence of dermal irritation or

sensitivity was found. Subsequently, the animals were also examined for pulmonary sensitization by challenge with aerosols containing 0.01 and 0.05 ml of 200 mg MDA/ml PEG. Lung insufflation pressures were measured as an indication of changes in lung distensibility. No evidence of pulmonary sensitization was found. After the pulmonary challenge, the animals were examined histopathologically, with emphasis on eye, lung, liver, and kidney toxicity. Ocular toxicity ranging from mild to more severe was observed in all MDA-treated animals, but in none of the control animals. Pigmented animals did not differ in sensitivity or effect compared to albino animals. Mild lesions were described as "retraction and thickening of the outer segments of the photoreceptor cells" while more severe effects included swelling "through the inner segments of the photoreceptor cells to the outer nuclear layer." Some evidence of inflammatory cell infiltration was also noted and the pigmented epithelial layer was also degenerated. The authors conclude that the effects were attributable to MDA because no retinal lesions have been associated with exposure to the PEG vehicle. Furthermore, the inhalation exposures to MDA are the likely cause rather than the dermal and lung sensitization study exposures because these subsequent studies were conducted on control as well as treated animals. Pulmonary granulomas consisting of "an aggregate of macrophages surrounded by a thin mantle of lymphocytes" were found in 7 of the 16 MDA-exposed animals and one of the 8 control animals (level of significance was not stated). Treated and control animals had a high background incidence of pulmonary lesions including slight to mild bronchitis. No liver or kidney effects were detected in treated animals.

Nine purebred beagle dogs were treated orally (by capsule) with 70 mg "crude" (4 dogs) or "purified" (5 dogs) MDA in corn oil three days per week for a period ranging from approximately 3 to 7 years (Deichmann *et al.*, 1978). No concurrent controls were included since untreated animals were regularly maintained in the laboratory. After 2 years, cystoscopic examination was performed at 15-month intervals. After 4½ years, clinical chemistry tests were performed at 4 month intervals on 3 dogs from each group. Microscopic examination of urinary bladder, liver, heart, ovaries, uterus, and lymph nodes was performed on moribund animals or at the end of the experimental period (7 years, 2 months). Liver toxicity was noted in all the treated animals. Effects were described as fatty change, cell degeneration and necrosis, and lymphoid cell infiltration. One dog from each treatment group died from the toxic effect on the liver. The kidneys of four treated animals (two from each group) showed toxic effects including granuloma, glomerular nephritis, and congestion with cloudy swelling. Two dogs treated with "purified" and one dog treated with "crude" MDA showed toxicity to the spleen described as hemosiderosis and swelling with lymphocyte infiltration.

Wistar rats (5/sex/dose) were treated orally with 0, 0.0083, and 0.083 g MDA/kg body weight in propylene glycol daily for 12 weeks (Pludro *et al.*, 1969). Doses were 1% and 10% of the experimentally determined median lethal dose. No significant changes in body weight or hematological parameters were found, although serum albumin, β-globulin, and γ-globulin were elevated in animals in the 0.083 mg/kg dose group. The livers of all the animals in the high dose group showed signs of degeneration, including atrophy of the parenchyma and stromal hyperplasia in the portal areas. Also in this dose group, all animals showed hypertrophy of the lymphatic nodules of the spleen. In the low dose group, one animal showed a liver lesion and one a lesion in the spleen.

Schoental (1968) treated rats (8/sex) with MDA in 25% aqueous ethanol by stomach tube. Rats were given 20 mg doses a total of 2-5 times over several weeks up to 7½ months (frequency not specified). Animals showed necrosis of the liver and kidney and congestion and edema of the lungs.

Visual toxicity was reported in 15 cats treated perorally with 25-100 mg MDA/kg body weight in a 1% aqueous suspension (Schilling von Canstatt *et al.*, 1966). In four animals treated once with 100 mg/kg, no blindness was reported. In all the other treated animals (four with one dose of 100 mg/kg, two with one dose of 150 mg/kg, and two with three doses of 25 mg/kg and 3 doses of 50 mg/kg), blindness occurred within 8 days. Three of the eight recovered sight within 4 days. Two other treated animals were examined microscopically, one treated with 25 and then 50 mg/kg and one treated once with 200 mg/kg. The first was examined after 7 days and showed signs of granular degeneration of the rods and cones with some proliferation of the pigmented epithelium. The second was examined after 4¼ years and showed atrophy of the retinal neuroepithelium. The authors noted that no visual disturbances were found in other MDA treated experimental animals, including dog, rabbit, guinea pig, and rat.

VI. Derivation of Chronic Reference Exposure Level (REL)

Study	Leong *et al.*, 1987
Study population	Guinea pigs
Exposure method	Discontinuous inhalation exposure (nose only) of aerosols
Critical effects	Degeneration of retinal epithelium
LOAEL	440 mg/m^3 (54 ppm)
NOAEL	Not observed
Exposure continuity	4 hours/day, 5 days/week
Exposure duration	2 weeks
Average experimental exposure	52 mg/m^3 for LOAEL group (440 x 4/24 x 5/7) (6.4 ppm)
Human equivalent concentration	52 mg/m^3 using the default assumption of RGDR = 1 for a gas with systemic effects
LOAEL uncertainty factor	10 (incidence = 100%)
Subchronic uncertainty factor	10
Interspecies uncertainty factor	3
Intraspecies uncertainty factor	10
Cumulative uncertainty factor	3,000
Inhalation reference exposure level	0.02 mg/m^3 (20 µg/m^3; 0.002 ppm; 2 ppb)

Two specific types of toxicity have been associated with exposure to MDA: hepatotoxicity and ocular toxicity. Several studies have demonstrated hepatotoxicity in experimental animals. The best study of long term toxicity of MDA was the report by Lamb *et al.* (1986). In addition to addressing the carcinogenicity of MDA, Lamb described non-cancer health effects, which resulted from lifetime exposure of two species, rats and mice, to MDA at two concentrations in the drinking water. The 150 ppm dose level was a LOAEL for fatty change and focal cellular

change to the livers of male and female rats as well as for liver degeneration in male mice. The corresponding effects were also observed in high-dose male rats and male mice. Nephropathy was observed in mice of both sexes at the 150 and 300 ppm. There is abundant evidence from both human and animal studies that MDA is hepatotoxic. Bastian (1984), Williams *et al.* (1974), and McGill and Motto (1974) reported hepatitis in people exposed by inhalation and dermal absorption routes. Kopelman *et al.* (1966a,b) demonstrated human hepatotoxicity from exposure by the oral route. However, limited data detailing exposure levels associated with adverse health effects in humans preclude the development of a chronic REL from studies in humans.

The other toxic effect of potential concern from MDA exposure is ocular toxicity. Leong *et al.* (1987) reported damage to the retinas of guinea pigs exposed for 2 weeks to MDA aerosols (0.44 g/m^3 for 4 hr/day, 5 days/week; average experimental exposure = 52 mg/m^3) by inhalation. Schilling von Canstatt *et al.* (1966) also reported blindness in cats treated orally with MDA. A single case of retinopathy and visual toxicity in humans was reported in a man who accidentally ingested MDA with potassium carbonate and γ-butyrolactone. The Leong *et al.* (1986) study was selected for the development of the chronic REL because, although conducted for a relatively short period of time, the study appears to address the most sensitive endpoint of toxicity by the most appropriate route of exposure (inhalation). The studies, which established the hepatotoxicity of MDA, were conducted by the oral route of exposure.

As a comparison with the proposed REL, the study by Lamb *et al*. (1986) found a LOAEL of 9 mg/kg-day for liver changes in male rats. Use of a LOAEL UF of 3, an interspecies UF of 10, and an intraspecies UF of 10 results in an oral chronic REL of 0.03 mg/kg-day. Use of route-to-route extrapolation with the assumption that a 70 kg person breathes 20 m^3 of air per day leads to an inhalation chronic REL estimate of 100 $\mu g/m^3$. The proposed chronic REL based on Leong *et al*. (1987) is lower by a factor of 5 than that obtaimed by using Lamb *et al*. (1986) and should be protective of hepatotoxicity.

VII. Data Strengths and Limitations for Development of the REL

The strengths of the inhalation REL for 4,4′-methylene dianiline include the availability of a controlled exposure inhalation study. Major areas of uncertainty are the lack of adequate human exposure data, the lack of chronic inhalation exposure studies, the lack of reproductive and developmental toxicity studies, and the lack of observation of a NOAEL. In addition the test animals were under additional stress due to the restraint used to obtain nose-only exposure, while the control animals were not restrained. Liver toxicity has been included as a potential critical effect because of uncertainty regarding the relative potency of this compound in causing liver toxicity in different species by different routes of exposure.

When assessing the health effects of methylene dianiline, its carcinogenicity must also be assessed.

VIII. Potential for Differential Impacts on Children's Health

No evidence to support a differential effect of methylene dianiline on infants and children was found in the literature.

IX. References

ACGIH. 1992. American Conference of Governmental Industrial Hygienists, Inc. Documentation of the threshold limit values and biological exposure indices. Sixth ed. Cincinnati, OH: ACGIH.

Bastian PG. 1984. Occupational hepatitis caused by methylenedianiline. Med. J. Aust. 141:533-535.

Brooks LJ, Neale JM, and Pieroni DR. 1979. Acute myocardiopathy following tripathway exposure to methylenedianiline. JAMA 242:1527-1528.

Bruynzeel DP, and van der Wegen-Keijser MH. 1993. Contact dermatitis in a cast technician. Contact Dermatitis 28:193-194.

CARB. 2000. California Air Resources Board. California Emissions Inventory Development and Reporting System (CEIDARS). Data from Data Base Year 1998. February 12, 2000.

CRC. 1994. CRC Handbook of Chemistry and Physics, 75th edition. Lide DR, ed. Boca Raton, FL: CRC Press Inc.

de Pablo P, Ortiz J, Borrego L, Romero G, and Iglesias L. 1992. Allergic contact dermatitis from diaminodiphenylmethane in an ostomy bag. Contact Dermatitis 27:260.

Deichmann WB, MacDonald WE, Coplan M, Woods F, and Blum E. 1978. Di-(4-aminophenyl)-methane (MDA): 4-7 year dog feeding study. Toxicology 11:185-188.

HSDB. 1995. Hazardous Substances Data Bank. National Library of Medicine, Bethesda, MD (TOMES® CD-ROM Version). Denver, CO: Micromedex, Inc. (Edition expires 7/31/96).

Kopelman H. 1968. The Epping Jaundice after two years. Postgrad. Med. J. 44:78-81.

Kopelman H, Robertson MH, Sanders PG, and Ash I. 1966a. The Epping Jaundice. Br. Med. J. 1:514-516.

Kopelman H, Scheuer PJ, and Williams R. 1966b. The liver lesion of the Epping Jaundice. Quebec Journal of Medicine 35:553-64.

Lamb JC, Huff JE, Haseman JK, Murthy ASK, and Lilja H. 1986. Carcinogenesis studies of 4,4'-methylenedianilinedihydrochloride given in drinking water to F344/N rats and $B6C3F_1$ mice. J. Toxicol. Environ. Health 18:325-337.

Leong BKJ, Lund JE, Groehn JA, Coombs JK, Sabatis CP, Weaver RJ, and Griffin RL. 1987. Retinopathy from inhaling 4,4'-methylenedianiline aerosols. Fundam. Appl. Toxicol. 9:645-658.

LeVine MJ. 1983. Occupational photosensitivity to diaminodiphenylmethane. Contact Dermatitis 9:488-490.

McGill DB, and Motto JD. 1974. An industrial outbreak of toxic hepatitis due to methylenedianiline. N. Engl. J. Med. 291:278-282.

Pludro G, Karlowski K, Mankowska M, Woggon H, and Uhde W-J. 1969. Toxicological and chemical studies of some epoxy resins and hardeners. I. Determination of acute and subacute toxicity of phthalic acid anhydride, 4,4'-diaminodiphenylmethane and of the epoxy resin: Epilox EG-34. Acta Pol. Pharmacol. 26:352-357.

Roy CW, McSorley PD, and Syme IG. 1985. Methylene dianiline: a new toxic cause of visual failure with hepatitis. Hum. Toxicol. 4:61-66.

Schilling von Canstatt B, Hofmann HT, Oettel H, and Zeller H. 1966. [Netzhautveränderungen der Katze bei der Vergiftung mit peroral oder perkutan verabreichten Chemikalien]. Verhandlungen der Deutschen Gesellschaft für Pathologie 50:429-535.

Schoental R. 1968. Pathological lesions, including tumors, in rats after 4,4'-diaminodiphenylmethane and gamma-butyrolactone. Isr. J. Med. Sci. 4:1146-58.

U.S.EPA. 1994. United States Environmental Protection Agency. Methods for derivation of inhalation reference concentrations and applications of inhalation dosimetry. EPA/600/8-90/066F. Office of Research and Development. Washington DC: U.S.EPA.

Van Joost T, Heule F, and de Boer J. 1987. Sensitization to methylenedianiline and para-structures. Contact Dermatitis 16:246-248.

Williams SV, Bryan JA, Burk JR, and Wolf FS. 1974. Toxic hepatitis and methylenedianiline [letter]. N. Engl. J. Med. 291:1256.

CHRONIC TOXICITY SUMMARY

METHYLENE DIPHENYL ISOCYANATE

(diphenylmethane diisocyanate)

CAS Registry Number: 101-68-8

I. Chronic Reference Exposure Level

Inhalation reference exposure level	**0.7 µg/m^3**
Critical effect(s)	Hyperplasia of the olfactory epithelium in rats
Hazard index target(s)	Respiratory system

II. Physical and Chemical Properties (HSDB, 1995)

Description	Light yellow solid
Molecular formula	$C_{15}H_{10}N_2O_2$ (monomer)
Molecular weight	Variable (monomer = 250.27 g/mol)
Density	1.197 g/cm^3 @ 70°C (monomer)
Boiling point	196°C (monomer)
Melting point	37°C (monomer)
Vapor pressure	0.001 torr @ 40°C (monomer)
Solubility	Soluble in acetone, benzene, kerosene, and nitrobenzene (monomer)
Conversion factor	Monomer: 1 ppm = 10.2 mg/m^3 at 25°C; Not applicable for polymer

III. Major Uses or Sources

Methylene diphenyl isocyanate (MDI) is used for bonding rubber to nylon. MDI is also used in the manufacture of lacquer coatings and in the production of polyurethane resins and spandex fibers (HSDB, 1995). It is often handled in a partially polymerized form ("MDI polymer"), which has a much lower vapor pressure than the monomer. The annual statewide industrial emissions from facilities reporting under the Air Toxics Hot Spots Act in California based on the most recent inventory were estimated to be 30,398 pounds of MDI (CARB, 2000).

IV. Effects of Human Exposure

A 5-year occupational study of 107 workers from a polyurethane plastic manufacturing plant examined pulmonary function, respiratory symptoms, and smoking habits (Musk *et al.*, 1982, 1985). No significant changes in pulmonary function or respiratory symptoms were observed

when controlled for smoking. Mean MDI concentrations measured ranged from 0.0003 to 0.0006 ppm.

Significantly increased prevalence of asthma in female workers and of chronic bronchitis in male and female workers was observed following occupational exposure to low levels of MDI (<0.02 ppm) (Pham *et al.*, 1988). Workers from two plants were grouped by job classification and evaluated in this study conducted in 1976; workers were grouped as unexposed (62 men, 21 women), indirectly exposed (61 men, 56 women), or directly exposed (91 men, 27 women). Further characterization of the exposure groups was not presented. Decrements in pulmonary function (measured by VC, FEV_1 and single-breath carbon monoxide diffusion tests) were observed in men in the direct and indirect exposure groups: decrements in men with a history of direct exposure to MDI were statistically significant. Workers were also grouped by duration of occupational exposure (<20 months, 20-60 months, >60 months). Workers with known (direct or indirect) occupational exposure to MDI for greater than 60 months exhibited statistically significant decrements in pulmonary function tests. The follow-up examination of this study describes data from male workers only. At the time of the 5-year follow-up, air levels had been reduced to below the maximum allowed air concentration of 0.005 ppm by a modification of the ventilation system. Statistically significant decrements in pulmonary function were observed again in workers with known direct occupational exposure to MDI. Workers who were exposed at the time of the 1976 study but had since been removed from exposure did not exhibit decrements in pulmonary function, leading the authors to conclude that the effects of low-level exposure to MDI are to some extent reversible. Flaws in study design, including lack of exposure characterization, attrition, and inclusion of asthmatics in cohorts, preclude a quantitative assessment of MDI exposure on lung function.

An epidemiologic study of foundry workers reported more respiratory symptoms and significantly lower mean FEV_1 and maximum mid-expiratory flow at 25-75% in exposed workers compared to controls (Johnson *et al.*, 1985). However, MDI-exposed workers also had unquantified exposure to silica, metal dust, phenol formaldehyde, and a pyridine derivative precluding the evaluation of respiratory effects resulting from MDI exposure.

A worker with 5 years occupational exposure and suspected MDI hypersensitivity was exposed continuously in a controlled chamber to 5 ppb for 15 minutes, then 10 ppb for 30 minutes, and 20 ppb for 15 minutes (Marczynski *et al.*, 1992). The worker had not been exposed to MDI in the workplace for 5 days prior to the test challenge. Exposure to MDI resulted in an immediate, moderate, asthmatic reaction associated with significant hypoxemia.

IgG antibodies recognizing MDI-human serum albumin conjugates were detected in 4 of 5 MDI-exposed workers (Aul *et al*., 1999). The levels of specific IgG antibodies were more elevated with polymeric MDI compared with monomeric MDI.

A workplace death of a 39-year-old foundry worker was ascribed to occupational asthma induced by MDI exposure (Carnio *et al*., 1997). Postmortem pulmonary findings included epithelial desquamation, mucosal eosinophilic/neutrophilic infiltration, bronchial vessel dilatation, and edema and hypertrophy of smooth muscle.

V. Effects of Animal Exposure

Rats were exposed to 0.2, 1.0, and 6.0 mg/m^3 aerosolized MDI polymer 6 hours per day, 5 days per week for 24 months (Reuzel *et al.*, 1990; 1994). Statistically significant increased incidences of basal cell hyperplasia, olfactory epithelial degeneration, alveolar duct epithelialization, localized alveolar bronchiolization, and adenomas were observed in male and female rats exposed to 6.0 mg/m^3 MDI. An accumulation of macrophages with yellow pigment was also noted in the lungs and mediastinal lymph nodes. Male rats exposed to this concentration also exhibited a statistically significant increase in the incidence of Bowman's gland hyperplasia. Male rats exposed to 1 mg/m^3 MDI also exhibited statistically significant increased incidences of basal cell hyperplasia and Bowman's gland hyperplasia. An accumulation of macrophages with yellow pigment was observed in the lungs of female rats and the lungs and mediastinal lymph nodes of male rats exposed to 1 mg/m^3. No adverse effects were noted in rats exposed to 0.2 mg/m^3 MDI.

Hyperplasia of the olfactory epithelium with MDI exposure (Reuzel *et al*., 1990; 1994)

Concentration (*mg/m^3*)	*Males*			*Females*			*Combined*		
	Responders	*N*	*Incidence*	*Responders*	*N*	*Incidence*	*Responders*	*N*	*Incidence*
0	14	60	0.23	4	60	0.067	18	120	0.15
0.2	13	60	0.22	8	60	0.13	21	120	0.18
1	26	60	0.43	8	60	0.13	34	120	0.28
6	32	60	0.53	49	59	0.83	81	119	0.68

Guinea pigs were exposed to 2 ppm MDI 3 hours per day for 5 days (Aizicovici *et al.*, 1990). Qualitative immunostaining techniques indicated that MDI was localized in the respiratory tract. The spleen, lymph nodes, and thymus had very little staining. However, another study exposed guinea pigs to 4 ppb radiolabelled toluene diisocyanate (TDI) for 1-hour and found measurable radioactivity in extrathoracic tissues and body fluids (Kennedy *et al.*, 1989). Therefore, there is a possibility that MDI may be transported to sites other than the respiratory tract, such as the ovaries and testes, following inhalation exposure.

Gravid Wistar rats, Crl:(WI)BR, were exposed by whole-body inhalation to clean air (control) and to 1, 3, and 9 mg/m^3 MDI, respectively, for 6 hr per day from days 6 to 15 post conception (Buschmann *et al*., 1996). Rats were killed on day 20. The lung weights in the high-dose group were significantly increased compared to the sham-treated control animals. Treatment did not influence any other maternal and/or fetal parameters investigated (including maternal weight gain, number of corpora lutea, implantation sites, pre-and postimplantation loss, fetal and placental weights, gross and visceral anomalies, and degree of ossification). A slight but

significant increase in litters with fetuses displaying asymmetric sternebra(e) was observed after treatment with the highest dose. Although the relevance of an increase of this minor anomaly in doses which maternal toxicity is limited and within the limits of biological variability, a substance-induced effect in the high-dose group cannot be excluded with certainty. Thus, the authors reported a NOAEL of 3 mg/m^3 for embryotoxic effects.

VI. Derivation of Chronic Reference Exposure Level (REL)

Study	Reuzel *et al.*, 1990; 1994
Study population	Rats
Exposure method	Inhalation of polymeric aerosolized MDI (0, 0.2, 1.0, and 6.0 mg/m^3)
Critical effects	Hyperplasia of the olfactory epithelium
LOAEL	1 mg/m^3
NOAEL	0.2 mg/m^3
Benchmark Concentration (BMC_{05})	0.25 mg/m^3 (95% lower confidence limit on concentration for a 5% incidence of response based on analysis of the combined male and female data with a linear model, the best-fitting of 6 models examined, p = 0.99)
Study continuity	6 hours per day, 5 days per week
Study duration	24 months
Average experimental exposure	0.046 mg/m^3 for BMC_{05} group (0.25 x 6/24 x 5/7)
Human equivalent concentration	0.020 mg/m^3 for BMC_{05} group (particle with extrathoracic respiratory effects, RDDR = 0.453, based on MMAD = 0.68 µm and sigma g = 2.93)
LOAEL uncertainty factor	1
Subchronic uncertainty factor	1
Interspecies uncertainty factor	3
Intraspecies uncertainty factor	10
Cumulative uncertainty factor	30
Inhalation reference concentration	0.7 µg/m^3

The data of Reuzel *et al*. (1990, 1994) were examined with six quantal dose-response models (linear, log-normal, Weibull, logistic, quadratic, gamma) using USEPA BMDS 1.2. All models except the quadratic gave a good fit to the combined male and female data set. The linear model was selected as the best-fitting model. Possible differences between male and female susceptibility are suggested by the gender-specific data, although the significance of these differences is uncertain.

USEPA used the same two studies and a BMC_{10} approach to develop an RfC of 0.6 µg/m^3. Since USEPA used a 3-fold database uncertainty factor, their BMC_{10}-based RfC is comparable to the BMC_{05}-based OEHHA REL.

VII. Data Strengths and Limitations for Development of the REL

Strengths of the REL for MDI include the use of a well-conducted, long-term inhalation study, the observation of a NOAEL, and the estimation of a benchmark concentration. A limitation of the REL is that it is based on data on exposures to MDI "polymer" which actually contains nearly 50% monomer. Monomers may in some cases be more toxic than polymers. Thus, effects of pure monomeric MDI may occur at concentrations somewhat lower than observed in the reported study on MDI polymer. However, the capacity of MDI polymer to induce immunologic sensitization is greater than that of MDI monomer (Aul *et al*., 1999). The relative potential of MDI monomer and polymer to induce hyperplasia of the olfactory epithelium is unknown.

VIII. References

Aizicovici S, Jin R, LaPietra D, Gottlieb F, and Karol MH. 1990. Use of immunohistochemistry to detect diphenyl methane 4,4-diisocyanate (MDI) in exposed guinea pigs. Toxicologist 10(1):286. (Abstr. #1144). [cited in U.S. EPA, 1995].

Aul DJ, Bhaumik A, Kennedy AL, Brown WE, Lesage J, Malo JL. 1999. Specific IgG response to monomeric and polymeric diphenylmethane diisocyanate conjugates in subjects with respiratory reactions to isocyanates. J. Allergy Clin. Immunol. 103(5 Pt 1):749-755.

Buschmann J, Koch W, Fuhst R, and Heinrich U. 1996. Embryotoxicity study of monomeric 4,4'-methylenediphenyl diisocyanate (MDI) aerosol after inhalation exposure in Wistar rats. Fundam. Appl. Toxicol. 32(1):96-101.

CARB. 2000. California Air Resources Board. California Emissions Inventory Development and Reporting System (CEIDARS). Data from Data Base Year 1998. February 12, 2000.

Carino M, Aliani M, Licitra C, Sarno N, and Ioli F. 1997. Death due to asthma at workplace in a diphenylmethane diisocyanate-sensitized subject. Respiration 64(1):111-113.

HSDB. 1995. Hazardous Substances Data Bank. National Library of Medicine, Bethesda, MD (TOMES® CD-ROM Version). Denver, CO: Micromedex, Inc. (Edition expires 1/31/95).

Johnson A, Chan-Yeng M, MacLean L, Atkins E, Dybuncio A, Cheng F, and Enarson D. 1985. Respiratory abnormalities among workers in an iron and steel foundry. Br. J. Ind. Med. 42(2):94-100. [cited in U.S. EPA, 1995].

Kennedy AL, Stock MF, Alarie Y, Brown WE. 1989. Uptake and distribution of 14C during and following inhalation exposure to radioactive toluene diisocyanate. Toxicol. Appl. Pharmacol. 100:280-292. [cited in U.S. EPA, 1995].

Marczynski B, Czuppon AB, Hoffarth HP, Marek W, and Baur X. 1992. DNA damage in human white blood cells after inhalative exposure to methylenediphenyl diisocyanate (MDI) - case report. Toxicol. Lett. 60:131-138.

Musk AW, Peters JM, and Berstein L. 1985. Absence of respiratory effects in subjects exposed to low concentrations of TDI and MDI: A reevaluation. J. Occup. Med. 27(12):917-920.

Musk AW, Peters JM, DiBerardinis L, and Murphy RLH. 1982. Absence of respiratory effects in subjects exposed to low concentrations of TDI and MDI. J. Occup. Med. 24(10):746-750.

Pham QT, Teculescu D, Meyer-Bisch C, and Mur JM. 1988. Effects of chronic exposure to diisocyantes. Bull. Eur. Physiopathol. Respir. 23:561-564.

Reuzel PGJ, Arts JHE., Lomax LG, Kuijpers MHM, Kuper CF, Gembardt C, Feron VJ, and Loser E. 1994. Chronic inhalation toxicity and carcinogenicity study of respirable polymeric methylene diphenyl diisocyanate (polymeric MDI) aerosol in rats. Fundam. Appl. Toxicol. 22:195-210.

Reuzel PGJ, Arts JHE, Kuijpers MHM, and Kuper CF. 1990. Chronic toxicity/carcinogenicity inhalation study of polymeric methylene diphenyl diisocyanate aerosol in rats (Final report). Prepared by Civo Institute for the International Isocyanate Institute. Report No. V88.122. [cited in U.S. EPA, 1995].

U.S. EPA. 2000. U. S. Environmental Protection Agency. Integrated Risk Information System (IRIS). Office of Health and Environmental Assessment, Environmental Criteria Assessment Office, Cincinnati, OH: U.S. EPA. Available online at http://www.epa.gov/ngispgm3/iris

CHRONIC TOXICITY SUMMARY

NAPHTHALENE

(naphthene, NCI-C5290, albocarbon, dezodorator, moth balls, moth flakes, tar camphor, white tar, naphthalin, naphthaline)

CAS Registry Number: 91-20-3

I. Chronic Toxicity Summary

Inhalation reference exposure level	**9 μg/m^3 (2 ppb)**
Critical effect(s)	Respiratory effects (nasal inflammation, olfactory epithelial metaplasia, respiratory epithelial hyperplasia) in mice
Hazard index target(s)	Respiratory system, blood systems

II. Physical and Chemical Properties (HSDB, 1995; 1999 except as noted)

Description	White crystalline powder; odor of mothballs
Molecular formula	$C_{10}H_8$
Molecular weight	128.6 g/mol
Density	4.42 g/cm^3 @ 20ºC
Boiling point	218ºC
Melting point	80.5 ºC
Vapor pressure	0.078 Torr @ 25ºC (Sonnenfeld *et al*., 1983); 0.10 Torr @ 27ºC (CRC, 1994)
Conversion factor	5.26 μg/m^3 per ppb at 25ºC

III. Major Uses or Sources

Naphthalene is a natural constituent of coal tar (approximately 11%) (HSBD, 1995). It is present in gasoline and diesel fuels. Naphthalene is used as a moth repellent, though this use is decreasing in favor of p-dichlorobenzene (HSDB, 1995). It has also been used in the manufacture of phthalic anhydride, phthalic and anthranilic acids, naphthols, naphthylamines, 1-naphthyl-n-methylcarbamate insecticide, beta-naphthol, naphthalene sulfonates, synthetic resins, celluloid, lampblack, smokeless powder, anthraquinone, indigo, perylene, and hydronaphthalenes (NTP, 1992; HSDB, 1995). The statewide emissions from facilities reporting under the Air Toxics Hot Spots Act in California, based on the most recent inventory, were estimated to be 164,459 pounds of naphthalene (CARB, 1999).

IV. Effects of Human Exposure

Nine persons (eight adults and one child) were exposed to naphthalene vapors from several hundred mothballs in their homes. Nausea, vomiting, abdominal pain, and anemia were reported (Linick, 1983). Testing at one home following the incident indicated an airborne naphthalene concentration of 20 ppb (105 $\mu g/m^3$). Symptoms abated after removal of the mothballs.

Workers occupationally exposed to naphthalene fumes or dust for up to five years were studied for adverse ocular effects (Ghetti and Mariani, 1956). Multiple pin-point opacities developed in 8 of 21 workers. Vision did not appear to be impaired.

Cataracts and retinal hemorrhage were observed in a 44 year old man occupationally exposed to powdered naphthalene, and a coworker developed chorioretinitis (van der Hoeve, 1906).

Wolf (1978) reported that a majority of 15 persons involved in naphthalene manufacture developed either rhinopharyngolaryngitis and/or laryngeal carcinoma.

Ingestion of naphthalene or p-dichlorobenzene mothballs is a frequent cause of accidental poisoning of children (Siegel and Wason, 1986). Infants exposed to naphthalene vapors from clothes or blankets have become ill or have died (U.S. EPA, 1990). The effects in infants have been associated with maternal naphthalene exposure during gestation (U.S. EPA, 1990).

Deaths have been reported following ingestion of naphthalene mothballs. A 17-year old male ingested mothballs, developed gastrointestinal bleeding, hematuria, and coma, and died after five days (Gupta *et al.*, 1979). A 30-year old female ingested 30 mothballs and died after five days (Kurz, 1987).

Acute hemolytic anemia was reported among 21 infants exposed to naphthalene vapors from nearby mothball-treated materials (Valaes *et al.*, 1963). Increased serum bilirubin, methemoglobin, Heinz bodies, and fragmented red blood cells were observed. Kernicterus was noted in eight of the children, and two of the children died. Ten of these children had a genetic deficiency in glucose-6-phosphate dehydrogenase.

A 12-year old male ingested 4 g of naphthalene and 20 hours later developed hematuria, anemia, restlessness, and liver enlargement (Manchanda and Sood, 1960). The patient recovered after 8 days.

A 69-year old female developed aplastic anemia two months after several weeks exposure to naphthalene and p-dichlorobenzene (Harden and Baetjer, 1978).

V. Effects of Animal Exposure

Male and female B6C3F1 mice were exposed to naphthalene (>99% pure) vapor for 6 hours per day, 5 days per week over 104 weeks (NTP, 1992). Concentrations used were 0 (150 mice), 10 (150 mice), or 30 ppm (300 mice) naphthalene. (Table 1). Lesions were observed in the nose and lungs of exposed mice, including increased incidences of chronic nasal inflammation, olfactory epithelial metaplasia, and respiratory epithelial hyperplasia.

Table 1. Incidence of respiratory tract lesions in mice (male and female combined) chronically exposed to naphthalene vapors (NTP, 1992).

	0 ppm	*10 ppm*	*30 ppm*
Nasal inflammation	3/139	34/134	108/270
Olfactory epithelial metaplasia	0/139	131/134	269/270
Respiratory epithelial hyperplasia	0/139	131/134	269/270

CD-1 mice were administered 5.3, 53, or 133 mg/kg/day naphthalene by gavage over 90 days (Shopp *et al.*, 1984). The only effect noted was inhibition of aryl hydrocarbon hydroxylase activity. No increase in mortality or changes in body weight were noted. Reduced spleen weights were noted in females exposed to the highest dose. No changes were noted in serum enzyme levels or electrolytes. The researchers did not conduct a histopathological examination.

B6C3F1 mice were administered 200 mg naphthalene/kg/day by gavage for 5 days per week over 13 weeks. No adverse effects were observed (U.S. EPA, 1990).

Developmental effects of naphthalene ingestion in Sprague-Dawley CD rats was studied by Navarro and associates (1991). The lowest dose tested (50 mg/kg/day by gavage) was associated with signs of CNS depression for the first 3 days. Fetal growth, survival, and morphological development were not significantly affected at 450 mg/kg/day compared with control animals, although a trend toward decreased fetal weight and increased malformations was observed.

Harris and associates (1979) intraperitoneally administered 395 mg/kg/day naphthalene to Sprague-Dawley rats over days 1 though 15 of gestation. Fetuses had a 50% increase in incidence in delayed cranial ossification and heart development.

New Zealand white rabbits were given 0, 40, 200, or 400 mg/kg/day by gavage over days 6 through 18 of gestation (U.S. EPA, 1986a). A dose-dependent increase in grooming, vocalization, aggression, diarrhea, dyspnea, and ocular and nasal discharge were noted at all doses. No statistically significant increase in malformations or developmental abnormalities was observed.

Sprague-Dawley rats were administered 0, 100, 300, or 1000 mg/kg/day of naphthalene via dermal application (U.S. EPA, 1986b). No effects were reported at 100 or 300 mg/kg/day. At the high dose a slight decrease in testes weight was noted.

VI. Derivation of Chronic Reference Exposure Level (REL)

Study	NTP (1992)
Study population	B6C3F1 mice (75 or 150/group/sex)
Exposure method	Discontinuous whole-body inhalation exposures to 0, 10, or 30 ppm naphthalene vapor
Critical effects	Nasal inflammation, olfactory epithelial metaplasia, and respiratory epithelial hyperplasia
LOAEL	10 ppm (96% incidence for males and 100% incidence for females)
NOAEL	Not observed
Exposure continuity	6 hours/day for 5 days/week
Average experimental exposure	1.8 ppm (10 ppm x 6/24 x 5/7) for LOAEL group
Exposure duration	104 weeks
Subchronic uncertainty factor	1
LOAEL uncertainty factor	10
Interspecies uncertainty factor	10 (see below)
Intraspecies uncertainty factor	10
Cumulative uncertainty factor	1000
Inhalation reference exposure level	0.002 ppm (2 ppb, 0.009 mg/m^3, 9 μg/m^3)

The NTP study was chosen for the REL derivation since it is the only available lifetime animal inhalation bioassay and because no adequate epidemiological studies of long-term human exposure are available. The study was judged to be of adequate study design. The complete lack of nasal effects among control animals and the nearly total effect among animals exposed at 2 different concentrations strongly indicates a causal relationship between naphthalene exposure and nasal effects. The effects seen are consistent with those reported among exposed workers, who developed rhinopharyngolaryngitis or laryngeal carcinoma (Wolf, 1978). However, the hematological effects observed in humans have not been reported in laboratory animals, which raises the possibility that humans may be significantly more sensitive to naphthalene.

The most important limitation of the study is that the lowest concentration tested caused adverse effects in most (≥96%) of the animals tested. Thus the study amply demonstrates the risk of lifetime exposures to 10 ppm, but is uninformative regarding the concentration-response relationship at lower concentrations. Only a general assumption can be drawn on the magnitude of uncertainty factor needed to predict a concentration at which adverse effects would most likely not be observed. Lacking specific guidance or relevant research for this situation, the default 10-fold factor was applied. U.S. EPA also used the NTP study to develop its RfC of 3 μg/m^3 with slightly different assumptions and a cumulative uncertainty factor of 3000 (U.S. EPA, 2000). OEHHA followed the U.S. EPA precedent in using an intraspecies UF of 10 for

naphthalene, rather than using the HEC/RGDR approach. According to U.S. EPA (2000), because of its low water solubility and low reactivity, naphthalene-related effects on the nasal epithelium are expected to result following absorption of naphthalene and its metabolism to reactive oxygenated metabolites, not from direct contact. This is supported by data on naphthalene metabolism indicating that toxic effects on the respiratory tract are due to a naphthalene metabolite that may be formed either in the liver or in the respiratory tract. Necrosis of bronchial epithelial (Clara) cells in mice and necrosis of olfactory epithelium in mice, rats, and hamsters occur following intraperitoneal injection of naphthalene. The nasal effects from inhalation exposure to naphthalene were considered to be extra-respiratory effects of a category 3 gas (U.S. EPA, 1994). The assumption is made that nasal responses in mice to inhaled naphthalene are relevant to humans; however, it is uncertain that the RfC for naphthalene based on nasal effects will be protective for hemolytic anemia and cataracts, the more well-known effects from naphthalene exposure in humans.

VII. Data Strengths and Limitations for Development of the REL

The strengths of the REL for naphthalene include the large number of animals in the key study on which the REL is based and the 2 year length of the study. The limitations include the very high incidence of lesions at the lowest level tested in the key study, the absence of a NOAEL in the key study, the absence of other animal studies by the inhalation route, and the paucity of human data.

VIII. References

ATSDR. 1990. Agency for Toxic Substances and Disease Registry. Toxicological Profile for Naphthalene and 2-Methylnaphthalene (Draft). Atlanta, GA: U.S. Public Health Service, U.S. Department of Health and Human Services.

CARB. 1999. Air toxics emissions data collected in the Air Toxics Hot Spots Program CEIDARS Database as of January 29, 1999.

CRC. 1994. CRC Handbook of Chemistry and Physics, 75th edition. Lide DR, ed. Boca Raton, FL: CRC Press Inc. p. 6-109.

Ghetti G, and Mariani L. 1956. [Eyes changes due to naphthalene]. Med. Lav. 47:533-538. [reviewed in ATSDR, 1995].

Gupta R, Singhal PC, Muthusethupathy MA, Malik AK, Chugh KS. 1979. Cerebral oedema and renal failure following naphthalene poisoning. J. Assoc. Physic. India 27:347-348.

Harden RA, and Baetjer AM. 1978. Aplastic anemia following exposure to p-dichlorobenzene and naphthalene. J. Occup. Med. 20: 820-822.

Harris J, Bond GP, and Niemeier RW. 1979. The effects of 2-nitropropane, naphthalene, and hexachlorobutadiene on fetal rat development. [Abstract]. Toxicol. Appl. Pharmacol. 48:A35.

HSDB. 1995. Hazardous Substances Data Bank. National Library of Medicine, Bethesda, MD (TOMES® CD-ROM Version). Denver, CO: Micromedex, Inc. (Edition expires 11/31/95).

HSDB. 1999. Hazardous Substances Data Bank. Available online at http://sis.nlm.nih.gov

Kurz JM. 1987. Naphthalene poisoning. Critical care nursing techniques. Dimens. Crit. Care Nurs. 6:264-270.

Linick M. 1983. Illness associated with exposure to naphthalene in mothballs - Indiana. MMWR 32:34-35.

Manchanda SS, and Sood SC. 1960. Accidental poisoning in children: with a case report of naphthalene poisoning. Ind. J. Child Health 9(2):113-119.

Navarro H, Proce C, Marr M, and Myers C. 1991. Developmental toxicity evaluation of naphthalene administered by gavage to Sprague-Dawley rats on gestational days 6 through 15. Research Triangle Institute. Research Triangle Park, NC: National Toxicology Program.

NTP. 1992. Toxicology & Carcinogenesis Studies of Naphthalene in B6C3F1 Mice. Technical Report Series No. 410. NIH Publication No. 92-3141.

Shopp GM, White KL, and Hosapple M. 1984. Naphthalene toxicity in CD-1 mice: General toxicology and immunotoxicology. Fundam. Appl. Toxicol. 4:406-429.

Siegel E, and Wason S. 1986. Mothball toxicity. Pediatr. Clin. North Am. 33:369-374.

Sonnenfeld WJ, Zoller WH, and May WE. 1983. Dynamic coupled-column liquid chromatographic determination of ambient temperature vapor pressures of polynuclear aromatic hydrocarbons. Anal. Chem. 55(2):275-280.

U.S.EPA. 1986a. U.S. Environmental Protection Agency. Developmental toxicity study in rabbits. PH 329-TX-001-85. 86-870000563. Office of Research and Development, Cincinnati, OH.

U.S.EPA. 1986b. U.S. Environmental Protection Agency. Ninety day (sub-chronic) dermal toxicity study with naphthalene in albino rats. 65-870000565. Cincinnati, OH: Office of Research and Development.

U.S.EPA. 1990. U.S. Environmental Protection Agency. Drinking Water Health Advisories for 15 Volatile Organic Chemicals. U.S. EPA/ODW. NTIS No. PB90-259821.

U.S. EPA. 1994. U.S. Environmental Protection Agency Methods for Derivation of Inhalation Reference Concentrations and Application of Inhalation Dosimetry. EPA/600/8-90/066F. Office of Research and Development. Washington, DC: U.S.EPA.

U. S. EPA. 2000. U.S. Environmental Protection Agency. Integrated Risk Information System (IRIS). Naphthalene. Available on-line at http://www.epa.gov/ngispgm3/iris

Valaes T, Doxiadis SA, and Fessas T. 1963. Acute hemolysis due to naphthalene inhalation. J. Pediatr. 63:904-915.

van der Hoeve, J. 1906. [Chorioretinitis in humans from the effects of naphthalene] [in German]. Arch. Augenheilkd. 56:259-262. [reviewed in ATSDR, 1995].

Wolf O. 1978. Cancer of the larynx in naphthalene cleaner. Z. Gesamte. Hyg. 24(10):737-739.

CHRONIC TOXICITY SUMMARY

NICKEL AND NICKEL COMPOUNDS: NICKEL OXIDE

Molecular Formula	*Molecular Weight*	*Synonyms*	*CAS Registry Number*
Ni	59	elemental nickel	7440-02-0
NiO	74.69	nickel oxide	1313-99-1
$NiCl_2$	129.6	nickel chloride nickel dichloride	7718-54-9
$NiSO_4$	154.75	nickel sulfate nickelous sulfate	7786-81-4
$NiCO_3$	118.7	nickel carbonate carbonic acid nickel salt	3333-67-3
Ni_3S_2	240.19	nickel subsulfide trinickel disulfide heazlewoodite	12035-72-2

I. Chronic Toxicity Summary

A. Nickel and Nickel Compounds (except nickel oxide)

Inhalation reference exposure level **0.05 μg Ni/m^3**

Critical effect(s) Lung, nasal epithelial and lymphatic pathology in male and female rats

Hazard index target(s) Respiratory system; hematopoietic system

B. Nickel Oxide

Inhalation reference exposure level **0.10 μg Ni/m^3**

Critical effect(s) Lung and lymphatic pathology in male and female rats

Hazard index target(s) Respiratory system; hematopoietic system

II. Physical and Chemical Properties (from HSDB, 1995)

Description	Ni metal: Silvery metal; $NiCl_2$: deliquescent crystals (U.S.EPA, 1985)
Molecular formula	See above
Molecular weight	See above
Density	8.9 g/cm^3 @ 20°C (Ni)
Boiling point	2730°C (Ni)
Vapor pressure	Not applicable
Solubility	Elemental nickel, nickel subsulfide, and nickel oxide are insoluble in water, but are soluble in dilute nitric, hydrochloric, and sulfuric acids. The chloride and sulfate forms of nickel are water soluble.
Conversion factor	Not applicable for fumes and dusts

III. Major Uses and Sources

The most common airborne exposures to nickel compounds are to insoluble nickel compounds such as elemental nickel, nickel sulfide, and the nickel oxides from dusts and fumes. Contributions to nickel in the ambient air are made by combustion of fossil fuels, nickel plating, and other metallurgical processes. The most common oxidation state of nickel is the divalent (Ni^{2+}) form (U.S.EPA, 1985). Elemental nickel is a malleable, silvery-white metal that is highly resistant to strong alkali. Because of its corrosion resistance, nickel is used in the production of stainless steel, permanent magnets, and other alloys that require resistance to extremes of temperature or stress (U.S.EPA, 1985). Nickel is also used in electroplating baths, batteries, textile dyes, and catalysts (U.S.EPA, 1985). Nickel dust or powder is flammable (CDTSC, 1985). Due to its unique toxicological and physico-chemical properties, nickel carbonyl is not included in this summary. The annual statewide industrial emissions from facilities reporting under the Air Toxics Hot Spots Act in California, based on the most recent inventory, were estimated to be 110,334 pounds of nickel (CARB, 1999).

IV. Effects of Human Exposure

Several studies have indicated that occupational inhalation exposure to nickel aerosols can result in development of asthma specific to nickel. Davies (1986) found 3 cases of asthma among 53 nickel-plating workers without a history of asthma prior to employment. Novey *et al.* (1983) described biphasic metal-specific bronchial responses in an individual metal-plating worker exposed to nickel and chromium salts. In another case, immunological studies conducted in a 24-year old man showed nickel-specific antibodies in the serum after several weeks of working in a nickel-plating shop using nickel sulfate (McConnell *et al.*, 1973). Dermatitis was observed on exposed areas of his skin, and pulmonary function, measured by FEV_1 with and without isoproterenol challenge, was significantly impaired compared with a control subject and normal

values. Dyspnea, non-productive cough, chest-tightness, and wheezing were reported as symptoms by this worker during the work period.

A group of 7 metal plating workers with occupational asthma were evaluated for atopy and pulmonary function challenge in response to inhalational challenge with nickel and other metals (Cirla *et al.*, 1985). Three of the asthmatics tested positive for the presence of nickel-specific IgE antibodies. Positive reactions to skin testing with nickel were found in 3 of the asthmatic workers who also had dermatitis. Six out of the 7 asthmatics exhibited significantly decreased FEV_1 (> 15%) when exposed to 0.3 mg/m^3 nickel sulfate for 30 minutes. Control challenges with other metal salts did not reveal similar deficits in FEV_1.

Although asthma has been described in the above studies, occupational inhalation of nickel dusts has not been found to be associated with pulmonary fibrosis (Muir *et al.*, 1993). An occupational epidemiology report by Broder *et al.* (1989) found no significant effects on pulmonary function in relation to nickel exposure in a nickel smelter, however a healthy worker effect was observed in this study.

V. Effects of Animal Exposure

Early studies on the chronic non-cancer effects of metallic nickel dust were complicated by early mortality and cancer in guinea pigs and rats (Hueper, 1958).

A 2-year inhalation study of nickel oxide in rats and mice (65 per sex, per group) was conducted by the National Toxicology Program (NTP, 1994a). In the first study, rats were exposed to 0, 0.62, 1.25, or 2.5 mg nickel oxide/m^3 (0, 0.5, 1.0, or 2.0 mg Ni/m^3) 6 hours/day, 5 days/week for 104 weeks. In addition to the carcinogenic effects of nickel oxide, a number of non-cancerous lesions were observed, particularly in the lungs. The incidence of inflammatory pigmentation in the alveoli was significantly greater in all exposed groups, compared to controls. The severity of the lesions reportedly increased with increasing exposure. Atypical alveolar hyperplasia was also seen in all exposed groups. Lymphoid hyperplasia in the bronchial lymph nodes was observed in males and females exposed to 1 mg Ni/m^3 or greater at 7 and 15 months and the incidence generally increased with increasing concentration at the end of the 2-year study. Females had an increased incidence of adrenal medullary hyperplasia at all exposures of nickel oxide. Body weights were significantly lower in the groups exposed to 2.0 mg Ni/m^3 for both sexes, and in males exposed to 1.0 mg Ni/m^3.

A companion study on nickel oxide in mice conducted by NTP showed similar lung inflammatory changes as seen in the rats, in addition to pigmentation of the alveolar region at all exposure concentrations, compared with controls (NTP, 1994a). The mice were exposed to 0, 1.0, 2.0, or 3.9 mg Ni/m^3. Bronchial lymph-node hyperplasia was also evident in all nickel-exposed animals. Body weights were slightly but significantly lower in the 3.9 mg Ni/m^3 group, compared with controls.

A continuous exposure of rats (20 - 40 per group) to 0, 60, or 200 μg Ni/m^3 as nickel oxide for 2 years resulted in severe pulmonary damage and premature mortality so that carcinogenesis could not be evaluated (Glaser *et al.*, 1986). Pulmonary alveolar proteinosis and septal fibrosis were

observed in the animals exposed to nickel. Only 1 rat per group survived the nickel exposures to the end of the experiment.

A 2-year study on the effects of nickel subsulfide in rats and mice was conducted by NTP (1994b). Rats (52-53 per sex per group) were exposed to 0, 0.15, or 1 mg Ni_3S_2/m^3 (0, 0.11, or 0.73 mg Ni/m^3) for 6 hours/day, 5 days/week for 104 weeks. Body weights were lowered in rats exposed to 0.73 mg Ni/m^3 compared with controls. Lung inflammation, alveolar hyperplasia, macrophage hyperplasia, and pulmonary fibrosis were observed with a significantly increased incidence at both nickel concentrations. Female rats exposed to nickel had significantly increased adrenal medullary hyperplasia. In addition to the pulmonary lesions, nasal inflammation and olfactory epithelial atrophy was observed in both sexes exposed to 0.73 mg Ni/m^3.

In the second phase of the NTP study (NTP, 1994b), mice were exposed to 0, 0.6, or 1.2 mg Ni_3S_2/m^3 (0, 0.44, or 0.88 mg Ni/m^3) for 6 hours/day, 5 days/week for 104 weeks. The same pathological lesions were observed in the lung and nasal passages as in the rats in the above study. These lesions were evident at both the 0.44 mg Ni/m^3 and the 0.88 mg Ni/m^3 concentrations. The adrenal medullary hyperplasia seen in female rats was not observed in the mice.

An exposure of rats to either 0 or 0.97 mg Ni_3S_2/m^3 (0 or 0.71 mg Ni/m^3) for 6 hours/day, 5 days/week for 78-80 weeks resulted in decreased body weight, hyperplasia, metaplasia, and neoplasia in the lungs due to Ni (Ottolenghi *et al.*, 1974).

The NTP (1994c) studied the chronic non-cancer and carcinogenic effects of nickel sulfate hexahydrate on rats and mice. Rats were exposed to 0, 0.12, 0.25, or 0.5 mg $NiSO_4/m^3$ (0, 0.03, 0.06, or 0.11 mg Ni/m^3) for 6 hours/day, 5 days/week for 104 weeks. Chronic effects of nickel exposure in rats included inflammatory lesions in the lung, lung macrophage hyperplasia, alveolar proteinosis, and fibrosis, in addition to bronchial lymph node hyperplasia and nasal epithelial atrophy. The above effects were seen at exposures of 0.06 mg Ni/m^3 or greater.

Mice were exposed to a similar regimen that included 0, 0.06, 0.11, and 0.22 mg Ni/m^3 as nickel sulfate hexahydrate (NTP, 1994c). Similar pulmonary, lymphatic and nasal changes were observed in the mice as with the rats. Fibrosis was not reported, but an increased incidence of interstitial infiltration and alveolar proteinosis were observed at exposures of 0.11 mg Ni/m^3 or greater. No clinical findings or hematological effects were observed, but body weights were significantly depressed in all groups of nickel-exposed female mice. The body weights of males were reduced only in the group exposed to 0.22 mg Ni/m^3.

Rats and mice (10 per group) were exposed to nickel sulfate, nickel subsulfide, or nickel oxide 6 hours/day, 5 days/week, for 13 weeks (Dunnick *et al.*, 1989). Exposure-related increases in lung weight and histological lesions were observed in both species for all nickel exposures. Histological lesions included inflammatory changes, fibrosis, and alveolar macrophage hyperplasia. Nasal lesions were also observed in animals treated with nickel sulfate or nickel subsulfide. Lung weight changes were observed at exposures of 0.05 mg Ni/m^3 or greater in female rats. Macrophage hyperplasia in the alveolar region was observed at concentrations as

low as 0.02 mg Ni/m^3. Additional inflammatory lesions in the lungs were observed at 0.1 mg Ni/m^3.

A similar study by Haley *et al.* (1990) found that exposure of mice to nickel sulfate, nickel subsulfide, or nickel oxide resulted in various immunological effects. Mice were exposed to 0, 0.11, 0.45, or 1.8 mg Ni/m^3 as Ni_3S_2; 0.47, 2.0, or 7.9 mg Ni/m^3 as NiO; and 0.027, 0.11, and 0.45 mg Ni/m^3 as $NiSO_4$ for 6 hours/day, 5 days/week for 13 weeks. Nickel exposures consistently decreased splenic antibody-forming cell (AFC) responses, with significant decreases occurring at 1.8 mg Ni/m^3 as nickel subsulfide. In contrast, AFC responses in the lung-associated lymph nodes were consistently increased, indicating a possible indirect influence of inflammatory mediators released in the lung on local lymph nodes.

Rabbits (8 nickel exposed and 8 controls) exposed to 0.24 mg Ni/m^3 as nickel chloride 6 hours/day, 5 days/week for 4 weeks exhibited significantly decreased macrophage lysozyme activity in pulmonary lavage fluid and in macrophage cultures, compared with control animals (Lundborg and Camner, 1984). Similar exposures of rabbits to chlorides of cadmium, cobalt, or copper did not reduce lysozyme activity.

VI. Derivation of Chronic Reference Exposure Level (REL)

A. Nickel and Nickel Compounds (except nickel oxide)

Study	National Toxicology Program, 1994c
Study population	Male and female F344/N rats (52-53 per group)
Exposure method	Discontinuous inhalation
Critical effects	Pathological changes in lung, lymph nodes, and nasal epithelium: (1) active pulmonary inflammation, (2) macrophage hyperplasia, (3) alveolar proteinosis, (4) fibrosis, (5) lymph node hyperplasia, (6) olfactory epithelial atrophy
LOAEL	60 μg Ni/m^3 (as nickel sulfate hexahydrate)
NOAEL	30 μg Ni/m^3
Exposure continuity	6 hours/day, 5 days/week
Exposure duration	104 weeks
Average experimental exposure	5.4 μg Ni/m^3 for NOAEL group (30 x 6/24 x 5/7)
Human equivalent concentration	1.6 μg Ni/m^3 for NOAEL group males (particulate with respiratory effects, RDDR = 0.29 based on MMAD = 2.5, sigma g = 1.26, male rat body weight = 380 g, SA(PU) = 0.34 m^2, DEP(PU) = 0.024)
LOAEL uncertainty factor	1
Subchronic uncertainty factor	1
Interspecies uncertainty factor	3
Intraspecies uncertainty factor	10
Cumulative uncertainty factor	30
Inhalation reference exposure level	0.05 μg Ni/m^3

Nickel Oxide

Study	National Toxicology Program, 1994c
Study population	Male and female F344/N rats (52-53 per group)
Exposure method	Discontinuous inhalation
Critical effects	Pathological changes in lung and lymph nodes: (1) active pulmonary inflammation, (2) lymph node hyperplasia Adrenal medullary hyperplasia (females)
LOAEL	500 μg Ni/m^3
NOAEL	Not observed
Exposure continuity	6 hours/day, 5 days/week
Exposure duration	104 weeks
Average experimental exposure	89.5 μg Ni/m^3 for LOAEL group (500 x 6/24 x 5/7)
Human equivalent concentration	30 μg Ni/m^3 for LOAEL group males (particulate with respiratory effects, RDDR = 0.29 based on MMAD = 2.5, sigma g = 1.26, male rat body weight = 380 g, SA(PU) = 0.34 m^2, DEP(PU) = 0.024)
LOAEL uncertainty factor	10
Subchronic uncertainty factor	1
Interspecies uncertainty factor	3
Intraspecies uncertainty factor	10
Cumulative uncertainty factor	300
Inhalation reference exposure level	0.10 μg Ni/m^3

The studies conducted by NTP (1994 a,b, & c) all showed similar non-carcinogenic effects in rats and mice, regardless of the form of nickel administered. It therefore appears that soluble and insoluble forms of nickel cause similar effects in rodents. The human epidemiological literature predominantly describes cancer mortality rates from occupational exposures to nickel compounds, but does not specifically examine non-cancer effects. However, it is clear from many case reports that allergies and dermatitis can occur in exposed workers. Hypersensitive reactions to nickel have not been quantitatively studied in humans or animals, therefore it is not possible to develop an REL based on immunological hypersensitivity at the present time. A host of subacute and subchronic animal studies have shown nickel to affect certain immunological responses unrelated to hypersensitivity, but the applicability of these results to chronic human exposures and responses involves considerable uncertainty. Furthermore, data show that nickel may precipitate onset of asthma in occupational settings.

The results of the NTP studies and these dose response analyses support the speciation of nickel oxide for noncancer effects. The health effects data for nickel oxide indicate that its adverse pulmonary effects were less severe (absence of fibrosis, lower chronic lung inflammation severity scores) at higher doses than the pulmonary effects observed for nickel sulfate and nickel

subsulfide. The higher chronic REL value for nickel oxide of 0.1 $\mu g/m^3$ reflects these dose response differences. Furthermore, while it is based upon a LOAEL, the lower severity of the adverse health effects at the LOAEL mitigates some of the uncertainty associated with use of a LOAEL rather than a NOAEL. OEHHA therefore concludes that 0.1 $\mu g/m^3$ is an appropriate REL for nickel oxide. However, in setting inhalation exposure RELs for groups of compounds, OEHHA uses the most sensitive strain, species, sex, chronic endpoint, and agent for each group of substances. Therefore, as the pulmonary toxicity of the relatively insoluble nickel subsulfide is greater than that of nickel oxide and closer to that of nickel sulfate, OEHHA proposes to use the chronic REL derived from nickel sulfate for all other nickel compounds.

VII. Data Strengths and Limitations for Development of the REL

The strengths of the inhalation REL include the availability of controlled lifetime exposure inhalation studies in multiple species at multiple exposure concentrations and with adequate histopathogical analysis and the observation of a NOAEL. The major areas of uncertainty are the lack of adequate human exposure data and the lack of lifetime toxicity studies in any non-rodent species.

In addition to being inhaled, airborne nickel can settle onto crops and soil and enter the body by ingestion. Thus an oral chronic refererence exposure level for nickel is also required.

Derivation of Oral Chronic Reference Exposure Level

Study	Ambrose *et al*., 1976
Study population	Rats
Exposure method	Diet
Critical effects	Decreased body and organ weights
LOAEL	1000 ppm (50 mg/kg-day)
NOAEL	100 ppm (5 mg/kg-day)
Exposure continuity	Continuous
Exposure duration	Lifetime
Average exposure	5 mg/kg-day
Human equivalent concentration	5 mg/kg-day
LOAEL uncertainty factor	1
Subchronic uncertainty factor	1
Interspecies uncertainty factor	10
Intraspecies uncertainty factor	10
Cumulative uncertainty factor	300
Oral reference exposure level	0.05 mg/kg-day

The oral REL for nickel used the same study used for the U.S. EPA's oral Reference Dose (RfD). U.S.EPA assumed that rat consumption of 1 ppm Ni in the feed resulted in a dose of 0.05 mg/kg/day. An uncertainty factor of 10 was used for interspecies extrapolation and another of 10 to protect sensitive human populations. An additional uncertainty factor of 3 was used by U.S. EPA to account for inadequacies in reproductive studies of nickel. OEHHA has not used such special uncertainty or modifying factor because the criteria for their use are not well presented.

In addition there is an extensive toxicologic database on nickel in general which includes studies on reproductive effects.

VIII. References

Ambrose AM, Larson PS, Borzelleca JF, and Hennigar GR, Jr. 1976. Long term toxicologic assessment of nickel in rats and dogs. J. Food Sci. Technol. 13: 181-187.

Broder I, Smith JW, Corey P, Holness L. Health status and sulfur dioxide exposure of nickel smelter workers and civic laborers. J. Occup. Med. 31(4):347-353.

CARB. 1999. Air toxics emissions data collected in the Air Toxics Hot Spots Program CEIDARS Database as of January 29, 1999.

CDTSC. 1985. California Department of Toxic Substances Control. Review: Nickel and compounds. 11/15/85.

Cirla AM, Bernabeo F, Ottoboni F, and Ratti R. 1985. Nickel induced occupational asthma: Immunological and clinical aspects. In: Brown SS, and Sunderman FW. (eds.) Progress in Nickel Toxicology. Boston, MA: Blackwell Sci. Pub.

Davies JE. 1986. Occupational asthma caused by nickel salts. J. Soc. Occup. Med. 36:29-31.

Dunnick JK, Elwell MR, Benson JM, Hobbs CH, Hahn FF, Haly PJ, Cheng YS, and Eidson AF. 1989. Lung toxicity after 13-week inhalation exposure to nickel oxide, nickel subsulfide, or nickel sulfate hexahydrate in F344/N rats and B6C3F1 mice. Fundam. Appl. Toxicol. 12:584-594.

Glaser U, Hochrainer D, Oldiges H, and Takenaka S. 1986. Long-term inhalation studies with NiO and As_2O_3 aerosols in Wistar rats. Int. Congr. Ser. Excerpta Med. 676: 325-328.

Haley PJ, Shopp GM, Benson JM, Cheng YS, Bice DE, Luster MI, Dunnick JK, and Hobbs CH. 1990. The immunotoxicity of three nickel compounds following 13-week inhalation exposure in the mouse. Fundam. Appl. Toxicol. 15:476-487.

HSDB. 1995. Hazardous Substances Data Bank. National Library of Medicine, Bethesda, MD (TOMES® CD-ROM version) Denver, CO: Micromedex, Inc. (Edition expires 4/30/95).

Hueper WC. 1958. Experimental studies in metal cancerigenesis. Arch. Pathol. 65:600-607.

Lundborg M, and Camner P. 1984. Lysozyme levels in rabbit lung after inhalation of nickel, cadmium, cobalt, and copper chlorides. Environ. Res. 34:335-342.

McConnell LH, Fink JN, Schlueter DP, and Schmidt MG. 1973. Asthma caused by nickel sensitivity. Ann. Int. Med. 78:888-890.

Muir DC, Julian J, Jadon N, Roberts R, Roos J, Chan J, Maehle W, Morgan WK. Prevalence of small opacities in chest radiographs of nickel sinter plant workers. Br. J. Ind. Med. 50(5):428-431.

NTP. 1994a. National Toxicology Program. Technical Report on the Toxicology and Carcinogenesis Studies of Nickel Oxide in F344/N Rats and B6C3F1 Mice. NTP TR 451, NIH Publication No. 94-3363. U.S. Department of Health and Human Services.

NTP. 1994b. National Toxicology Program. NTP Technical Report on the Toxicology and Carcinogenesis Studies of Nickel Subsulfide in F344/N Rats and B6C3F1 Mice. NTP TR 453, NIH Publication No. 94-3369. U.S. Department of Health and Human Services.

NTP. 1994c. National Toxicology Program. NTP Technical Report on the Toxicology and Carcinogenesis Studies of Nickel Sulfate Hexahydrate in F344/N Rats and B6C3F1 Mice. NTP TR 454, NIH Publication No. 94-3370. U.S. Department of Health and Human Services.

Novey HS, Habib M, and Wells ID. 1983. Asthma and IgE antibodies induced by chromium and nickel salts. J. Allergy 72(4):407-412.

Ottolenghi AD, Haseman JK, Payne WW, Falk HL, and MacFarland HN. 1974. Inhalation studies of nickel sulfide in pulmonary carcinogenesis of rats. J. Natl. Cancer Inst. 54(5):1165-1172.

U.S. EPA. 1985. United States Environmental Protection Agency. Health Assessment Document for Nickel. EPA/600/8-83/012F, pp. 3-3.

CHRONIC TOXICITY SUMMARY

PHENOL

(Carbolic acid, phenylic acid, phenyl hydroxide)

CAS Registry Number: 108-95-2

I. Chronic Toxicity Summary

Inhalation reference exposure level	**200 µg/m^3 (50 ppb)**
Critical effect(s)	Twitching, muscle tremors, neurological impairment; elevated serum liver enzymes in rats
Hazard index target(s)	Alimentary system; circulatory system; kidney; nervous system

II. Physical and Chemical Properties (From HSDB, 1995, 1999; ATSDR, 1989)

Description	Colorless to light pink solid
Molecular formula	C_6H_5OH
Molecular weight	94.11 g/mol
Density	1.0576 g/cm^3 @ 20° C
Boiling point	181.75° C
Melting point	40.9° C
Vapor pressure	0.3513 torr @ 25° C
Odor threshold	40 ppb (150 µg/m^3) (Amoore and Hautala, 1983)
Solubility	86,000 ppm in water, very soluble in alcohol, carbon tetrachloride, acetic acid and liquid sulfur dioxide; soluble in chloroform, ethyl ether, carbon disulfide; slightly soluble in benzene
Henry's Law Constant	3.97 x 10^{-7} ATM-m^3 /mol (25 °C)
Conversion factor	1 ppm = 3.85 mg/m^3

III. Major Uses or Sources (HSDB, 1995)

Phenol is obtained from coal tar and is widely used as a disinfectant for industrial and medical applications. It also serves as a chemical intermediate for manufacture of nylon 6 and other man-made fibers and for manufacture of epoxy and other phenolic resins and as a solvent for petroleum refining. Approximately half of the U.S. consumption is directly related to the housing and construction industries, in applications such as germicidal paints and slimicides. Phenol is present in the atmosphere as an emission from motor vehicles and as a photooxidation product of

benzene. The annual statewide industrial emissions from facilities reporting under the Air Toxics Hot Spots Act in California, based on the most recent inventory, were estimated to be 234,348 pounds of phenol (CARB, 1999).

IV. Effects of Human Exposures

The information that is available concerning the health effects of phenol exposure to humans is almost exclusively limited to case reports of acute effects of oral exposure (Bruce *et al.*, 1987), dermal exposure (Griffiths, 1973), or occupational exposures, including some exposure by inhalation (Dosemeci *et al.*, 1991; Ohtsuji and Ikeda, 1972; Connecticut Bureau of Industrial Hygiene, undated). Data in animals are consistent with human data and show phenol to be well absorbed by oral, dermal, and inhalation routes of exposure. Severe chronic poisoning manifests in systemic disorders such as digestive disturbances including vomiting, difficulty swallowing, ptyalism (excess secretion of saliva), diarrhea, and anorexia (Bruce *et al.*, 1987; Baker *et al.*, 1978). Phenol poisoning is associated with headache, fainting, vertigo, and mental disturbances (Bruce *et al.*, 1987; Gosselin *et al.* 1984) which are likely symptoms of neurological effects well documented in animal studies. Ochronosis, or discoloration of the skin, and other dermatological disorders may result from dermal phenol exposure (Deichmann and Keplinger, 1962; Bruce *et al.*, 1987). Several investigators (Truppman and Ellenby, 1979; Warner and Harper, 1985) have reported that the use of phenol in the surgical procedure of skin peeling can produce cardiac arrhythmias although specifics of dose received were not determined and would be expected to be high.

Human exposure studies in which populations were exposed to phenol over longer periods of time (subchronic and chronic) are limited and have serious deficiencies including multiple chemical exposures, in many cases small size of exposed populations, and lack of information on dose received.

Occupational studies make up the majority of subchronic/chronic studies available on human health effects associated with phenol exposure. Merliss (1972) described muscle pain and weakness of unknown etiology, enlarged liver, and elevated serum enzymes (LDH, GOT, and GPT) characteristic of liver damage in an individual with intermittent inhalation and dermal exposures to phenol, cresol and xylenol. Bruze (1986) noted that a number of phenol-formaldehyde based resins are dermal irritants and contact sensitizers. Johnson *et al.* (1985) examined 78 iron and steel foundry workers with multiple chemical and aerosol exposures that included phenol and found more respiratory symptoms in the phenol exposed group. However, multiple exposure to diphenyl methane diisocyanate, formaldehyde, and silica containing aerosols prevented determination of the effects of phenol. Baj *et al.* (1994) examined twenty-two office workers exposed for six months via inhalation to a commercial product containing formaldehyde, phenol and chlorohydrocarbons. At the end of the six month period the indoor air of the workers contained 1,300 $\mu g/m^3$ of formaldehyde and 800 $\mu g/m^3$ of phenol. The eight workers with the highest concentrations of phenol in their urine had decreased erythrocyte and T-helper lymphocyte numbers and increased numbers of eosinophils and monocytes compared to controls. The multiple chemical exposure of this study prevents concluding that these effects are attributable to phenol exposure. In a study of hospital workers Apol and Cone (1983) documented dermal effects in workers exposed to a number of chemicals including phenols

contained in disinfectants. This study however could not document any differences in urinary levels of phenol metabolites between control populations and exposed populations and could not assign any of the dermal effects seen to phenol or other substances in the work environment. Dosemeci *et al*. (1991) conducted a follow-up study to evaluate mortality in 14,861 workers in five manufacturing facilities producing or using phenol and formaldehyde. Arteriosclerotic heart disease, emphysema, disease of the digestive system, and cirrhosis of the liver were inversely related to the extent of phenol exposure. Due to multiple chemical exposures the effects of phenol alone could not be identified with any certainty.

Baker *et al.* (1978) completed a study of 39 individuals exposed to drinking water contaminated with phenol for a period of 4-8 weeks. Doses of phenol were estimated to range between 10 mg/day and 240 mg/day. Effects seen included increased incidence of diarrhea, mouth sores and irritation of the oral cavity.

Two occupational studies are of note since they reported NOAELs. Workers exposed continuously for an unspecified period of time to an average air concentration of 4 ppm phenol experienced no respiratory irritation (Connecticut Bureau of Industrial Hygiene, undated). No adverse effects were reported among workers in a Bakelite factory who were exposed to levels of phenol up to 12.5 mg/m^3 (3.3 ppm) (Ohtsuji and Ikeda, 1972). In this study urinary phenol levels were measured and were observed to return to pre-exposure levels within 16 hours after exposure indicating a relatively rapid clearance of phenol from the body that was confirmed in a study by Piotrowski (1971). Ohtsuji and Ikeda (1972) did not clearly indicate the number of workers sampled or the duration of exposure.

V. Effects of Animal Exposures

In animal studies a number of subchronic and chronic studies employing oral and inhalation routes of exposure are available as well as shorter term studies using the dermal route of exposure. Responses observed in animal studies include: pulmonary damage (inhalation exposure), myocardial injury (inhalation and dermal exposure), liver damage (inhalation exposure), renal damage (inhalation exposure), neurological effects (inhalation exposure), developmental effects (oral exposure) and dermal effects (dermal exposure). Comparison of the three routes of exposure found that oral exposure was less effective at producing systemic toxic effects possibly due to the rapid metabolism of phenol to sulfate and glucuronide conjugates by the gastrointestinal tract. Comparison of health effects among studies using dermal, oral and inhalation routes of exposure finds that inhalation is a sensitive route of exposure for laboratory animals.

Several subchronic inhalation studies of health effects from phenol exposure are available but no inhalation studies longer than 90 days could be identified. Deichmann *et al.* (1944) exposed guinea pigs, rats, and rabbits to concentrations of phenol between 26 and 52 ppm for 28-88 days depending on species. Guinea pigs exposed for 7 hours per day, five days per week, for four weeks, displayed signs of respiratory difficulty and paralysis primarily of the hind quarters, indicating neurological effects. Five of twelve animals exposed at this concentration died at 28 days. At necropsy, extensive myocardial necrosis, lobular pneumonia, fatty degeneration of the

liver, and centrilobular hepatocellular necrosis were observed in all animals exposed at this level. Guinea pigs that were necropsied at 41 days also exhibited pulmonary inflammation, pneumonia, bronchitis, endothelial hyperplasia, and capillary thrombosis. Rabbits exposed at these same concentrations did not exhibit any signs of discomfort, but showed similar findings at necropsy at 88 days. Rats were less sensitive in this study with an apparent NOAEL of 26 ppm phenol for these effects. In this study, guinea pigs were the most sensitive species. Limitations of the Deichmann study include the range of exposure concentrations and the lack of a control group.

Sandage (1961) exposed Sprague-Dawley rats, mice and rhesus monkeys for 90 days continuously to 5 ppm phenol. Sandage found no effects on pulmonary, cardiovascular, hematological, hepatic, or renal systems, thus defining free-standing NOAELs for these systemic effects in these species. Limitations of this study include absence of guinea pigs (previously identified as the most sensitive species in the Deichmann study) and lack of a demonstrated dose response to the effects of phenol.

Dalin and Kristofferson (1974) examined the effects of phenol on the nervous system in rats exposed continuously for 15 days to a concentration of 26 ppm phenol and found muscle tremors, twitching and disturbances in walking rhythm and posture after 3-5 days exposure. After 15 days exposure, severe neurological impairment as measured by decreased performance on tilting plane test was found. The Dalin and Kristofferson (1974) study also documented elevated serum concentrations of LDH, GOT, GPT, and GDH indicative of liver damage in animals exposed to 26 ppm phenol continuously for 15 days.

The NCI (1980) study of the carcinogenicity of phenol is the most complete chronic study using the oral route of exposure. Mice and rats were exposed for 103 weeks to concentrations of phenol in their drinking water of 100, 2500, 5000, and 10,000 ppm. NOAELs in the mouse of 523 mg/kg/day (5000 ppm in drinking water) and NOAELs in the rat of 630 mg/kg/day (5000 ppm in drinking water) were observed for effects on the respiratory system, cardiovascular system, gastrointestinal system, hepatic system, renal system, and the brain based on histological examination of tissues. Male rats exposed to the 5000 ppm had a higher incidence of kidney inflammation (94%) than controls (74%). No tests of kidney function were performed in this study.

Boutwell and Bosch (1959) reported on the results of a chronic study in mice involving skin painting of 1.2 mg phenol or 2.5 mg phenol for a 52 week period. A NOAEL of 1.2 mg/animal for a 52 week exposure for dermal effects was found.

No multi-generational studies evaluating reproductive or developmental effects under chronic exposure conditions could be identified. Jones-Price *et al.* (1983a) reported that pregnant rats dosed orally with 0, 30, 60, and 120 mg/kg/day on gestation days 6-15 exhibited reduced fetal weight in a dose-related manner. However, no teratogenic effects or fetal deaths were observed. In a following study Jones-Price *et al.* (1983b) reported that pregnant mice dosed orally with 0, 70, 140, and 280 mg/kg/day on gestation days 6-15 exhibited decreased maternal weight gain, tremors, and increased maternal mortality at the 280 mg/kg/day dose. In the fetus reduced growth, decreased viability, and increased incidence of cleft palate were seen at the 280 mg/kg/day dose.

VI. Derivation of Chronic Reference Exposure Level (REL)

Study	Sandage, 1961; Dalin and Kristofferson, 1974
Study population	Mice, Sprague Dawley rats and rhesus monkeys
Exposure method	Continuous inhalation
Critical effects	Systemic effects including liver and nervous system effects
LOAEL	26 ppm (Dalin and Kristofferson, 1974)
NOAEL	5 ppm (Sandage, 1961)
Exposure continuity	Continuous
Average exposure concentration	5 ppm for NOAEL group
Human equivalent concentration	5 ppm for NOAEL group (gas with systemic effects, based on RGDR = 1.0 using default assumption that lambda (a) = lambda (h))
Exposure duration	90 days
Subchronic uncertainty factor	3
LOAEL uncertainty factor	1
Interspecies uncertainty factor	3
Intraspecies uncertainty factor	10
Cumulative uncertainty factor	100
Inhalation reference exposure level	0.05 ppm (50 ppb; 0.2 mg/m^3 (200 μg/m^3)

No suitable human studies were available for use since exposures were short term or occupational in nature with insufficient ancillary information (e.g., duration of exposure) or did not determine dose. Of the three routes of exposure available, inhalation appears to be the most sensitive based on the number and intensity of systemic effects noted (Deichmann *et al.*, 1944) relative to oral exposure (NCI, 1980). In support of this, ATSDR (1989) notes that the gastrointestinal tract has a large capacity to metabolize phenol to sulfate and glucuronide conjugates which appear likely to be less toxic than the parent compound, thus NOAELs derived from oral studies may not be applicable for other routes of exposure. The Deichmann *et al.* (1944) study identified guinea pigs as the most sensitive species. However, this study had a number of serious deficiencies including absence of controls, significant variability in the concentrations of phenol used in their exposure, and exposure that was not continuous. Since alternative studies using guinea pigs could not be identified, the rat was chosen as an alternative species since the rat has the most similar metabolic profile for metabolism of phenol to that of humans (ATSDR, 1989; Capel *et al.*, 1972). The Sandage (1961) study was chosen over other available studies since it was the longest in duration (90 days), had a continuous exposure, and evaluated three species (rats, mice, monkey). NOAELs determined in the Sandage study for systemic effects in all three species examined were 5 ppm, consistent with the idea that 5 ppm is a NOAEL for a number of species. Although this is a free-standing NOAEL, a subsequent study in rats indicated that nervous system and hepatic effects occur at a concentration of 26 ppm after several days (Dalin and Kristofferson, 1974).

The 5.0 ppm standard for phenol in the workplace (ACGIH, 1988; OSHA, 1985; NIOSH, 1976) is considered protective of the health of workers exposed occupationally but does not consider

sensitive populations and is not for continuous exposure conditions. The workplace standard is consistent with reports indicating that no respiratory irritation occurred among workers exposed regularly to 4 ppm phenol (Connecticut Bureau of Industrial Hygiene, undated) and no adverse effects were mentioned among workers exposed to 3.3 ppm (Ohtsuji and Ikeda, 1972). Neither report was considered appropriate to be the basis of a REL. However, for the sake of comparison adjusting the reported NOAEL of 4 ppm to continuous exposure and dividing by an intraspecies uncertainty factor of 10 results in an estimated chronic REL of 140 ppb, in reasonable agreement with the proposed REL of 50 ppb.

VII. Data Strengths and Limitations for Development of the REL

The major strength of the key study is the observation of a NOAEL from a continuous exposure study involving exposure of several different species. The primary uncertainties are the lack of adequate human health effects data, the lack of multiple concentration inhalation exposure studies demonstrating a dose-response relationship, the lack of animal studies longer than 90 days, and the lack of studies with guinea pigs, which have previously been identified as a sensitive species for phenol.

VIII. References

ACGIH. 1988. American Conference of Governmental Industrial Hygienists. TLVs - Threshold Limit Values and Biological Exposure Indices for 1988-1989. Cincinnati, OH: ACGIH.

Amoore JE, Hautala E. 1983. Odor as an aid to chemical safety: odor thresholds compared with threshold limit values and volatilities for 214 industrial chemicals in air and water dilution. J. Appl. Toxicol. 3(6):272-290.

Apol AG, and Cone J. 1983. Bay Area Hospital, Coos Bay, Oregon. Health Hazard Evaluation Report. HETA 82-053-1263. National Institute of Occupational Safety and Health.

ATSDR. 1989. Agency for Toxic Substances and Disease Registry. Toxicological Profile for Phenol. NTIS Report PB90-181249. Atlanta, Georgia: ATSDR.

Baj Z, Majewska E, Zeman K, Pokoca L, Dworniak D, Paradowski M, and Tchorzewski H. 1994. The effect of chronic exposure to formaldehyde, phenol and organic chlorohydrocarbons on peripherial blood cells and the immune system in humans. J. Investig. Allergol. Clin. Immunol. 4(4):186-191.

Baker EL, Landrigan PJ, Bertozzi PE, Field PH, Basteyns BJ, Skinner HG. 1978. Phenol poisoning due to contaminated drinking water. Arch. Environ. Health 33:89-94.

Boutwell RK, and Bosch DK. 1959. The tumor-promoting action of phenol and related compounds for mouse skin. Cancer Res. 19:413-424.

Bruce RM, Santodonato J, and Neal MW. 1987. Summary review of the health effects associated with phenol. Toxicol. Ind. Health 3:535-568.

Bruze M. 1986. Sensitizing capacity of 2-methylol phenol, 4-methylol phenol and 2,4,6-trimethylol phenol in the guinea pig. Contact Dermatitis 14:32-38.

CARB. 1999. Air toxics emissions data collected in the Air Toxics Hot Spots Program CEIDARS Database as of January 29, 1999.

Capel ID, French MR, Millburn P, Smith RL, Williams RT. 1972. Fate of [14C]-phenol in various species. Xenobiotica 2:25-34.

Connecticut Bureau of Industrial Hygiene. (Year not reported) unpublished data. [as cited by American Conference of Governmental Industrial Hygienists, 1984]

Dalin NM, and Kristoffersson R. 1974. Physiological effects of a sublethal concentration of inhaled phenol on the rat. Ann. Zool. Fenn. 11:193-199.

Deichmann WB, Kitzmiller KV, and Witherup BS. 1944. Phenol studies. VII. Chronic phenol poisoning, with special reference to the effects upon experimental animals of the inhalation of phenol vapor. Am. J. Clin. Pathol. 14:273-277.

Deichmann WB, and Keplinger ML. 1962. Phenols and phenolic compounds. In: Industrial Hygiene and Toxicology, Patty FA (ed.) Vol II. Toxicology, 2nd ed., pp.1363-1408. New York: Interscience Publishers, Inc.

Dosemeci M, Blair A, Stewart PA, Chandler J, and Trush MA. 1991. Mortality among industrial workers exposed to phenol. Epidemiology 2(3):188-193.

Gosselin RE, Smith RP, Hodge HC, and Braddock JE. (1984) Phenol. In: Clinical Toxicology of Commerical Products. Baltimore: Williams and Wilkins. pp. III-345-346.

Griffiths GJ. 1973. Fatal acute poisoning by intradermal absorption of phenol. Med. Sci. Law 13:46-48.

HSDB. 1995. Hazardous Substances Data Bank. On-line data base. Profile No. 113.

HSDB. 1999. Hazardous Substances Data Bank. Available online at http://sis.nlm.nih.gov

Johnson A, Moira CY, Maclean L, Atkins E, Dybuncio A, Cheng F, and Enarson D. 1985. Respiratory abnormalities among workers in an iron and steel foundry. Br. J. Ind. Med. 42(2):94-100.

Jones-Price C, Ledoux TA, Reel JR, *et al.* 1983a. Teratologic evaluation of phenol (CAS No. 108-95-2) in CD-1 rats. Research Triangle Park, NC: Research Triangle Institute.

Jones-Price C, Ledoux TA, Reel JR, *et al.* 1983b. Teratologic evaluation of phenol (CAS No. 108-95-2) in CD-1 mice. Laboratory study: September 18, 1980 to January 12, 1981. Research Triangle Park, NC: Research Triangle Institute.

Merliss RR. 1972. Phenol marasmus. Occup. Med. 14:55-56

NCI. 1980. National Cancer Institute. Bioassay of phenol for possible carcinogenicity. Technical Report Series No. NCI-CG-TR-203. Bethesda, MD: U.S. Department of Health and Human Services.

NIOSH. 1976. National Institute for Occupational Safety and Health. Criteria for a recommended standard … occupational exposure to phenol. DHEW Pub 76-196.

Ohtsuji H, and Ikeda M. 1972. Quantitative relation between atmospheric phenol vapor and phenol in the urine of workers in Bakelite factories. Br. J. Ind. Med. 29:70-73.

OSHA. 1985. Occupational Safety and Health Administration.Occupational standards permissible exposure limits. 29 CFR 1910.1000.

Piotrowski JK. 1971. Evaluation of exposure to phenol: Absorption of phenol vapor in the lungs through the skin and excretion of phenol in urine. Br. J. Ind. Med. 28:172-178.

Sandage C. 1961. Tolerance criteria for continuous inhalation exposure to toxic material. I. Effects on animals of 90-day exposure to phenol, CCl_4 and a mixture of indole, skatole, H_2S and methyl mercaptan. ASD technical report 61-519(I). Wright Patterson Air Force Base, OH: U.S. Air Force Systems Command, Aeronautical Systems Division.

Truppman ES, and Ellenby JD. 1979. Major electrocardiographic changes during chemical face peeling. Plast. Reconstr. Surg. 63:44-48.

Warner MA, and Harper JV. 1985. Cardiac dysrhythmias associated with chemical peeling with phenol. Anesthesiology 62:366-367.

CHRONIC TOXICITY SUMMARY

PHOSPHINE

(hydrogen phosphide; phosphorus trihydride; Celphos; Phostoxin)

CAS Registry Number: 7803-51-2

I. Chronic Toxicity Summary

Inhalation reference exposure level	**0.8 μg/m^3** (0.6 ppb)
Critical effect(s)	Decreased body weight gain in mice
Hazard index target(s)	Respiratory system; alimentary system; nervous system; kidney; hematopoietic system

II. Chemical Property Summary (HSDB, 1995, except as noted)

Description	Colorless gas
Molecular formula	PH_3
Molecular weight	34 g/mol
Vapor density	1.17 (air = 1)
Boiling point	–87.7°C
Vapor pressure	20 atm @ –3°C (Weast, 1980)
Solubility	0.26 volumes in water @ 20°C; soluble in alcohol, ether (Sax and Lewis, 1989)
Conversion factor	1.39 mg/m^3 per ppm at 25ºC

III. Major Uses and Sources

Phosphine is used as an agricultural fumigant against insects and is among the most acutely toxic of the fumigant gases (HSDB, 1995). In its use as a fumigant, application of aluminum, magnesium, or zinc phosphide pellets generates phosphine gas upon exposure to moisture. Because of high volatility, phosphine residue dissipates from treated material upon ventilation. Inadequate sealing of materials during the course of treatment can result in unplanned environmental exposure.

Phosphine is also used by the semiconductor industry as a chemical doping agent for electronic components (n-type semiconductors) (HSDB, 1995). Other minor sources/uses of phosphine are in chemical syntheses: specifically, in preparations of phosphonium halides, for polymerization initiation, and as condensation catalysts. The annual statewide industrial emissions from facilities reporting under the Air Toxics Hot Spots Act in California based on the most recent inventory were estimated to be 3312 pounds of phosphine (CARB, 1999). In 2000, more than 120,000 pounds of phosphide compounds (including 119,519 pounds of aluminum phosphide and 1607 pounds of zinc phosphide) were applied in California agriculture (CDPR, 2001). In the

presence of water these phosphides break down to phosphine. However, the fraction emitted as phosphine is not known.

IV. Effects of Exposures to Humans

Toxicity among 22 workers intermittently exposed to phosphine levels of 0.17-2.11 ppm in air from fumigation activity ranging over 0.5 to 29 years (mean = 11.1 years) was evaluated (Misra *et al.*, 1988). The subjects were interviewed within one day of fumigation activity and reported that symptoms subsided when phosphine was not in use. The most frequently reported symptoms include dyspnea (31.8%), headache (31.8%), chest tightness (27.3%), cough (18.2%), anorexia and epigastric pain (18.2%), finger paresthesia and numbness (13.6%), and giddiness, numbness, and lethargy (13.6%). No change in motor or sensory nerve conduction velocity was found.

A similar spectrum of toxic effects among workers involved in grain storage at a seaboard terminal has been reported (Jones *et al.*, 1964). Among 69 men exposed to breathing zone phosphine levels of 0-35 ppm for as long as 16 hours per day, the authors report symptoms of multiple origins. These include gastrointestinal (diarrhea, nausea, epigastric pain, vomiting), cardio-respiratory (chest tightness, dyspnea, pain in chest, palpitations, retrospinal pain), and central nervous (headache, dizziness, staggering gait) systems. Symptoms were reported to appear only at the time of exposure and apparently were reversible.

In another report of chronic occupational exposure, authors cited the appearance of chronic bronchitis, anemia, and digestive disorders (Eichler, 1934).

Most literature reports of human toxic health effects of phosphine, however, come from case reports of acute exposures. Some are suggestive of potential chronic toxicity endpoints because of the irreversible nature of the effect. In a case report of phosphine poisoning of 29 people exposed by inhalation on a grain freighter, pathological findings included evidence of urinary tract injury (occult blood), liver damage (bilirubinuria and increased SGPT, GGPT, and LDH), and myocardial damage (increased MB fraction of CPK, abnormal ECG) (Wilson *et al.*, 1980). A-two-year old child who died as a result of the exposure showed myocardial necrosis with mononuclear infiltrates, pulmonary edema with damaged epithelia, pleural effusion, and an enlarged spleen. In another case report exposure of a 7-months pregnant, 24-year-old woman to aluminum phosphide from a nearby grain storage site was lethal (Garry *et al.*, 1993). There was evidence of severe pulmonary edema, necrosis of individual hepatic cells, and anoxic change in Purkinje cells of the cerebellum. These reported deaths of a small child and a pregnant woman exposed together with individuals who survived exposure to phosphine suggest that there may be sensitive human subpopulations. In another case report of acute phosphine poisoning by inhalation, Schoonbroodt *et al.*, (1992) observed necrosis of the nasal mucosa, delayed onset of pulmonary edema, and myocardial injury. Chopra *et al*. (1986) treated sixteen patients with aluminium phosphide poisoning during 1985. Findings included mucosal necrosis and cardiac abnormalities (due to hypoxemia). Renal failure (1/16), proteinuria (1/16), and increased blood transaminases (2/16) resulted from oral exposure to phosphine. The multi-organ involvement in toxicity suggests that phosphine is a broad-spectrum toxicant.

In a 1994 two deaths and three illnesses were reported due to phosphine fumigation of agricultural products in railroad cars (Perrotta *et al*., 1994). The annual report for the year 2000 of the American Association of Poison Control Centers' Toxic Exposure Surveillance System does not list fumigants as a separate category of pesticides (Litovitz *et al*., 2001). However, two of the case reports of fatalities, that were presented in abstract form, were due to aluminum phosphide.

V. Effects of Exposures to Animals

A subchronic inhalation toxicity study of phosphine was conducted in Balb-c mice (Barbosa *et al.*, 1994). Twelve animals/sex/dose group were exposed for 6 hours/day, 5 days/week for 13 weeks to 0, 0.3, 1.0, or 4.5 ppm phosphine. Non-cancer toxicity endpoints included reduction in weight gain and changes in relative organ weights of kidneys, lungs, liver, heart, brain and spleen. In the highest dose group, itching and scratching of the eyes, feet and tail, and decreased overall activity were observed. No diarrhea, loss of equilibrium, convulsions, seizures, or other neurological disturbances were noted. A dose-dependent decrease in total body weight gain was observed at all exposure levels with a greater effect observed in females ($p<0.0001$). Statistically significant decreases in relative organ weights (kidney, heart, and brain) were observed in males only at the 0.3 ppm exposure level ($p<0.001$). On the other hand, female mice showed increased relative organ weights (lungs, heart, and spleen) predominantly at higher doses (1.0 and 4.5 ppm; $p<0.001$). At 4.5 ppm phosphine absolute kidney and spleen weights were significantly increased in females ($p<0.01$). Increased frequencies of micronuclei in polychromatic erythrocytes from bone marrow and spleen were also seen at 4.5 ppm. This group also conducted a short-term repeated dose experiment. Six mice/sex/group were exposed to 5.5 ppm phosphine for 2 weeks (6 hrs/day, 5 days/wk). No statistically significant changes in weight gain were observed at the end of this exposure period.

In another subchronic inhalation toxicity study, male and female Fischer 344 rats (10/sex/group) were exposed to levels of 0, 0.37, 1.0, and 3.1 ppm phosphine for 6 hours per day, 5 days per week, for 13 weeks (Newton *et al.*, 1993). A higher dose group at 10 ppm was terminated prematurely (at 3 days) because of high mortality. A satellite group exposed to 5.1 ppm for 2 weeks was terminated after 13 days recovery. Observations of overt toxicity and viability were made at the time of each exposure; body weight and food consumption were monitored weekly; ophthalmic examination was done the day before termination; and hematological and clinical chemistry indices were measured after 4 and 13 weeks. Postmortem examination included gross necropsy, with particular attention to orifices, the cranial cavity, surfaces of the brain and spinal cord, nasal cavity and sinuses, the thoracic, abdominal, and pelvic cavities and viscera, and the cervical tissue and organs. Histopathology was performed on 10% buffered formalin-fixed/hematoxylin-eosin-stained tissues. Significant observations after 13 weeks of phosphine exposure included decreased hemoglobin, hematocrit, and erythrocytes in males in the 3.1 ppm dose group. Male rats in the 1 ppm dose group showed decreased weight gain. Increased incidence of small seminal vesicles was noted at 1 and 3.1 ppm, although no histological correlate was observed. Absolute and relative decreases in liver weight were observed in all exposed groups, but there was no evidence that this effect was dose-related. A significant

decrease in serum glutamic pyruvic transaminase (SGPT) was observed at 3.1 ppm, although the authors noted unusually high control levels. None of these effects were observed after the 4 week recovery period. Other effects of a transient nature noted during the exposure include decreased weight gain in female rats at 1 ppm, decreased food consumption at 0.37 ppm in males and females, and increased blood urea nitrogen (BUN) at 3.1 ppm. Observations in the 10 ppm group necropsied after 3 days of exposure included decreased erythrocytes, increased alkaline phosphatase, and increased kidney weight with coagulative necrosis of the tubular epithelium of the outer cortex. In a subchronic study of CD male and female rats under similar conditions (exposure to 0, 0.3, 1, or 3 ppm phosphine 6 h/day, 5 days/week for 13 weeks), no neurotoxicity was observed (Schaefer *et al*., 1998).

Newton *et al.* (1993) also examined developmental toxicity by exposing 24 pregnant female CD^R rats per group to 0, 0.03, 0.33, 2.8, 4.9, and 7.0 ppm phosphine. The highest dose group was terminated prematurely because of high mortality; all other animals were necropsied after 20 days for evaluation of maternal and fetal toxicity. Maternal toxicity endpoints included weight of ovaries and uteri, number of corpora lutea, pregnancy, and implantation rate. Fetal toxicity was evaluated by weight, number, and location of fetuses and resorptions, visceral malformations and variations, and skeletal changes after alizarin staining. No statistically significant differences from control animals were observed for any parameter at any dose, with the exception of a change in mean number of resorption sites ($p \leq 0.01$), mean resorption/implant ratio ($p \leq 0.05$), and incidence of females with resorption ($p \leq 0.05$), all at 0.03 ppm only. In the absence of this effect at higher dose levels, these observations are not considered useful in establishing a low adverse effect level.

A 35-day phosphine inhalation study was conducted exposing rats continuously to 0, 0.05, 0.2, 1.5, and 8.0 mg/m^3 phosphine (0, 0.036, 0.14, 1.1, and 5.8 ppm) in which hematological endpoints and histopathological changes of the lungs and kidneys were examined (Pazynich *et al.*, 1984). Observations include a statistically significant change in erythrocytes (increase followed by a decrease at day 35) and decreased hemoglobin at the 0.05 and 0.2 mg/m^3 dose levels, although the 1.5 mg/m^3 dose group did not show this change. Other significant changes, noted in the lowest dose group, included decreased peroxidase activity after 35 days exposure, decreased sulfhydryl group content in blood after 27 days, and decreased phagocytotic index after 21 days. Some histological changes were noted in the lungs, kidneys, and to a lesser extent, the liver, particularly in the higher dose groups, although the exact nature of the degenerative change is not well described. Unclear dose-response relationships and temporal aspects of the endpoints also make establishment of a low adverse effect level unreliable.

Two rats were exposed to 20 ppm phosphine for 14 days (4 hours/day) (Waritz and Brown, 1975). Animals were monitored for weight gain, and organs/tissues fixed in Bouin's solution and stained with trichrome were examined histopathologically. There were no reported histopathological effects, although there was slightly reduced weight gain in the exposed animals.

In a chronic study of phosphine (Newton *et al*., 1999), 60 male and female F344 rats per group were exposed via whole-body inhalation for 6 h/day, 5 days/wk for up to 104 wk to mean concentrations of 0, 0.3, 1, or 3 ppm phosphine. Three ppm (4.17 mg/m^3) was the maximum

exposure level because of lethality seen at the high exposure level (7 ppm = 9.73 mg/m^3) in previous repeat dose studies (Newton *et al*., 1993). Ten rats per sex per group were killed after 52 weeks of exposure. Survivors were killed after 104 weeks of exposure. There were no phosphine-related effects seen on clinical observations, body weight, food consumption, hematology, clinical chemistry, urinalysis, or ophthalmology. There were no phosphine-related macroscopic findings or effect on absolute or relative organ weights. No histologic or morphologic alterations attributable to phosphine exposure were seen in the more than 40 organs and tissues examined. Under the conditions of this study, the authors found no treatment-related changes suggestive of a toxic or carcinogenic effect in rats following 52 weeks or 2 years of whole-body inhalation exposure to 0.3, 1, or 3 ppm phosphine. Thus 3 ppm is a chronic NOAEL for rats.

VI. Derivation of Chronic Reference Exposure Level

Study	Barbosa *et al*., 1994
Study population	Balb-c mice (12 animals/sex/group)
Exposure method	Discontinuous whole body inhalation exposure (0, 0.3, 1, or 4.5 ppm)
Critical effects	Decrease in body weight gain, increase in relative organ weights; increase in micronuclei
LOAEL	4.5 ppm
NOAEL	1 ppm
Exposure continuity	6 hr/day, 5 days/ week
Average experimental exposure	0.178 ppm for NOAEL group (1 x 6/24 x 5/7)
Human equivalent concentration	0.178 ppm for NOAEL group (gas with systemic effects, based on RGDR = 1.0 using default assumption that lambda (a) = lambda (h))
Exposure duration	13 weeks
LOAEL uncertainty factor	1
Subchronic uncertainty factor	3
Interspecies uncertainty factor	10 (see below)
Intraspecies uncertainty factor	10
Cumulative uncertainty factor	300
Reference exposure level	0.0006 ppm (0.6 ppb; 0.0008 mg/m^3; 0.8 μg/m^3)

Supportive study	Newton *et al*., 1999
Study population	Rats (60 animals/sex/exposure level)
Exposure method	Discontinuous whole body inhalation exposure (0, 0.3, 1, or 3 ppm)
Critical effects	None
LOAEL	None detected
NOAEL	3 ppm
Exposure continuity	6 hours/day, 5 days/week
Average experimental exposure	0.53 ppm (3 x 6/24 x 5/7)
Human equivalent concentration	0.53 ppm (gas with systemic effects, based on RGDR = 1.0 using default assumption that lambda (a) = lambda (h))
Exposure duration	2 years
LOAEL uncertainty factor	1
Subchronic uncertainty factor	1
Interspecies uncertainty factor	10 (see below)
Intraspecies uncertainty factor	10
Cumulative uncertainty factor	100
Reference exposure level	0.005 ppm (5 ppb, 0.007 mg/m^3, 7 μg/m^3)

Newton *et al*. (1999) found no treatment-related changes suggestive of a toxic effect in F344 rats following 52 weeks or 2 years of whole-body inhalation exposure to 0.3, 1, or 3 ppm phosphine. Thus in this study 3 ppm is a chronic NOAEL for rats. Three ppm was set as the maximum level because in an earlier subchronic study in rats Newton *et al*. (1993) found lethality at 7 ppm. However, the chronic results of Newton *et al*. (1999) differ from the subchronic results of Newton *et al*. (1993), in which at least transient effects were seen in the hematopoietic system after 13 weeks at 3.1 ppm. In a subchronic study in mice (Barbosa *et al*., 1994), 4.5 ppm phosphine was a LOAEL and 1 ppm was a NOAEL for decrease in body weight gain. The results of Barbosa *et al*. (1994) indicated that mice may be more sensitive than rats. Thus, it was selected as the key study, and decrease in body weight gain was selected as the critical effect.

OEHHA has applied a subchronic uncertainty factor of 3 to account for the short duration of the Barbosa *et al*. (1994) study and an intraspecies uncertainty factor of 10 to account for human variability. Due to the general inconsistencies among the various studies in the database on phosphine, and in particular with the observation of mortality at 7 ppm in a short-term developmental study in rats (Newton *et al.*, 1993), it was considered prudent to include the full interspecies uncertainty factor of 10 (even though the HEC adjustment procedure could be applied) to acknowledge the severity of effect in at least one comparison study, and the additional uncertainty associated with the apparent wide and unpredictable variability between species and between different studies in the same species (rats). This results in a cumulative uncertainty factor of 100 to be applied to the NOAEL of 1 ppm in the subchronic study by Barbosa *et al*. (1994) and a chronic REL for phosphine of 0.8 μg/m^3 (0.6 ppb).

The U.S. EPA based its RfC of 0.3 μg/m^3 on the Barbosa *et al.* (1994) study, an adequate subchronic animal study for the derivation of a REL, and included a Modifying Factor (MF) of 3

for database deficiencies (lack of multigenerational reproduction studies). The criteria for use of modifying factors are not well specified by U.S. EPA. USEPA used its default interspecies uncertainty factor of 10 for a 13 week study.

The lack of adequate data on levels of chronic phosphine exposure to humans precludes development of a REL from human studies. The endpoint used in the determination of the REL (total body weight gain) showed a dose-related decrease with phosphine exposure in Balb-c mice. This endpoint is also consistent with that found by Newton *et al.* (1993), who noted dose-dependent decreases in body weight gain in Fischer 344 rats after a 13 week exposure regimen at 1 ppm, and Waritz and Brown (1975), who reported slightly decreased weight gain in rats exposed for 14 days to 20 ppm. Surprisingly Newton *et al*. (1999) did not find differences in body weight gain at either 1 ppm or 3 ppm and they did not comment on the discrepancy between their 2 reports. Although body weight changes or changes in food consumption were not addressed in human studies, the scant human data do relate phosphine exposure to a broad spectrum of toxic effects (gastrointestinal, cardio-respiratory, CNS). The decrease in weight gain found in the animal studies and reported changes in some relative organ weights (Barbosa *et al.*, 1994) suggest systemic toxicity.

VII. Data Strengths and Limitations for Development of the REL

The strengths of the inhalation REL for phosphine include the availability of data on multiple inhalation exposure concentrations and the observation of a NOAEL in a lifetime animal study. Major areas of uncertainty are the lack of adequate human exposure data, the lack of reproductive and developmental toxicity studies, and the inconsistency of the dose-response relationship across rodent studies.

VIII. Potential for Differential Impacts on Children's Health

Based on the lack of a dose-response in developmental toxicity caused by exposing pregnant female rats to 4.9 ppm phosphine (Newton *et al*., 1993) , the proposed REL of 0.8 $\mu g/m^3$ (0.6 ppb) is likely to be protective of developing humans in utero. However, there is no direct evidence in the literature to quantify a differential effect of phosphine on infants and children.

IX. References

Barbosa A, Rosinova E, Dempsey J, and Bonin AM. 1994. Determination of genotoxic and other effects in mice following short term repeated-dose and subchronic inhalation exposure to phosphine. Environ. Mol. Mutag. 24:81-88.

CARB. 2000. California Air Resources Board. California Emissions Inventory Development and Reporting System (CEIDARS). Data from Data Base Year 1998. February 12, 2000.

CDRR. 2001. California Department of Pesticide Regulation. Summary of Pesticide Use Report Data 2000 Indexed by Chemical. Preliminary Data. Available on-line at http://www.cdpr.ca.gov/docs/pur/pur00rep/chmrpt00.pdf

Chopra JS, Kalra OP, Malik R, Sharma R, and Chandna A. 1986. Aluminum phosphide poisoning: a prospective study of 16 cases in one year. Postgrad. Med. J. 62:1113-1115.

Eichler O. 1934. Phosphine poisoning: chronic, occupational? Sammlung von Vergiftungsfällen, 5:23.

Garry VF, Good PF, Manivel JC, and Perl DP. 1993. Investigation of a fatality from nonoccupational aluminum phosphide exposure: measurement of aluminum in tissue and body fluids as a marker of exposure. J. Lab. Clin. Med. 122:739-747.

HSDB. 1995. Hazardous Substances Data Bank. National Library of Medicine, Bethesda, MD (TOMES® CD-ROM Version). Denver, CO: Micromedex, Inc. (Edition expires 7/31/96).

Jones AT, Jones RC, and Longley EO. 1964. Environmental and clinical aspects of bulk wheat fumigation with aluminum phosphide. Ind. Hyg. J. 25:376-379.

Litovitz TL, Klein-Schwartz W, White S, Cobaugh DJ, Youniss J, Omslaer JC, Drab A, Benson BE. 2001. 2000 Annual report of the American Association of Poison Control Centers Toxic Exposure Surveillance System. Am. J. Emerg. Med. 19(5):337-395.

Misra UK, Bhargava SK, Nag D, Kidwai MM, and Lal MM. 1988. Occupational phosphine exposure in Indian workers. Toxicol. Lett. 42:257-263.

Newton PE, Schroeder RE, Sullivan JB, Busey WM, and Banas DA. 1993. Inhalation toxicity of phosphine in the rat: acute, subchronic, and developmental. Inhal. Toxicol. 5:223-239.

Newton PE, Hilaski RJ, Banas DA, Wilson NH, Busey WM, Shaheen DG. 1999. A 2-year inhalation study of phosphine in rats. Inhal. Toxicol. 11(8):693-708.

OEHHA, 1997. Draft Air Toxics Hot Spots Program Risk Assessment Guidelines. Part III. Technical Support Document for the Determination of Noncancer Chronic Reference Exposure Levels.

OEHHA, 1999. Draft Air Toxics Hot Spots Program Risk Assessment Guidelines. Part III. Technical Support Document for the Determination of Noncancer Chronic Reference Exposure Levels.

OEHHA. 2000. Office of Environmental Health Hazard Assessment. Air Toxics Hot Spots Program Risk Assessment Guidelines. Part III. Technical Support Document for the Determination of Noncancer Chronic Reference Exposure Levels. Available on-line at http://www.oehha.ca.gov

Pazynich VM, Mazur IA, Podloznyi AV, Chinchevich VI, and Mandrichenko BE. 1984. Experimental substantiation and prediction of time-related maximum permissible concentration of phosphine in the air. Gig. Sanit. Jan(1):13-15.

Perrotta D, Willis T, Salzman D *et al*. 1994. Deaths associated with exposure to fumigants in railroad cars – United States. MMWR 43(27):489-491.

Sax NI, and Lewis RJ (eds). 1989. Dangerous Properties of Industrial Chemicals. 7th ed. New York: Van Nostrand Reinhold.

Schaefer GJ, Newton PE, Gruebbel MM, Busey WM, and Shaheen DG. 1998. Acute and subchronic inhalation neurotoxicity of phosphine in the rat. Inhal. Toxicol. 10:293-320.

Schoonbroodt D, Guffens P, Jousten P, Ingels J, and Grodos J. 1992. Acute phosphine poisoning? A case report and review. Acta Clin. Belg. 47:280-284.

Waritz RS, and Brown RM. 1975. Acute and subacute inhalation toxicities of phosphine, phenylphosphine and triphenylphosphine. Am. Ind. Hyg. Assoc. J. 36:452-458.

Weast RC (ed.). 1980. CRC Handbook of Chemistry and Physics. 61st ed. Boca Raton, FL: CRC Press.

Wilson R, Lovejoy FHJr, Jaeger RJ, and Landrigan PL. 1980. Acute phosphine poisoning aboard a grain freighter. JAMA 244:148-150.

CHRONIC TOXICITY SUMMARY

PHOSPHORIC ACID

(Orthophosphoric acid)

CAS Registry Number: 7664-38-2

I. Chronic Toxicity Summary

Inhalation reference exposure level	**7 µg/m^3**
Critical effect(s)	Bronchiolar fibrosis of the respiratory tract in rats
Hazard index target(s)	Respiratory system

II. Chemical Property Summary (HSDB, 1995; 1999)

Description	Clear syrupy liquid or unstable crystals; odorless
Molecular formula	H_3PO_4
Molecular weight	98
Boiling point	213°C
Melting point	42.35°C
Vapor pressure	0.03 torr @ 20°C
Solubility	Very soluble in hot water; 548 g/100 ml cold water; soluble in alcohol
Conversion factor	4.0 µg/m^3 per ppb at 25ºC

III. Major Uses and Sources

Phosphoric acid has varied uses (HSDB, 1995). In manufacturing, it is a chemical intermediate or reagent in the production of numerous phosphate fertilizers, agricultural feeds, waxes, polishes, soaps, and detergents. It is added to foods as a preservative, acidifying agent, flavor enhancer, and clarifying agent. Phosphoric acid is also used in processes such as the coagulation of rubber latex, electropolishing, soil stabilization, and as a catalyst in the production of propylene and butene polymers, ethylbenzene, and cumene. By far, largest use of phosphoric acid comes in the production of fertilizers (>75%). The annual statewide industrial emissions from facilities reporting under the Air Toxics Hot Spots Act in California, based on the most recent inventory, were estimated to be 81,103 pounds of phosphoric acid (CARB, 1999).

Airborne phosphoric acid can be produced by the hydrolysis of phosphorus oxides generated from either the spontaneous ignition of white phosphorus in air or the combustion of red phosphorus (Burton *et al.*, 1982; US Department of Defense (US DOD), 1981).

IV. Effects of Human Exposures

The toxic effects to 48 workers exposed (28 unexposed control workers) to oxidation products of phosphorus during the course of phosphorus production were reported (Hughes *et al.*, 1962). Exposure duration ranged from 1 to 17 years. No differences were observed between exposed and control workers with respect to leukocyte count, an effect observed in acute intoxications, or hand bone density, an effect observed in experimentally exposed animals (Inuzuka, 1956).

A prospective study of 131 workers exposed to several compounds including phosphoric acid, phosphorus pentoxide, fluorides and coal tar pitch in the air was conducted at an industrial refinery (Dutton *et al.*, 1993). Mean duration of exposure (employment) was 11.4 years and the maximum exposure level measured was 2.23 mg/m^3 (phosphorus pentoxide). Pulmonary function tests were performed annually over a 3 to 7 year period. No significant residual effect was found after adjusting for age and smoking status.

V. Effects of Animal Exposures

Two 13-week inhalation studies of the effects of exposure to the combustion products of 95% red phosphorus and 5% butyl rubber were conducted in male Sprague-Dawley rats, with the first group exposed to 0, 300, 750, or 1200 mg/m^3 combustion products, and the second exposed to 0, 50, 180, or 300 mg/m^3 combustion products (Aranyi *et al.*, 1988a; Aranyi *et al.*, 1988b). Group numbers in the first study were 176, 84, 176, and 176, respectively. The second study used 40 animals/group. Animals were exposed for 2¼ hours/day on 4 consecutive days/week. Control animals were exposed to filtered air only. Daily particle measurements showed MMADs of 0.49-0.65 μm and σ_gs of 1.56-1.83. Fractional content of phosphoric acid in the aerosol was 71-79%. Nineteen of the 176 animals in the 1200 mg/m^3 dose group died of treatment related effects. Post-mortem examination of animals that died during the course of the study showed damage to the laryngeal mucosa, which was probably contributory to mortality. The two highest dose groups in the first study also showed decreased weight gain. Twelve animals from each dose group in the first study were examined histologically and neurobehavioral studies were conducted on other animals. Half the animals in the second study were examined strictly for toxic effects on the respiratory tract, with examination of the trachea, 2 sections of the nasal turbinates, and 5 lobes of the lung. Surviving animals in the high-dose study were observed to have moderate to severe fibrosis of the terminal bronchioles, with minimal severity of this effect in the animals in the low-dose study. The reported incidence of this lesion was 9/20 at 300 mg/m^3, 4/20 at 180 mg/m^3, and 0/20 at 50 mg/m^3. Little to no involvement of pulmonary tissue was observed.

The effects of acid aerosols (particularly sulfuric and phosphoric acid) were studied by U.S. EPA (1989). The respiratory tract was the primary target of toxicity resulting from the irritational effect of the acid on the tissues of the larynx and trachea. The nature of the effect was dependent upon the aerosol particle size, duration of exposure, and the hygroscopic character of the acid.

Sprague-Dawley rats were exposed to the smoke and combustion products of white phosphorus in felt pellets at 192.5 (18 animals/sex), 589 (24 animals/sex), or 1161 mg/m^3 (34 males, 43

females) phosphoric acid equivalents for 15 minutes/day, 5 days/week, for 13 weeks (US Department of Defense (US DOD), 1981). Control animals numbering half the size of the treated groups were exposed to air only. Groups of animals were sacrificed at 6 and 13 weeks, and 4 weeks post-exposure. Endpoints examined included: hematology, clinical chemistry, gross- and histo-pathology, ECG, pulmonary function, and behavior. Of the animals in the highest dose group, 56% died as a result of exposure, with the only other death occurring in the control group. Findings were restricted to effects on the respiratory system, with tracheitis and laryngitis incidences of 2/35, 32/47, and 28/31among surviving animals in the three dose groups. In the post-exposure examination, bronchiolitis occurred with a frequency of 0/12, 5/24, and 6/16in the three dose groups.

The toxicity of the combustion products of 95% amorphous red phosphorus and 5% polyvinyl butyral BL18 to female Wistar rats, Porton-strain mice, and guinea pigs was reported (Marrs *et al.*, 1989). Rats (50/group), mice (100/group), and guinea pigs (42-48/group) were exposed to concentrations of 0, 16, or 128 mg/m^3 for 1 hour/day, 5 days/week for 36 weeks (mice) or 40 weeks (rats and guinea pigs), with an examination conducted at 19 months or when animals appeared unhealthy. All groups, including controls, showed high mortality. Mice showed accumulation of alveolar macrophages with incidences of 2/41, 9/37, and 9/22 in the control, low-, and high-dose groups, respectively. Guinea pigs appeared to be particularly intolerant to the effects of the smoke.

Female rabbits and rats (10/group) were examined for acute toxic effects of smoke generated by the combustion of either 95% red phosphorus / 5% butyl rubber (Smoke I) or 97% red phosphorus / 3% butadiene styrene (Smoke II) (Marrs, 1984). Animals were exposed for 30 minutes and examined one and 14 days later. Smoke I produced inflammation of the larynx and trachea in rats at 1 day with some inflammation still observed at 14 days. Tracheal inflammation was also reported in rabbits exposed to Smoke I. Four of the rats exposed to Smoke II died within the first day, with severe pulmonary congestion observed in the animals.

One hour exposure to the combustion products of 95% red phosphorus / 5% butyl rubber (plus 1% mineral oil) produced epiglottal deformation, laryngeal edema, and laryngeal and tracheal lesions in rats (Burton *et al.*, 1982). A four-hour exposure produced more severe effects of a similar nature plus some hemorrhaging.

Rats (number unspecified) exposed to 150-160 mg/m^3 elemental phosphorus for 30 minutes/day for 60 days were examined for toxic effects (Inuzuka, 1956). Limb bone abnormalities were noted and effects included delayed ossification, widening of the epiphysis, and abnormal axial development.

Two studies have addressed the reproductive and developmental toxicity from exposure to the combustion products of white phosphorus and felt for 15 minutes/day during gestational days 6-15 in rats (24/group) (US Department of Defense (US DOD), 1981; US Department of Defense (US DOD), 1982). Fetal effects included increased incidence of some visceral variations and hypoplasia of the xiphoid process although data were incompletely reported. Another study, which exposed dams 3 weeks prior to mating, throughout gestation, and through lactation and males for 10 weeks prior to and during mating, showed decreased pup body weight, 24-hour and

21-day survival, and lactation. An oral study in which elemental phosphorus was administered to male and female rats by gavage in corn oil showed no statistically significant effects (Condray, 1985).

VI. Derivation of the Chronic Reference Exposure Level

Study	Aranyi *et al.*, 1988a
Study population	Male Sprague-Dawley rats (40-176/group)
Exposure method	Discontinuous whole body inhalation
Critical effects	Bronchiolar fibrosis of the respiratory tract
LOAEL	180 mg/m^3
NOAEL	50 mg/m^3
BMC_{05}	64 mg/m^3
Exposure continuity	2¼ hours/day, 4 days/week
Exposure duration	13 weeks
Average experimental exposure	2.7 mg/m^3 for NOAEL group (estimated as 3.5 mg/m^3 at BMC_{05})
Human equivalent concentration	2.2 mg/m^3 at BMC_{05} (particle with respiratory effects, RDDR = 0.63) (3.5 x 0.63)
LOAEL uncertainty factor	1 (BMC_{05} assumed to be similar to NOAEL)
Subchronic uncertainty factor	10
Interspecies uncertainty factor	3
Intraspecies uncertainty factor	10
Cumulative uncertainty factor	300
Reference exposure level	0.007 mg/m^3 (7 µg/m^3)

OEHHA has used the same study which U.S. EPA used in the development of its Reference Concentration (RfC) of 10 µg/m^3. The U.S. EPA has used a benchmark dose methodology for the derivation of the RfC for phosphoric acid from the toxicity data in the Aranyi *et al.* (1988) study (U.S. EPA, 1995). The RfC is restricted to "aerosols of phosphoric acid and phosphorus oxidation products and does not apply to elemental phosphorus or other forms of phosphorus, such as phosphorus salts".

The U.S. EPA, using the Weibull model, estimated the lower 95% confidence level bound on the maximum likelihood estimate (MLE = 150 mg/m^3) resulting in 10% incidence of lesions in the tracheo-bronchiolar region to be 100 mg/m^3 (the BMC_{10}). The U.S. EPA considered 10% incidence level to be a correlate to the NOAEL, based on a precedent in the analysis of data with developmental toxicity endpoints (Allen *et al.*, 1994; Faustman *et al.*, 1994). After correction for exposure continuity, a regional deposited dose ratio (RDDR) for the tracheobronchial region of 0.64 was applied due to the availability of data concerning the growth and deposition of phosphoric acid aerosol particles in humans and the similarities in the effects of phosphoric and better-characterized sulfuric acid aerosols. Key assumptions in the generation of this factor include: (1) the lowest σ_g of 1.56 µm cited in the study was used in the calculation; (2) geometric rather than aerodynamic diameter approximations were used; (3) particles of this size reach the deposition / lesion site (bronchioles); 4) these hygroscopic particles become more uniform with

growth; and (5) particle growth is similar in humans and rodents. An uncertainty factor of 10 was applied because of the subchronic duration of the study. A factor of 3 was applied for interspecies extrapolation in light of the fact that some correction for human equivalency was made with the RDDR. Finally, a factor of 10 was applied for protection of potentially sensitive human subpopulations. The resulting RfC for phosphoric acid is 0.01 mg/m^3.

OEHHA uses a BMC_{05} for development of acute Reference Exposure Levels (OEHHA, 1999; Fowles *et al*., 1999). OEHHA staff believe that the BMC_{05} is more likely to approximate a NOAEL than a BMC_{10} since 5% is closer than 10% to the lower end of average risk levels associated with a NOAEL (Leisenring and Ryan, 1992). A BMC_{05} is more likely to represent a value close to the limit of most studies to detect an effect, and is therefore more like a NOAEL. In contrast, a BMC_{10} is more likely to represent a LOAEL since it is usually in the detectable range of responses. In the specific case of phosphoric acid the BMC_{10} of 100 mg/m^3 was twice the NOAEL of 50 mg/m^3. The BMC_{05} was calculated to be 64 mg/m^3, much closer to the NOAEL. Use of the BMC_{05} results in a chronic REL of 7 $\mu g/m^3$.

VII. Data Strengths and Limitations for Development of the REL

The strengths of the inhalation REL for phosphoric acid include the availability of subchronic inhalation exposure data from a well-conducted study with histopathological analysis and the observation of a NOAEL. Major areas of uncertainty are the lack of adequate human exposure data, the lack of chronic inhalation exposure studies, and the discontinuous nature of exposures (only 2 1/4 hours per day).

The Aranyi *et al.* (1988a) study represents the most adequate study for the quantitative evaluation of the toxicity of phosphoric acid. It was conducted with a large number of animals with multiple doses, produced good dose-response data, and examined likely targets of toxicity (respiratory system) of smoke generated from the combustion of phosphorus and butyl rubber. Uncertainties associated with these data, however, include that (1) the study used combustion products of phosphorus rather than phosphoric acid itself, (2) the total exposure time was relatively short and discontinuous over the duration of the experiment, and (3) only one species/strain/sex was studied.

VIII. References

Allen BC, Kavlock RJ, Kimmel CA, and Faustman EM. 1994. Dose-response assessment for developmental toxicity. II. Comparison of generic benchmark dose estimates with no observed adverse effect levels. Fundam. Appl. Toxicol. 23:487-495.

Aranyi C, Henry MC, Vana SC, and Iverson WO. 1988a. Effects of multiple intermittent inhalation exposures to red phosphorus/butyl rubber obscurant smokes in Sprague-Dawley rats. Inhal. Toxicol. 1:65-78.

Aranyi C, Vana S, Bradof J, and Sherwood R. 1988b. Effects of inhalation of red phosphorus-butyl rubber combustion products on alveolar macrophage responses in rats. J. Appl. Toxicol. 8:393-398.

Burton F, Clark M, Miller R, and Schirmer R. 1982. Generation and characterization of red phosphorus smoke aerosols for inhalation exposure of laboratory animals. Am. Ind. Hyg. Assoc. J. 43:767-72.

CARB. 1999. Air toxics emissions data collected in the Air Toxics Hot Spots Program CEIDARS Database as of January 29, 1999.

Condray J. 1985. Elemental yellow phosphorus one-generation reproduction study in rats. IR-82-215. IRD No. 401-189. St. Louis, MO: Monsanto Company.

Dutton CB, Pigeon MJ, Renzi PM, Feustel PJ, Dutton RE, and Renzi GD. 1993. Lung function in workers refining phosphorus rock to obtain elementary phosphorus. J. Occup. Med. 35:1028-33.

Faustman EM, Allen BC, Kavlock RJ, and Kimmel CA. 1994. Dose-response assessment for developmental toxicity. I. Characterization of database and determination of no observed effect levels. Fundam. Appl. Toxicol. 23:478-86.

Fowles JR, Alexeeff GV, Dodge D. 1999. The use of benchmark dose methodology with acute inhalation lethality data. Regul. Toxicol. Pharmacol. 29(3):262-278.

HSDB. 1995. Hazardous Substances Data Bank. National Library of Medicine, Bethesda, Maryland (TOMES® CD-ROM Version). Denver, CO: Micromedex, Inc. (Edition expires 7/31/96).

HSDB. 1999. Hazardous Substances Data Bank. Available online at http://sis.nlm.nih.gov

Hughes JPW, Baron R, Buckland DH, *et al.* 1962. Phosphorus necrosis of the jaw: a present-day study. Br. J. Ind. Med. 19:83-99.

Inuzuka H. 1956. A study on the experimentally-created chronic poisoning of yellow phosphorus to bones. Igaku Kenkyu, 26:2848-61.

Leisenring W, Ryan L. 1992. Statistical properties of the NOAEL. Regul. Toxicol. Pharmacol. 15(2 Pt 1):161-171.

Marrs T. 1984. Histological changes produced by exposure of rabbits and rats to smokes produced from red phosphorus. Toxicol. Lett. 21:141-6.

Marrs T, Colgrave H, Edginton J, Rice P, and Cross N. 1989. The toxicity of a red phosphorus smoke after repeated inhalation. J. Haz. Mat. 22:269-82.

OEHHA. 1999. Air Toxics Hot Spots Program Risk Assessment Guidelines. Part I. Technical Support Document for Determination of Acute Reference Exposure Levels for Airborne Toxicants. Oakland, CA: Office of Environmental Health Hazard Assessment, Air Toxicology & Epidemiology Section. Available online at http://www.oehha.ca.gov

U.S. DOD. 1981. US Department of Defense. AD-B058-048. Available from Defense Technical Information Center, Alexandria, VA 22314 (703) 274-7633.

U.S. DOD. 1982. US Department of Defense. AD-A118-682. Available from Defense Technical Information Center, Alexandria, VA 22314 (703) 274-7633.

U.S. EPA. April 1989. An acid aerosols issue paper: health effects and aerometrics. Prepared by the Office of Health and Environmental Assessment, Environmental Criteria and Assessment Office. EPA/600/8-88/005F. Research Triangle Park, NC: U.S. EPA.

U.S. EPA. 1995. United States Environmental Protection Agency. Documentation of the reference concentration for chronic inhalation exposure (RfC) for phosphoric acid. Integrated Risk Information System (IRIS on-line). Washington, DC: U.S. EPA.

CHRONIC TOXICITY SUMMARY

PHTHALIC ANHYDRIDE

(1,3-isobenzofurandione; phthalic acid anhydride)

CAS Registry Number: 85-44-9

I. Chronic Toxicity Summary

Inhalation reference exposure level	**20 μg/m^3**
Critical effect(s)	Eye and respiratory irritation, asthma, and bronchitis in occupationally exposed workers
Hazard index target(s)	Respiratory system

II. Chemical Property Summary (HSDB, 1995; CRC, 1994)

Description	White or pale yellow crystals
Molecular formula	$C_8H_4O_3$
Molecular weight	148.11 g/mol
Boiling point	295°C
Melting point	130.8°C
Vapor pressure	5.14×10^{-4} torr @ 25°C; 1 torr @ 96.5°C
Solubility	Soluble in 162 parts water, 125 parts carbon disulfide; soluble in hot benzene
Conversion factor	1 μg/m^3 per ppb at 25ºC

III. Major Uses and Sources

The primary use of phthalic anhydride (PA) is as a chemical intermediate in the production of plastics from vinyl chloride. Phthalate esters, which function as plasticizers, are derived from phthalic anhydride. Phthalic anhydride has another major use in the production of polyester resins and other minor uses in the production of alkyd resins used in paints and lacquers, certain dyes (anthraquinone, phthalein, rhodamine, phthalocyanine, fluorescein, and xanthene dyes), insect repellents, and urethane polyester polyols. It has also been used as a rubber scorch inhibitor and retarder (HSDB, 1995; National Cancer Institute (NCI), 1979). The annual statewide industrial emissions from facilities reporting under the Air Toxics Hot Spots Act in California based on the most recent inventory were estimated to be 11,442 pounds of phthalic anhydride (CARB, 2000).

IV. Effects of Human Exposure

Symptoms in workers exposed to phthalic anhydride by inhalation in two plants (A and B) manufacturing alkyd and unsaturated polyester resins were studied (Nielsen *et al.*, 1988). Two groups of exposed workers were identified in each plant. One group worked directly loading the reactors from bags of phthalic anhydride ("heavy" exposure – 35 workers) and the other group was involved with "other work" which led to "low" exposure (25 workers). Mean employment times for the "heavy" and "low" exposure groups were 13.3 and 11.9 years, respectively. Time-weighted average air concentrations for workers from the loading of PA was 6.1 (range: 1.8-14.9) and 6.8 mg PA/m^3 (range: 1.5-17.4) in plants A and B, respectively. Similar exposure levels in both plants led to pooling of data. The exposure duration of the "heavy" group was estimated at approximately 30 minutes two times a day, corresponding to the time of loading, and resulted in a full-day time weighted exposure estimate of 0.4 mg PA/m^3. For those engaged in "other work" exposure levels were estimated at < 0.1 mg PA/m^3 (the limit of detection). Other chemicals in use in smaller amounts included maleic anhydride, isophthalic anhydride, and trimellitic anhydride. Comparison of symptom incidence between the "heavy" and "low" exposure groups included conjunctivitis (46% vs. 20%), rhinitis (40% vs. 20%), rhinoconjunctivitis (17% vs. 12%), asthma (17% vs. 0%), and chronic bronchitis (17% vs. 4%). Serum antibodies were measured in both groups of workers and compared to 22 nonexposed workers (employed at a food processing factory). The only significantly changed level was an increase in specific IgG in the "heavy" exposure group. A correlation was also noted between specific IgG level and exposure level, although not all individuals with elevated specific IgG reported symptoms.

In a study conducted at another plant manufacturing alkyd and/or unsaturated polyester resins, serum immunoglobulins and lung function were examined in 23 workers exposed to phthalic anhydride and 18 control subjects (Nielsen *et al.*, 1991). Estimated exposure levels were 6.6 mg PA/m^3 (range: 1.5-17) (Nielsen *et al.*, 1988). Workers were examined for sensitization to PA and other allergens and possible development of small airways disease. Among the exposed workers, there was significantly increased reporting of conjunctivitis and rhinoconjunctivitis. One worker showed an asthmatic response to anhydrides. No significant differences in lung function tests were observed between exposed and unexposed groups.

Symptoms in workers occupationally exposed to PA during the course of producing alkyd and/or polyunsaturated polyester resins were described (Wernfors *et al.*, 1986). Exposure estimates of breathing zone PA levels ranged from 3 to 13 mg/m^3 for workers engaged directly with the handling of PA. In other areas the estimated level was <0.3 mg/m^3. The study examined 48 workers who were employed at the time of the study and 70 former employees who responded to a survey of symptoms related to exposure. No unexposed control group was included in the study. Workers who were employed for at least two months reported symptoms of rhinitis (28%), chronic bronchitis (11%), and asthma (28%). Among a subset of 11 workers with asthma, 3 had positive skin tests for PA sensitivity. Bronchial provocation tests with 6 or 0.5 mg/m^3 PA for 5 or 10 minutes were positive in 2 workers.

V. Effects of Animal Exposure

Male albino rats (6/treatment group) were exposed to phthalic anhydride vapors at 0, 0.02, 0.2, and 1 mg/m^3 continuously for 45 days (Protsenko, 1970). After a two week recovery period the testes were examined for spermatozoa motility time as well as for ascorbic acid, dehydroascorbic acid, and nucleic acid content. Motility time was defined as the time it took for spermatozoa to cease motion completely under microscopic examination. Spermatozoa motility time was decreased ~50% in the 1 mg/m^3 dose group and ~25% in the 0.2 mg/m^3 dose group. Significant decreases in ascorbic acid and dehydroascorbic acid levels were found in animals exposed to 0.2 and 1.0 mg/m^3 phthalic anhydride, and dehydroascorbic acid levels were decreased in the 0.02 mg/m^3 dose group. At 1 mg/m^3, RNA levels and combined RNA and DNA levels were significantly increased over controls. No significant changes were observed in the 0.02 mg/m^3 dose group.

Five and six female Hartley guinea pigs were exposed to 0.05-0.2 mg/m^3 and 0.6-6 mg/m^3 phthalic anhydride dust, respectively, for 3 hours/day for 5 consecutive days (Sarlo and Clark, 1992). Exposures were expressed as ranges due to difficulty in regulating dust levels in the chambers. Sampling of dust showed particles were 65-80% < 10 μm diameter and had a mean mass diameter of 5.8-9.8 μm. Eight control animals were exposed to filtered air only. Two weeks after the last exposure, animals were challenged for 30 minutes with aerosolized PA-guinea pig serum albumin conjugate. All animals in the "high" dose group showed immediate bronchoconstriction and transiently increased respiratory rate. Animals in this dose group also showed elevated IgG antibody titers. No detectable increase in antibody levels was found in the "low" dose group.

Type I hypersensitivity was examined in female Hartley guinea pigs exposed to phthalic anhydride dust (Sarlo *et al.*, 1994). Two groups of 8 animals were exposed to 0.5 or 1.0 mg/m^3, and two groups of 16 animals were exposed to 0 (filtered air only) or 5.0 mg/m^3 phthalic anhydride dust (respirable size – 5 μm) in stainless steel chambers for 3 hours/day for 5 consecutive days. Groups of 8 animals from the control and 5 mg/m^3 groups were challenged after a two week recovery period for 30 minutes with 5.0 mg/m^3 phthalic anhydride dust. Respiratory data were collected using a plethysmograph from 30 minutes before the exposure to 60 minutes after the exposure. No significant difference (defined as a change of 3 standard deviations from the same parameter in the control animals) in respiration rate or plethysmograph pressures was found between the exposed and unexposed animals. Eight animals in each of the four exposure groups were also challenged after two weeks of recovery with 2.0 mg/m^3 aerosolized PA-guinea pig serum albumin (GPSA) conjugate as described above. Respiratory rate was increased in 4/8 of the high-dose group animals and 1/8 of the low-dose animals. Plethysmograph pressures were increased in 3/8 animals in the high-dose group and one animal each in the low- and mid-dose groups. Serum IgG antibodies to PA-GPSA were elevated in all exposed animal groups and the effect showed a dose-response. Passive cutaneous anaphylaxis testing for anti-phthalic anhydride-GPSA IgG1a immunoglobulins showed positive results for 3/8, 1/8, and 5/8 animals in the 0.5, 1.0, and 5.0 mg/m^3 dose groups, respectively. Results in control animals were not described. Three of eight animals in the highest dose group had >189 hemorrhagic foci in their lungs. No control animal had more than 2 such foci. No foci were

observed in animals challenged with albumin conjugate. Serum IgG titer correlated with the presence of these foci.

Slavgorodskiy (1969) studied the toxicity of phthalic anhydride to animals from inhalation exposure. Sixty white male rats (strain not reported; group distribution not stated, but presumed to be 15 animals/treatment group) were exposed in 100 L chambers to 0, 0.18, 0.54, and 1.52 mg PA/m^3 aerosol continuously for 70 days. General condition and behavior, body weight, motor chronaxy of flexor and extensor muscles (every 10 days), cholinesterase activity (every two weeks), and hematological parameters were monitored during the course of the study. (Chronaxy is the minimum time for which a current must flow, at a voltage twice the minimal current necessary to produce muscle stimulation, in order to cause a muscle to contract.) No changes in body weight or behavior were observed in the treated animals. In animals in the high-dose group, the chronaxy ratio of flexors and extensors differed from the controls beginning on day 31 of exposure and continued until two weeks after exposure ceased. Significantly decreased whole blood cholinesterase activity occurred in the high- and mid-dose groups, with the change occurring after 42 days of exposure. An increase in thrombocyte count occurred in the high- and mid-dose groups after 70 days of exposure, but returned to normal during the two-week recovery period. Thus, 0.18 mg/m^3 PA appears to be a NOAEL in this study.

A chronic feeding study was conducted with phthalic anhydride in rats and mice to evaluate the carcinogenicity of the compound (National Cancer Institute (NCI), 1979). F344 rats (50/sex/dose group plus 20/sex control animals) were treated with diet containing 0, 7500, or 15,000 ppm phthalic anhydride for 105 weeks (which corresponds to approximately 0, 300, and 600 mg/kg-day, assuming that food consumption is 4% body weight/day). Animals were monitored for changes in body weight and for survival, and, upon death or the end of the study, were examined histopathologically. The only group showing significantly lower body weights was male rats in the high-dose group after week 13. No significant change in mortality was observed. Adverse non-cancer effects observed in the dosed groups, but not in the control animals, included “arched back, rough hair coat, ulceration, and corneal opacity”, however, incidences were described as “low”. No significant histopathological effects were found to be associated with exposure to phthalic anhydride. $B6C3F_1$ mice (50/sex/dose group plus 20/sex control animals) were initially treated with diet containing 0, 25,000, or 50,000 ppm phthalic anhydride (approximately 0, 3000, and 6000 mg/kg-day, assuming that food consumption is 12% body weight/day). Because of excessive weight loss after week 32, exposure levels were reduced during the course of the study such that the time-weighted average exposure for males was 16,346 and 32,692 ppm and for females was 12,019 and 24,038 ppm phthalic anhydride. Evaluation of toxicity was conducted at 104 weeks as with the rats. Mean body weight was reduced in male and female mice in a dose-related manner. No other significant treatment-related adverse effects were observed in the mice.

Pregnant female CD-1 mice (10/dose group) were treated intraperitoneally with phthalic anhydride in 0.5%(w/v) carboxymethyl cellulose solution on gestational days 8-10 (Fabro *et al.*, 1982). Dosing was variable, beginning within the 95% confidence limits of the LD_{01} and progressing geometrically downward until no effect was observed. Animals were terminated on Day 18 and examined for teratogenic effects including fetal viability and number, resorption, and gross malformations. The 95% lower confidence limit on the dose producing teratogenicity

(grossly observable malformations and fetal internal malformations) in 5% and 50% of animals were 0.40 and 1.37 mmol/kg-day (59 and 203 mg/kg-day), respectively.

VI. Derivation of Chronic Reference Exposure Level (REL)

Study	Neilsen *et al.* (1988; 1991)
Study population	23 occupationally-exposed workers
Exposure method	Discontinuous occupational inhalation exposures
Critical effects	Increased incidence of conjunctivitis, rhinitis, asthma, and chronic bronchitis
LOAEL	6.5 mg/m^3 (mean of 6.1 and 6.8)
NOAEL	Not observed
Exposure continuity	8 hours/day, 5 days/week
Exposure duration	Mean of 13.3 years
Average experimental exposure	2.3 mg/m^3 for LOAEL group (6.5 mg/m^3 × 10/20 × 5/7)
LOAEL uncertainty factor	10
Subchronic uncertainty factor	1
Interspecies uncertainty factor	1
Intraspecies uncertainty factor	10
Cumulative uncertainty factor	100
Inhalation reference exposure level	0.02 mg/m^3 (20 μg/m^3)

Adverse effects were demonstrated to occur in humans occupationally exposed to phthalic anhydride in the workplace over long periods of time (Nielsen *et al.*, 1988). The symptoms reported primarily affected the respiratory system, with increased incidence of rhinitis, rhinoconjunctivitis, asthma, and chronic bronchitis. Conjunctivitis was also reported in exposed workers. Specific anti-PA IgG was significantly elevated compared to a non-exposed group. Increased incidences of rhinoconjunctivitis, conjunctivitis, or chronic bronchitis have also been reported in workers exposed to similar levels of PA dust (Nielsen *et al.*, 1991; Wernfors *et al.*, 1986). In these reports, adverse effects were clearly observed at the exposure level reported (6.5 mg PA/m^3; full-day time weighted exposure of 0.4 mg PA/m^3). Although symptoms were reported by Nielsen (1988) in the lower exposure level group, the significance is not clear since a true control group (unexposed workers) was not included in the symptomatology section of the study. The low exposure group's level of exposure was less than the detection limit for phthalic anhydride cited in the study, and this group was considered as a control group.

VII. Data Strengths and Limitations for Development of the REL

The strengths of the inhalation REL for phthalic anhydride include the use of human exposure data from workers exposed over a period of years. Major areas of uncertainty are (1) the uncertainty in estimating exposure, (2) the potential variability in exposure concentration, (3) the

potential low exposures of the group considered as controls, (4) potential confounding by exposures to other chemicals, (5) the limited nature of the study, (6) the lack of reproductive and developmental toxicity studies, and (6) the lack of observation of a NOAEL in the key study. Another area of uncertainty is the apparent 10-fold greater sensitivity to bronchoconstriction from PA exposure in guinea pigs (a model for human asthmatics) in comparison to occupationally exposed workers.

The study in rats by Protsenko (1970) identified a LOAEL of 0.2 mg/m^3 and a NOAEL of 0.02 mg/m^3 for decreased sperm motility. However, this result from 1970 has not been verified or further explored in more recent toxicological or epidemiological studies. The small sample size of 6/group further weakens confidence in this result. Therefore, the study in workers by Nielson *et al.* (1988, 1991) was chosen as the basis for the REL for PA.

VIII. References

CARB. 2000. California Air Resources Board. California Emissions Inventory Development and Reporting System (CEIDARS). Data from Data Base Year 1998. February 12, 2000.

CRC. 1994. CRC Handbook of Chemistry and Physics, 75th edition. Lide DR, ed. Boca Raton, FL: CRC Press Inc.

Fabro S, Shull G, and Brown NA. 1982. The relative teratogenic index and teratogenic potency: proposed components of developmental toxicity risk assessment. Teratog. Carcinog. Mutag. 2:61-76.

HSDB. 1995. Hazardous Substances Data Bank. National Library of Medicine, Bethesda, MD (TOMES® CD-ROM Version). Denver, CO: Micromedex, Inc.

NCI. 1979. National Cancer Institute. Bioassay of Phthalic Anhydride for Possible Carcinogenicity. Springfield, VA: NTIS. PB 293-594.

Nielsen J, Bensryd I, Almquist H, Dahlqvist M, Welinder H, Alexandersson R, and Skerfving S. 1991. Serum IgE and lung function in workers exposed to phthalic anhydride. Int. Arch. Occup. Environ. Health 63:199-204.

Nielsen J, Welinder H, Schütz A, and Skerfving S. 1988. Specific serum antibodies against phthalic anhydride in occupationally exposed subjects. J Allergy Clin. Immunol. 82:126-133.

Protsenko EI. 1970. [Effect of phthalic anhydride on gonads]. Gig. Sanit. 35:127-130. [in Russian]

Sarlo K, and Clark ED. 1992. A tier approach for evaluating the respiratory allergenicity of low molecular weight chemicals. Fundam. Appl. Toxicol. 18:107-114.

Sarlo K, Clark ED, Ferguson J, Zeiss CR, and Hatoum N. 1994. Induction of type I hypersensitivity in guinea pigs after inhalation of phthalic anhydride. J. Allergy Clin. Immunol. 94:747-756.

Slavgorodskiy LP. 1969. [Biological effect and hygienic evaluation of pollution of atmospheric air with phthalic anhydride.] Akademiya Meditsinskikh Nauk SSSr 10:86-96. [in Russian]

Wernfors M, Nielsen J, Schütz A, and Skerfving S. 1986. Phthalic anhydride-induced occupational asthma. Int. Arch. Allergy Immunol. 79:77-82.

CHRONIC TOXICITY SUMMARY

PROPYLENE

(1-propene; 1-propylene; propene; methylethene; methylethylene)

CAS Registry Number: 115-07-1

I. Chronic Toxicity Summary

Inhalation reference exposure level	**3,000 μg/m^3 (2,000 ppb)**
Critical effect(s)	Squamous metaplasia (males and females), epithelial hyperplasia (females only), and inflammation (males only) of the nasal cavity in Fischer 344/N rats
Hazard index target(s)	Respiratory system

II. Chemical and Physical Properties (HSDB, 1995; CRC, 1994)

Description	Colorless gas; practically odorless.
Molecular formula	C_3H_6
Molecular weight	42.08
Boiling point	–47.6 °C
Melting point	–185.2°C
Vapor pressure	8690 torr at 25°C
Solubility	Soluble in alcohol and ether.
Conversion factor	1.72 μg/m^3 per ppb at 25°C

III. Major Uses and Sources (HSDB 1995)

Propylene is produced primarily as a by-product of petroleum refining and of ethylene production by steam cracking of hydrocarbon feedstocks. Propylene is a major chemical intermediate. The most important derivatives of chemical and polymer grade propylene are polypropylene, acrylonitrile, propylene oxide, isopropanol and cumene. Use of polypropylene in plastics (injection moulding) and fibers (carpets) accounts for over one-third of U.S. consumption. It is also used in the production of synthetic rubber and as a propellant or component in aerosols. In 1994, propylene was ranked seventh among the top 50 chemicals produced domestically (C&EN, 1995). In the environment, propylene occurs as a natural product from vegetation. It is also a product of combustion of organic matter (biomass burning, motor vehicle exhausts and tobacco smoke) and is released during production and use. The most probable route of exposure to humans is by inhalation. Propylene has been detected in the atmosphere over both metropolitan (2.6 to 23.3 ppb) and rural (0.007 to 4.8 ppb) areas (Cox *et al.*, 1976; Leonard *et al.*, 1976). The annual statewide emissions from facilities reporting under

the Air Toxics Hot Spots Act in California, based on the most recent inventory, were estimated to be 696,350 pounds of propylene (CARB, 1999).

IV. Effects of Human Exposures

No data were available on the absorption, distribution or excretion of propylene in humans. However, hemoglobin (Hb) adducts of the metabolite intermediate propylene oxide have been used to monitor the internal dose of propylene (Tornqvist and Ehrenberg, 1990). The background level of the 2-hydroxylpropyl adduct to the N-terminal valine of hemoglobin was found to be about 2 pmol/g Hb. This was estimated to be equivalent to smoking 10 cigarettes per day; cigarette smoking is a source of propylene. Occupational exposure to propylene at 1 ppm (1.72 mg/m^3) was assumed to be associated with an increment of 5 pmol/g Hb (Kautiainen and Tornqvist, 1991).

No data were available on the chronic effects of propylene in humans.

V. Effects of Animal Exposures

In rats and mice, most propylene inhaled into the lungs is exhaled again and does not reach the blood to become systemically available (Golka *et al.*, 1989; Svensson and Osterman-Golkar, 1984). Once absorbed, a major route of metabolism for propylene is through the cytochrome P-450 system to propylene oxide, a known carcinogen in experimental animals. Cytochrome P-450 enzymes in both the liver and nasal epithelium (Maples and Dahl, 1991) can convert propylene to its toxic metabolite. However, in rats, propylene metabolism becomes increasingly saturated at concentrations above 50 ppm (86 mg/m^3) in the atmosphere (Golka *et al.*, 1989), which limits the amount of propylene oxide produced. Therefore, the amount of absorbed propylene may not reach high enough levels in classical long-term inhalation studies (Quest *et al.*, 1984) to show positive carcinogenic or serious chronic effects.

The only chronic toxicity investigation found for propylene was a comprehensive 2-year study in F344/N rats and B6C3F$_1$ mice (Quest *et al.*, 1984; NTP, 1985). Groups of 50 rats and 50 mice of each sex were exposed to concentrations of 0, 5000, and 10,000 ppm for 6 hr/day, 5 days/week, for 103 weeks. (Mean daily concentrations were 0, 4985, and 9891 ppm, respectively, for the rat study; and 0, 4999, and 9957 ppm, respectively, for the mouse study.) In exposed rats, treatment-related chronic effects were observed in the nasal cavity. In female rats, epithelial hyperplasia occurred in the high dose group and squamous metaplasia occurred in both dosage groups. In male rats, squamous metaplasia was seen only in the low dose group, but both dosage groups had inflammatory changes characterized by an influx of lymphocytes, macrophages and granulocytes into the submucosa and granulocytes into the lumen (see below). Nasal lesions were not observed in mice. The inflammatory lesions were more severe in the high dose group. Very mild focal inflammation was observed in the kidneys of treated mice but the relationship to propylene exposure was unclear. No other treatment-related effects, including clinical signs, mortality, mean organ and body weights, and histopathology, were observed.

Incidences of epithelial changes in nasal cavities of rats (Table 2 from Quest *et al*., 1984)

Observation	Control	5000 ppm	10,000 ppm
Epithelial hyperplasia			
Male	2/50 (4%)	2/50 (4%)	5/50 (10%)
Female	0/49 (0%)	4/50 (8%)	9/50 (18%)*
Squamous metaplasia			
Male	2/50 (4%)	19/50 (38%)*	7/50 (14%)
Female	0/49 (0%)	15/50 (30%)*	6/50 (12%)*
Inflammation			
Male	11/50 (22%)	21/50 (42%)*	19/50 (38%)
Female	8/49 (16%)	10/50 (20%)	13/50 (26%)

* Significantly ($p < 0.05$) higher than control values

In a long-term carcinogenicity study, Sprague-Dawley rats and Swiss mice (100-120 animals/group/sex) were exposed by inhalation to 0, 200, 1000 and 5000 ppm propylene 7 hr/day, 5 days/week, for 104 weeks (rats) or 78 weeks (mice) (Ciliberti *et al.*, 1988). No body weight differences were observed between treated and control animals of either species. Mortality was reported to be slightly increased in male rats in the 1000 and 5000 ppm groups and in male mice in the 5000 ppm group, but numerical values of mortality were not presented in the report. Therefore, it is assumed that mortality differences were insignificant. Other possible general body system or nonneoplastic effects were not reported and assumed to have not been investigated.

VI. Derivation of Chronic Reference Exposure Level (REL)

Study	Quest *et al.*, 1984
Study population	50 rats/group/sex, 300 total.
Exposure method	Discontinuous whole body inhalation exposure (0 or 4,985 or 9,891 ppm).
Critical effects	Respiratory system; squamous metaplasia (males and females), epithelial hyperplasia (females only), and inflammation (males only) of the nasal cavity
LOAEL	4,985 ppm (8,570 mg/m^3)
NOAEL	Not observed
Exposure continuity	6 hr/day, 5 days/week
Exposure duration	2 years
Average experimental exposure	890 ppm for LOAEL group (4985 x 6/24 x 5/7)
Human equivalent concentration	190 ppm (gas with extrathoracic respiratory effects, RGDR = 0.21, based on BW = 305 g, MV = 0.21 L/min, SA(ET) = 15 cm^2)
LOAEL uncertainty factor	3 (low severity)
Subchronic uncertainty factor	1
Interspecies uncertainty factor	3
Intraspecies factor	10
Cumulative uncertainty factor	100
Inhalation reference exposure level	2 ppm (2,000 ppb, 3 mg/m^3, 3,000 μg/m^3)

VII. Data Strengths and Limitations for Development of the REL

Strengths of the propylene REL include the availability of a long-term, controlled exposure study in large groups of experimental animals that included extensive histopathological analyses.

Lifetime exposure of rats and mice to propylene resulted in adverse effects in the nasal cavity of rats at both exposure levels. Therefore, a NOAEL was not observed. However, the effects observed were mild.

Other weaknesses of the database for propylene include the lack of lifetime toxicity studies in any non-rodent species. Also, no long-term human toxicity or epidemiology studies were located in the literature. Human pharmacokinetic studies to compare with experimental animal pharmacokinetic studies were absent. Another uncertainty is the lack of reproductive and developmental toxicity studies. A comprehensive multi-generation study in an experimental animal species would enhance the development of a propylene REL.

VIII. References

CARB. 1999. Air toxics emissions data collected in the Air Toxics Hot Spots Program CEIDARS Database as of January 29, 1999.

C&EN. 1995. Chemical & Engineering News. Production by the U.S. chemical industry. M. Heylin, ed. American Chemical Society, Washington DC, June 26:38-44.

CRC. 1994. CRC Handbook of Chemistry and Physics, 75th edition. Lide DR, ed. Boca Raton, FL: CRC Press Inc.

Ciliberti A, Maltoni C, and Perino G. 1988. Long-term carcinogenicity bioassays on propylene administered by inhalation to Sprague-Dawley rats and Swiss mice. Ann. N.Y. Acad. Sci. 534:235-245.

Cox RA, Derwent RG, and Sandalls FJ. 1976. Some air pollution measurements made at Harwell, Oxfordshire, during 1973-1975, AERE-R 8324, Harwell, U.K. Atomic Energy Research Establishment, Environmental and Medical Sciences Division.

Golka K, Peter H, Denk B, and Filser JG. 1989. Pharmacokinetics of propylene and its reactive metabolite propylene oxide in Sprague-Dawley rats. Arch. Toxicol. Suppl. 13: 240-242.

HSDB. 1995. Hazardous Substances Data Bank. National Library of Medicine, Bethesda, MD (TOMES® CD-ROM version). Denver, CO: Micromedex, Inc. (edition expires 11/31/95).

Kautiainen A, and Tornqvist M. 1991. Monitoring exposure to simple epoxides and alkenes through gas chromatographic determination of hemoglobin adducts. Int. Arch. Occup. Environ. Health 63:27-31.

Leonard MJ, Fisher EL, Brunelle MF, and Dickinson JE. 1976. Effects of the motor vehicle control program on hydrocarbon concentrations in the Central Los Angeles atmosphere. J. Air Pollut. Contr. Assoc. 26:359-363.

Maples KR, and Dahl AR. 1991. Blood levels of propylene oxide during propylene inhalation and effect on hepatic and nasal cytochrome P-450 concentrations. Drug Metab. Dispos. 19(4):835-837.

NTP. 1985. National Toxicology Program. Toxicology and carcinogenesis studies of propylene in F344/N rats and $B6C3F_1$ mice. Technical Report Series NTP-TR-272, NIH publication no. 86-2528.

Quest JA, Tomaszewski JE, Haseman JK, Boorman GA, Douglas JF, and Clarke WJ. 1984. Two-year inhalation toxicity study of propylene in F344/N rats and $B6C3F_1$ mice. Toxicol. Appl. Pharmacol. 76:288-295.

Svensson K, and Osterman-Golkar S. 1984. Kinetics of metabolism of propene and covalent binding to macromolecules in the mouse. Toxicol. Appl. Pharmacol. 73:363-372.

Tornqvist M, and Ehrenberg L. 1990. Approaches to risk assessment of automotive engine exhausts. In: Vainio H, Sorsa M, and McMicheal AJ, eds. Complex Mixtures and Cancer Risks. IARC Scientific Publications No. 104. Lyon: IARC, pp. 272-287.

CHRONIC TOXICITY SUMMARY

PROPYLENE GLYCOL MONOMETHYL ETHER

(1-Methoxy-2-propanol; 1-methoxypropanol; Propapsol solvent M)

CAS Registry Number: 107-98-2

I. Chronic Toxicity Summary

Inhalation reference exposure level	**7,000 µg/m^3** (2000 ppb)
	.
Critical effect(s)	Liver effects in rats
Hazard index target(s)	Alimentary system (liver)

II. Physical and Chemical Properties (HSDB, 1995)

Description	Colorless liquid
Molecular formula	$C_4H_{10}O_2$
Molecular weight	90.14
Density	0.962 g/cm^3 @ 20° C
Boiling point	118-118.5°C
Melting point	-96.7°C
Vapor pressure	11.8 torr @ 25°C
Solubility	Soluble in water, methanol, ether, and other organic solvents
Conversion factor	1 ppm = 3.69 mg/m^3 at 25° C

III. Major Uses or Sources

Propylene glycol monomethyl ether (PGME) is used as a solvent for cellulose, acrylics, dyes, inks and stains (HSDB, 1995). Thus, the primary use of PGME is in lacquers and paints. Use of PGME is anticipated to increase due to its low systemic toxicity. The annual specific statewide industrial emissions of PGME from facilities reporting under the Air Toxics Hot Spots Act in California, based on the most recent inventory, were estimated to be 205,769 pounds (CARB, 1999). (Many industries did not estimate emissions of specific glycol ethers so that in 1998 there were also emitted 2,922,744 pounds of the general category glycol ethers, some of which may be PGME.)

IV. Effects of Exposures on Humans

No reports or studies of human toxicity following chronic exposure to PGME were located in the literature. Slight eye irritation was reported by two of six human volunteers exposed to 100 ppm PGME for 2 hours (Stewart *et al.*, 1970). These subjects were exposed for a total of 3 1/2 hours during which no decrement in visual acuity, coordination, neurological responses or reaction time was measured. The same experiment exposed 23 subjects to 250 ppm PGME. After 15 to 30 minutes of exposure, 8/23 reported eye irritation and 3/23 reported throat irritation; lacrimation was observed in 3/23 subjects. Three subjects each reported one of the following symptoms: irritation, headache, and nausea. While the subjects frequently reported the odor to be objectionable upon first entering the chamber, the odor was usually undetectable by the end of the exposure. Clinical chemistry and urinalysis completed following exposure was not altered as compared to pre-exposure measurements.

V. Effects of Exposures on Animals

Male and female rats (10 per sex per concentration) and rabbits (7 per sex per concentration) were exposed by inhalation to 300, 1000, or 3000 ppm PGME 5 hours per day, 5 days per week for 13 weeks (Landry *et al.*, 1983). Relative liver weights were statistically significantly higher than controls in both male and female rats exposed to 3000 ppm PGME. Hepatocellular hypertrophy was observed upon histopathologic examination of high dose females. The authors conclude that these effects are the result of physiologic adaptation rather than a manifestation of toxicity. The key observation in this study was sedation of rats and rabbits exposed to 3000 ppm PGME. The sedative effects were no longer apparent after 1-2 weeks of exposure.

Similar findings of mild CNS depression were observed by Hanley *et al.* (1984). Pregnant rats and rabbits were exposed to 500, 1500, or 3000 ppm PGME 6 hours per day either days 6-15 or days 6-18 of gestation, respectively. During the first 4-5 days of exposure, rats in the 3000 ppm PGME exposure group were lethargic and moderately ataxic. Statistically significant decreases in food consumption and maternal body weight gain were also observed during this period. A statistically significant increase in the incidence of delayed sternebral ossification was observed in the 3000 ppm exposure group. Rabbits exposed to 3000 ppm exhibited mild lethargy during the first 1-2 days of exposure with rapid post-exposure recovery. Overall maternal weight gain during the exposure (days 6-18 of gestation) was statistically significantly lower than controls.

No significant effect on fetal birth weight or on pup survival indices (e.g., proportion of pups surviving to day 3 post-delivery) was noted following exposure of pregnant rats to 200 or 600 ppm PGME 6 hours per day on days 6-17 of gestation (Doe *et al.*, 1983). Male rats were exposed to 200 or 600 ppm PGME 6 hours per day for 10 consecutive days. No significant effects on testicular weight or pathology were observed.

Increased liver and kidney weights were observed in male and female rats (10 per sex per concentration) following exposure to 6000 ppm for 7 hours per day, for 81 exposures over a 114-day period (Rowe *et al.*, 1954). No histopathological abnormalities were observed at necropsy.

Ciezlak et al. (1998) evaluated the potential chronic toxicity/oncogenicity and the response of liver and kidney tissue of Fischer 344 rats to propylene glycol monomethyl ether (PGME) at targeted vapor concentrations of 0, 300, 1000 or 3000 ppm. Groups of 50 male and female rats per sex were whole-body exposed under dynamic airflow conditions for 6 hours/day, 5 days/week for up to 2 years. Parameters evaluated included the general appearance and demeanor of animals, in-life body weights, survival, hematology, urinalysis and clinical chemistry determinations, survival, selected organ weights, gross and microscopic pathologic changes and tumor incidence. (The metabolic and morphological bases for PGME-induced sedation, hepatic hypertrophy and renal toxicity were characterized in separate groups of male and female rats exposed to PGME for 6, 12 or 18 months. Hepatic enzyme induction and cellular proliferation, as well as renal cellular proliferation and accumulation of $alpha_{2u}$-globulin (males only) in the kidneys, were conducted in these separate groups of animals.)

PGME-induced sedation at 3000 ppm resolved in all animals during the second week of exposure in conjunction with the appearance of adaptive changes in the liver (cytochrome P450 induction and hepatocellular proliferation). Cytochrome P450 (pentoxyresorufin O-demethylase) activities dropped to near control concentrations by week 52, coinciding with a return of sedation at 3000 ppm PGME. In male rats, the loss of metabolic adaptation was followed by a dose-related increase in altered hepatocellular foci after two years of exposure to 1000 or 3000 ppm PGME. The kidney toxicity observed in male rats was confirmed immunohistochemically as an $alpha_{2u}$-globulin nephropathy. No statistically-identified increases in tumors were observed in any tissue. The authors established a NOEL of 300 ppm PGME for the study.

Ethylene glycol methyl ether (EGME), a structurally related compound, exerts considerable toxicity on the blood, thymus, testes, and developing fetus. The toxicity of EGME has been linked to its primary metabolite, methoxyacetic acid. Recent comparative toxicity and metabolism studies (Miller *et al.*, 1983, Miller *et al.*, 1984) indicate that the relatively low systemic toxicity exerted by PGME is due to its different metabolites. Following a single oral dose of PGME, the key urinary metabolites identified in rats were propylene glycol and the sulfate and glucuronide conjugate of PGME (Miller *et al.*, 1983).

VI. Derivation of Reference Exposure Level

Study	Ciezlak *et al.*, 1998
Study population	Fischer 344 rats (50/sex/concentration)
Exposure method	Discontinuous whole-body inhalation (0, 300, 1000, or 3000 ppm)
Critical effects	Increased eosinophilic foci of altered hepatocytes
LOAEL	1000 ppm
NOAEL	300 ppm
Exposure continuity	6 hours per day, 5 days per week
Average experimental exposure	54 ppm for NOAEL group (300 x 6/24 x 5/7)
Human equivalent concentration	54 ppm for NOAEL group (gas with systemic effects, based on RGDR = 1.0 using default assumption that lambda (a) = lambda (h))
Exposure duration	104 weeks
LOAEL factor	1
Subchronic uncertainty factor	1
Interspecies uncertainty factor	3
Intraspecies uncertainty factor	10
Cumulative uncertainty factor	30
Inhalation reference exposure level	2 ppm (2000 ppb, 7 mg/m^3, 7000 µg/m^3)

VII. Data Strengths and Limitations for Development of the REL

Strengths of the PGME RfC include the observation of a NOAEL and a LOAEL in the same study, and the availability of chronic exposure studies involving multiple concentrations. A major area of uncertainty is the lack of human data.

VIII. References

AIHA. 1989. American Industrial Hygiene Association. Odor thresholds for chemicals with established occupational health standards. Akron, OH: AIHA.

CARB. 1999. Air toxics emissions data collected in the Air Toxics Hot Spots Program CEIDARS Database as of January 29, 1999.

Ciezlak FS, Crissman JW, Stott WT, and Corley RA. 1998. Propylene glycol monomethyl ether: a two-year vapor inhalation chronic toxicity/oncogenicity study and evaluation of hepatic and renal cellular proliferation, P450 enzyme induction and protein droplet nephropathy in Fischer 344 rats. Dow Chemical Company, Midland, MI.

Doe JE, Samuels DM, Tinston DJ, de Silva Wickramaratne GA. 1983. Comparative aspects of the reproductive toxicology by inhalation in rats of ethylene glycol monomethyl ether and propylene glycol monomethyl ether. Toxicol. Appl. Pharmacol. 69:43-47.

Gingell R, Boatman RJ, Bus JS, Cawley TJ, Knaak JB, Krasavage WJ, Skoulis NP, Stack CR, and Tyler TR. 1994. Chapter 31: Glycol ethers and other selected glycol derivatives. In: Patty's Industrial Hygiene and Toxicology. Fourth Edition, Vol. 2, Part D. Clayton GD, and Clayton FE, eds. New York: John Wiley and Sons, Inc.

Hanley TR, Calhoun LL, Yano BL, and Rao KS. 1984. Teratologic evaluation of inhaled propylene glycol monomethyl ether in rats and rabbits. Fundam. Appl. Toxicol. 4:784-794.

HSDB. 1995. Hazardous Substance Data Bank. National Library of Medicine, Bethesda, Maryland (TOMES® CD-ROM Version). Denver, CO: Micromedex, Inc., (Edition expires 1/31/95).

IRIS. 1995. Integrated Risk Information System. (CD-ROM Version). Cincinnati, OH: US Environmental Protection Agency, Office of Health and Environmental Assessment, Environmental Criteria Assessment Office.

Landry TD, Gushow TS, and Yano BL. 1983. Propylene glycol monomethyl ether: A 13-week inhalation toxicity study in rats and rabbits. Fundam. Appl. Toxicol. 3:627-630.

Miller RR, Hermann EA, Langvardt PW, McKenna MJ, and Schwetz BA. 1983. Comparative metabolism and disposition of ethylene glycol monomethyl ether and propylene glycol monomethyl ether in male rats. Toxicol. Appl. Pharmacol. 67:229-237.

Miller RR, Hermann EA, Young JT, Landry TD, and Calhoun LL. 1984. Ethylene glycol monomethyl ether and propylene glycol monomethyl ether: Metabolism, disposition and subchronic inhalation toxicity studies. Environ. Health Perspect. 57:233-239.

Rowe VK, McCollister DD, Spencer HC, Oyen F, Hollingsworth RL, and Drill VA. 1954. Toxicology of mono-, di-, and tri-propylene glycol methyl ethers. Arch. Ind. Hyg. Occup. Med. 9:509-525.

Stewart RD, Baretta ED, Dodd HC, and Torkelson TR. 1970. Experimental human exposure to vapor of propylene glycol monomethyl ether: Experimental human exposure. Arch. Environ. Health 20:218-223.

U.S. EPA. 1986. U.S. Environmental Protection Agency. Health and Environmental Effects Profile for Glycol Ethers. Prepared by the office of Health and Environmental Assessment, Environmental Criteria and Assessment Office, Cincinnati, OH for the Office of Solid Waste and Emergency Response, Washington, DC. EP/540/1-86/052. [cited in U.S. EPA, 1995].

CHRONIC TOXICITY SUMMARY

PROPYLENE OXIDE

(1-,2-propylene oxide; methyl ethylene oxide; propene oxide)

CAS Registry Number: 75-56-9

I. Chronic Toxicity Summary

Inhalation reference exposure level	**30 µg/m^3 (9 ppb)**
Critical effect(s)	Degenerative and hyperplastic changes in the respiratory epithelium of rats
Hazard index target(s)	Respiratory system

II. Physical and Chemical Properties (HSDB, 1994)

Description	Colorless liquid
Molecular formula	C_3H_6O
Molecular weight	58.08
Density	0.83 g/cm^3 @ 20° C
Boiling point	34.23° C
Melting point	-112.13° C
Vapor pressure	445 torr @ 20° C
Solubility	Soluble in water, miscible in acetone, benzene, carbon tetrachloride, methanol, ether
Conversion factor	2.38 mg/m^3 per ppm at 25° C

III. Major Uses or Sources

Propylene oxide is used as a fumigant such as in the sterilization of packaged foods. It is also used as a chemical intermediate in the production of propylene glycol and glycol ethers and as a solvent. Propylene oxide is used in the preparation of surfactants and oil demulsifiers (HSDB, 1994). The annual statewide emissions from facilities reporting under the Air Toxics Hot Spots Act in California, based on the most recent inventory, were estimated to be 619,494 pounds of propylene oxide.

IV. Effects of Human Exposures

Conclusive data regarding the effects of occupational exposure to propylene oxide were not located.

An epidemiological study examining mortality among workers with exposure to asbestos and several chemicals, including propylene oxide, identified three deaths due to mesothelioma, a rare cancer associated with asbestos exposure, and a statistically significant increase in the number of deaths attributed to forms of heart disease other than ischemia and hypertension (Egedahl *et al.*, 1989). The latter finding was explained by the authors to be the result of differences in diagnostic accuracy between rural and urban, and primary and tertiary medical care settings. A statistically significant decrease in observed deaths was found for all respiratory cancers, cancer of the bronchus and lung, circulatory disease, digestive diseases, cirrhosis and other liver disease, and death due to accidents, poisonings, and violence. These observations may be partially attributed to a "healthy worker effect".

V. Effects of Animal Exposures

Male and female rats were exposed for 124 or 123 weeks (respectively) to 30, 100 or 300 ppm propylene oxide 6 hours per day, 5 days per week (Kuper *et al.*, 1988). Interim sacrifices were performed at 12, 18, and 24 months. Cumulative mortality was statistically significantly different from controls at 115 weeks in rats of both sexes exposed to 300 ppm propylene oxide. Cumulative mortality was also significantly different from controls at 119 weeks in female rats exposed to 100 ppm. However, a contributing factor to the increased mortality in female rats was the presence of mammary tumors. Atrophy of the olfactory epithelium and degenerative changes in the respiratory epithelium were observed in both male and female rats following 28 months of exposure to 30, 100, or 300 ppm propylene oxide. Severe hyperplastic changes in the olfactory epithelium were observed in male and female rats following 28 months exposure to 300 ppm propylene oxide. Mild hyperplastic changes were observed in the olfactory epithelium of female rats exposed to 100 ppm propylene oxide.

Rats and mice were exposed to 200 and 400 ppm propylene oxide 6 hours per day, 5 days per week for 103 weeks (NTP, 1985). Survival in mice was adversely affected in all groups exposed to propylene oxide; a statistically significant decrease in survival was observed in male and female mice exposed to 400 ppm propylene oxide. Survival in rats was not adversely affected by propylene oxide exposure. Rats exhibited exposure-related increases in suppurative inflammation of the nasal cavity, epithelial hyperplasia and squamous metaplasia.

Rats were exposed to 1500 ppm propylene oxide 6 hours per day, 5 days per week for 7 weeks (Ohnishi *et al.*, 1988). After 3-4 weeks of exposure the rats exhibited an awkward gait; the rats were ataxic by the seventh week. Histopathological examination revealed axonal degeneration of myelinated fibers of the hindleg nerve and fasciculus gracilis indicating central-peripheral distal axonopathy.

Eldridge *et al*. (1995) exposed male F344 rats to 0, 10, 20, 50, 150, or 525 ppm propylene oxide vapor for up to 4 weeks (with up to 4 weeks of recovery). Histopathology showed that the incidence and severity of respiratory epithelial hyperplasia increased with exposure time and regressed after termination of exposure, with complete recovery after 4 weeks. Cell proliferation (determined by bromodeoxyuridine incorporation) was elevated following 1 and 4 weeks of exposure, but decreased to control values after 1 week of recovery. Degeneration of the olfactory epithelium was found after 4 weeks of exposure with a decrease in incidence and severity after termination of exposure. Proliferation of olfactory epithelium was elevated during the 4-week exposure period and 1 week post-exposure and returned to control values after 4 weeks of recovery. The authors report a 4-week NOAEL for propylene oxide effects in nasal epithelium of 50 ppm.

Artificially inseminated rabbits were exposed to 500 ppm propylene oxide on days 1-19 or 7-19 of gestation (Hardin *et al.*, 1983). Maternal toxicity as indicated by a significant reduction in food intake and a significant decrease in maternal body weight gain was observed in both exposed groups. An increased number of resorptions per litter, with no change in total resorptions, was observed in rabbits exposed on days 1-19 of gestation. Sternebral and limb anomalies (considered minor by U.S. EPA and the authors) were significantly increased in the offspring of rabbits exposed on days 1-19 of gestation.

The same study also reported similar findings in sperm-positive rats exposed to 500 ppm propylene oxide on either days 1-16 or 7-16 of gestation or daily for 3 weeks prior to mating and then daily on days 1-16 of gestation. Reproductive capacity was impaired in rats exposed prior to breeding; the number of corpora lutea, implantation sites, and live fetuses were reduced. Those dams exposed pregestationally to propylene oxide also exhibited more resorptions. Maternal toxicity as indicated by decreased food intake and decreased body weight gain was observed in all exposed rats. Significant reductions in fetal body weight and fetal crown-rump length were observed in all exposed groups. An increased incidence of wavy ribs and reduced ossification were observed in the offspring of rats exposed from days 1-16 of gestation.

Harris *et al*. (1989) evaluated the developmental toxicity potential of propylene oxide in Fischer 344 rats. Four groups of 25 mated female rats were exposed to 0, 100, 300, and 500 ppm for 6 hours per day on gestation days 6 through 15. Cesarean sections were performed on all females on gestation day 20 and the fetuses were removed for morphological evaluation. Exposure to propylene oxide did not adversely affect survival, appearance, or behavior at any level. Maternal body weight gain and food consumption were reduced significantly at the 500 ppm level during exposure. Only one exposure-related effect was noted with respect to maternal water consumption, organ weights, cesarean section, or fetal morphological observations: increased frequency of seventh cervical ribs in fetuses at the maternally toxic exposure level of 500 ppm. Thus 300 ppm was considered the NOAEL.

VI. Derivation of Chronic REL (U.S. EPA Reference Concentration (IRIS, 1995))

Study	Kuper *et al.*, 1988
Study population	Rats (male and female)
Exposure method	Inhalation (0, 30, 100 or 300 ppm)
LOAEL	30 ppm
Critical effects	Degenerative and hyperplastic changes in the respiratory epithelium
NOAEL	Not observed
Exposure continuity	6 hr/day for 5 days/week
Exposure duration	124 weeks
Average experimental exposure	5.4 ppm for LOAEL group (30 x 6/24 x 5/7)
Human equivalent concentration	1.2 ppm for LOAEL group (gas with extrathoracic respiratory effects, RGDR = 0.23, based on MV = 0.3 m^3/day, SA(ET) = 11.6 cm^2)
LOAEL uncertainty factor	3 (mild effects only observed during last 4 months of exposure)
Subchronic uncertainty factor	1
Interspecies uncertainty factor	3
Intraspecies uncertainty factor	10
Cumulative uncertainty factor	100
Inhalation reference exposure level	0.009 ppm (9 ppb, 0.03 mg/m^3, 30 µg/m^3)

VII. Data Strengths and Limitations for Development of the REL

The chronic REL is equivalent to the US EPA RfC. The major strength of the REL for propylene oxide is the use of a well-conducted, long-term, multi-concentration study with adequate histopathological analyses. Weaknesses include the lack of adquate human data and the lack of a chronic NOAEL observation.

VIII. References

CARB. 1999. Air toxics emissions data collected in the Air Toxics Hot Spots Program CEIDARS Database as of January 29, 1999.

Egedahl RD, Olsen GW, Coppock E, Young ML, Arnold IM. 1989. An historical prospective mortality study of the Sarnia Division of Dow Chemical Canada Inc., Sarnia, Ontario (1950-1984).Can. J. Public Health 80(6):441-446.

Eldridge SR, Bogdanffy MS, Jokinen MP, Andrews LS. 1995. Effects of propylene oxide on nasal epithelial cell proliferation in F344 rats. Fundam. Appl. Toxicol. 27(1):25-32.

HSDB. 1994. Hazardous Substance Data Bank. National Library of Medicine, Bethesda, Maryland. (TOMES® CD-ROM Version). Denver, CO: Micromedex, Inc. (Edition expires 11/31/94).

Hardin B, Niemeier R, Sikov M, and Hackett P. 1983. Reproductive-toxicologic assessment of the epoxides ethylene oxide, propylene oxide, butylene oxide, and styrene oxide. Scand. J. Work Environ. Health 9:94-102.

Kuper CF, Reuzel GJ, and Feron VJ. 1988. Chronic inhalation toxicity and carcinogenicity study of propylene oxide in Wistar rats. Food Chem. Toxicol. 26(2):159-167.

NTP. 1985. National Toxicology Program. Technical Report on the Toxicology and Carcinogenesis Studies of Propylene Oxide in F344/N Rats and B6C3F$_1$ Mice (Inhalation Studies). NTP TR 267. U.S. Department of Health and Human Services, Public Health Service, National Institutes of Health. Research Triangle Park, NC: NTP.

Ohnishi A, Yamamoto T, Murai Y, Hayashida Y, and Hori Tanaka I. 1988. Propylene oxide causes central-peripheral distal axonopathy in rats. Arch. Environ. Health 43(5):353-356.

IRIS. 1995. Integrated Risk Information System (IRIS). US Environmental Protection Agency, Washington, D.C. (CD-ROM Version).

CHRONIC TOXICITY SUMMARY

SELENIUM AND SELENIUM COMPOUNDS

(other than Hydrogen Selenide**)**

Molecular Formula	*Synonyms*	*Molecular Weight* (g/mol)	*CAS Reg. No.*
Se	elemental selenium	78.96	7782-49-2
SeO_2	selenium dioxide; selenium oxide; selenious anhydride	110.96	7446-08-4
H_2SeO_3	selenious acid	128.97	7783-00-8
$SeOCl_2$	seleninyl chloride; selenium oxychloride; selenium oxichloric	165.86	7791-23-3
Na_2SeO_3	disodium selenite	263.01	10102-18-8
Na_2SeO_4	disodium selenate	188.94	13410-01-0
SeS	selenium sulfide; sulfur selenide	111.02	7446-34-6

I. Chronic Toxicity Summary

Inhalation reference exposure level	**20 μg/m^3**
Oral reference exposure level	**0.005 mg/kg/day** (USEPA RfD)
Critical effect(s)	Clinical selenosis
Hazard index target(s)	Alimentary system; cardiovascular system; nervous system

II. Chemical Property Summary (HSDB, 1995; Weast, 1980; Canady and Hodes, 1994; ACGIH, 1992)

Description	Se^0 crystal: metallic gray H_2SeO_4, Na_2SeO_3: white crystals H_2SeO_3, Na_2SeO_4: colorless crystals SeO_2: lustrous crystals; yellow vapor SeS: yellow to orange powder
Molecular formula	see above
Molecular weight	see above
Vapor pressure	0.001 torr @ 20°C
Melting point	SeO_2: 340°C SeS: decomposes at 118-119°C
Solubility	Se^0: insoluble in water, alcohol; slightly soluble in CS_2; soluble in ether H_2SeO_4: sol. in water; decomposes in alcohol H_2SeO_3: sol. in hot water, alcohol Na_2SeO_3: sol. in water Na_2SeO_4: 84 g/100 ml water at 35°C SeO_2: 38.4 g/100 ml water at 14°C SeS: insoluble in water
Conversion factor	Se^0: not applicable (particulate) SeO_2: 4.5 μg/m^3 per ppb at 20°C

III. Major Uses and Sources

Selenium occurs in four valence states: selenates (Se^{6+}), selenites (Se^{4+}), selenides (Se^{2-}), and elemental selenium (Se^0) (Goyer, 1991) which include compounds formed with oxygen, sulfur, metals, and/or halogens. Selenium compounds are used in the glass industry as decolorizing agents and in the rubber industry as vulcanizing agents. Selenium compounds are also found in toning baths used in photography and xerography, and in insecticides and photoelectric cells. Selenious acid is a component of gun cleaning chemicals (Quadrani *et al*., 2000). Selenium sulfide is used in shampoos as an anti-dandruff agent. The most widely used selenium compound in industry is selenium dioxide (SeO_2) which catalyzes reactions of organic compounds and is produced by the oxidation of selenium with nitric acid followed by evaporation or by burning selenium in oxygen (HSDB, 1995). The largest anthropogenic sources of atmospheric selenium are from the combustion of fossil fuels and the production/refining of copper; particulates are the primary expected form of the compound (National Academy of Sciences (NAS), 1976; U.S. EPA, 1984). The annual statewide industrial emissions from facilities reporting under the Air Toxics Hot Spots Act in California based on the most recent inventory were estimated to be 12,417 pounds of selenium and 4846 pounds of selenium sulfide (CARB, 1999).

Selenium is an essential trace element in humans and other species; selenium deficiency leads to cardiomyopathy in humans (Goyer, 1991). For dietary intake, the National Research Council has

set a U.S. Recommended Daily Allowance (RDA) of 0.87 μg/kg (55-70 μg/person/day) (Subcommittee on the Tenth Edition of the RDAs, 1989). The average daily oral intake of selenium is 125 μg/person (U.S. EPA, 1991). Organic selenium compounds (e.g., dimethyl selenide) are known to occur as metabolites and as microbial degradation products in the environment. These compounds appear to have relatively low toxicity.

IV. Effects of Human Exposures

Acute occupational exposure to SeO_2 resulted in bronchospasm, irritation of the upper respiratory passages, violent coughing, and gagging with nausea and vomiting (Wilson, 1962).

The relationship between inhalation exposure to selenium and the presence of selenium in the urine was investigated in a five year study of workers at a selenium rectifying plant (Glover, 1967). Workers were exposed to fumes and dusts of elemental red selenium, which, the author reported, is converted 80% to SeO_2 in the presence of air. Average air concentrations of selenium were reported to be 3.6 mg/m^3 in grinding processes, 0.04 mg/m^3 in annealing processes, and a range of averages of 0.23-0.87 mg Se/m^3 in various "special" processes, e.g., punching, scraping, sorting, refining, and testing. The same author previously reported symptoms among selenium exposed workers including garlic-like odor of the breath, skin rashes, indigestion, and poorly-defined "socio-psychological" effects including lassitude and irritability (Glover, 1954).

Clinical signs of toxicity were observed among a population exposed to high levels of selenium in soils and food supplies in China (Yang *et al.*, 1983). Approximately half of 248 people in this region showed symptoms including hair and nail loss, discoloration and decay of the teeth, and CNS disturbances including pain and anesthesia of the extremities. Animals in the region were also affected, with hoof damage and horn sloughing reported in water buffalo, cattle, and pigs. Populations in low-, medium-, and high-selenium areas of China were later studied to associate the symptoms with selenium intake. Estimated daily intake for adults in these areas were 70, 195, and 1438 μg Se for males and 62, 198, and 1238 μg for females, respectively (Yang *et al.*, 1989). Selenium intake was highly correlated with whole blood, breast milk, and 24-hour urine selenium levels. The authors also suggested the possibility of liver dysfunction as indicated by a delay in prothrombin time among persons with intake of 750-850 μg Se/day. More clearly recognized and characteristic clinical signs, however, were only observed in a group exposed to greater than 1261 μg Se/day and not among those exposed to less than 853 μg Se/day. Assuming a 55 kg body weight, these respective daily dose rates were 0.023 and 0.015 mg/kg-day.

A population of 142 subjects in seleniferous areas of western South Dakota and eastern Wyoming was examined for signs of selenosis over a two-year period with monitoring of selenium levels in diet, whole blood, serum, urine, and toenails (Longnecker *et al.*, 1991). Subjects completed health questionnaires, underwent physical examinations, provided blood samples for clinical assessment, and provided blood, urine, toenails, and duplicate-plate food collections for selenium analysis. About half of the 142 free-living subjects had selenium intakes greater than 2.54 mμmol/day (200 μg/day) (range 0.86-9.20 mμmol/day, or 68-724 μg/day). Average intake among the population was estimated at 239 μg Se/day. No clinical

signs and no changes in hematological function, clinical chemistry, or liver function were observed in the population, even in subjects whose intake was as high as 9.20 mμmol/day (724 μg/day).

V. Effects of Animal Exposures

Toxic effects from acute inhalation exposure to selenium dust were examined in rats, guinea pigs, and rabbits (Hall *et al.*, 1951). Twenty female rats were exposed once for 8 hours to 33 ± 10 mg Se/m^3. Many animals showed signs of pulmonary effects at both one week and 4 weeks after exposure; however, no control group was included in the experiment with which to compare incidence. Similarly, six female rabbits and 10 male guinea pigs were exposed to the same level of selenium dust for four 4-hour periods every 48 hours (8 days total duration). The animals showed signs of interstitial pneumonitis at one week (2 animals of each species) and lung congestion and alveolar infiltration of large macrophages.

Guinea pigs exposed one time to concentrations "less than 0.021 mg H_2Se/L" (22 mg Se/m^3 as hydrogen selenide) for 2, 4, or 8 hours exhibited difficulty breathing and a red-tinged discharge from the nose (Dudley and Miller, 1941). Mortality studies were conducted with guinea pigs (16/group) using the same exposure duration and selenium concentrations ranging from 1 to 43 mg Se/m^3. Fifty percent mortality was observed at 30 days among animals exposed once for 2 hours to 12 mg Se/m^3. Mortality after 30 days was 50% among animals exposed once to 1 mg Se/m^3 for 8 hours. Histopathological evaluation of guinea pigs exposed once for 4 hours to 8 mg Se/m^3 showed fatty change to the liver, pneumonia, lymphoid hyperplasia, and increased reticuloendothelial tissue in the spleen. These effects did not begin to resolve until more than 17 days after the exposure.

Several studies have addressed the toxicity of selenium compounds to animals when administered in either food or drinking water. Mice (50/group) treated with 0, 1, 4, or 8 ppm Na_2SeO_3 in drinking water over 50 weeks showed decreased growth rates at 8 ppm (Jacobs and Forst, 1981). The same group reported gross liver pathology in male mice treated by oral gavage for 3 days with 0.5 ml of 64 ppm Na_2SeO_3. Hamsters (8/sex/group) treated with 0.1 (unsupplemented), 1, 5, 10, or 20 ppm Na_2SeO_3 in the diet for 42 days showed histopathological changes to the liver (Beems and van Beek, 1985). Rats (6-8/group) treated in the diet with SeS_2, Na_2Se, Na_2SeO_3, or Na_2SeO_4 showed increased relative liver weights and/or decreased body weight gain at 10 ppm (for each compound) over a 5 week exposure (Dausch and Fullerton, 1993). A 13-week drinking water study of Na_2SeO_3, and Na_2SeO_4 in rats and mice showed increased mortality, decreased body weights, and histopathological changes to the kidneys in rats and decreased body weight and decreased water consumption in mice (Abdo, 1994). Decreased body weights were observed in rats treated for 6 weeks in drinking water with 2 ppm Na_2SeO_3 or Na_2SeO_4 (Palmer and Olson, 1974).

Decreased percentage of live spermatozoa, altered sperm morphology, and decreased body weight gain were observed in rats (6/group) treated for 5 weeks with 2 ppm Na_2SeO_3 in the diet (Kaur and Parshad, 1994). Rats (7-12/group) exposed to 0, 4, 8, or 16 ppm Na_2SeO_3 in drinking

water for 240 days showed alterations in testicular LDH and β-glucuronidase activity at 4 ppm (Nebbia *et al.*, 1987).

Developmental toxicity endpoints were examined in hamsters (5-10/group) exposed by oral gavage on gestational day 8 to Na_2SeO_3 and Na_2SeO_4 at concentrations ranging from 0 - 110 μmol/kg body weight (Ferm *et al.*, 1990). Effects observed at 100 μmol Na_2SeO_3/kg included decreased fetal crown-rump length and increased percentage of abnormal litters. At 90 μmol Na_2SeO_4/kg, an increased percentage of abnormal litters was observed. Mice (10 or 14/group) treated with 0, 3, or 6 ppm Na_2SeO_3 in drinking water from 30 days pre-gestation through gestation showed altered estrus cycle length, decreased fetal growth, and a decreased number of ossified vertebrae in offspring (Nobunaga *et al.*, 1979).

VI. Derivation of Chronic Reference Exposure Level (REL) (for selenium and selenium compounds other than hydrogen selenide)

Study	Yang *et al*., 1989
Study population	400 people in China
Exposure method	Low, medium, & high environmental levels of Se
Critical effects	Clinical selenosis (liver, blood, skin, CNS)
LOAEL	0.023 mg/kg-day* (1.261 mg/day / 55 kg)
NOAEL	0.015 mg/kg-day* (0.853 mg/day / 55 kg)
Exposure continuity	Continuous
Exposure duration	Lifetime
Average experimental exposure	70, 195, and 1438 μg/day for adult males; 62, 198, and 1238 μg/day for adult females
LOAEL uncertainty factor	1
Subchronic uncertainty factor	1
Interspecies factor	1
Intraspecies factor	3
Cumulative uncertainty factor	3
Oral reference exposure level	0.005 mg/kg/day (USEPA RfD)
Inhalation extrapolation factor	3,500 $\mu g/m^3$ per mg/kg-day
Inhalation reference exposure level	20 $\mu g/m^3$

*Factors: NOAEL (0.853 mg/day) and LOAEL (1.261 mg/day) calculated from regression analysis (log Y = 0.767 log X - 2.248, where Y = blood selenium and X = selenium intake) based upon the correlation (r = 0.962) between dietary selenium intake and blood selenium level for data showing incidence of clinical selenosis in adults based on an average adult body weight of 55 kg.

The inhalation chronic REL is based on the oral chronic REL, which is the same as the USEPA's oral reference dose (RfD) (U.S. EPA, 1996). In addition to being inhaled, airborne selenium can settle onto crops and soil and enter the body by ingestion. Thus an oral chronic reference exposure level for selenium is also required for Air Toxics Hot Spots health risk assessments. The chronic inhalation REL was derived by route-to-route extrapolation of the RfD. The

principal study used for the REL/RfD was that of Yang *et al*. (1989). Yang *et al*. (1989), in a follow-up to an earlier study (Yang *et al*., 1983), studied a population of approximately 400 individuals living in an area of China with unusually high environmental concentrations of selenium (Se). The subjects were evaluated for clinical and biochemical signs of Se intoxication. Three geographical areas with low, medium, and high selenium levels in the soil and food supply were chosen for comparison in the studies. The earlier study was conducted in response to endemic selenium intoxication in two separate areas with sample sizes of only 6 and 3. Comparisons were then made to a selenium-adequate area (n=8) and low-selenium area (n=13). The Yang *et al*. (1989) studies provide a much larger sample size and include additional analysis of tissue selenium levels. This allows a more accurate estimation of the dose-response relationship observed for selenium toxicity. Selenium levels in soil and approximately 30 typical food types commonly eaten by the exposed population showed a positive correlation with blood and tissue Se levels. The daily average Se intakes, based on lifetime exposure, were 70, 195, and 1438 μg for adult males and 62, 198, and 1238 μg for adult females in the low-, medium- and high-selenium areas, respectively. Significant correlations, demonstrated between Se concentrations of various tissues, were used to estimate the minimal daily Se intake values that elicited various alterations in biochemical parameters indicative of possible Se-induced liver dysfunction (i.e., prolongation of clotting time and serum glutathione titer) and clinical signs of selenosis (i.e., hair or nail loss, morphological changes of the nails, etc.). In this manner, a marginal safe level of daily Se intake was estimated. Analysis of the results indicated that persistent clinical signs of selenosis were observed only in 5/349 adults, a potentially sensitive subpopulation. The blood selenium concentration in this group ranged from 1.054 to 1.854 mg/L with a mean of 1.346 mg/L. Clinical signs observed included the characteristic "garlic odor" of excess selenium excretion in the breath and urine, thickened and brittle nails, hair and nail loss, lowered hemoglobin levels, mottled teeth, skin lesions, and CNS abnormalities (peripheral anesthesia, acroparesthesia, and pain in the extremities). Alterations in the measured biochemical parameters occurred at dietary intake levels of 750-850 μg/day. These alterations were described as a delay in prothrombin time, i.e., increase in blood coagulation time and reduction in blood glutathione concentration. However, these indicators were poorly characterized and are not typically used as an index for clinical selenosis resulting from chronic exposure to selenium (NAS, 1989). Based upon the blood selenium levels shown to reflect clinical signs of selenium intoxication, a whole blood selenium concentration of 1.35 mg/L corresponding to 1.261 mg of daily selenium intake is indicative of the lowest correlative selenium intake causing overt signs of selenosis. The next lowest whole blood selenium concentration of 1.0 mg/L, corresponding to 0.853 mg selenium/day, produces no clinical signs of selenosis. The NOAEL for this study is 0.85 mg Se/day and the LOAEL is 1.26 mg Se/day.

An intraspecies uncertainty factor of 3 was applied to the NOAEL to account for sensitive individuals. A full factor of 10 was not deemed necessary since similar NOAELs were identified in two moderately-sized human populations exposed to selenium levels in excess of the RDA throughout a lifetime without apparent clinical signs of selenosis. No modifying factor was applied by USEPA. OEHHA accepted the USEPA analysis.

Route-to-route extrapolation assumes by default that a chemical is equally absorbed by the inhalation and the oral routes and that the first pass effect due to metabolism by the liver is not important for the chemical. The latter assumption is applicable to most metals. There are

limited data to evaluate the assumption of equal absorption across the gastrointestinal tract and the lungs. Limited data indicate that 60% (range = 44-100%) of ingested Se is absorbed by the gastrointestinal tract, while in one study 30% (single estimate) of inhaled selenium was deposited in the respiratory tract (Owen, 1990). Deposition is dependent on particle size. The available data are not adequate to depart from the default assumption.

The USEPA stated its confidence in the RfD as: Study - Medium; Data Base - High; and RfD - High. Confidence in the chosen principal study is medium. Although this is a human epidemiological study in which a sizable population with sensitive subpopulations was studied, there are still several possible interactions that were not fully accounted for, e.g., fluoride intake and protein status. Also, except for clinical signs of selenosis there are no other reliable indicators, biochemical or clinical, of selenium toxicity. Confidence in the database is high because many animal studies and epidemiologic studies support the principal study. An additional human study with a freestanding NOAEL (Longnecker et al., 1991) provides support for the NOAEL identified in the principal study. Longnecker *et al*. (1991) found no effects at 238 μg Se per day, which would equate to 0.004 mg/kg-day for a 55 kg person. Therefore, high confidence in the RfD is selected based upon support of the critical study and the high level of confidence in the database.

There are insufficient data relating human inhalation exposure to selenium compounds to adverse health effects to use for the development of a chronic REL although toxicity has been reported from occupational exposure to gases of both H_2Se and SeO_2 (Buchan, 1947; Wilson, 1962). Experiments in animals have shown that H_2Se is toxic following inhalation exposure, with 8-hour exposures to concentrations as low as 1 mg H_2Se/m^3 causing "irritation sufficiently damaging to cause pneumonitis" and subsequently increasing 30-day mortality (Dudley, 1937; Dudley and Miller, 1941). Thus the selenium chronic REL is not meant to be applied to H_2Se, which may be considerably more toxic than other selenium compounds. At this time there are inadequate data to develop a REL for H_2Se. It is also not intended to be applied to organic metabolites of selenium.

VII. Data Strengths and Limitations for Development of the REL

The strengths of the REL for selenium include its basis on a study with a large number of human subjects in a non-occupational setting that determined both a NOAEL and a LOAEL. The weaknesses include its basis on a route of exposure other than inhalation and its lack of applicability to hydrogen selenide, the most toxic selenium compound.

VIII. Potential for Differential Impacts on Children's Health

The key study (Yang *et al*., 1989) included evaluation of children as young as one year old. Thus the chronic REL should be protective of infants and children. No adverse reproductive outcomes were reported, although only 400 people were studied. However, the inhalation REL is based on an oral REL of 0.005 mg/kg-day (0.06 μmol/kg-day). Ferm *et al*. (1990) did not find adverse effects on hamster development with Se doses below 34 μmol/kg. Thus the chronic REL should also be protective of infants and children.

IX. References

Abdo KM. 1994. NTP (National Toxicology Program) technical report on toxicity studies of sodium selenate and sodium selenite (CAS No. 13410-01-0 and 10102-18-8) administered in drinking water to F344/N rats and B6C3F1 mice. PB94-215753. Springfield, VA: NTIS.

ACGIH. 1992. American Conference of Governmental Industrial Hygienists, Inc. Documentation of the threshold limit values and biological exposure indices. Sixth ed. Cincinnati, OH: ACGIH.

Beems RB, and van Beek L. 1985. Short-term (6-week) oral toxicity study of selenium in Syrian hamsters. Food Chem. Toxicol. 23:945-947.

Buchan RF. 1947. Industrial selenosis: A review of the literature, report of five cases and a general bibliography. Occup. Med. 3:439-456.

CARB. 1999. California Air Resources Board. Air toxics emissions data collected in the Air Toxics Hot Spots Program CEIDARS Database as of January 29, 1999.

Canady RA, and Hodes CS. August, 1994. In: Draft Toxicological Profile for Selenium. Agency for Toxic Substances and Disease Registry, Public Health Service, US Department of Health and Human Services, Washington, DC: USDHHS.

Dausch JG, and Fullerton FR. 1993. Increased levels of S-adenosylmethionine in the livers of rats fed various forms of selenium. Nutr. Cancer 20:31-39.

Dudley HC. 1937. Toxicology of selenium. IV. Effects of exposure to hydrogen selenide. U.S. Public Health Reports 52:1217-1231.

Dudley HC, and Miller JW. 1941. Toxicology of selenium. VI. Effects of subacute exposure to hydrogen selenide. J. Ind. Hyg. Toxicol. 23:470-477.

Ferm VH, Hanlon DP, Willhite CC, Choy WN, and Book SA. 1990. Embryotoxicity and dose-response relationships of selenium in hamsters. Reprod. Toxicol. 4:183-190.

Glover JR. 1954. Some medical problems concerning selenium in industry. Trans. Assoc. Industr. Med. Offrs. 4:94-96.

Glover JR. 1967. Selenium in human urine: a tentative maximum allowable concentration for industrial and rural populations. Ann. Occup. Hyg. 10:3-14.

Goyer RA. 1991. Toxic effects of metals. In: Casarett and Doull's Toxicology. The Basic Science of Poisons. Fourth ed. Amdur MO, Doull J, and Klaassen CD (eds.). New York: Pergamon Press, pp. 658-660.

Hall RH, Laskin S, Frank P, Maynard EA, and Hodge HC. 1951. Preliminary observations on toxicity of elemental selenium. Arch. Ind. Hyg. Occup. Med. 4:458-464.

HSDB. 1995. Hazardous Substances Data Bank. National Library of Medicine, Bethesda, MD (TOMES® CD-ROM Version). Denver, CO: Micromedex, Inc. (Edition expires 7/31/96).

Jacobs M, and Forst C. 1981. Toxicological effects of sodium selenite in Swiss mice. J. Toxicol. Environ. Health 8:587-598.

Kaur R, and Parshad VR. 1994. Effects of dietary selenium on differentiation, morphology and functions of spermatozoa of the house rat, Rattus rattus L. Mutat. Res. 309:29-35.

Longnecker MP, Taylor PR, Levander OA, Howe M, Veillon C, McAdam PA, Patterson KY, Holden JM, Stampfer MJ, Morris JS, and Willett WC. 1991. Selenium in diet, blood, and toenail in relation to human health in a seleniferous area. Am. J. Clin. Nutr. 53:1288-1294.

NAS. 1976. National Academy of Sciences. Selenium. Committee on Medical and Biological Effects, Environmental Pollution Subcommittee on Selenium. Washington, DC: NAS.

Nebbia C, Brando C, Burdino E, and Rasero R. 1987. Effects of the chronic administration of sodium selenite on rat testis. Res. Commun. Chem. Pathol. Pharmacol. 58:183-197.

Nobunaga T, Satoh H, and Suzuki T. 1979. Effects of sodium selenite on methylmercury embryotoxicity and teratogenicity in mice. Toxicol. Appl. Pharmacol. 47:79-88.

Owen BA. 1990. Literature-derived absorption coefficients for 39 chemicals via oral and inhalation routes of exposure. Regul. Toxicol. Pharmacol. 11(3):237-252.

Palmer IS, and Olson OE. 1974. Relative toxicities of selenite and selenate in the drinking water of rats. J. Nutr. 104:306-314.

Quadrani DA, Spiller HA and Steinhorn D. 2000. A fatal case of gun blue ingestion in a toddler. Vet. Hum. Toxicol. 42(2):96-98.

Subcommittee on the Tenth Edition of the RDAs 1989. Food and Nutrition Board: Recommended Dietary Allowances. Tenth ed. Commission on Life Sciences, National Research Council. Washington, DC: National Academy Press.

U.S. EPA. 1984. United States Environmental Protection Agency. Health effects assessment for selenium (and compounds). EPA/540/1-86/058. NTIS Doc. No. PB86-134699. Washington, DC: U.S. EPA.

U.S. EPA. 1991. United States Environmental Protection Agency. Title 40, Code of Federal Regulations, Parts 141, 142, and 143. National Primary Drinking Water Regulations; Final Rule. Federal Register, 56:3538-3539.

U.S. EPA. 1996. United States Environmental Protection Agency. Selenium. IRIS (Integrated Risk Information System). Available online at http://www.epa.gov/ngispgm3/iris

Weast RC. (ed.) 1980. CRC Handbook of Chemistry and Physics. 61st ed. Boca Raton, FL: CRC Press.

Wilson HM. 1962. Selenium oxide poisoning. N.C. Med. J. 23:73-75.

Yang G, Wang S, Zhou R, and Sun S. 1983. Endemic selenium intoxication of humans in China. Am. J. Clin. Nutr. 37:872-881.

Yang G, Zhou R, Yin S, and Gu L. 1989. Studies of safe maximal daily dietary selenium intake in a seleniferous area in China. I. Selenium intake and tissue selenium levels of the inhabitants. J. Trace Elem. Electrolytes Health Dis. 3:77-87.

CHRONIC TOXICITY SUMMARY

SILICA (CRYSTALLINE, RESPIRABLE)

(silicon dioxide, quartz, tridymite, cristobalite)

CAS Registry Number: 7631-86-9

I. Chronic Toxicity Summary

Inhalation Reference Exposure Level	**3 µg/m^3** [respirable, as defined occupationally by ACGIH (2004)/ISO (1995)
Critical effect(s)	Silicosis in miners and other workers
Hazard index target(s)	Respiratory system

II. Physical and Chemical Properties (HSDB, 2001)

Description	Transparent crystals
Molecular formula	SiO_2
Molecular weight	60.09 g/mol
Density	2.65 g/cm^3 @ 0 °C (quartz)
Melting point	1610 °C
Boiling point	2230 °C (2503.20 °K)
Vapor pressure	10 torr @ 1732 °C
Solubility	Practically insoluble in water or acids, except hydrofluoric acid; very slightly sol. in alkali.
Conversion factor	Not applicable

In crystalline silica, the silicon and oxygen atoms are arranged in a definite regular pattern throughout the crystal. The characteristic crystal faces of a crystalline form of silica are the outward expression of this regular arrangement of the atoms (HSDB, 2001). This REL is meant to be applied only to particles of crystalline silica (quartz, cristobalite, tridymite), of respirable size, as defined by the occupational hygiene methods described by ACGIH (2004)/ISO (1995) which has a 50% cut-point at 4 µm particle aerodynamic diameter. This occupational definition of respirable differs from the environmental definition of respirable, which is PM_{10}. (The occupational particle category "thoracic" has a 50% cut-point at 10 µm particle diameter (ACGIH, 2004) and the category "inhalable" has a 50% cut-point at 100 µm particle diameter (ACGIH, 2004).)

III. Major Uses and Sources

At least 11 chemically identical forms (polymorphs) have been described for crystalline silica. Alpha-quartz is the most abundant polymorph and constitutes 12% of the earth's crust (Elzea, 1997). Silica is also found in the amorphous (non-crystalline) state. The amorphous silica in diatomaceous earth (composed mainly of the cell walls of diatoms) can be converted to the crystalline form cristobalite by heating to 1000-1100 ºC (calcining). Silica is often associated

with silicates, which, in addition to silicon and oxygen, contain other metals such as iron, magnesium, aluminum, calcium, potassium, and sodium.

The major uses of silica are in the manufacture of glass, abrasives, ceramics, and enamels, in scouring and grinding compounds, and in molds for castings. Silica is also used in decolorizing and purifying oils and petroleum products; as a clarifying agent; in filtering liquids; and in the manufacture of heat insulators, firebrick, and fire- and acid-proof packing materials. As diatomite (naturally occurring diatomaceous earth), silica is used as a filtration agent, as an abrasive, and as an industrial filler. Sources of ambient respirable crystalline silica in California include mines, quarries, diatomaceous earth calcining plants, sand blasting, and entrained fines (e.g., PM10) from surface soil. The annual statewide industrial emissions from facilities reporting under the Air Toxics Hot Spots Act in California based on the most recent inventory were estimated to be 2,514,981 pounds of crystalline silica (CARB, 2001. The fraction, which is respirable as defined either occupationally or environmentally, is not known.

Measurement of crystalline silica has evolved. Instrumentation has varied by country. In South Africa since the 1930s, dust was collected with a konimeter (Le Roux, 1970; Cherrie and Aitken, 1999). A small volume of air (e.g., 5 cm3 captured in less than a second) was collected (impacted) onto a small area of a glass slide coated with adhesive. Total dust particles were counted and expressed as dust particles per cubic centimeter. Later, slides were heated to 500-550 ºC (ignition) to remove carbonaceous materials and immersed in hot 50% hydrochloric acid followed by a second ignition to remove acid-soluble materials. The remainder was mostly silica particles, which could be counted. The konimeter was superseded by the thermal precipitator, which also deposited particles onto glass but could sample larger air volumes at high flow rates (> 1 L/minute) for several hours. With time, particle counting was replaced by estimation of a particle's surface area, initially by examining slides but more recently by an automated method (Kitto, 1960; 1970).

In the United States the impinger method was used from 1922 until 1984 (Lippmann, 2001). Air was drawn into a trap containing fluid, particles in an aliquot of the fluid were counted under magnification, and concentrations were expressed as million particles per cubic foot of air sampled. Later, gravimetric analysis was introduced. Gravimetric analysis is dominated by the larger particles in any given size range.

When it was realized that only a fraction of the dust was responsible for silicosis, respirable dust was collected onto filters using size-specific dust collectors, such as horizontal plate elutriators in South Africa and cyclones in the United States. The sizes of particles collected on the filter were a function of the apparatus used and the rate of airflow through the apparatus. Quartz dust was quantified by examining filters in an electron microscope with a specific X-ray diffraction beam absorbed by crystalline silica. The National Institute of Occupational Sciences and Health (NIOSH, 2003) has approved Method 7500, which uses one of three approved cyclones and a 5 μm PVC membrane filter to sample, and X-ray diffraction to measure crystalline silica. The ARB has used Method 7500 in research projects.

In order to harmonize respirable particulate sampling methodology in workers, an international agreement has been reached to use dust samplers that have a 50% cut point for particles of 4 μm aerodynamic diameter (ISO, 1995; ACGIH, 2004).

Various attempts have been made to estimate the changes in silica levels in workplaces over time (e.g., Seixas et al., 1997 for diatomaceous earth facilities in California; Verma et al., 1989 for Ontario hard rock miners). However, although some conversion factors have been proposed, correlation between dust particle number in earlier studies, when dust concentrations were higher, and dust particle weight in the later studies, when the dust concentrations have been lowered, is imprecise so it is difficult to compare the earlier silica measurements with the more recent ones.

IV. Effects of Human Exposures

Inhalation of crystalline silica initially causes respiratory irritation and an inflammatory reaction in the lungs (e.g., Vallyathan et al., 1995). Acute exposures to high concentrations cause cough, shortness of breath, and pulmonary alveolar lipoproteinosis (acute silicosis). After chronic but lower workplace exposures to silica for six to sixteen years, the small airways become obstructed as measured by pulmonary function tests (e.g., decreased FEV1) in granite quarry workers (no measurement of silica levels reported; Chia et al., 1992). In a report on the hazards of exposure to crystalline silica, the American Thoracic Society (1997) stated: "Studies from many different work environments suggest that exposure to working environments contaminated by silica at dust levels that appear not to cause roentgenographically visible simple silicosis can cause chronic airflow limitation and/or mucus hypersecretion and/or pathologic emphysema." Hnizdo and Vallyathan (2003) also concluded that "chronic levels of silica dust that do not cause disabling silicosis may cause the development of chronic bronchitis, emphysema, and/or small airways disease that can lead to airflow obstruction, even in the absence of radiological silicosis." Fibrotic lesions associated with crystalline silica have also been found at autopsy in the lungs of granite workers who lacked radiological evidence of silicosis (Craighead and Vallyathan, 1980).

Silicosis results from chronic exposure; it is characterized by the presence of histologically unique silicotic nodules and by fibrotic scarring of the lung. The histological progression of silicosis has been described as: (1) granuloma composed of histiocytic cells, collagen, and lymphocytes; (2) cellular fibrotic nodule with irregular collagen at the center and circular collagen at the periphery; (3) more mature nodule with acellular and avascular center; and (4) late mature nodule composed of dust and collagen including a calcified center (Green and Vallyathan, 1996). Lung diseases other than cancer associated with silica exposure include silicosis, tuberculosis/silicotuberculosis, chronic bronchitis, small airways disease, and emphysema (Oxman et al., 1993; Park et al., 2002; Hnizdo and Vallyathan, 2003; Balmes et al., 2003). Silica exposure has been implicated in autoimmune diseases (rheumatoid arthritis, scleroderma, systemic lupus erythematosus) in gold miners and granite workers (Steenland and Goldsmith, 1995; Parks et al., 1999) and in the causation of kidney disease in some occupations (Goldsmith and Goldsmith, 1993; Stratta et al., 2001), possibly by an immune mechanism.

At the cellular level, silica particles are engulfed in the lung by alveolar macrophages (AM). According to the generally assumed pathological model, the AM subsequently release various growth factors and reactive oxygen species (ROS; superoxide anion, hydrogen peroxide, hydroxyl radical) (Lapp and Castranova, 1993; Mossman and Churg, 1998; Ding et al., 2002). ROS and some growth factors (e.g., activator protein-1, platelet activating factor) are inflammatory and attract neutrophils to the site of inflammation, while other factors (fibronectin,

alveolar macrophage-derived growth factor) stimulate fibroblasts to proliferate and to make collagen. Since silica particles cannot be digested by the macrophage, the inflammatory process becomes chronic (frustrated phagocytosis). An increased silica burden leads to more foci of inflammation, nodule formation, and fibrosis. The internal process can continue after external exposure ends. Silica particles also enter into alveolar Type I epithelial cells (Churg, 1996), which can lead to cell death of Type I cells and to hypertrophy and proliferation of Type II epithelial cells to replace the Type I cells. The epithelial repair process is associated with a subsequent increase in collagen formation.

The initial diagnosis of silicosis is often based on chest radiographs. Recent papers have used the 1980 classification by the International Labor Organization (ILO, 1980) to identify and classify silicosis into categories and subcategories of seriousness by comparison of patient radiographs with ILO-supplied reference radiographs taken at various stages of silicosis (Table 1):

Table 1. International Labor Organization categorization of silicosis (ILO, 1980).

ILO Category	***Qualitative Description***
0/0	No small (up to 1 cm) silicotic opacities (nodules) are present
0/1	Probably no nodules, but some areas of radiograph are suspect [possible silicosis]
1/0	Small silicotic nodules are most likely present, but not certainly [probable silicosis]
1/1	Small silicotic nodules are definitely present
1/2	Small silicotic nodules are definitely present; other areas of the radiograph may indicate more advanced lesions including large opacities (> 1 cm), pleural thickening, etc.
2/1, 2/2, 2/3, 3/2, 3/3	More advanced stages of silicosis/increasing certainty of the presence of lung abnormalities

Some reports (e.g., Kreiss and Zhen, 1996; Hughes et al., 1998) use 1/0 (probable) as the basis of classification of silicosis, since many cases of silicosis are not detected by chest radiographs, yet silicotic nodules and other lesions are found at autopsy (Craighead and Vallayathan, 1980; Hnizdo et al., 1993). Other reports (e.g., Hnizdo and Sluis-Cremer, 1993) use the definite 1/1 as the lowest category indicating silicosis. Some disease is missed by radiography and is determined only by autopsy (Hnizdo et al., 1993). The ILO criteria are intended as an epidemiologic classification and comparison tool, not as a diagnostic classification on an individual basis. In occupational medicine practice, a group of tests is used to clinically diagnose silica-related lung disease including physical examination, X-rays, and high resolution computed tomography (CT) scans of the lung (e.g., Begin et al., 1991; Olivetti et al., 1993).

A. Environmental silicosis

Several studies have reported "environmental silicosis", cases where the silicosis occurs in the absence of an industry usually associated with the disease (reviewed by USEPA, 1996). In one of the stronger examples, Saiyed et al. (1991) investigated non-occupational pneumoconiosis in Ladakh, India, high in the western Himalayas where there are no mines or industries. Among 449 randomly selected inhabitants of three villages, there were many cases of pneumoconiosis

associated with progressive massive fibrosis (nodules > 1 cm) and "egg shell" calcification of hilar glands. The prevalence of pneumoconiosis was 2.0% (3/150) in the village of Saboo, 20.1% (31/149) in Shey, and 45.3% (68/150) in Chushot, and corresponded with the severity of dust storms and the presence or absence of chimneys in the kitchens (i.e., ventilated cooking). Without chimneys (Chushot), dust concentrations in kitchens averaged 7.5 mg/m3 during cooking periods. The free silica content of the dust storms was 60-70%. The authors suggested that exposure to free silica from dust storms and to soot from cooking with domestic fuels caused the pneumoconiosis. Perhaps the interaction of silica and soot led to the disease. Such exposures in this and other studies, such as Bar-Ziv and Goldberg (1974), might be considered to be non-industrial but occupational, since the subjects studied by Saiyed et al. (1991) were involved in the domestic work of cleaning and cooking (USEPA, 1996). In any case, the exposures were very high and thus similar to some occupational exposures.

B. Occupational silicosis

Several relatively recent reports have presented data that allow a quantitative relationship between occupational dust exposure and the development of silicosis in workers to be calculated.

Hard rock miners in Ontario, Canada (Muir *et al*., 1989)

Muir *et al.* (1989) examined the relationship between cumulative exposure to silica (free crystalline silica, specifically alpha-quartz) and the development of silicosis in 2109 male hard rock (uranium, gold, mixed metals) miners in Ontario, Canada. The miners began work between 1940 and 1959 and were followed either until they ended their dust exposure or until December 31, 1982 (whichever came first). Five X-ray readers examined chest radiographs; one or more readers identified 32 cases of silicosis, defined as ILO category 1/1 or greater with round opacities. All five readers agreed on only six cases, while 12 cases were identified by only one reader (Table 2). A Weibull model of the form

$$R(x) = 1 - \exp[-(\alpha x)^{\beta}] \quad (x \geq 0, \beta > 0)$$

gave the best fit to the data for cumulative risk R of silicosis as a function of cumulative exposure in units of (mg/m^3)-yr. In this model x is the cumulative exposure (lagged five years), α is the Weibull scale parameter, and β is the Weibull shape parameter (Table 2). Estimates of α and β for each reader are given in Table II of Muir *et al*. (1989).

Table 2. Silicosis Risk *vs*. Cumulative Respirable Silica in (mg/m^3)-y (Table IV of Muir *et al.*)

Reader	***Cases (n)***	***1% risk*** [a]	***2% risk***	***5% risk***	***10% risk***
1	14	3.5 (2.4-5.1)	5.7 (3.9-8.4)	11.2 (6.8-18.2)	18.6 (9.9-35.0)
2	24	2.7 (2.0-3.6)	4.1 (3.2-5.3)	7.1 (5.5-9.1)	10.9 (8.1-14.8)
3	24	3.0 (2.3-3.9)	4.3 (3.4-5.3)	6.9 (5.6-8.5)	9.9 (7.8-12.7)
4	14	3.7 (2.6-5.2)	5.6 (4.1-7.7)	9.8 (6.7-14.3)	15.1 (9.3-24.4)
5	7	5.7 (4.0-8.0)	7.8 (5.5-11.0)	11.9 (7.8-18.3)	16.5 (9.7-28.2)
Any reader	32	2.1 (1.6-2.9)	3.3 (2.6-4.2)	6.0 (4.8-7.5)	9.6 (7.3-12.5)
At least 3	15	3.5 (2.5-4.9)	5.4 (4.0-7.3)	9.5 (6.6-13.6)	14.6 (9.3-23.2)
All readers	6	6.1 (4.1-8.9)	8.5 (5.6-12.8)	13.2 (7.8-22.5)	18.7 (9.7-36.1)

[a] In parentheses is the 95% confidence interval (CI) for each risk estimate.

The Ontario cohort gives the shallowest dose-response relationship for silicosis of the several cohorts examined (see Summary Table 15 below) due in part to the lack of follow-up of members who left the mines (either for another type of work or for retirement). Silicosis often develops after leaving employment (Hnizdo and Sluis-Cremer, 1993; Chen *et al.*, 2001). In Hnizdo and Sluis-Cremer (1993), for more than half the cases of silicosis radiographic signs developed at an average of 7.4 years after mining exposure ended. In addition, some of the Ontario miners in the Muir et al. study may have changed to a less dusty job if their physician told them that their (annual) radiograph showed abnormalities. The lack of follow-up, leading to under-ascertainment of silicosis, is a serious limitation of this study.

Gray iron foundry workers (Rosenman et al., 1996)

Rosenman et al. (1996) evaluated 1,072 (96.8% males) current and retired workers in a Mid-western gray iron foundry, which produces engine blocks for the automotive industry. Medical records and silica exposure data were analyzed for those with at least 5 years of employment as of June 1991. Nearly half had worked at the foundry for 20 years. Sixty had radiographic evidence of pneumoconiosis (ILO categories 1/0 and greater). Twenty-eight workers had radiographs consistent with silicosis; of these 25 had simple silicosis and three had progressive massive fibrosis. The prevalence of radiographic changes consistent with silicosis increased with years at the foundry, work area, quantitative silica exposure, and cigarette smoking. In regard to quantitative silica exposure, the authors stated that 0.3-2.7% of workers at the OSHA standard (90-100 $\mu g/m^3$) were silicotic, as were 4.9-9.9% of workers above 100 $\mu g/m^3$. After controlling for confounders, Rosenman et al. (1996) used a logistic regression analysis based on cumulative silica exposure to determine an odds ratio of 1.45 for developing a radiograph consistent with silicosis after 20 years of work at 100 $\mu g/m^3$ and an odds ratio of 2.10 after 40 years of work at 100 $\mu g/m^3$ (Tables 3 and 4). This study probably underestimates risk due to lack of follow-up of the current workers. Although silica is not the only toxic chemical in a foundry, the unique nature of the silicotic nodule diminishes the likelihood of confounding by other exposures.

Table 3. Silicosis risk based on Rosenman *et al*. data (Finkelstein, 2000)

Cumulative silica exposure	***Prevalence of silicosis***
< 2 (mg/m^3)-y	0.4%
2-6 (mg/m^3)-y	2.7%
> 6 (mg/m^3)-y	10%

Table 4. Odds ratios for silicosis (from Table 8 of Rosenman *et al.*)[a]

Time-weighted average silica exposure (mg/m^3)	*20-year cumulative exposure [(mg/m^3)-y]*	*Odds ratio (95% C.I.)*	*40-year cumulative exposure [(mg/m^3)-y]*	*Odds ratio (95% C.I.)*
0.010	0.2	1.04 (1.02-1.15)	0.4	1.08 (1.05-1.11)
0.025	0.5	1.10 (1.06-1.14)	1.0	1.20 (1.12-1.30)
0.050	1.0	1.20 (1.12-1.30)	2.0	1.45 (1.25-1.68)
0.075	1.5	1.32 (1.18-1.47)	3.0	1.74 (1.40-2.17)
0.100	2.0	1.45 (1.25-1.68)	4.0	2.10 (1.15-2.82)
0.150	3.0	1.74 (1.40-2.17)	6.0	3.04 (1.96-4.72)
0.200	4.0	2.10 (1.56-2.82)	8.0	4.40 (2.45-7.93)
0.300	6.0	3.04 (1.96-4.72)	12.0	9.24 (3.83-22.3)

[a] Additional mean silica exposures, their calculated odds ratios, and 95% confidence intervals (C.I.) are given in the paper.

Diatomaceous earth workers in California (Hughes *et al.*, 1998; Park *et al.*, 2002)

Hughes *et al.* (1998) investigated 1,809 Caucasian male diatomaceous earth workers in Lompoc, California, who had at least one year of exposure to cristobalite between 1942 and 1987. The crystalline silica isomorph cristobalite is formed when the amorphous silica in diatomaceous earth is calcined at 1000-1100 ºC. Quantitative estimates of dust exposure were made and published in the peer-reviewed literature by Seixas *et al*. (1997) based on 6395 air sampling records taken from 1948-1988. The average estimated respirable dust concentrations for 135 jobs were 3.55 ± 1.25 mg/m^3 prior to 1949, 1.37 ± 0.48 mg/m^3 from 1949-1953, 0.47 ± 0.16 mg/m^3 from 1954-1973, and 0.29 ± 0.10 mg/m^3 from 1974-1988. The workers had periodic chest radiographs. Based on the median of radiographic readings by three independent readers, 81 workers (4.5%) were judged to have opacities on chest radiographs (small opacities, ILO profusion ≥ 1/0, and/or large opacities). Age-adjusted relative risk of opacities increased significantly with cumulative exposure to crystalline silica. The concentration of respirable crystalline silica was an important determinant of risk after accounting for cumulative exposure. The workers were split into two categories: those exposed to < 0.50 mg/m^3 (or hired after 1950) and those exposed to > 0.50 mg/m^3 (or hired before 1950). The risk of opacities for a cumulative exposure to crystalline silica of 2.0 mg/m^3-yr is shown in Table 5.

Table 5. Silica exposure and silicosis based on data of Hughes *et al*. (1998)

Average crystalline silica exposure	*Cumulative risk of silicotic opacities*
< 0.50 mg/m^3 (or hired after 1950)	1.1%
> 0.50 mg/m^3 (or hired before 1950)	3.7%

The findings of Hughes *et al.* (1998) indicate an exposure-response relationship between cumulative exposure to crystalline silica as cristobalite and radiographic opacities. The relationship was substantially steeper among those exposed at the highest average concentrations of crystalline silica. The authors believe that the data do not support the regulatory assumption that cristobalite is more fibrogenic than quartz (i.e., prior to 2000 the occupational limit for cristobalite was half that for quartz), since at average silica levels comparable to other

epidemiologic studies quartz gave a higher incidence of silicosis than did cristobalite in this study. However, since radiography can under-diagnose silicosis, complete accounting for silicosis will require evaluation at autopsy. The ACGIH recently lowered the TLV for alpha-quartz from 100 to 50 μg/m^3, so that it has the same TLV as cristobalite (ACGIH, 2000).

Park *et al*. (2002) carried out a quantitative risk assessment, by Poisson regression methods, of the onset of silicosis among the diatomaceous earth workers in Lompoc. A linear relative risk model gave the best fit to the data. They estimated an excess lifetime risk for radiographic silicosis of 68-75 cases per thousand workers exposed to 50 μg/m^3 silica (cristobalite) for a 45 year work-life, then living to age 85. At 1 μg/m^3 silica the excess lifetime risk was estimated to be 1.6 cases of lung disease other than cancer per thousand workers exposed (Table 6).

Table 6. Excess lifetime risk of silicosis predicted by Park *et al*. (2002)

Silica concentration (mg/m^3)	***45 year cumulative exposure in mg/m^3-y***	***Radiographic silicosis - all workers***	***Radiographic silicosis in workers with < 10 mg/m^3-y***
0.001	0.045	6.2/1000*	1.6/1000
0.005	0.225	17/1000	7.8/1000
0.01	0.45	26/1000	16/1000
0.02	1.8	39/1000	31/1000
0.05	2.25	68/1000	75/1000
0.1	4.5	100/1000	140/1000
0.2	9	150/1000	260/1000

* Excess risk estimates assume that workers were exposed to a constant silica concentration for up to 45 years (ages 20-65). Annual risks are accumulated up to age 85.

White South African gold miners (Hnizdo and Sluis-Cremer, 1993)

Hnizdo and Sluis-Cremer (1993) investigated silicosis risk retrospectively in a cohort of 2,235 white male South African gold miners. Exposure estimates were made for nine separate occupational categories based on a special study of dust levels in these mines done by Beadle in the 1960s (Beadle, 1971). To compensate for the fact that the average hours working in dust ranged among the 9 categories from 4 hours for “other officials” to 8 hours for “shaft sinkers and developers,” exposure was “normalized” to 8-hour shifts. The workers had a minimum of 10 years and an average of 24 years service from 1940 until the early 1970s. Dust levels were fairly constant during this period (see, e.g., Table 2 in Gibbs and DuToit (2002)). The miners had an annual chest radiograph while mining; they were followed until 1991 for radiographic signs of the onset of silicosis. An ILO category 1/1 (definite silicosis) or greater was selected to designate silicosis. Two independent readers initially read the chest films, but only the reader whose interpretations correlated better with autopsy results was used for additional analysis; the use of one reader is a limitation of the study. There were 313 miners (14% of the cohort) who developed radiographic signs of silicosis at an average age of 55.9 years. The latency period was largely independent of the cumulative dust exposure (CDE). In 57% of the silicotics, the radiographic signs developed at an average of 7.4 years after mining exposure ceased. The risk of silicosis determined by chest radiographs increased exponentially with cumulative dust dose. At the highest level of 15-(mg/m^3)-years CDE (approximately 37 years of gold mining at an

average respirable dust concentration of 0.4 mg/m^3), the cumulative risk for silicosis reached 77% as estimated by the accelerated failure time model using the log-logistic distribution (SAS Proc LIFEREG):

$$CR(t) = 1 - \{1/[1 + \exp(-\mu/\sigma) \times t^{(1/\sigma)}]\}$$

where CR(t) = cumulative risk at time t, and μ (2.439) is the intercept and σ (0.2199) is the scale parameter estimated by SAS's LIFEREG procedure. The authors concluded that the risk of silicosis was strongly dose-dependent, but that the latency period was largely independent of dose. The life table analysis (SAS Proc LIFETEST) below (Table 7) shows the number of miners who developed silicosis ("cases"), the number of miners considered by the authors to be at risk, and the risk per unit of CDE (also as calculated by the authors). In the table in column 1 (in parentheses) are OEHHA's determination of the mg/m^3–yr respirable silica exposure, based on Hnizdo and Sluis-Cremer's estimate of 30% silica in the dust, and in column 4 is the total number of miners actually at each midpoint level of CDE or silica. The values in column 4 of Table 7 are the number of workers in the group with the temporally integrated dust exposure in column 1.

Table 7. Life table results - Risk of silicosis per unit Cumulative Dust Exposure (CDE)
(from Table IV of Hnizdo and Sluis-Cremer, 1993)

Midpoint in (mg/m^3)-y of CDE (silica)	***Cases of silicosis***	***Number of workers at risk based on life table***	***Number of workers remaining at this CDE midpoint***	***"Risk/ unit CDE"***	***Mean years in dust***	***Mean dust conc. (mg/m^3)***
1 (0.3)	0	2218	204			
3 (0.9)	9	2014	474	0.002	20.5	0.17
5 (1.5)	48	1540	556	0.016	23.5	0.24
7 (2.1)	85	984	469	0.045	27.2	0.30
9 (2.7)	93	515	318	0.099	28.0	0.33
11 (3.3)	53	197	142	0.156	29.4	0.38
13 (3.9)	20	55	44	0.222	31.5	0.41
15 (4.5)	5	11	11	0.227	37.0	0.42

[a] CDE = Σ number of dusty shifts x mean mass respirable dust conc. x average number of hours spent underground / (270 shifts/year x 8 h/shift)

A plot of risk of silicosis per unit of Cumulative Dust Exposure (CDE) versus the mid-point unit CDE, as given in Figure 1 of the Hnizdo and Sluis-Cremer report, and a plot of % silicosis among the workers actually exposed to a given level of silica (Figure 2), as determined by OEHHA staff, respectively, are given below.

Figure 1. Risk of silicosis per unit CDE vs. CDE mid-point

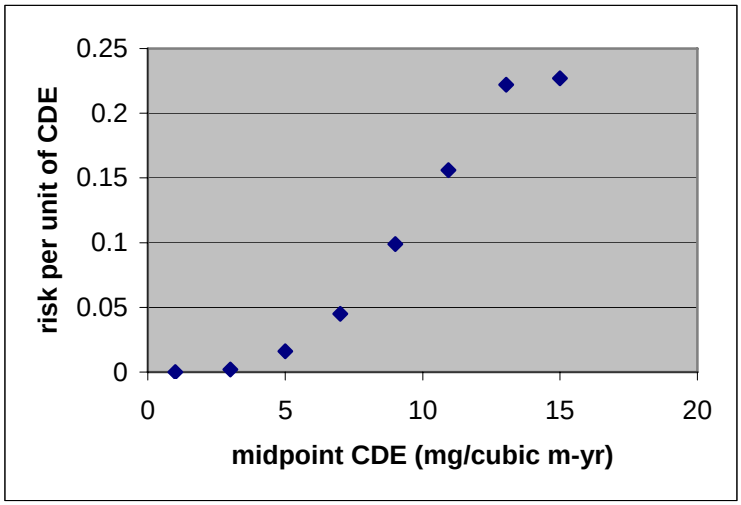
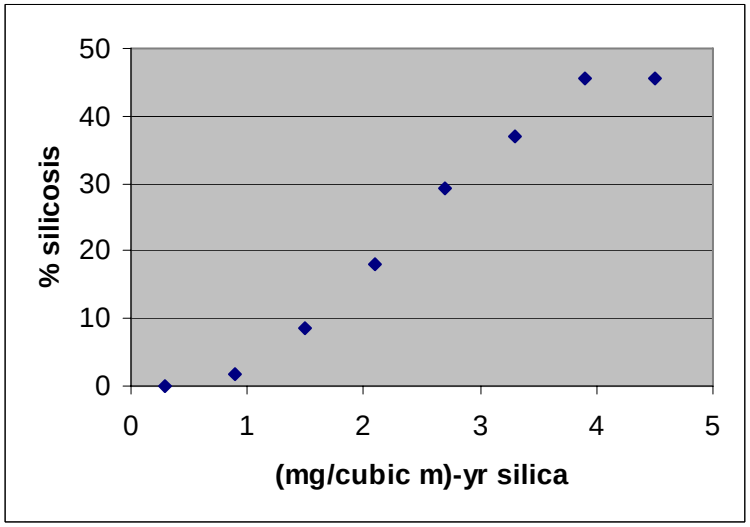


Figure 2. Percent silicosis among workers at each silica level



Black South African gold miners (Churchyard *et al*., 2004; Murray *et al*., 1996)

Black migrant contract workers constitute a large majority (85 - 90%) of South African gold miners. In a cross sectional study, Churchyard *et al*. (2004) interviewed and took chest radiographs of 520 black gold miners (mean age = 46.7 years, range = 37.1 – 59.9) who were still mining (average service = 21.8 years, range 6.3-34.5). Two readers examined the radiographs. As in the Hnizdo and Sluis-Cremer study, silicosis was defined as an ILO (1980) profusion of ≥ 1/1. The mean respirable dust concentration was 0.37 mg/m^3 (0 - 0.70); the mean quartz concentration was 0.053 mg/m^3 (0 - 0.095). The prevalence of silicosis was determined to be 18.3% by one reader and 19.9% by the other (mean 19.1%) (Table 8). This included several workers with more serious silicosis as indicated by ILO profusions ≥ 2/1 (see Table 1).

Significant trends were found between the prevalence of silicosis and: (1) length of service (OR = 1.69 per 5 years), (2) mean intensity of exposure (OR = 1.18 per 0.01 mg/m^3), and (3) cumulative exposure to quartz (OR = 3.2). The study confirms the large burden of silicosis among older black workers in this industry (see next paragraph). The burden is likely to worsen with continuous employment in dusty jobs. For this cohort the prevalence of silicosis will increase even if the miners stop mining immediately. If, as assumed by the authors, the dust levels during the working life of these black miners were constant, silicosis developed while they were exposed to a quartz level below the workplace limit of 0.100 mg/m^3.

Table 8. Silicosis in black gold miners (Churchyard *et al*., 2003; 2004)

Cumulative quartz exposure in mg/m^3-yr	***Mid-point of cumulative quartz exposure***	***Number in quintile****	***Cases of silicosis***	***Percent silicosis***
0 – 0.80	0.4	103	11	10.7
0.80 – 0.99	0.9	97	8	8.2
0.99 – 1.24	1.12	103	18	17.5
1.24 – 1.48	1.36	104	23	22.1
1.48 –3.08	2.28	103	33	32.0
(Total)		(510)**	(93)	(18.2)

* Personal communication from Dr. J. teWaterNaude, December 2, 2004.
** Ten of the 520 films were unreadable.

Murray *et al*. (1996) analyzed data from 16,454 black South African gold miners dying from unnatural causes between 1975 and 1991 in order to study change in prevalence in silicosis and pulmonary tuberculosis (TB). TB prevalence increased from 0.9% in 1975 to 3.9% in 1991, while that for silicosis increased from 9.3% to 12.8%. The prevalence of both increased with age and duration of service. Silicosis was the most significant predictor of TB (OR = 1.78, CI = 1.27 - 2.30, p = 0.0001). A highly significant trend for TB, for year of autopsy, remained after adjustment for other variables, such as age and duration of service (OR = 1.04, CI = 1.01 – 1.06, p = 0.0046). (Another 21,202 black gold miners died of natural causes during the study period.)

<u>Hong Kong granite workers (Ng and Chan, 1994)</u>

Ng and Chan (1994) investigated silicosis among 338 male workers, who had worked at least one year between 1967 and 1985 in two granite quarries in Hong Kong. Three readers examined the chest radiographs. Silicosis was defined as an ILO classification of at least 1/1 (for small rounded opacities) or greater, assigned by at least two of the three readers. Exposure was estimated for each worker based on job category and particle counts. Thirty-six workers (10.6%) were designated silicotic. Both a logistic and a linear model fit the data well. The study suffered because only about half of the previously employed granite workers were studied, which probably led to an underestimate of silicosis risk in at least the highest exposure category and maybe in others. The data are summarized in Table 9.

Table 9. Silica exposure and silicosis in Ng and Chan (Finkelstein, 2000)

Mean cumulative exposure (mg/m^3)-y	*Prevalence of silicosis* [a]
< 1	0%
3.1	13%
7.1	25%
22	22%

[a] rounded opacities determined by at least 2 of 3 readers (Table 3 of Ng and Chan)

Gold miners in South Dakota (Steenland and Brown, 1995)

Steenland and Brown (1995) studied a very large cohort (3330) of white male gold miners in South Dakota, who had worked at least 1 year underground between 1940 and 1965 (average = 9 years underground). The mine dust contained on average 13% silica (range = 1-48%). A job-exposure matrix was created for full-time underground workers grouped into five categories. The authors estimated that most miners were exposed to a median silica level of 0.05 mg/m^3, but that those hired before 1930 were exposed to a median level of 0.15 mg/m^3. A total of 170 cases of silicosis (5.1% of the cohort) was determined from death certificates only (n = 128 cases), from two cross-sectional radiographic surveys in 1960 and 1976 (n = 29 cases; ILO category 1/1 or greater), or from both (n = 13 cases). Unfortunately, only 25% of living cohort members were surveyed radiographically. The life-time risk of silicosis was less than 1% with a cumulative exposure under 0.5 mg/m^3-years and increased to 68% to 84% for the highest cumulative exposure category (more than 4 (mg/m^3)-years) (Table 10).

Table 10. Risk of silicosis for cohort by cumulative exposure (Table 3, Steenland and Brown)

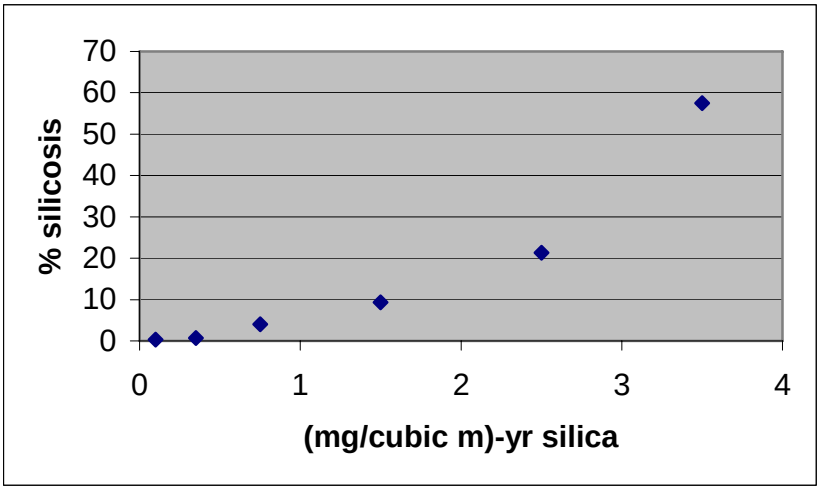
Silica exposure in (mg/m^3)-yrs: range (midpoint)	*Miners with silicosis*	*Number entering exposure category (from life table)*	*Number remaining at this exposure level*	*Cumulative* [a] *Risk*	*Mean years of exposure*	*Mean year first exposed*
0-0.2 (0.10)	5	3330	1530	0.002	2.9	1953
0.2-0.5 (0.35)	5	1800	740	0.005	9.7	1948
0.5-1.0 (0.75)	15	1060	376	0.017-0.022[b]	15.4	1942
1.0-2.0 (1.50)	33	684	353	0.060-0.084[b]	13.2	1931
2.0-3.0 (2.50)	44	331	206	0.167-0.245[b]	18.8	1926
3.0-4.0 (3.50)	42	125	73	0.403-0.534[b]	25.5	1921
>4.0	26	52	52	0.678-0.844[b]	30.6	1914

[a] Cumulative risk = 1−exp[−sum of (hazards * interval width)], where the hazards for each category of cumulative exposure are:
no. cases/(width*(no. entering category – 0.5*no. cases – 0.5*no. withdrawals))
[b] Cumulative risk adjusted for age and calendar time (Steenland and Brown, 1995)

The best predictor of disease was cumulative exposure ((mg/m^3) – years), followed by duration of exposure (years), and then by average exposure (mg/m^3). Figure 1 of Steenland and Brown indicates that a plot of their data for silicosis risk versus cumulative silica exposure was similar

to a plot of the data of Hnizdo and Sluis-Cremer (1993). After adjustment for competing risks of death, Steenland and Brown estimate that a 45-year exposure to 90 - 100 μg/m^3 silica would lead to a lifetime risk of silicosis for gold miners of 35% to 47%. A limitation of this study is the reliance on death certificates rather than on ILO interpretation of radiographs. In addition, no mention was made of validating the data on the death certificates. It was also not clear what, if any, autopsy data were available. A plot of silicosis incidence among the workers (as determined by OEHHA staff) actually exposed to the estimated level of silica is given in Figure 3 below. An accompanying editorial (Wagner, 1995) commended the article for estimating both the risk of silicosis while working and the lifetime risk of silicosis resulting from exposure during work.

Figure 3. % Silicosis vs. silica exposure in Steenland and Brown (see Table 10)



Miners in Leadville, Colorado (Kreiss and Zhen, 1996)

Kreiss and Zhen (1996) investigated the exposure-response relationships for silicosis among 134 male miners over 40 years old in Leadville, Colorado. The men had been studied three years earlier in a random sample of respiratory disease in their community (Kreiss *et al*., 1989). Of 100 dust-exposed miners, 32 had radiological profusions of small opacities of ILO category 1/0 or greater at a mean of 36.1 years since their first silica exposure. Of miners with cumulative silica, exposures of 2 (mg/m^3)-years or less, 20% had silicosis while 63% of miners accumulating greater than 2 (mg/m^3)-years had silicosis. Average silica exposure was also strongly associated with silicosis prevalence rates (Table 11).

Table 11. Miners studied by Kreiss and Zhen (1996)

Average silica exposure	***% silicotics***
0.025-0.05 mg/m^3	13% (5/38)
> 0.05-0.1 mg/m^3	34% (15/44)
> 0.1 mg/m^3	75% (9/12)
Cumulative silica exposure	*% silicotics*
≤ 2 (mg/m^3)-y	20% (14/70)
2 – 4 (mg/m^3)-y	63% (15/24)

Based on logistic regression models of the form $R(x) = [1 + \exp(-\alpha - B'x]^{-1}$, Kreiss and Zhen concluded that the risk of silicosis was best predicted by elapsed time since last silica exposure together with either (1) cumulative silica exposure or (2) a combination of average silica exposure and duration of exposure. Exposure-response relationships were substantially higher using measured silica exposures (compared to using estimated silica exposures based on measured total dust exposures and assuming a constant silica proportion of dust). The risk of silicosis in this study is higher than in workforce studies having no follow-up of those leaving the mining industry (e.g., Muir *et al*., 1989) and in studies without job title-specific silica measurements (e.g., Hnizdo and Sluis-Cremer, 1993). However, the risk is comparable to several recent studies of exposure-response relationships for mining dust (e.g., Ng and Chan, 1994; Steenland and Brown, 1995) (see Summary Table 15 below). A limitation relative to other studies is the small number of subjects (100) in the group.

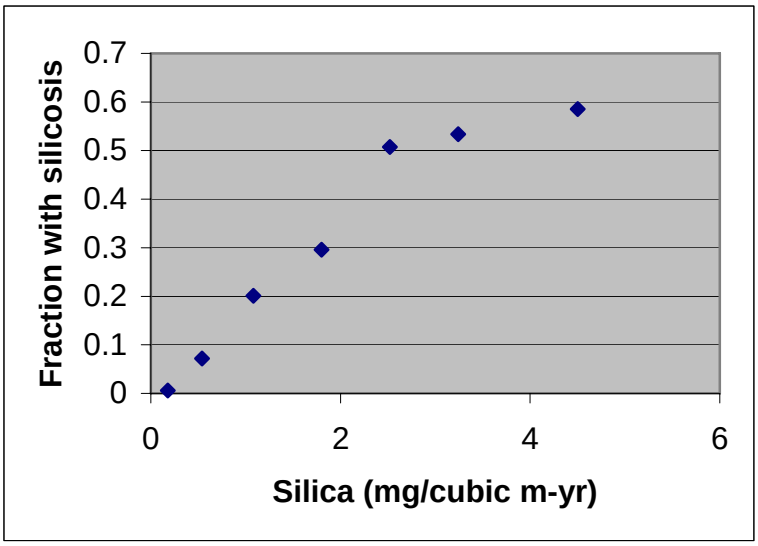
Chinese tin miners (Chen *et al.*, 2001)

Chen *et al.* (2001) found a clear exposure-response relationship between silica dust exposure and silicosis in a cohort of 3010 (2795 male and 215 female) miners employed for at least 1 year during the period 1960-1965 in any of four Chinese tin mines. No other diseases due to silica or tin were mentioned. Each cohort member was followed through 1994. Historical Chinese total dust (CTD) data were used to create a job exposure matrix for each facility, job title, and calendar year. The CTD data were converted to estimates of respirable crystalline silica for comparison with findings from other epidemiological studies of silicosis (including some of those above). Each miner's work history was abstracted from employment records. The diagnosis of silicosis was based on 1986 Chinese Roentgen diagnostic criteria for pneumoconiosis. The criteria classified silicosis as stages I-III, similar to an ILO classification of 1/1 or greater. Of the 3010 miners, 1015 (33.7%) were identified as silicotic (mean age = 48.3 years, with a mean of 21.3 years after first exposure) (Table 12). Among the silicotics, 684 (67.4%) developed silicosis after their tin mine exposure had ended (mean = 3.7 years after). The risk of silicosis was strongly related to cumulative exposure to silica. The Weibull distribution gave a very good fit to the data. The risk of silicosis was less than 0.1% when CTD was less than 10 (mg/m^3)-yr (= 0.36 (mg/m^3)-yr of respirable crystalline silica). The risk of silicosis increased to 68.7% when CTD exposure was equal to 150 (mg/m^3)-yr (= 5.4 (mg/m^3)-yr of respirable crystalline silica). Latency period was not correlated to the risk of silicosis or to cumulative dose. From their data, the authors predicted a 55% risk of silicosis for 45 years exposure to 0.1 mg/m^3 respirable crystalline silica, the workplace exposure limit (4.5-(mg/m^3)-years silica). Figure 4 plots the fraction of the workers in Chen et al. with silicosis (column 2 in Table 12 divided by column 4) exposed to a given level of silica (mid-point – in parentheses in column 1 of Table 12), as calculated by OEHHA staff.

Table 12. Cumulative silicosis risk based on cumulative total dust (CTD) (Table 5, Chen *et al*., 2001)

Range of CTD exposure in (mg/m^3)-y/ (silica mid-point)	*Cases of silicosis (n)*	*Workers entering category*	*Workers at this level of CTD/silica*	*Cumulative risk based on Weibull model*	*Mean net exposure (years)*	*Mean latency (years)*
<10 (0.18)	2	3010	333	0.001	2.2	14.7
10-19.99 (0.54)	24	2677	334	0.010	5.3	21.3
20-39.99 (1.08)	126	2343	626	0.070	9.3	22.0
40-59.99 (1.80)	127	1717	429	0.145	11.9	21.5
60-79.99 (2.52)	196	1288	386	0.285	9.9	20.3
80-99.99 (3.24)	141	902	264	0.405	10.8	19.0
100-149.99 (4.50)	244	638	417	0.663	13.1	20.4
≥ 150 (≥ 5.4)	155	221	221	0.917	15.7	25.4

Figure 4. Percent silicosis vs. silica level from Chen *et al*.



<u>Industrial sand workers (McDonald et al., 2001; Hughes et al., 2001; Rando et al., 2001)</u>

McDonald *et al*. (2001) studied a cohort of 2670 men employed before 1980 for 3 years or more and followed through 1994 in one of nine North American sand-producing plants and in a large associated office complex (since most of the office employees had previously worked in the mines). They found 37 deaths due to silicosis and silicotuberculosis. The mean exposure of the cohort was 42 µg/m^3 silica (Rando *et al.*, 2001). Odds ratios for silicosis mortality, determined using conditional multiple logistic regression (SAS software), were significantly related to cumulative silica exposure (Hughes *et al.*, 2001) (Table 13). The odds ratios are in general agreement with those in the gray foundry workers of Rosenman *et al.* (1996) (Table 4).

Table 13. Median cumulative silica exposure and odds ratio (Table 3 in Hughes *et al*., 2001)

No lagging			*Lagged 15 yr*		
Median exposure in (mg/m^3)-y	***Silicotics (n)***	***Odds ratio [a] for mortality***	***Median exposure in (mg/m^3)-y***	***Silicotics (n)***	***Odds ratio [a,b] for mortality***
0.832	7	1.00	0.142	7	1.00
2.744	7	1.27	1.229	7	2.54
6.916	8	2.62	2.583	7	4.55
12.084	7	2.13	7.990	8	5.16

[a] Matched odds ratio relative to lowest cumulative exposure category. Although labeled a cohort study, the data analysis compared cases of silicosis with non-silicotic controls.
[b] Significant increasing trend across exposure categories (see Hughes *et al*. for more details)

Ceramic workers (Cavariani *et al*., 1995; Legrand-Cattan *et al*. (1998)

Cavariani *et al*. (1995) investigated the incidence of silicosis among 2,480 men in the ceramics industry in central Italy. The workers were surveyed during the period 1974-1987 and followed through 1991 with annual chest radiographs. The cumulative risk of silicosis (ILO category 1/1 or greater) was 48% after 30 years of employment. A multivariate Cox's proportional hazards model indicated that silicosis increased linearly up to the period of 25-29 years employment. A hazard risk of 14.6 was found comparing those with ≥ 30 years exposure to those employed 10 years. Smoking significantly contributed to the model, but its role was unclear.

Legrand-Cattan *et al*. (1998) examined the dose-response relationship in two French ceramic plants. A 1992 cross-sectional study included more than 200 silica-exposed workers. Three ILO certified B readers read chest radiographs. Silica was sampled in the airborne dust. The results are tabulated below (Table 14).

Table 14. Silicosis in two French ceramic plants (Legrand-Cattan *et al*., 1998)

Cumulative exposure to silica in (mg/m^3 – years)	***Number of workers at this level***	***Number with small opacities with ILO profusion ≥ 1/0***	***Percent***
< 0.35	50	2	4
0.35 – 1.08	57	8	14
1.09 – 1.77	55	11	20
> 1.77	55	17	31
Total	217	38	(18)

A dose response relationship is clear; the authors reported a p value of 0.002. However, the study is limited by the lack of follow-up of the workers.

Slate workers (Glover *et al*., 1980; Saiyed *et al*., 1985; Saiyed and Bannerjee, 1985)

Slate contains calcium carbonate, iron oxides, silicates, amorphous silica, and crystalline silica. Glover *et al*. (1980) studied slate workers in North Wales. The respirable slate dust contained 13-32% crystalline silica. In the study group were 725 current and former workers exposed only to slate dust, while the controls were 530 men from the same area who had never been exposed to dust. Pneumoconiosis was found in 239 slate workers (33 %), and 10% had degrees of pneumoconiosis (category 2 or higher using the 1971 ILO scheme) that would bring worker's compensation. The prevalence of respiratory symptoms (cough, phlegm, dyspnea) was high. There was evidence of an effect of both simple and complicated pneumoconiosis on lung function (declines in FVC and FEV_1) additional to the effect of age. The high prevalence (40-50%) of radiological lesions suggested the presence of healed tubercular lesions in men over 55. Either pneumoconiosis or old tubercular lesions (or both) could account for the symptomatology and disability of the men.

Saiyed *et al*. (1985) surveyed the slate-pencil industry in India. An industrial hygiene survey revealed very high levels of free silica (2-10 mg/m^3), while a medical survey showed that 324 of 593 workers (54.6%) had silicosis. Of these, 105 had "conglomerate" silicosis (progressive massive fibrosis, PMF). Some lung lesions were detectable after less than five years of exposure to slate dust. Saiyed and Bannerjee (1985) conducted a follow-up examination 16 months later. The progression of silicosis was very rapid, and a total of 23 workers had died during this period (mean age = 34.7 years; mean exposure = 11.9 years). The authors attributed the high mortality to high levels of silica leading to early onset of PMF. The progression of silicosis was related to the intensity and duration of dust exposure, and to the severity of silicosis found initially.

Silicosis has been reported in other groups of slate workers in Norway (Bang and Suhr, 1998; Suhr *et al*., 2003) and in Germany (Mehnert *et al*., 1990).

Silica particle size

Data on silica particle size in the various workplaces are limited. According to Witschi and Last (2001), silica particles with a diameter of 1 μm (range = 0.5 - 3 μm) appear to be the most fibrotic in humans. NIOSH (1974) reviewed the existing literature and found that in five diatomite plants the mean silica diameter was 1.1 μm (range = 0.5 - 2 μm). For nine potteries, the particle size was 1.2 μm. For 18 foundries, more than 90% of the particles were less than 3 μm. The majority of particles to which shipyard sandblasters were exposed was also less than 3 μm. In the Vermont granite sheds, 10 mppcf (million particles per cubic foot) granite dust were initially estimated to be equal to 0.1 mg/m^3 respirable quartz. Steenland and Brown (1995) used this estimate for silica in South Dakota gold mines. Assuming that the density of quartz is 2.65 g/cm^3 and that the quartz particles are spherical, the data indicate that the particles have a diameter of 0.59 μm. NIOSH (1974) listed 0.94 μm as the median particle size in metal mines. No indication was given of the dispersion of the particle sizes around the average value. Davis *et al*. (1983) used the value of 10 mppcf in granite sheds as equal to 0.075 mg/m^3 silica. For that estimation, OEHHA staff calculated the particle diameter to be 0.53 μm. Thus, existing data indicate that the majority of silica in the workplace is respirable. In most of the occupational studies examined, the exposures were measured using a calibrated cyclone sampler similar to that recommended in the current NIOSH (2003) method. This allows collection of particles

primarily in the 0.5 – 5 μm range, with a collection efficiency profile intended to match the penetration of particles into the alveolar region of the human lung. In the case of the South African gold mine studies (Beadle, 1971; Page-Shipp and Harris, 1972; Hnizdo and Sluis-Cremer, 1993), particle number was determined by an optical method selecting respirable particles (range of 0.5 to 5 μm). Thus, the risk estimates obtained from these studies refer to particles in the size range where penetration occurs into the respiratory region of the lung. This corresponds to the size range of particles thought to be responsible for silicosis. It differs from the definition of "respirable" particles (*i.e.* PM_{10}) commonly used in environmental measurements, which refers to particles capable of penetrating anywhere in the lower respiratory tract (described as "thoracic" particles in occupational studies).

Risk estimation for silicosis from epidemiologic studies

The data from the above studies have been used by a number of investigators (Finkelstein, 2000; Chen *et al*., 2001; Hughes, 1995) and by OEHHA staff to estimate percent silicosis based on cumulative silica exposure in units of (mg/m^3)-yr. The results are summarized in Table 15.

In Table 15, more than 14,000 workers were studied, of whom approximately 12% were classified as silicotic. The 12% is likely an underestimate of the incidence of silicosis due to lack of follow-up by chest radiographs during life in some cohorts and to the lack of an autopsy after death.

Table 15. Summary - Estimates of % silicosis based on cumulative silica exposure in (mg/m^3)-y

Study	***Population (number with silicosis)***	***Exposure of 2 (mg/m^3)-y***	***Exposure of 4 (mg/m^3)-y***	***Exposure of 4.5 (mg/m^3)-y***
Muir *et al*., 1989	2109 male Ontario hard rock miners (“15”)	0.4[a,c]	1.2[a,c]	2[b]
Rosenman *et al*., 1996	1072 Midwestern foundry workers (28)	2[a]	10[a]	3[b]
Graham *et al*., 1991	408 Vermont granite workers (35)	~3[c]	—	—
Hughes *et al*., 1998	1809 white male diatomaceous earth workers (81)	1.1 (low intensity) 3.7 (high intens.)[a]	4 (low) 12 (high)[a]	—
Park *et al*., 2002	2342 white male diatomaceous earth workers (80)	~7[e]	13[e]	14[e]
Hnizdo & Sluis-Cremer 1993	2235 white male South African gold miners (313)	5[a] 10[c]	52[a] 60[c]	77[b]
Ng & Chan, 1994	338 male Hong Kong granite workers (36)	6[a]	15[a]	15-20[b]
Steenland & Brown, 1995	3330 male S. Dakota gold miners (170)	8[a]	53[a]	70[b]
Kreiss & Zhen, 1996	100 miners in Leadville, CO (32)	11[a]	53[a]	92[b]
Chen *et al*., 2001	3010 Chinese tin miners (1015)	14[d]	47[d]	55[b]
Churchyard *et al*., 2004	510 black gold miners (93)	~28	-	-

[a] From Table II of Finkelstein (2000)
[b] From Table 6 of Chen *et al*. (2001)
[c] From Tables 3 and 4 of Hughes (1995)
[d] Interpolated by OEHHA staff from Fig. 2 of Chen *et al*. (2001).
[e] Estimated by OEHHA staff from Table 4 of Park *et al*. (2002)
[f] 158 had an ILO reading ≥ 1/0, while 103 had an ILO reading ≥ 1/1.

Determination of LOAEL and NOAEL for silicosis (Rice and Stayner, 1995)

In another approach to the data, Rice and Stayner (1995) identified the NOAEL and LOAEL for silicosis in several studies (Table 16). The study of Hnizdo and Sluis-Cremer (1993) yielded both a LOAEL and a NOAEL.

Table 16. Estimates of NOAELs and LOAELs for silicosis (Rice and Stayner, 1995)

Study	***Subjects***	***NOAEL in μg/m³***	***LOAEL in μg/m³***
Davis *et al*., 1983	969 granite workers	67.5	
Hnizdo and Sluis-Cremer, 1993	2235 gold miners	7	20
McDonald and Oakes, 1984	1321 gold miners	-	8[a]
	64 gypsum miners	35	49
Muir *et al*., 1989	2109 gold miners	Could not determine	Could not determine
Rice *et al*., 1986	888 dusty trade workers	80-100	200-252

[a] McDonald and Oakes (1984) considered this value to be only an approximation.

Proposals to change the occupational exposure limit

Silicosis is still being diagnosed at death in workers who were supposed to be exposed to occupational levels of 50-100 μg/m^3. Thus, there have been recommendations that the occupational exposure limit for respirable, crystalline silica (specifically alpha-quartz) be lowered from the current level of 100 μg/m^3 to 50 μg/m^3 (NIOSH, 1974; Rosenman *et al*., 1996; ACGIH, 1999; Finkelstein, 2000). In 2000, the ACGIH lowered its TLV for quartz from 100 to 50 μg/m^3. In 1986, WHO recommended that the occupational level be set at 40 μg/m^3 (WHO, 1986). Greaves (2000) recommended that the TLV be lowered to 10 μg/m^3. Based on existing data Greaves (2000) estimated that at 10 μg/m^3 the incidence rate for ILO grade 1/0 silicosis would be less than 5%, while for grade 1/1 it would be less than 2%. Chen *et al*. (2001) recommended that the TLV be lowered to 5 μg/m^3. "If the lifetime risk of silicosis is to be under 1 in 1000 (a criterion used by OSHA) for a lifetime exposure of 45 years, then the mean Chinese total dust concentration must be lower than 0.14 mg/m^3 (or lower than 0.005 mg/m^3 respirable crystalline silica)" (Chen *et al*., 2001). Mannetje *et al*. (2002) pooled data from six occupational cohorts. These included four groups discussed above: diatomaceous earth workers, Vermont granite workers, U.S. industrial sand workers, and South Dakota gold miners. Among them 170 deaths from silicosis were reported. The estimated mortality risk from silicosis to age 65 after 45 years of exposure at 100 μg/m^3 silica was 13 per 1000, while the risk of death at 50 μg/m^3 was estimated at 6 per 1000. Both estimates are above the 1 per 1000 risk acceptable to OSHA. Mannetje *et al*. also concluded that the occupational standards for silica should be lowered, but they did not specify a level. They further state that their estimates of silicosis mortality are probably underestimates due to exposure misclassification and to outcome misclassification, since deaths due to silicosis might have been coded to tuberculosis or chronic obstructive pulmonary disease.

C. Silica exposure and lung cancer in workers

In 1997, IARC classified respirable crystalline silica in Class 1, a Known Human Carcinogen, based on occupational epidemiologic studies. However, chronic RELs are not based on cancer endpoints. Further, there is no approved cancer potency factor for silica.

V. Effects of Animal Exposures

Several papers have reported that freshly fractured quartz, which has increased surface activity, causes greater inflammation than "aged" quartz. Vallyathan *et al*. (1991) reported that “fresh” silica was 4.2-fold more potent than silica aged for 1-2 days in decreasing the membrane integrity of male rat macrophages; 50% more potent in activating hydrogen peroxide secretion by macrophages; and 4.6-fold more potent in stimulating cellular chemiluminescence. Vallyathan *et al*. (1995) reported that inhalation of 19.3 mg/m^3 aged (for 2 months) quartz for five hours/day for 10 days by male Fischer 344 rats increased the number of cells recoverable by bronchoalveolar lavage (BAL) (Table 17). Aged quartz also gave histopathological evidence of increased pulmonary infiltrates, showed higher levels of biochemical markers of lung injury, increased lipid peroxidation, and increased the ability of pulmonary phagocytes to produce more oxygen radicals than air-exposed controls. These pulmonary responses were significantly more pronounced after inhalation of 22.4 mg/m^3 freshly fractured quartz.

Table 17. Cells recovered in bronchoalveolar lavage from rats (Vallyathan *et al*., 1995)

Cell type	*Room air*	*Aged quartz*	*Freshly fractured*	*Fresh/aged*
Total cells	7.1±0.78*	9.3±1.2	20.4±2.2	2.2
Macrophages	6.7±0.69	4.7±0.79	5.4±0.78	1.1
Neutrophils	≥ 0.038	5.3±0.66	10.4±1.44	2.0
Lymphocytes	≥ 0.038	1.7±0.25	3.6±0.27	2.1
Red blood cells	≥ 0.038	1.7±0.26	6.0±0.57	3.5

* Cell counts are in millions. Each value is the mean ± standard error of 5 rats.

Burns *et al.* (1980) exposed female Balb/c mice for up to 39 weeks to 4.9 mg/m^3 Min-U-Sil brand crystalline silica. By 24 weeks, silica-laden macrophages were present in the lungs. After 39 weeks of exposure, silicotic lesions were seen in the lungs and adjacent lymph nodes (Table 18).

Davis *et al*. (1998) exposed mice to an aerosol of cristobalite silica (mass median aerodynamic diameter (MMAD) = 1.7 μm) for five hours/day in order to examine (1) the effects of exposure dose, (2) the evolution of disease over time, and (3) the variation in responses among strains. In C3H/HeN mice, incremental, cumulative exposure doses of cristobalite (10 mg/m^3 for 8 days, 43 mg/m^3 for 9 days, and 70 mg/m^3 for 12 days) caused (1) increased initial lung dust burden at 12 to 16 weeks post-exposure, (2) progressively intense pathological responses, and (3) increased total lung collagen (as measured by hydroxyproline).

The histopathological changes and total lung collagen increased with time after exposure. Silicosis was compared in four inbred strains of mice (BALB/c, C3H/HeN, MRL/MpJ, New Zealand Black) 16 weeks after aerosol inhalation exposure to cristobalite (70 mg/m^3, 5 hours/day, 12 days). C3H/HeN mice had histopathological silicotic lesions, enlarged

intrapulmonary lymphoid tissue, and increased lung wet weight, increased bronchoalveolar lavage (BAL) recoverable macrophages, lymphocytes, and neutrophils, and increased total lung collagen (hydroxyproline analyses). BALB/c mice developed slight pulmonary lesions. MRL/MpJ mice showed prominent pulmonary infiltrates with lymphocytes. New Zealand Black (NZB) mice developed extensive alveolar proteinaceous deposits, inflammation, and fibrosis. The authors found both dose-time-response relationships and a substantial variation of responses among mouse strains to the high level, short duration exposure.

At Brookhaven National Laboratory, groups of Fischer 344 rats were exposed to 0, 2, 10, and 20 mg/m^3 Min-U-Sil brand silica (alpha-quartz) for six months (Kutzman, 1984a; as summarized by USEPA, 1996). Other groups of rats had the same exposure, but were allowed to "recover" in air for an additional 6 months (Kutzman, 1984b; as summarized by USEPA, 1996). Significant alterations in total lung weight, total lung collagen, total elastin per unit lung dry weight, and total protein per unit lung dry weight at 2 mg/m^3 silica and microscopic evidence of silicotic lesions at the higher silica levels indicated that 2 mg/m^3 was a LOAEL for silica effects. After six months in clean air, the silica-induced lesions appeared to worsen.

Muhle *et al*. (1989) exposed groups of 50 male and 50 female rats to 1 mg/m^3 DQ12 quartz six hours/day, five days/week for 24 months. DQ12 contains 87% crystalline alpha-quartz, has a mass median aerodynamic diameter (MMAD) of 1.3 µm, and is 74% respirable. Moderate fibrosis was seen in 85 animals, slight fibrosis in 13, and very slight fibrosis in 1 (total rats with fibrosis = 99/100). Varying amounts of peribronchial granulomatous foci were noted in 95 rats.

Muhle *et al*. (1998) reported lung fibrosis in hamsters exposed to 3 mg/m^3 DQ12 silica. After 18 months of exposure to DQ12 for 6 h/day, 5 days/week, all hamsters in the group of 15-19 animals necropsied had very slight fibrosis. Approximately 100 silica-exposed animals were exposed for five more months to air only. Afterward 22.2% had very slight fibrosis, 68.7 % had slight fibrosis, and 1% had moderate fibrosis (i.e., more than 90/100 hamsters had lung fibrosis). No collagen measurements were reported. Thus, rats, mice, and hamsters show pulmonary fibrosis after crystalline silica exposure at and above 1 mg/m^3.

Wagner *et al*. (1968) exposed dogs up to 2.5 years, guinea pigs up to 18 months, and rats up to 2 years for 6 hours/day, 5 days/week to 61% cristobalite (in calcined diatomaceous earth). Dust exposures were 2 and 5 million particles per cubic foot (mppcf), equivalent to 0.2 and 0.5 mg/m^3 cristobalite (USEPA, 1996), with occasional excursions to 50 mppcf. No lung fibrosis was detected at these levels but all levels caused accumulation of inflammatory cells in the lung parenchyma. However, in dogs fibrotic nodules developed in the hilar lymph nodes with more nodules at 5 mppcf than at 2 mppcf.

Scheuchenzuber *et al*. (1985) examined immunologic responses in Balb/c mice following inhalation of 1.954 mg/m^3 silica for 150, 300, or 570 days. Mice exposed for 570 days were tested immediately post-exposure. Those exposed for 150 or 300 days were tested immediately or were rested for 30 or 150 days to allow for possible recovery from effects of dust inhalation. Silica inhalation suppressed the number of specific plaque-forming cells (PFC) in the spleen produced in response to aerosolized *E. coli*. After 570 days of inhalation, silica also reduced the ability of alveolar macrophages to phagocytize *Staphylococcus aureus in vitro* and impaired the ability to lyse allogeneic tumor cells (from mice other than Balb/c) *in vitro*. Silica inhalation did

not affect antibody-dependent cell-mediated cytotoxic and mitogenic responses by splenic lymphocytes. (Fibrosis was not an endpoint measured, but the effect level is similar to the LOAELs in other animal studies.)

Table 18. Animal studies of silica inhalation analyzed by USEPA (1996)

Study	***Species***	***Duration*** [a]	***LOAEL***
Muhle *et al*., 1989	Rat	24 mo	1.0 mg/m^3
Scheuchenzuber *et al*., 1985	Mice	150-570 d	2.0
Burns *et al*., 1980	Mice	3-39 wk	4.9
Kutzman, 1984a	Rat	6 mo	2.0
Kutzman, 1984b	Rat	6 mo + 6 mo recovery	2.0
Wagner *et al*., 1986	Dog	Up to 2.5 yr	0.2

[a] Inhalation exposure was generally for 6 h/day, 5 d/wk.

Quartz has the ability to induce the generation of free radicals and to cause oxidative stress in tissues. Many substances that affect the quartz surface can modify this ability. Some of these modifiers could originate from other minerals, which exist together with quartz in nature. Donaldson and Borm (1998) proposed that the hazard posed by quartz may vary widely depending on the origin of the silica sample or on its contact with other chemicals/minerals. Such mechanistic data could assist in the interpretation of epidemiological studies such as those above. Experimentally their group found that DQ12 quartz, a European quartz standard which is often used in experimental studies of silica effects, is much more inflammatory in rat lung than respirable silica collected from two workplaces (Clouter *et al*., 2001).

Humans appear to show adverse effects of silica exposure at lower levels than animals (compare LOAELs in Table 18 to LOAELs/NOAELs in Table 16). Rodents tend to be obligate nose-breathers and to have extensive nasal turbinates, which may result in less silica reaching the lower lung. For silica, results in animals may not be a good predictor of human effect levels.

VI. Derivation of Chronic Reference Exposure Level (REL)

Key study	Hnizdo and Sluis-Cremer, 1993
Study population	2235 white South African gold miners
Exposure method	Workplace inhalation
Critical effects	Silicosis (313 miners) (14 %)
LOAEL	3 mg/m^3-years CDE (9 miners with silicosis)
NOAEL	2 mg/m^3-years CDE (0 miners with silicosis) or 600 μg/m^3-years silica (dust = 30% silica)
$BMCL_{01}$	2.12 (mg/m^3)–yr CDE or 0.636 (mg/m^3)–yr silica
Exposure continuity	8 h/day, 5 d/wk
Exposure duration	Average of 24 years dust exposure (10-39 years)
Average experimental exposure	235 μg/m^3-yr silica at BMC_{01} (636 x 10 m^3/20 m^3 x 270 shifts/365 days) 235 μg/m^3-yr/24 yr = 9.8 μg/m^3
Human Equivalent Concentration (HEC)	9.8 μg/m^3
LOAEL uncertainty factor	Not needed in BMC approach
Subchronic uncertainty factor	1
Interspecies uncertainty factor	1
Intraspecies uncertainty factor	3
Cumulative uncertainty factor	3
Inhalation Reference Exposure Level	3 μg/m^3 (based on 30% silica in mine dust) [respirable, as defined occupationally by ACGIH/ISO]

First supportive study	Steenland and Brown, 1995
Study population	3330 S. Dakota gold miners
Exposure method	Workplace inhalation
Critical effects	Silicosis (170 miners) (5.1%)
LOAEL	0-0.2 mg/m^3-years (5 miners with silicosis)
NOAEL	Not found
$BMCL_{01}$	0.34 (mg/m^3)–yr (see text below)
Exposure continuity	8 h/day, 5 d/wk
Exposure duration	3-36 years (average 9 years underground)
Average experimental exposure	112 μg/m^3-y (340 x 10 m^3/20 m^3 x 5 d/7 d x 48 wk/52 wk) 112 μg/m^3-y/9 y = 12.4 μg/m^3
Human Equivalent Concentration (HEC)	12.4 μg/m^3
LOAEL uncertainty factor	Not needed in BMC approach
Subchronic uncertainty factor	1
Interspecies uncertainty factor	1
Intraspecies uncertainty factor	3
Cumulative uncertainty factor	3
Inhalation Reference Exposure Level	4 μg/m^3 [respirable, as defined occupationally]

Second supportive study	Hughes *et al*., 1998
Study population	1809 California diatomaceous earth workers
Exposure method	Workplace inhalation
Critical effects	Silicosis (81 workers) (4.5%)
LOAEL	> 1, ≤ 3 mg/m^3-years (17 workers with silicosis)
NOAEL	≤ 1 mg/m^3-years (6 cases). (Six cases were observed, but Hughes *et al*. assigned the group a Relative Risk = 1 for silicosis.)
Exposure continuity	8 h/day, 5 d/wk
Exposure duration	1-45 years (mean = 11.5 years)
Average experimental exposure	≤ 330 μg/m^3-y (1000 x 10/20 x 5/7 x 48/52) ≤ 330 μg/m^3-y/ 11.5years = ≤ 29 μg/m^3
Human Equivalent Concentration (HEC)	29 μg/m^3
LOAEL uncertainty factor	3 (authors' NOAEL actually is a LOAEL)
Subchronic uncertainty factor	1
Interspecies uncertainty factor	1
Intraspecies uncertainty factor	3
Cumulative uncertainty factor	10
Inhalation Reference Exposure Level	3 μg/m^3 [respirable, as defined occupationally]

Third supportive study	Chen *et al*. (2001)
Study population	3010 Chinese tin miners
Exposure method	Workplace inhalation
Critical effects	Silicosis (1015 workers) (33.7 %)
LOAEL	10-19.99 mg CTD/m^3-years (24 cases)
NOAEL	≤ 10 mg CTD/m^3-years (2 cases) ≤ 360 μg silica/m^3 - years
BMCL$_{01}$	132 μg silica/m^3 - years
Exposure continuity	8 h/day, 5 d/wk
Exposure duration	2.2 years for NOAEL group
Average experimental exposure	40 μg/m^3-y (132 x 10/20 x 5/7 x 48/52) 40 μg/m^3-y/2.2 years = 18 μg/m^3
Human Equivalent Concentration (HEC)	18 μg/m^3
LOAEL uncertainty factor	Not needed in BMC approach
Subchronic uncertainty factor	1
Interspecies uncertainty factor	1
Intraspecies uncertainty factor	3
Cumulative uncertainty factor	3
Inhalation Reference Exposure Level	6 μg/m^3 [respirable, as defined occupationally]

Fourth supportive study	Churchyard *et al*., 2004
Study population	510-520 black South African gold miners
Exposure method	Workplace inhalation
Critical effects	Silicosis (93 cases)
LOAEL	0-0.80 mg/m^3-yr (11 cases)
NOAEL	Not identified
BMCL$_{05}$	0.673 (mg/m^3)-yr
Exposure continuity	270 shifts/year
Exposure duration	21.8 yr (6.3-34.5)
Average experimental exposure	249 (μg/m^3)-yr (673 x 10/20 x 270shifts/365) 249 (μg/m^3)-yr/21.8 yr= 11.4 μg/m^3
Human equivalent concentration (HEC)	11.4 μg/m^3
LOAEL uncertainty factor	Not needed in BMC approach
Subchronic uncertainty factor	1
Interspecies uncertainty factor	1
Intraspecies uncertainty factor	3
Cumulative uncertainty factor	3
Inhalation Reference Exposure Level	4 μg/m^3 [respirable, as defined occupationally]

The study of 2235 white South African gold miners by Hnizdo and Sluis-Cremer (1993) not only determined a NOAEL of 2 (mg/m^3)-yr CDE (600 μg/m^3-yr silica), but also had sufficient dose-response data for a BMC derivation. This study was powerful enough to detect a 1.9% incidence of silicosis (9 cases out of 474 exposed) at 0.9 mg/m^3-yr silica (0/204 vs. 9/474, p = 0.064 by Fisher exact test, two-tailed). Because this incidence represents approximately the sensitivity limit of the data, and silicosis is a severe irreversible endpoint, the BMCL$_{01}$ (*i.e.*, the lower bound estimate of the concentration at which 1% of the population develops silicosis) was selected as the basis of the chronic REL. In benchmark analysis of chronic animal studies, BMCL$_{05}$ is typically regarded by OEHHA as equivalent to a NOAEL. However, the power of this large-scale study is sufficient to demonstrate measurable responses below the 5% incidence level (which cannot then be logically considered a no-effect level). Furthermore, the endpoint measured in this epidemiological study is considered to be severe, since it represents the occurrence of clinically recognizable and irreversible disease, rather than an adverse physiological or biochemical response or a histopathological result seen at autopsy.

Benchmark Concentration (BMC) models, developed by the USEPA (BMDS versions 1.3, 1.3.1, and 1.3.2), were fit to the human data in Hnizdo and Sluis-Cremer (1993) (Table 7 and Figure 2 above). Fitting the probit model to the log dose of the Hnizdo and Sluis-Cremer (1993) data yielded an MLE$_{01}$ of 2.45 (mg/m^3)–yr CDE and a BMCL$_{01}$ of 2.12 (mg/m^3)–yr CDE (χ^2 = 0.64; p value for fit = 0.9957) (Figure 5, Table 19). (For comparison the BMCL$_{05}$ was 3.73 (mg/m^3)–yr CDE.) Fitting the logistic model to the same data yielded a BMCL$_{01}$ of 1.73 (mg/m^3)–yr CDE (χ^2 = 2.71; p value for fit = 0.8446) (Table 19). The BMCL$_{01}$ from these data is about the same as the apparent NOAEL. In general, a BMC is preferred to a NOAEL because the BMC takes into account all the dose response data in a study. The apparent NOAEL may be either above or below an actual effect level, depending on the study design and distribution of the data.

Figure 5. Probit model fit to the log dose of the Hnizdo and Sluis-Cremer data.

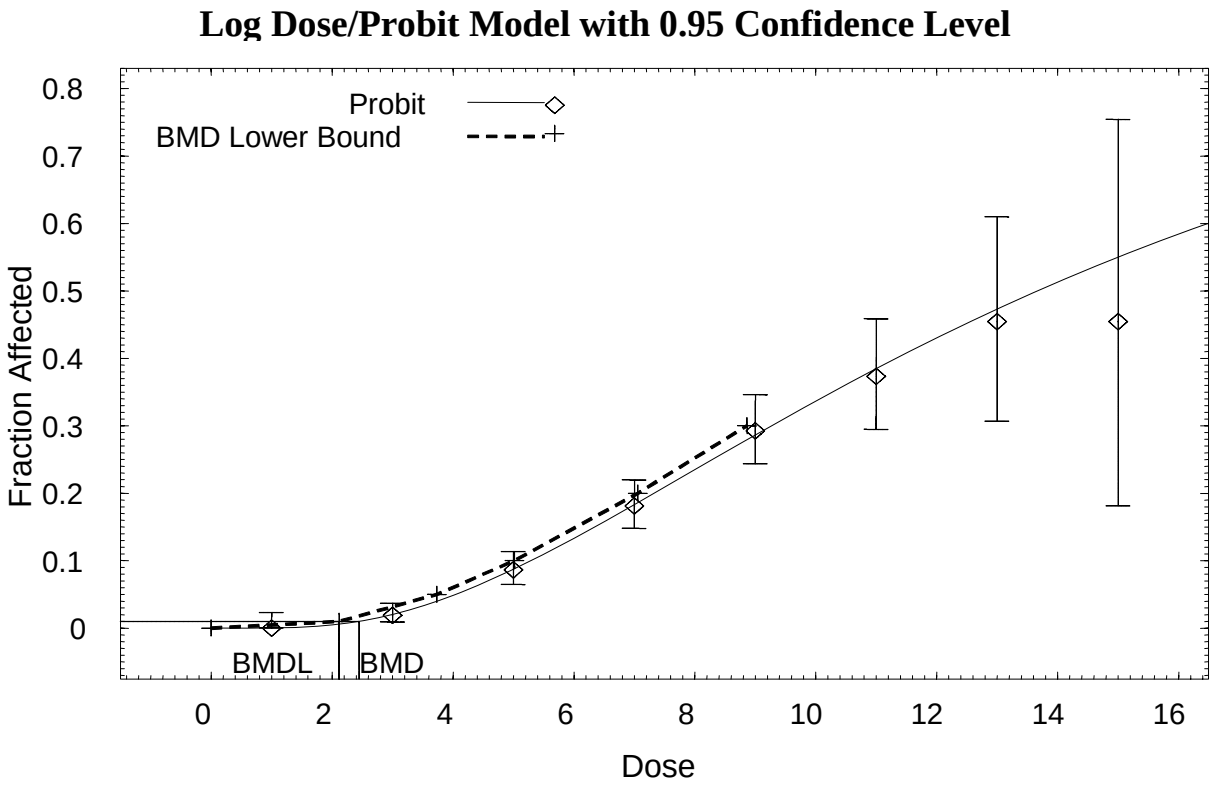


Table 19. Fits of benchmark models to the Hnizdo and Sluis-Cremer (1993) data

BMDS Model	*MLE_{01}*	*$BMCL_{01}$*	*p value for fit*
Probit-log-dose	2.45 (mg/m^3)–yr CDE	2.12 (mg/m^3)–yr CDE	0.9957
Logistic-log-dose	2.07	1.73	0.8446
Multistage (n=2)	2.47	1.89	0.7213
Quantal-quadratic	1.62	1.54	0.5017
Probit	1.56	1.32	0.0079
Logistic	1.48	1.28	0.0003
Quantal-linear	0.37	0.34	0.0000

For the estimate of 30% silica in the South African gold mine dust, Hnizdo and Sluis-Cremer (1993) relied on estimates for the years 1956-1960 by Beadle (Beadle and Bradley, 1970; Beadle, 1971). The original data, obtained by Corner House Laboratories for the South African Bureau of Mines, are partly presented by Beadle and Bradley (1970), but a more detailed presentation of exposures for various classes of workers is given by Page-Shipp and Harris

(1972). The latter paper also describes in some detail the methodology used to obtain the particle counts, and to convert those data into either respirable surface area or respirable mass values. Gibbs and Du Toit (2002) reviewed the data and methodology used by Hnizdo and Sluis-Cremer (1993) to estimate silica exposures of workers, which apparently depended on an unpublished analysis of the Corner House Laboratories' data done by Du Toit in 1991. Gibbs and Du Toit state that the exact relationship between the observed particle counts and theoretically derived mass concentrations cannot be determined, but that the uncertainties in this conversion do not appear to be severe for the dust characteristics observed in the South African mines. They accept the estimates by Beadle and Bradley (1970) of the quartz percentages in the dust, *i.e.* 54% for incinerated and acid-washed dust and 30% for unmodified dust.

However, Gibbs and Du Toit (2002) assert that Hnizdo and Sluis-Cremer (1993) incorrectly applied the 30% (total dust) silica content to figures for acid-treated dust in calculating the silica exposures of each occupational group. This contention is supported by the footnote to Table II in Hnizdo and Sluis-Cremer (1993) where the respirable dust concentration is described as "After heat and acid treatment". In order to clarify this point, OEHHA reviewed the independent reporting of the underlying data by Page-Shipp and Harris (1972). For most occupational groups, the silica exposures (shown in Table 20) calculated from Appendix I of Page-Shipp and Harris (1972), using the 54% silica content appropriate for acid-washed dust, correspond more closely to those calculated by Hnizdo and Sluis-Cremer (1993) (applying the 30% quartz content to their reported "respirable dust concentrations," i.e., the untreated dust), than to the modified, and higher, quartz exposures proposed by Gibbs and Du Toit (2002). For example, 113 exposure samples were taken for stopers.

Table 20. Estimates of silica exposures in mg/m^3 for different occupational groups in South African gold mines.

Occupation	*Shaft Sinkers*	*Developers*	*Stopers*	*Assistant miners/ Trammers*	*Shift Bosses*	*Other Officials*	*Banks/ Skips*	*Workers Near shafts*	*Boiler-makers*	*Other Artisans*	*Miscellan-eous*
Page-Shipp and Harris (1972) (Table III and Appendix I)											
Hours/shift (t)	7.70	8.00	7.80	7.70	5.20	4.00	7.50	6.50	6.30	5.70	7.20
Number of samples	10	37	113	157	43	106	33	34	41	61	11
RM x t	4.44	1.96	1.57	1.20	0.87	0.77	1.31	0.56	1.00	0.64	1.01
s.d.	3.94	1.59	1.00	0.93	0.71	0.53	1.38	0.57	0.71	0.51	0.79
Respirable Mass (RM)	0.58	0.25	0.20	0.16	0.17	0.19	0.17	0.09	0.16	0.11	0.14
Silica (54%)	0.31	**0.13**	**0.11**	**0.08**	**0.09**	**0.10**	**0.09**	**0.05**	**0.09**	**0.06**	0.08
(after acid treatment)											
Hnizdo and Sluis-Cremer (1993) (Table II)											
RM		0.48	0.37	0.27	0.30	0.30	0.13	0.10	0.19	0.19	
Silica (30%)		**0.14***	**0.11***	**0.08***	**0.09***	**0.09***	0.04	0.03	0.06	**0.06***	
(before acid treatment)											
Gibbs and Du Toit (2002) (Table 4)											
RM		0.48	0.37	0.27	0.30	0.30	0.13	0.10	0.19	0.19	
Silica (54%)		0.26	0.20	0.15	0.16	0.16	**0.07***	**0.05***	**0.10***	0.10	
(after acid treatment)											

* denotes that value is equal to or closer to the value based on Page-Shipp and Harris

The last line of Appendix I of Page-Shipp and Harris (1972) gives a mean value for stopers of 1.57 (mg/m^3)-hours respirable dust mass after acid treatment. Since the average work shift for stopers was 7.8 hours (Page-Shipp and Harris, 1972, Table III, last row), the average exposure level was 0.20 mg/m^3. If 54% of this were quartz, the quartz level would be 0.11 mg/m^3. Table II of Hnizdo and Sluis-Cremer (1993) lists 0.37 mg/m^3 respirable dust for stopers. Thirty % of 0.37 mg/m^3 equals 0.11 mg/m^3, the same value reported by Page-Shipp and Harris. In Table 4 of Gibbs and Du Toit (2002) stopers are also reported to be exposed to 0.37 mg/m^3 respirable dust. If 54% were quartz, as Gibbs and Du Toit contend, the quartz level would be 0.2 mg/m^3. For 6 of the 9 categories of workers comprising 83% of the samples taken the silica levels correspond more closely to values used by Hnizdo and Sluis-Cremer than to those suggested by Gibbs and Du Toit.

Several more recent analyses of quartz content of South African mining rock have been reported (Table 21). Kielblock *et al*. (1997) give the overall silica content of the dust as 15% for the late 1980s to early 1990s. Dr. Eva Hnizdo (personal communication, 2003), now with the U.S. National Institute of Occupational Safety and Health (NIOSH), provided a summary of various other estimates that have been made. "Past surveys indicate that the amount of airborne respirable dust in SA gold mines in 1980's and in 1970's was on average around 0.4 mg/m^3 with average quartz concentration of 0.08 mg/m^3" (about 20%). In a Ph.D. thesis submitted by the late R.E.G. Rendall (1999) on dust in the air of gold mines, the silica percentage averaged 22% during the period from 1964 to 1988. In summary,

(1) Notwithstanding some apparent contradictions in the various accounts, the silica concentrations in air proposed by Hnizdo and Sluis-Cremer, based on the Corner House Laboratory data, are a reasonable contemporary estimate of the exposures experienced by the workers examined in the study by Hnizdo and Sluis-Cremer (1993).

(2) Other, more recent estimates of percent silica in the mine dust were lower than the value of 30% used by Hnizdo and Sluis-Cremer (1993). Newer studies, which using more sophisticated methods to measure silica in the dust, indicate lower silica concentrations in the various occupational settings. Since dust levels in the mines were fairly constant for decades and quantification of silica was improving, 30% is more likely to be an overestimate than an underestimate of silica levels.

(3) Analysis of the data of Page-Shipp and Harris (1972) by OEHHA staff indicated that Hnizdo and Sluis-Cremer (1993) used the correct silica content, despite an erroneous statement in a footnote to Table II of their paper[1].

[1] Dr. Eva Hnizdo reviewed this analysis of the silica content of the dust and agrees with the assessment. ("I am very pleased that you studied carefully all the reports and came to the conclusion that our study was after all reasonably correct. Based on the Churchyard study and the measurements data I have seen in SA during the 1990s, I am also convinced that our results are reasonable estimates of the exposure of the cohort." (Hnizdo, personal communication October 2004)

Table 21. Estimates of respirable silica fraction of South African gold mine dust

Authors	*Time frame*	*% silica*		*Number of samples*	*Methods*
Beadle and Bradley, 1970	1958-1967	total dust: 25.7%; gravimetric: 28.5%; microscopy. acid-washed: 54%		142 grav; 143 elect ppt	gravimetric; precipitator + microscopy
Hnizdo and Sluis-Cremer (1993)	1956-1960	30%			precipitator + microscopy
Rendall (unpublished thesis)					
Survey 1	1987-8	17%		588	gravimetric
Survey 2	1977	20%		166	gravimetric
Survey 3	1977	17%		90	gravimetric
Survey 4a	1964-7	22%		112	gravimetric
Hnizdo (personal communication)	1970-1989	20%			
Kielblock (1997)	~1990	15.08%			Not stated
Churchyard (2004)	2000-1	14.3%			Gravimetric + X-ray diffraction

In the first supportive study Steenland and Brown (1995) found five cases of silicosis in the lowest dose group of 0 – 0.2 (mg/m^3)-yr and considered the group to be a LOAEL (Table 10 above). None of the BMDS models gave an acceptable fit at the $p \geq 0.05$ level using six or seven silica levels. The closest was the quantal quadratic model ($\chi^2 = 9.62$; $p = 0.0473$), which resulted in a BMC_{01} for silica of 0.43 (mg/m^3)–yr using the six lowest levels of silica. In risk assessment, the highest dose or doses are often dropped in order to obtain an acceptable fit of the model to the data. This is reasonable with the benchmark approach since the highest doses should be least informative and the doses in the low dose region near the benchmark should be most informative for the benchmark concentration (USEPA, 1995; Filipsson *et al*., 2003). Fitting the probit model to the log dose of the five lowest silica levels from Steenland and Brown yielded a $BMCL_{01}$ of 0.34 (mg/m^3)–yr CDE ($\chi^2 = 1.32$; p value for fit = 0.5177). [For comparison, $BMCL_{05} = 0.85$ (mg/m^3)–yr CDE.] Fitting the quantal quadratic model gave a $BMCL_{01}$ of 0.45 (mg/m^3)–yr ($\chi^2 = 3.36$; $p = 0.3395$). Use of the BMC_{01} value of 0.34 (mg/m^3)–yr CDE from the log dose probit model resulted in a chronic REL estimate for crystalline silica of 4 μg/m^3. Steenland and Brown stated that "silicosis has no background rate for non-exposed populations that changes with age or calendar time" and thus they assumed that the five silicotics in the 0 – 0.2 (mg/m^3)-yr were exposed to silica in the mines.

In a second supportive study, Hughes *et al*. (1998) found six cases of silicosis in the lowest exposure group of ≤ 1 mg/m^3-yr but considered that group to be a NOAEL, not a LOAEL. If the lowest exposure group is used as a NOAEL, a chronic REL of 10 μg/m^3 is calculated from the

data. Hughes *et al*. (1998) cite examples of possible non-occupational chest radiograph opacities (due, for example, to age or smoking) to explain the six cases in the lowest exposure group. However, due to the rarity of silicosis the six cases are biologically significant. OEHHA considers that the six cases may be work related, not cases of environmental or background silicosis. When a LOAEL to NOAEL UF of 3 is applied to the data of Hughes *et al*. (1998), the estimated REL is 3 $\mu g/m^3$.

In a third supportive study, Chen *et al*. (2001) found two cases of silicosis in the lowest exposure group of ≤ 10 mg CTD/m^3-years and considered that exposure level to be a NOAEL. One of the advantages of the benchmark dose analysis is that a NOAEL/LOAEL controversy, such as the one above with the Hughes *et al*. (1998) data, does not impact the procedure. The chart of the Chen *et al*. data above (Figure 4) indicates that the dose response is linear at low doses. Fitting the probit model to the log dose of the four lowest data points yielded a BMC_{01} of 0.132 (mg/m^3) - yr CDE (χ^2 = 2.19; p value for fit = 0.335). Use of five, six, or seven data points gave BMC_{01}s of 0.14 to 0.17, but the p values were less than 0.1. For comparison, fitting the logistic model to the log dose of the four lowest data points yielded a $BMCL_{01}$ of 0.093 (mg/m^3) - yr CDE (χ^2 = 4.86; p value for fit = 0.0879). An inhalation chronic Reference Exposure Level for crystalline silica of 6 $\mu g/m^3$ was estimated from the Chen *et al*. data.

The fourth supportive study is that of black South African gold miners by Churchyard *et al*. (2003, 2004). A problem with this data set is the statistical "noise" in the lower exposure groups; e.g., the lowest exposure group has a higher incidence of silicosis (11/103) than the next group (8/97). This noise causes problems in estimating a low benchmark such as the $BMCL_{01}$ used with the Hnizdo and Sluis-Cremer (1993) data and with data from Steenland and Brown (1995) and Chen *et al*. (2001). The calculation therefore uses a 5% BMCL of 0.673 (mg/m^3)-yr from the probit log dose model as the benchmark, which is reasonably well within the range of the reliably observed data and which does not differ too widely from the MLE_{05} estimate of 0.955 (mg/m^3)-yr for that parameter. This $BMCL_{05}$ is not strictly comparable to the $BMCL_{01}$ calculated from the data of Hnizdo and Sluis-Cremer, but the concerns about the severity of the effect noted in the discussion of that derivation apply with equal or greater force here.

On the other hand, the variability in the low-dose data in this study implies that an unobserved factor is affecting the data. All the models predict that the "background" is substantially (as much as 5 – 10%) above zero, which is intrinsically implausible for silicosis unless there is an unrecorded additional source of silica exposure. Possibly, there were occasional excursions in the exposure of the workers in less exposed jobs, which were not captured by the systematic assessments for these job classifications. Alternatively, perhaps the assignment of an assumed zero exposure value to "non-dusty" job classifications noted in the paper was in fact inaccurate for some individuals. There may also be some distortion of the curve resulting from the "binning" of the exposure categories; certainly, the bin widths hinder the attempt to calculate a $BMDL_{01}$ in this case. In relation to the model fit, other models (including the quantal linear model, which emphasizes the likely more reliable incidences at higher dose levels) are consistent with the $BMCL_{05}$ results from the log probit model used here. Finally, the total number of cases and controls examined is fewer than in Hnizdo and Sluis-Cremer's study, which reduces the precision.

None of these issues can be resolved without recourse to the individual data, which were not available for this analysis, and may not be resolved even then. However, the derivation of an HEC of 11.2 $\mu g/m^3$ (and thus a comparison REL of 4 $\mu g/m^3$) indicates at least that the results of the earlier analysis are unlikely to have underestimated the proper value of the REL. Although the uncertainties in the Churchyard *et al*. data prevent us from being more precise, we cannot eliminate the possibility that the REL should be set lower. However, the California ambient monitoring data, although subject to considerable uncertainty as to the relevant particle size distributions, suggest a plausible lower bound on the REL, which is consistent with our analysis of Hnizdo and Sluis-Cremer's data.

Other investigators have approached the possibility that some opacities on radiographs may be due to background influences such as age and smoking. In regard to smoking, Blanc and Gamsu (1988) reviewed the literature and concluded that smoking would not interfere with the determination of silicosis by the ILO system. Based on reading 1422 films of unexposed blue-collar workers, Castellan *et al*. (1985) stated that the use of the median result of 3 readers (the same number used by Hughes *et al*.) rarely results in interpreting a chest radiograph as ILO category $\geq$ 1/0 in workers who were not exposed to dust (and regardless of smoking status).

The USEPA (1996) did a benchmark analysis with the Hnizdo and Sluis-Cremer (1993) data. They estimated that the lower bound for a 1% risk for silicosis ($BMCL_{01}$) was 1.31 (mg/m^3)-yr, which by their methods is equivalent to a continuous, 70-year exposure to 6.7 $\mu g/m^3$ silica. However, USEPA did not do a formal Reference Concentration (RfC) derivation for silica by either the BMC/UF or NOAEL/UF approach.

The key (Hnizdo and Sluis-Cremer, 1993) and supporting (Steenland and Brown, 1995; Hughes *et al*., 1998; Chen *et al*., 2001) studies were of human adults, nearly all males, who were presumably healthy, at least initially, since they were able to work. Thus there is need to protect the sensitive members of the population, especially children, in whose airways penetration of silica particles will be greater (Phalen *et al*., 1985; Schiller-Scotland *et al*., 1994; Oldham *et al*., 1997; Bennett and Zeman, 1998). In addition, women may be more sensitive than men to the development of silicosis (Gerhardsson and Ahlmark, 1985; Katsnelson *et al*., 1986). The selection of three as the intraspecies uncertainty factor (UF_H) was based on several considerations.

(1) The workers who developed silicosis at low silica concentrations are by definition the most sensitive workers to silica-induced silicosis. Because of the large population of workers examined in these studies (more than 14,000), the sensitive individuals represent at least part of the range of sensitivity to be expected in the general population. This may justify reducing the UF_H from the default value of 10. Since these workers did not include children, the elderly, or females (except for the 215 females in Chen *et al*.), some uncertainty related to inter-individual variability remains. Therefore, a UF_H of 3 rather than 1 is chosen.

(2) Mukherji *et al*. (1993) reported mean ambient silica levels (in PM_{10}) at three locations in the northern part of Santa Barbara County, California (see the Appendix to this report). At Santa Maria (an urban site) the level was 2.3 $\mu g/m^3$; in Santa Ynez (a rural site) 0.6 $\mu g/m^3$; and in Buellton (a remote background site) 0.2 $\mu g/m^3$ crystalline silica. Thus, use

of a human intraspecies uncertainty factor (UF_H) of 10 with the data from the key study would result in an estimated chronic REL of 0.9 μg/m^3 (ACGIH method), a level in the range of ambient levels in California. Although the reported levels at the urban site may (according to the authors) have reflected some anthropogenic contributions such as disturbance and tracking of siliceous road dust, the rural and remote site values are apparently (perhaps conservatively) reflective of the natural background to which all California residents are exposed. (U.S. EPA (1996) found slightly higher average ambient levels of silica in PM_{10}; this average may include some sites affected by disturbance and emissions.) There is no evidence that these background levels of silica are causing silicosis. On the other hand, silicosis in the general population is not a target for medical attention, and autopsy rates are very low, so the possibility of a low frequency of response at these levels cannot be entirely dismissed. On balance, it appears plausible that a REL of 3 μg/m^3 (benchmark + UF_H = 3) would be protective of the general population.

(3) The dose-response curve for silicosis due to inhalation of crystalline silica is steep, and an upward curvature of this dose response was seen in some studies (Figure 7-1 in USEPA, 1996). It is notable that, whereas exposures in the 1-3 μg/m^3 range are apparently without effect (based on the benchmark calculations and the California ambient background data), Rice and Stayner (1995) described a LOAEL for silicosis of 8 μg/m^3 in gold miners (Table 16; based on data from McDonald and Oakes [1984]). This finding may partly reflect differences in physical state of the silica, and co-exposures, but it might indicate that, although the chronic REL should be protective of public health, chronic exposures only moderately exceeding the REL may lead to clinically observable disease.

The animal studies gave LOAELs for silica of 0.2 mg/m^3 in dogs and from 1 to 4.9 mg/m^3 in rodents. After extrapolation to equivalent continuous time and application of LOAEL to NOAEL, interspecies, and intraspecies UFs, the estimated chronic RELs from animal data are all less than 1 μg/m^3. This reflects in part the greater uncertainty in extrapolating from animal studies to predicted human health effects.

The silica particles of concern in the causation of silicosis are those of respirable size. California EPA defines 'respirable' as particles 10 μm or less MMAD. This reflects one usual type of sampler (for "PM_{10}") used for ambient air sampling in the general environment. The other usual type of environmental sampler, $PM_{2.5}$, collects even smaller particles. There are differences in the size range distribution between a typical PM_{10} measuring device and the NIOSH type personal samplers, or other devices with similar size selection properties, used by the investigators in the epidemiological studies. The NIOSH-type samplers capture 50% of particles with a MMAD of 4 μm, and higher percentages of smaller particles. A smaller proportion of larger particles between 4 and 10 μm in aerodynamic diameter will also be collected. Figure 5, from Volume I of U.S. EPA's Third External Review Draft of Air Quality Criteria for Particulate Matter (April 2002), includes particle penetration curves for PM_{10}, $PM_{2.5}$, and occupational samplers.

Figure 5. Size cut curves of particle penetration

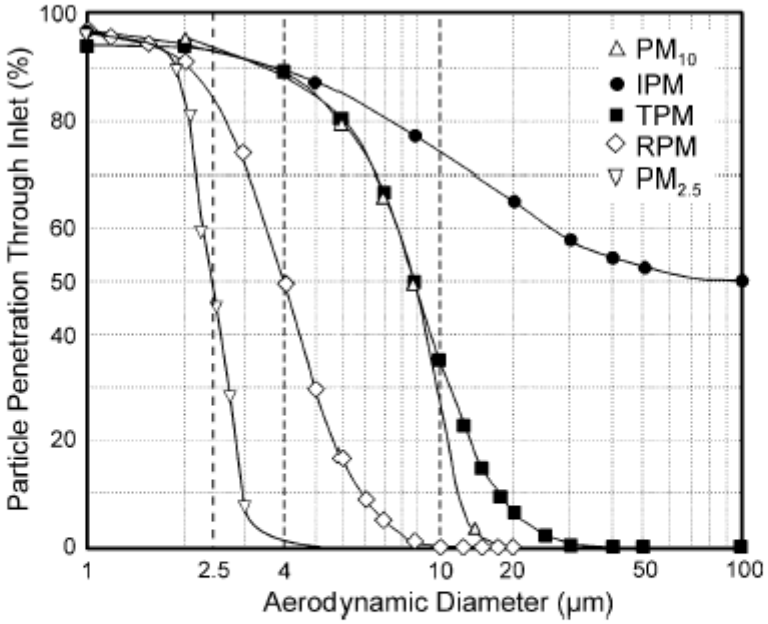


Figure 2-6. Specified particle penetration (size-cut curves) through an ideal (no-particle-loss) inlet for five different size-selective sampling criteria. Regulatory size cuts are defined in the Code of Federal Regulations; $PM_{2.5}$ (2001c), PM_{10} (2001a). $PM_{2.5}$ is also defined in the Federal Register (1997). Size-cut curves for inhalable particulate matter (IPM), thoracic particulate matter (TPM) and respirable particulate matter (RPM) size cuts are computed from definitions given by American Conference of Governmental and Industrial Hygienists (1994).

The NIOSH samplers are designed to mimic the size range of particles that reach into the bronchiolar and alveolar spaces (what the occupational community calls respirable). PM_{10} samplers are meant to capture particles that penetrate the entire length of the lower respiratory tree, including those that penetrate to the tracheobronchial and alveolar regions. Penetration (and therefore presumably deposition) by particle size is complex, and is dependent on the aerodynamic diameter, hygroscopicity, and electrostatic charge of the particles, and on a number of host factors including airway structure and geometry, as well as depth, rate, and mode of breathing (nasal vs. oronasal). The fractional penetration in the various regions of the respiratory tract is not linear with respect to size. Generally, though, larger particles impact higher in the respiratory tree (the extrathoracic and tracheobronchial regions), while smaller particles show greater penetration to the lower tracheobronchial and alveolar regions. There are a number of models of regional penetration and deposition in the respiratory tract, as well as some measurements. Chan and Lippmann (1980) showed peak alveolar deposition for particles about 3 μm MMAD with deposition dropping above and below that. Their data and model indicate that tracheobronchial deposition rises rapidly above about 3 μm MMAD. Available data also

indicate significant inter-individual variability in fractional deposition. The ICRP (1994) model used in evaluating risk from radioactive particles indicates that total deposition in the respiratory tract for particles 3 μm in activity median thermodynamic diameter (AMTD) is about 0.78 with a regional deposition fraction of 0.077 for the alveolar region for a reference male worker during nasal breathing. The same model predicts a total deposition in the respiratory tract of 0.77 for 10 μm AMTD particles and a deposition fraction of 0.024 in the alveolar region. Thus, many particles with a 10 μm MMAD get into the alveolar space. A smaller difference in regional penetration and deposition is predicted for mouth breathers. Therefore, if only the size range measured by the samplers used in the studies were considered, the measurement might underestimate the amount of silica that reaches the gas exchange regions of the lung, depending on the actual particle size distributions in the occupational studies and in the environments in which the REL is to be applied. Unfortunately, neither the occupational nor the environmental silica particle size distributions are known in detail; measurements have been reported only in terms of NIOSH sampler results or PM_{10} cutoff values.

It is generally assumed that the silicosis is induced by that fraction of the silica that reaches the alveoli. Nevertheless, no actual data exonerate the coarser particles in the 4 - 10 μm range. A fraction of these particles can enter the bronchioles and alveoli. However, some data from South African gold mines indicate that more than 99% of the crystalline silica dust can be in the $PM_{2.5}$ fraction (Sichel, 1957). Thus, the samplers used in the key study appear to be collecting the biologically relevant range of particles in that situation.

In the absence of comprehensive data on the silicosis-inducing activity of different particle sizes, it is not possible to adjust the REL for different particle size distributions, which might be found in the general environment, or for different measurement methods. The REL is therefore specified as applicable to concentrations of particles having a size range (and reactivity) similar to those measured in the occupational studies [respirable as defined occupationally (ISO, 1995; NIOSH, 2003; ACGIH, 2004)]. Results obtained by other sampling methods would need to be corrected for any difference in size selectivity of the method used. Such a correction factor would be specific to the particle size distribution present at the site studied, so no general correction factors can be proposed. A more inclusive sampling procedure, such as that used for PM_{10}, would overestimate the relevant exposure in any situation, and so would be inappropriate for precise risk quantification. However, PM_{10} would be useful as a screening method to establish that a particular situation is unlikely to present a hazard. For example, if the silica concentration in PM_{10} modeled at a receptor is less than the REL (3 $\mu g/m^3$), occupationally respirable silica will also be less than 3 $\mu g/m^3$, so a facility would not pose a risk due to silica at that receptor. If the silica concentration in $PM_{2.5}$ modeled at a receptor is less than 3 $\mu g/m^3$ but PM_{10} is greater than 3 $\mu g/m^3$, further testing would be needed. If both $PM_{2.5}$ and PM_{10} exceeded the REL, the chronic Hazard Index would exceed 1 to an undetermined extent, suggesting a need for risk management. More precise determination of the amount of material in the respirable size fraction for environmental samples may require further work on measurement methodology, since ISO (1995) and similar occupational methods have not been validated for the lower levels encountered in environmental samples.

VII. Data Strengths and Limitations for Development of the REL

The strengths of the inhalation REL for silica include:

(1) The availability of several long-term studies of inhalation in workers at varying exposure concentrations (see Summary Table 15 above), with adequate histopathological and radiological analysis, and with adequate follow-up.

(2) The finding of a dose-response effect for silicosis in several of the studies (e.g., Hnizdo and Sluis-Cremer, 1993; Steenland and Brown, 1995; Chen *et al*., 2001).

(3) The observation of a NOAEL in some studies including the key study (summarized by Rice and Stayner, 1995).

(4) The power of the Hnizdo and Sluis-Cremer (1993) data to detect a small effect.

Major areas of uncertainty are:

(5) The limited follow-up of the cohort members in some studies (e.g., Muir *et al*., 1989; Rosenman et al., 1996) with consequent under-ascertainment of silicosis (even to the extent that such studies are useless for determining exposure-response).

(6) The general underestimation of silicosis by radiography alone (Hnizdo *et al*., 1993), which results in higher, less health-protective chronic REL estimates.

(7) The possible underreporting of silicosis where complete radiographic data and autopsy data are not available (Steenland and Brown, 1995).

(8) The uncertainties in exposure estimation, especially when reconstructing historical levels of silica exposure (Seixas et al., 1997; Gibbs and Du Toit, 2002) including the variability in the estimates of percent quartz in the South African mine dust (Beadle, 1971; Hnizdo and Sluis-Cremer, 1993; Kielblock *et al*., 1997; Gibbs and Du Toit, 2002; Hnizdo, personal communication) and when converting particle counts to mass.

(9) The differences in percent silicosis in different studies at what were considered similar silica levels and similar exposure duration (see Summary Table 15 above).

(10) The variability in toxicity of various forms of silica (e.g., freshly fractured vs. aged quartz; cristobalite vs. quartz) although all forms have toxicity (Table 17).

(11) The limited information on silica particle size (including its variability) in the epidemiological studies, other than that the silica was respirable, and the variability in particle penetration and deposition as a function of particle size in the respiratory tract in the human population (e.g., Heyder *et al*., 1982; ICRP, 1994; Hattis *et al*., 2001).

(12) The use of area samplers rather than personal samplers to estimate exposure, which usually results in an underestimation of silica exposure (Cherrie, 1999).

VIII. Potential for Differential Impacts on Children's Health

Silica is a respiratory irritant and a modifier of immune function. Since the key study involved over 2000 men, some were likely to be more sensitive to silica than others. In addition, we used a benchmark of 1% adverse effect, rather than the usual 5 %. Thus, use of the human intraspecies uncertainty factor (UF_H) of 3 should result in a REL that adequately protects most members of the general population. Exacerbation of asthma, which has a more severe impact on children than on adults, is a known response to some respiratory irritants. However, there is no data on such a response to silica in infants or children. The epidemiological studies used in the derivation of the REL did not include children. If children's susceptibility were much greater than that of adults, it would be expected that clinical disease would be evident in children following exposures in the upper range of the respirable silica levels measured in ambient air in California. No such reports have been identified in the literature. There are no data on silica's effects on the immune system of children.

OEHHA is currently evaluating its risk assessment methodology, in particular the UF_H, for its adequacy in protecting infants and children. Since children have smaller airways than adults and breathe more air on a body weight basis, penetration and deposition of particles in the airways and alveoli in children is likely greater than that in adults exposed to the same concentration (Phalen *et al*., 1985; Schiller-Scotland *et al*., 1994; Oldham *et al*., 1997; Bennett and Zeman, 1998).

IX. References

ACGIH. 1999. American Conference of Governmental Industrial Hygienists. 1999 TLVs and BEIs. Threshold Limit Values for chemical substances and physical agents and Biological Exposure Indices. Cincinnati: ACGIH.

ACGIH. 2000. American Conference of Governmental Industrial Hygienists. 2000 TLVs and BEIs. Threshold Limit Values for chemical substances and physical agents and Biological Exposure Indices. Cincinnati: ACGIH.

ACGIH. 2004. American Conference of Governmental Industrial Hygienists. 2004 TLVs and BEIs. Threshold Limit Values for chemical substances and physical agents and Biological Exposure Indices. Cincinnati: ACGIH. pp. 73-6.

American Thoracic Society. 1997. Adverse effects of crystalline silica exposure. Am Respir Crit Care Med. 155:761-5.

Balmes J, Becklake M, Blanc P, Henneberger P, Kreiss K, Mapp C, Milton D, Schwartz D, Toren K, Viegi G. Environmental and Occupational Health Assembly, American Thoracic Society. 2003. American Thoracic Society Statement: Occupational contribution to the burden of airway disease. Am J Respir Crit Care Med. 167(5):787-97.

Bar-Ziv J, Goldberg JM. 1974. Simple siliceous pneumoconiosis in Negev Bedouins. Arch Environ Health. 29:121-6.

Beadle DG. 1971. The relationship between the amount of dust breathed and the development of radiological signs of silicosis: an epidemiologic study of South African gold miners. In: Walton WH (ed). Inhaled particles III. Oxford: Pergamon Press, pp. 953-64.

Beadle DG, Bradley AA. 1970. The composition of airborne dust in South African gold mines. In: Shapiro HA (ed). Pneumoconiosis. Proceedings of the International Conference. Johannesburg 1969. Cape Town: Oxford University Press. pp. 462-6.

Begin R, Ostiguy G, Fillion R, Colman N. 1991. Computed tomography scan in the early detection of silicosis. Am Rev Respir Dis. 144(3 Pt 1):697-705.

Bennett WD, Zeman KL. 1998. Deposition of fine particles in children spontaneously breathing at rest. Inhal Toxicol. 10:831-42.

Blanc PD, Gamsu G. 1988. The effect of cigarette smoking on the detection of small radiographic opacities in inorganic dust diseases. J Thorac Imaging. 3(4):51-6.

Burns CA, Zarkower A, Ferguson FG. 1980. Murine immunological and histological changes in response to chronic silica exposure. Environ Res. 21(2):298-307.

CARB. 2001. California Air Resources Board. California Emissions Inventory Development and Reporting System (CEIDARS). Data from Data Base Year 2001.

Castellan RM, Sanderson WT, Petersen MR. 1985. Prevalence of radiographic appearance of pneumoconiosis in an unexposed blue collar population. Am Rev Respir Dis. 131(5):684-6.

Cavariani F, Di Pietro A, Miceli M, Forastiere F, Biggeri A, Scavalli P, Petti A, Borgia P. 1995. Incidence of silicosis among ceramic workers in central Italy. Scand J Work Environ Health. 21 (Suppl 2):58-62

Chan TL, Lippmann M. 1980. Experimental measurements and empirical modeling of the regional deposition of inhaled particles in humans. Am Ind Hyg Assoc J. 41:399-409.

Chen W, Zhuang Z, Attfield MD, Chen BT, Gao P, Harrison JC, Fu C, Chen JQ, Wallace WE. 2001. Exposure to silica and silicosis among tin miners in China: exposure-response analyses and risk assessment. Occup Environ Med. 58(1):31-7.

Chia KS, Ng TP, Jeyaratnam J. 1992. Small airways function of silica-exposed workers. Am J Ind Med. 22(2):155-62.

Cherrie JW. 1999. The effect of room size and general ventilation on the relationship between near and far-field concentrations. Appl Occup Environ Hyg. 14(8):539-46.

Cherrie JW, Aitken RJ. 1999. Measurement of human exposure to biologically relevant fractions of inhaled aerosols. Occup Environ Med. 56(11):747-52.

Churchyard GJ, Ehrlich R, teWaterNaude JM, Pemba L, Dekker K, Vermeijs M, White N, Myers J (2004). Silicosis prevalence and exposure-response relations in South African goldminers. Occup Environ Med. 61(10):811-6.

Churchyard G, Pembe L, Magadla B, Dekker K, Vermeijs M, Ehrlich R, teWaterNaude J, Myers J (2003). Silicosis prevalence and exposure response relationships in older black miners on a South African goldmine. Final report. Safety in Mines Research Advisory Committee. Simhealth 606. March 7, 2003.

Churg A. 1996. The uptake of mineral particles by pulmonary epithelial cells. Am J Respir Crit Care Med. 154(4 Pt 1):1124-40.

Clouter A, Brown D, Hohr D, Borm P, Donaldson K. 2001. Inflammatory effects of respirable quartz collected in workplaces versus standard DQ12 quartz: particle surface correlates. Toxicol Sci. 63(1):90-8.

Craighead JE, Vallyathan NV. 1980. Cryptic pulmonary lesions in workers occupationally exposed to dust containing silica. JAMA. 244(17):1939-41.

Davis GS, Leslie KO, Hemenway DR. 1998. Silicosis in mice: effects of dose, time, and genetic strain. J Environ Pathol Toxicol Oncol. 17(2):81-97.

Davis LK, Wegman DH, Monson RR, Froines J. 1983. Mortality experience of Vermont granite workers. Am J Ind Med. 4(6):705-23.

Ding M, Chen F, Shi X, Yucesoy B, Mossman B, Vallyathan V. 2002. Diseases caused by silica: mechanisms of injury and disease development. Int Immunopharmacol. 2(2-3):173-82.

Donaldson K, Borm PJ. 1998. The quartz hazard: a variable entity. Ann Occup Hyg. 42(5):287-94.

Elzea JM. 1997. The regulation of crystalline silica: an industry perspective. J Expo Anal Environ Epidemiol. 7(3):377-84.

Filipsson AF, Sand S, Nilsson J, Victorin K. 2003. The benchmark dose method--review of available models, and recommendations for application in health risk assessment. Crit Rev Toxicol. 33(5):505-42

Finkelstein MM. 2000. Silica, silicosis, and lung cancer: a risk assessment. Am. J. Ind. Med. 38(1):8-18.

Gerhardsson L, Ahlmark A. 1985. Silicosis in women. Experience from the Swedish Pneumoconiosis Register. J Occup Med. 27(5):347-50.

Gibbs GW, Du Toit RS. 2002. Estimating the quartz exposure of South African gold miners. Ann Occup Hyg. 46(7):597-607.

Glover JR, Bevan C, Cotes JE, Elwood PC, Hodges NG, Kell RL, Lowe CR, McDermott M, Oldham PD. 1980. Effects of exposure to slate dust in North Wales. Br J Ind Med. 37(2):152-62.

Goldsmith JR, Goldsmith DF. 1993. Fiberglass or silica exposure and increased nephritis or ESRD (end-stage renal disease). Am J Ind Med. 23(6):873-81.

Graham WG, Ashikaga T, Hemenway D, Weaver S, O'Grady RV. 1991. Radiographic abnormalities in Vermont granite workers exposed to low levels of granite dust. Chest. 100(6):1507-14.

Greaves IA. 2000. Not-so-simple silicosis: a case for public health action. Am J Ind Med. 37(3):245-51.

Green FHY, Vallyathan V. 1996. Pathologic responses to inhaled silica. In: Castranova V, Vallyathan V, Wallace WE (eds.). Silica and Silica-Induced Lung Diseases. Boca Raton: CRC Press, 1996, pp. 39-59.

Hattis D, Russ A, Goble R, Banati P, Chu M. 2001. Human interindividual variability in susceptibility to airborne particles. Risk Anal. 21(4):585-99.

HSDB. 2001. Hazardous Substances Data Bank. National Library of Medicine, Bethesda, MD. Available at: http://toxnet.nlm.nih.gov.

Heyder J, Gebhart J, Stahlhofen W, Stuck B. 1982. Biological variability of particle deposition in the human respiratory tract during controlled and spontaneous mouth-breathing. Ann Occup Hyg. 26(1-4):137-47.

Hnizdo E, Murray J, Sluis-Cremer GK, Thomas RG. 1993. Correlation between radiological and pathological diagnosis of silicosis: an autopsy population based study. Am J Ind Med. 24(4):427-45.

Hnizdo E, Sluis-Cremer GK. 1993. Risk of silicosis in a cohort of white South African gold miners. Am J Ind Med. 24(4):447-57.

Hnizdo E, Vallyathan V. 2003. Chronic obstructive pulmonary disease due to occupational exposure to silica dust: a review of epidemiological and pathological evidence. Occup Environ Med. 60(4):237-43.

Hughes JM. 1995. Radiographic evidence of silicosis in relation to silica exposure. Appl Occup Environ Hyg. 10(12) 1064-9.

Hughes JM, Weill H, Checkoway H, Jones RN, Henry MM, Heyer NJ, Seixas NS, Demers PA. 1998. Radiographic evidence of silicosis risk in the diatomaceous earth industry. Am J Respir Crit Care Med. 158(3):807-14.

Hughes JM, Weill H, Rando RJ, Shi R, McDonald AD, McDonald JC. 2001. Cohort mortality study of North American industrial sand workers. II. Case-referent analysis of lung cancer and silicosis deaths. Ann Occup Hyg. 45(3):201-7.

IARC Monographs on the Evaluation of Carcinogenic Risks to Humans. Volume 68. Silica, Some Silicates, Coal Dust and para-Aramid Fibrils. Lyon: IARC, 1997.

ICRP. 1994. International Commission for Radiological Protection. Human Respiratory Tract Model for Radiological Protection. ICRP Publication 66. Annals of the ICRP. 24(1-3):1-482.

International Labour Office. 1980. Guidelines for the use of ILO International Classification of radiographs of pneumoconiosis. Rev. Ed. Occupational Safety and Health Services, No. 22 (Rev.). ILO, Geneva.

ISO. 1995. International Organization for Standardization. Air quality – Particle size fractionation definitions for health-related sampling. ISO No. 7708:1995. ISO, Geneva.

Katsnelson BA, Polzik EV, Privalova LI. 1986. Some aspects of the problem of individual predisposition to silicosis. Environ Health Perspect. 68:175-85.

Kielblock AJ, Franz RM, Unsted AD, vander Linde A, Ashworth SGE. 1997. Quantitation of occupational health risks in the South African mining industry and assessment of sources of uncertainty in the estimates. SIMRAC Safety in Mines Advisory Committee final project report, project no. SIMRISK 401. Johannesburg: CSIR Division of Mining Technology.

Kitto PH. 1960. Methods of dust measurement. In: Orenstein AJ (ed). Proceedings of Pneumoconiosis Conference held at the University of Witwatersrand, Johannesburg 9th – 24th February, 1959. London: J & A Churchill Ltd. pp. 13-20.

Kitto PH. 1970. South African methods for the assessment of dust in gold and coal mines. In: Shapiro HA (ed). Pneumoconiosis. Proceedings of the International Conference. Johannesburg 1969. Cape Town: Oxford University Press. pp. 457-61.

Kreiss K, Greenberg LM, Kogut SJ, Lezotte DC, Irvin CG, Cherniack RM. 1989. Hard-rock mining exposures affect smokers and nonsmokers differently. Results of a community prevalence study. Am Rev Respir Dis. 139(6):1487-93.

Kreiss K, Zhen B. 1996. Risk of silicosis in a Colorado mining community. Am J Ind Med. 30(5):529-39.

Kutzman RS. 1984a. A study of Fischer 344 rats exposed to silica dust for six months at concentrations of 0, 2, 10 or 20 mg/m^3. Upton, NY: Brookhaven National Laboratory; report no. BNL 34617.

Kutzman RS. 1984b. A study of Fischer 344 rats exposed to silica dust for six months at concentrations of 0, 2, 10 or 20 mg/m^3, then maintained for six months prior to assessment. Upton, NY: Brookhaven National Laboratory; report no. BNL 35735.

Lapp NL, Castranova V. 1993. How silicosis and coal workers' pneumoconiosis develop--a cellular assessment. Occup Med. 8(1):35-56.

Legrand-Cattan K, Vuillaume M, Iwatsubo Y, Ameille J, Brochard P, Letourneux M, Housset B, Laureillard J, and Pairon J. 1998. Silicosis in the ceramic industry: dose-response relationship. In: Advances in the Prevention of Occupational Respiratory Disease. Chiyotani K, Hosoda Y, Aizawa Y (eds.). Amsterdam: Elsevier. Pp. 113-117.

Le Roux WL. 1970. Recorded dust conditions and possible new sampling strategies on South African gold mines. In: Shapiro HA (ed). Pneumoconiosis. Proceedings of the International Conference. Johannesburg 1969. Cape Town: Oxford University Press..

Lippmann N. 2001. Size-selective health hazard sampling. In: Cohen BS, McCammon Jr CS (eds.). Air Sampling Instruments for Evaluation of Atmospheric Contaminants. 9th ed. Cincinnati: ACGIH. pp. 93-134.

't Mannetje A, Steenland K, Attfield M, Boffetta P, Checkoway H, DeKlerk N, Koskela RS. 2002. Exposure-response analysis and risk assessment for silica and silicosis mortality in a pooled analysis of six cohorts. Occup Environ Med. 59(11):723-728.

McDonald AD, McDonald JC, Rando RJ, Hughes JM, Weill H. 2001. Cohort mortality study of North American industrial sand workers. I. Mortality from lung cancer, silicosis and other causes. Ann Occup Hyg. 45(3):193-9.

McDonald JC, Oakes D. 1984. Exposure-response in miners exposed to silica. In: Sixth International Pneumoconiosis Conference. 1983. Bochum, Germany. Vol 1. Geneva: International Labour Office (ILO). Pp. 114-23.

Mehnert WH, Staneczek W, Mohner M, Konetzke G, Muller W, Ahlendorf W, Beck B, Winkelmann R, Simonato L. 1990. A mortality study of a cohort of slate quarry workers in the German Democratic Republic. IARC Sci Publ. Vol. 97. pp. 55-64.

Mossman BT, Churg A. 1998. Mechanisms in the pathogenesis of asbestosis and silicosis. Am J Respir Crit Care Med. 157(5 Pt 1):1666-80.

Muhle H, Bellman B, Creutzenberg O, Koch W, Dasenbrock C, Ernst H, Mohr U, Morrow P, Mermelstein R. 1998. Pulmonary response to toner, TiO_2, and crystalline silica upon chronic inhalation exposure in Syrian golden hamsters. Inhal Toxicol. 10:699-729.

Muhle H, Takenaka S, Mohr U, Dasenbrock C, Mermelstein R. 1989. Lung tumor induction upon long-term low-level inhalation of crystalline silica. Am J Ind Med. 15(3):343-6.

Muir DC, Julian JA, Shannon HS, Verma DK, Sebestyen A, Bernholz CD. 1989. Silica exposure and silicosis among Ontario hardrock miners: III. Analysis and risk estimates. Am J Ind Med. 16(1):29-43.

Mukherji S, Petrini J, Murphy T. 1993. North Santa Barbara County Crystalline Silica Study. Prepared for U.S. Environmental Protection Agency Region IX. Goleta, CA: Santa Barbara County Air Pollution Control District.

Murray J, Kielkowski D, Reid P (1996). Occupational disease trends in black South African gold miners. An autopsy-based study. Am J Respir Crit Care Med. 153(2):706-10.

NIOSH. 1974. National Institute for Occupational for Safety and Health. Criteria for a Recommended Standard. Occupational Exposure to Crystalline Silica. DHEW (NIOSH) Publication No. 75-120.

NIOSH (1994, 1996, 1998, 2003) Manual of Analytical Methods, 4th ed. DHHS (NIOSH) Publication 94-113 (August, 1994), 1st Supplement Publication 96-135, 2nd Supplement Publication 98-119, 3rd Supplement 2003-154. Schlecht PC, O'Connor PF, eds.

Ng TP, Chan SL. 1992. Lung function in relation to silicosis and silica exposure in granite workers. Eur Respir J. 5(8):986-91.

Ng TP, Chan SL. 1994. Quantitative relations between silica exposure and development of radiological small opacities in granite workers. Ann Occup Hyg. 38 Suppl 1:857-63.

OEHHA. 1999. Air Toxics Hot Spots Program Risk Assessment Guidelines. Part I. The Determination of Acute Reference Exposure Levels for Airborne Toxicants. Available online at http://www.oehha.ca.gov.

Oldham MJ, Mannix RC, Phalen RF. 1997. Deposition of monodisperse particles in hollow models representing adult and child-size tracheobronchial airways. Health Phys. 72(6):827-34.

Olivetti L, Grazioli L, Milanesio L, Provezza A, Chiodera P, Tassi G, Bergonzini R. 1993. Anatomo-radiologic definition of minimal interstitial silicosis and diagnostic contribution of high-resolution computerized tomography [in Italian]. Radiol Med (Torino). 85(5):600-5.

Oxman AD, Muir DC, Shannon HS, Stock SR, Hnizdo E, Lange HJ. 1993. Occupational dust exposure and chronic obstructive pulmonary disease. A systematic overview of the evidence. Am Rev Respir Dis. 148(1):38-48.

Page-Shipp RJ, Harris E. 1972. A study of dust exposure of South African white gold miners. South Afr Inst Mining Metall. 73:10-24.

Park R, Rice F, Stayner L, Smith R, Gilbert S, Checkoway H. 2002. Exposure to crystalline silica, silicosis, and lung disease other than cancer in diatomaceous earth industry workers: a quantitative risk assessment. Occup Environ Med. 59(1):36-43.

Parks CG, Conrad K, Cooper GS. 1999. Occupational exposure to crystalline silica and autoimmune disease. Environ Health Perspect. 107 Suppl 5:793-802.

Phalen RF, Oldham MJ, Beaucage CB, Crocker TT, Mortensen JD. 1985. Postnatal enlargement of human tracheobronchial airways and implications for particle deposition. Anat Rec. 212(4):368-80.

Rando RJ, Shi R, Hughes JM, Weill H, McDonald AD, McDonald JC. 2001. Cohort mortality study of North American industrial sand workers. III. Estimation of past and present exposures to respirable crystalline silica. Ann Occup Hyg. 45(3):209-16.

Rendall REG. 1999. The nature of dusts in the air of gold mines and foundries and the risk of silicosis. Thesis submitted (posthumously) to the University of the Witwatersrand.

Rice CH, Harris RL, Checkoway H, Symons MJ. 1986. Dose-response relationships for silicosis from a case-control study of North Carolina dusty trades workers. In: Goldsmith DF, Winn DM, Shy CM (eds). Silica, Silicosis, and Cancer. Controversy in Occupational Medicine. New York: Prager. Pp. 77-86.

Rice FL, Stayner LT. 1995. Assessment of silicosis risk for occupational exposure to crystalline silica. Scand J Work Environ Health. 21 Suppl 2:87-90.

Rosenman KD, Reilly MJ, Rice C, Hertzberg V, Tseng CY, Anderson HA. 1996. Silicosis among foundry workers. Implication for the need to revise the OSHA standard. Am J Epidemiol. 144(9):890-900. Saiyed HN, Chatterjee BB. 1985. Rapid progression of silicosis in slate pencil workers: II. A follow-up study. Am J Ind Med. 8(2):135-42.

Saiyed HN, Parikh DJ, Ghodasara NB, Sharma YK, Patel GC, Chatterjee SK, Chatterjee BB. 1985. Silicosis in slate pencil workers: I. An environmental and medical study. Am J Ind Med. 8(2):127-33.

Saiyed HN, Sharma YK, Sadhu HG, Norboo T, Patel PD, Patel TS, Venkaiah K, Kashyap SK. 1991. Non-occupational pneumoconiosis at high altitude villages in central Ladakh. Br J Ind Med. 48(12):825-9.

Scheuchenzuber WJ, Eskew ML, Zarkower A. 1985. Effects of prolonged inhalation of silica and olivine dusts on immune functions in the mouse. Environ Res. 38(2):389-99.

Schiller-Scotland CF, Hlawa R, Gebhart J. 1994. Experimental data for total deposition in the respiratory tract of children. Toxicol. Lett. 72(1-3):137-44.

Seixas NS, Heyer NJ, Welp EA, Checkoway H. 1997. Quantification of historical dust exposures in the diatomaceous earth industry. Ann Occup Hyg. 41(5):591-604.

Sichel HS (1957). On the size distribution of airborne mine dust. J S Afr Inst Min Metal. 58(5):171-225.

Steenland K, Brown D. 1995. Silicosis among gold miners: exposure--response analyses and risk assessment. Am J Public Health. 85(10):1372-7.

Steenland K, Goldsmith DF. 1995. Silica exposure and autoimmune diseases. Am J Ind Med. 28(5):603-8.

Stratta P, Canavese C, Messuerotti A, Fenoglio I, Fubini B. 2001. Silica and renal diseases: no longer a problem in the 21st century? J Nephrol. 14(4):228-47.

Suhr H, Bang B, Moen BE. 2003. Respiratory health among quartz-exposed slate workers--a problem even today. Occup Med (Lond). 53(6):406-7.

Theriault GP, Burgess WA, DiBerardinis LJ, Peters JM. 1974. Dust exposure in the Vermont granite sheds. Arch Environ Health. 28(1):12-7.

USEPA. 1995. U.S. Environmental Protection Agency. The Use of the Benchmark Dose Method in Health Risk Assessment. EPA/630/R-94/007. Washington, DC: U.S. EPA.

USEPA. 1996. U.S. Environmental Protection Agency. Ambient Levels and Noncancer Health Effects of Inhaled Crystalline and Amorphous Silica: Health Issue Assessment. EPA/600/R-95/115. Office of Research and Development. Washington, DC: U.S. EPA.

Vallyathan V, Kang JH, Van Dyke K, Dalal NS, Castranova V. 1991. Response of alveolar macrophages to in vitro exposure to freshly fractured versus aged silica dust: the ability of Prosil 28, an organosilane material, to coat silica and reduce its biological reactivity. J Toxicol Environ Health. 33(3):303-15.

Vallyathan V, Castranova V, Pack D, Leonard S, Shumaker J, Hubbs AF, *et al*. 1995. Freshly fractured quartz inhalation leads to enhanced lung injury and inflammation. Potential role of free radicals. Am J Respir Crit Care Med. 152(3):1003-9.

Verma DK, Sebestyen A, Julian JA, Muir DC, Schmidt H, Bernholz CD, Shannon HS. 1989 Silica exposure and silicosis among Ontario hardrock miners: II. Exposure estimates. Am J Ind Med. 16(1):13-8.

Wagner G. (1995). The inexcusable persistence of silicosis. Am J Public Health. 85(10):1346-7.

Wagner WD, Fraser DA, Wright PG, Dobrogorski OJ, Stokinger HE. 1968. Experimental evaluation of the threshold limit of cristobalite--calcined diatomaceous earth. Am Ind Hyg Assoc J. 29(3):211-21.

Witschi HR, Last JA. 2001. Toxic responses of the respiratory system. In: Casarett and Doull's Toxicology. The Basic Science of Poisons. 6th Ed. Klaassen CD (ed). New York: McGraw-Hill.

World Health Organization (WHO). 1986. Recommended health-based limits in occupational exposure to selected mineral dusts (silica, coal). Geneva, Switzerland: World Health Organization, Technical Report Series 734.

X. Appendix

Particulate Levels of Interest for Exposure to Respirable Crystalline Silica Isomorphs

150 μg/m^3	Federal 24 hour PM_{10} standard
65 μg/m^3	Federal 24 hour $PM_{2.5}$ standard
50 μg/m^3	California 24 hour PM_{10} standard
50 μg/m^3	Federal PM_{10} annual standard (chronic exposure)
50 μg/m^3	8 hour TLV for quartz, cristobalite, and tridymite for workers (ACGIH Method)
50 μg/m^3	estimated workplace LOAEL for silicosis from studies by Theriault *et al*. (“)
20 μg/m^3	CA annual PM_{10} standard (chronic exposure) (arithmetic mean)
15 μg/m^3	Federal annual $PM_{2.5}$ standard (chronic exposure)
12 μg/m^3	CA annual $PM_{2.5}$ standard (chronic exposure) (arithmetic mean)
12 μg/m^3	current silica TLV adjusted to equivalent continuous exposure (50 μg/m^3 x 8 h/24 h x 5 d/7d) (ACGIH)
10 μg/m^3	TLV for silica proposed by Greaves (2000) (ACGIH)
8 μg/m^3	current silica TLV further adjusted by 46/70 years occupational exposure (ACGIH)
8 μg/m^3	estimated high-end ambient crystalline silica level in US (USEPA, 1996) (PM_{10})
6.7 μg/m^3	lower bound on 1% risk of silicosis estimated by USEPA (1996) (PM_{10})
5 μg/m^3	TLV for silica proposed by Chen *et al*. (2001) (ACGIH)
5 μg/m^3	“acceptable” ambient level for silica (10% of PM_{10}) (USEPA, 1996)
5 μg/m^3	RfC for diesel exhaust particulate, a respirable PM ($PM_{2.5}$)
3 μg/m^3	estimated average ambient exposure to crystalline silica (USEPA, 1996) (PM_{10})
3 μg/m^3	draft silica chronic REL proposed by OEHHA (ACGIH)
2.3 μg/m^3 (1.17-3.46; n=12)*	silica level during 1989 in Santa Maria, CA (urban site) (PM_{10})
0.6 μg/m^3 (0-1.44; n=16)*	silica level during 1989 in Santa Ynez, CA (rural site) (“)
0.2 μg/m^3 (0-1.15; n=18)*	silica level during 1989 in Buellton, CA (remote background)(“)

* mean, range, and number of crystalline silica measurements (Mukherji *et al*., 1993)

CHRONIC TOXICITY SUMMARY

STYRENE

(ethenylbenzene, phenylethylene, vinylbenzene)

CAS Registry Number: 100-42-5

I. Chronic Toxicity Summary

Inhalation reference exposure level	**900 μg/m^3 (200 ppb)**
Critical effects(s)	Neuropsychological deficits in humans as measured by memory and sensory/motor function tests
Hazard index target(s)	Nervous system

II. Chemical Property Summary

Description	Colorless to slightly yellow liquid with sweet, floral odor (HSDB, 1999)
Molecular formula	C_8H_8
Molecular weight	104.16
Boiling point	145.2 ^oC
Melting point	-31^oC (HSDB, 1999)
Vapor pressure	10 torr at 31^oC, polymerizes at 82^oC and above (Weast, 1979)
Solubility	310 μg/ml (Dean, 1985)
Conversion factor	4.26 μg/m^3 per ppb at 25^oC

III. Major Uses and Sources

The major source of styrene is industrial synthesis in which ethylbenzene is the starting material (ATSDR, 1992). The major uses of styrene are in polystyrene manufacturing, the butadiene-styrene rubber industry, and in the reinforced plastics industry (RPI) (WHO, 1983). Major non-styrene contaminants in the butadiene-styrene rubber industry are butadiene, benzene, carbon disulfide, and trichloroethylene, whereas the main co-contaminants associated with the RPI are glass fibers and acetone (WHO, 1983). Environmental exposures to styrene may result from mainstream cigarette smoke (Newhook and Caldwell, 1993) and newly installed carpets containing a styrene-butadiene rubber latex adhesive (Hodgson *et al.*, 1993). The Third National Health and Nutrition Examination Survey (NHANES) (Ashley *et al.*, 1994) reported a mean blood styrene level among ≥ 600 individuals as 0.074 ppb. In 1996, the latest year tabulated, the statewide mean outdoor monitored concentration of styrene was less than 0.1 ppb (CARB, 1999a). The annual statewide industrial emissions of styrene from facilities reporting under the

Air Toxics Hot Spots Act in California based on the most recent inventory were estimated to be 2,365,873 pounds (1999b).

IV. Effects of Human Exposure

Chronic exposures to styrene (to be discussed below) result in central nervous system (CNS) and peripheral nervous system effects, although the latter are not as pronounced (ATSDR, 1992; Rebert and Hall, 1994; Murata *et al.*, 1991). Irritation or discomfort of the upper respiratory tract resulting from styrene exposure has not been reported in long-term occupational studies (Foureman, 1994). However, sensory irritation and neurological impairment does occur in acute human studies at concentrations above 100 ppm (Stewart *et al*., 1968). The evidence for styrene induced hepatic changes is either negative or equivocal (ATSDR, 1992). Evidence for nephrotoxicity due to long-term occupational exposure is also negative or equivocal (ATSDR, 1992; Verplanke and Herber, 1998; Kolstad *et al.*,1995). Some human studies suggest that chronic exposure to styrene results in reproductive effects, but the limited data are difficult to interpret because of the small sample numbers (Brown, 1991; Lindbohm, 1993). Immunologic alterations (e.g., altered phenotypic profiles among lymphocyte subsets, decreased natural killer cell activity, and decreased chemotaxis) have also been observed, but the limited data prevent quantitative interpretation (Bergamaschi *et al.*, 1995; Governa *et al.*, 1994).

The CNS depressant effects of acute exposures to high styrene levels are probably mediated by the direct effect of the lipophilic, unmetabolized styrene on nerve cell membranes. Long-term effects of styrene exposure may result from the action of one or more metabolites of styrene (Savolainen, 1977; Mutti *et al.*, 1988). In humans, styrene metabolism is initiated by cytochrome P450 (P450)-mediated oxidation of styrene to a reactive metabolite, styrene oxide. The reaction takes place in human liver and, to a minor extent, in lung (Nakajima *et al.*, 1994). The P450 enzymes responsible for the epoxidation of styrene to stryene oxide are also found in human brain, but the brain isozymes have not been tested specifically with styrene as a substrate (Bhamre *et al.*, 1993). Styrene may also be oxidized to styrene oxide by enzymes which share specific iron and porphyrin components with P450 and those that utilize active oxygen species (Belvedere *et al.*, 1983; Tursi *et al.*, 1983; Miller *et al.*, 1992).

The major end product of styrene metabolism in humans is urinary mandelic acid (MA) and phenylglyoxylic acid (PGA) (Bardodej and Bardodejova, 1970; Leibman, 1975; Guillemin and Bauer, 1979). Other pathways that may be present in other animals are either absent or are quantitatively negligible in humans, except when high styrene levels are encountered (Guillemin and Berode, 1979; Chakrabarti *et al.*, 1993; Hallier *et al.*, 1995). Confounders of the quantitative relationship between styrene exposure and urinary MA+PGA are the consumption of ethanol (Berode *et al.*, 1986) and exposure to ethylbenzene (Bardodej and Bardodejova, 1970). An important consequence of ethanol related decreased levels of urinary mandelic acid is the potential underestimation of exposure to styrene (Guillemin and Bauer, 1979; Berode *et al.*, 1986). However, the urinary metabolite levels return to control values 4-5 hours after the ethanol consumption (Berode *et al.*, 1986).

Indicators of human styrene exposure include exhaled styrene, blood styrene, urinary MA, and urinary MA+PGA (Guillemin and Berode, 1988). Exposure to styrene by inhalation results in 89 percent absorption (Guillemin and Berode, 1988). In the occupational studies that are the basis

for quantifying the relationship between chronic styrene exposure and health effects, end-of-shift or next-morning MA+PGA have been used. The next-morning measurements are more reflective of past exposures due to the high fat solubility of styrene (fat:blood partition coefficient = 94 (Csanady *et al.*, 1994)), the presence of a second, long biological half-life for MA = 25 hours., and a long biological half-life for PGA = 11 hours (Guillemin and Bauer, 1979). Following inhalation, the half-life for styrene is 41 minutes in blood (Wigaeus *et al.* 1983) and 32-46 hours in fat tissue (Perbellini *et al.*, 1988).

One postulated mechanism for the chronic non-cancer toxicity of styrene is the binding of the highly reactive styrene oxide to components of nervous tissue. Another postulated mechanism is an alteration in the levels of circulating catecholamines (e.g., dopamine) due to the binding of PGA to these biogenic amines (Mutti, 1993; Mutti *et al.*, 1984a; Checkoway, 1994) and the subsequent changes in physiological functions that are under biogenic amine control. Although long-term exposures to styrene are associated with decrements in physiological functions, the exact mechanism(s) for these effects have not been clearly established (see reviews by ATSDR, 1992; Mutti, 1993; Rebert and Hall, 1994).

Kolstad *et al.* (1995) estimated excess deaths due to four major non-malignant disease groups for 53,847 male workers in the Danish RPI. Low and high styrene exposures were based on companies with less than 50% (low) and those with 50% or more (high) employees involved with reinforced plastics. An internal comparison was made with workers unexposed to styrene to account for more similar activities and lifestyles. Statistically significant ($p < 0.05$) excess deaths due to pancreatitis and degenerative disorders of the myocardium and non-significant excess deaths due to degenerative diseases of the nervous system were observed. Non-significant excess deaths due to glomerulonephritis were also observed.

Checkoway *et al.* (1994) described a cross-sectional study of 59 male boat plant workers exposed to <1 to 144 (mean = 37.2) ppm styrene. Monoamine oxidase B (MAO-B) activity in platelets was measured as an indicator of catecholamine metabolism. When the styrene exposed workers were divided into quartile exposures, a dose dependent decrease in MAO-B activity was observed after adjustments were made for age, smoking, alcohol and medication use.

Female workers employed in the reinforced plastics industry (RPI) were studied for levels of substances associated with neuroendocrine function (Mutti *et al.*, 1984a**).** Serum prolactin, thyroid stimulating hormone, human growth hormone, follicle stimulating hormone, and luteinizing hormone were measured in 30 women who were between the 5th and 15th day of the menstrual cycle. Exposure was based on the next-morning MA+PGA, and levels of the neuroendocrine substances were measured in venous blood samples taken the next morning before the start of work. On the basis of a relationship (not detailed in the report) between urinary metabolites and styrene air concentration, the authors estimated that the average styrene TWA/8 hr was about 130 ppm. Controls consisted of women factory workers living in the same area as the styrene-exposed women, but not knowingly exposed to styrene. After controlling for age and exposure time, the increased prolactin and thyroid stimulating hormone levels were correlated with the concentration of next-morning urinary MA+PGA, although only the increased prolactin levels were statistically significant.Numerous occupational studies have noted CNS disturbances in styrene-exposed workers. Decreased manual dexterity, increased reaction times, and/or abnormal vestibuloocular reflex (ability to track moving objects) were observed by Gotell *et al*. (1972), Gamberale *et al*. (1975), Lindstrom *et al*. (1976), Mackay and Kelman (1986), Flodin *et al.* (1989), Moller *et al*. (1990), and Cherry and Gautrin (1990) for air

styrene levels of about 12 ppm to more than 100 ppm. However, in each of these studies, there were difficulties in quantifying the effect. The difficulties included small sample size, unknown exposure duration, lack of concurrent control group, lack of dose-response data, and either unknown ethanol consumption or lack of adjustment for ethanol consumption. In the Cherry and Gautrin (1990) investigation, however, the authors determined that accounting for ethanol consumption did not reduce the correlation between increased reaction time and exposure.

Decrements in other CNS functions were observed among workers in the well controlled studies of Fallas *et al.* (1992), Chia *et al.* (1994), and Mutti *et al.* (1984b). Fallas *et al.* (1992) studied 60 male workers (average age = 29.5 years, average air styrene = 24.3 ppm). The styrene-exposed population was compared to non-exposed worker controls and matched for age, intellectual level, and ethnic origin. The results from a standardized test battery showed decrements in the aiming response and 22/60 styrene exposed workers exhibited increased reaction times compared to 7/60 controls. Acquired color vision loss (dyschromatopsia) was also observed in the styrene-exposed workers compared to controls. Chia *et al.* (1994) also observed decrements in CNS function as defined by altered visual retention, audio-digit recognition, and digit recognition. However, a dose-response relationship did not exist. These workers also exhibited a statistically nonsignificant dose-dependent dyschromatopsia.

In the most comprehensive occupational study to date on CNS effects of styrene exposure, Mutti *et al.* (1984b) assessed memory and sensory/motor function in a group of 50 male styrene-exposed workers (average exposure = 8.6 years) and a control group of 50 manual workers. In addition to matching for age, sex, and educational level, a vocabulary test was included to match for general intelligence. Eligibility criteria included absence of metabolic, neurologic, or psychiatric disorders, limited ethanol intake, and limited cigarette usage. All subjects were instructed to avoid intake of alcohol and drugs for two days prior to testing. Styrene exposure was assessed from urinary MA+PGA levels the morning after the last workday in the week, followed immediately by participation in a battery of 8 neuropsychological tests designed to measure CNS function. The tests included reaction time, short and long term logic memory, short and long term verbal memory, digit-symbol association (using a reference code), block design (reproducing a displayed design using colored blocks), and embedded figures (timed identification of figures in Rey's table). The mean $\pm$ 2 SDs of the values found in the control group was set as the normal range limit for each neuropsychological test. The results were expressed as continuous and quantal data. Expressed as continuous data, styrene-exposed workers exhibited significantly poorer performances than controls in all tests, except in the digit-symbol test. Also, urinary metabolite concentration and duration of exposure were found to be significantly correlated with the scores of several tests. As a subgroup, workers with metabolite levels of up to 150 mmoles MA+PGA/mole creatinine (mean = 75 mmoles/mole creatinine $\pm$ 33 [SD], which is equivalent to a mean styrene concentration of 15 ppm) appeared to have no significant effects. The authors state that this level of urinary metabolites corresponds to a mean daily 8-hour exposure to air styrene of 25 ppm (106 mg/m^3). Based on greater urinary excretion of styrene metabolites, significantly poorer performances in four or more neuropsychological tests were recorded in the other three subgroups (150-299, 300-450, and > 450 mmoles MA + PGA/mole creatinine).

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Mutti *et al.* (1984b) expressed the quantal data as the fraction of tested subjects who responded abnormally to ≥ 1, ≥ 2, and ≥ 3 tests (see Table 1). Positive dose-response relationships existed between intensity of styrene exposure (mmoles MA + PGA/mole creatinine) and abnormal scores, whether it was expressed as abnormal responses in at least one, at least two, or at least three neuropsychological tests. The chi-square test and validity calculations were performed by constructing 2 x 2 tables selecting different levels of urinary excretion of MA and PGA as a cut-off point. The highest values for chi-square and predictive validity were found when the cut-off of 150 mmol/mol creatinine was chosen, suggesting that the quantal isolation of the low dose subgroup from the next subgroup is appropriate. When the quantal data for the low dose subgroup were analyzed by OEHHA using the Fisher's Exact Test, a significant level of abnormal responses were observed for ≥1 ($p = 0.005$) and ≥3 ($p = 0.04$) tests. The abnormal responses for ≥2 tests were statistically marginal ($p = 0.06$). For each of the remaining exposure groups, the p-values were <0.05. Unlike the assumptions made concerning the continuous data, quantal data results suggest that the low dose subgroup represents a LOAEL, and that a NOAEL is not available from the data. Mutti *et al*. (1984b) also expressed the data in a quantal three-way representation including prevalence (number of respondents for at least one, two or three abnormal tests), duration (years at work), and intensity (metabolite level). This representation revealed a positive correlation of neuropsychological deficits with duration as well as intensity.

Table 1. Subjects Classified Positive on Neuropsychological Tests as a Function of Styrene Exposure [a].

MA+PGA, mmoles per mole creatinine[b]	Total Subjects	Number of Abnormal Tests		
		≥ 1	≥ 2	**≥ 3**[c]
Controls	50	4/50	2/50	**0/50**
< 150 (mean = **75** ± 33)[d]	14	6/14	3/14	**2/14**
150-299 (mean = **216** ± 45)	9	6/9	5/9	**3/9**
300 - 450 (mean = **367** ± 49)	14	10/14	7/14	**5/14**
> 450 (mean = **571** ± 108)	13	11/13	8/13	**6/13**

[a] Data from Table IV in Mutti *et al.* (1984b).
[b] "Next-morning" styrene urinary metabolites.
[c] The quantal grouping of the number of subjects that performed abnormally in ≥3 tests based on their styrene urinary metabolite concentrations, both shown in bold, were used in a benchmark concentration (BMC) analysis for the derivation of the REL (see Section VI below).

[d] Based on Guillemin *et al.* (1982), a linear relationship exists for converting the urinary metabolite concentrations to ppm air styrene levels (4.97 mmoles MA+PGA/mole creatinine is equivalent to 1 ppm styrene). Thus, the mean styrene concentrations per group are 0, 15, 44, 74,

and 115 ppm.In addition to dyschromatopsia observed by Chia *et al*. (1994), Gobba and Cavalleri (1993) and Campagna *et al*. (1995) also reported this visual dysfunction among styrene workers in the reinforced plastics industry. Workers (n=36) exposed to an average of 16 ppm styrene exhibited significantly greater dyschromatopsia than controls, matched for age, ethanol consumption and tobacco smoking (Gobba and Cavalleri, 1993). Among the study population, only 1/36 styrene-exposed workers (compared to 16/36 controls) performed the test with 100 percent accuracy. When a different group of styrene-exposed workers was tested, those exposed to > 50 ppm styrene exhibited greater dyschromatopsia than those exposed to ≤ 50 ppm, and within this group, a subset exhibited a similar decrement after returning from a one month vacation. In the Campagna *et al.* (1995) study, the test for dyschromatopsia was given to 81 reinforced plastics industry workers (79 male and 2 female) exposed to 4.6, 10.1, and 88.8 ppm styrene (first quartile, median, and third quartile, respectively). No control group was used in this study. Statistical analysis revealed a correlation of color vision loss with exposure to styrene (defined as next-morning urinary mandelic acid), age, and ethanol consumption.

Exposure to styrene may affect the peripheral nervous system (PNS). In a case report (Behari *et al.*, 1986), a man working for 5 years with a photostat process that used styrene was diagnosed with peripheral neuropathy. However, in occupational studies, the relationship between exposure to styrene and PNS effects has been inconsistent (Triebig *et al.*, 1985; Cherry and Gautrin, 1990). A major difficulty in understanding the potential for this relationship is the lack of knowledge about the appropriate surrogate for dose that leads to PNS disturbance (Murata *et al.*, 1991). In one study, however, chronic exposure indices were developed which included work method, years at work, time spent laminating (source of high exposure), styrene air concentration, and end-of-shift urinary mandelic acid (Matikainen *et al.* (1993). Numbness in the extremities increased with the exposure index, although statistically the effect was marginally insignificant ($p < 0.1$). The styrene TWA/8 hr was 32 ppm for the 100 study subjects.

Female reproductive toxicity has been inconsistently reported among humans (Brown, 1991; Lindbohm, 1993). These studies are difficult to interpret because of the high background rates of endpoints such as spontaneous abortion and menstrual disorders in combination with confounding exposures. In those studies that showed no reproductive effects due to styrene exposure, the power of the studies was low due to the small numbers of women. Hence the evidence for any adverse effects of exposure to styrene on female reproductive function is inconclusive.

Male workers employed in the reinforced plastics industry were examined for effects on sperm chromatin structure and semen quality (Kolstad *et al*., 1999a) and time to pregnancy (Kolstad et al., 1999b). No indications of an exposure-response relationship were seen when individual changes in semen quality were related to the postshift urinary mandelic acid concentrations among 23 exposed workers. A weak increase in sperm DNA-susceptibility to *in situ* denaturation as a function of mandelic acid concentration was indicated, but was within the interassay variability. No detrimental effect of styrene exposure was observed with regard to male fecundity among 188 exposed workers when compared to 353 unexposed workers.

Immune system alterations were reported in a study conducted by Bergamaschi *et al.* (1995). Reinforced plastics industry workers (n=32 female/39 male, average age = 32 years, average exposure duration = 7 years) were compared with non-styrene exposed factory workers and matched for age, sex, tobacco use and ethanol consumption. Air styrene levels, among the different factories, varied between 10 - 50 ppm, and individual worker exposure was measured

by urinary metabolites the morning after the last shift (15 hours post-exposure). Among all workers in the study (median exposure = 16 ppm - according to the data of Guillemin *et al.* (1982)), the proportion of 12/18 lymphocyte subsets and the prevalence of abnormal values of immunologic phenotypes for 11/18 subsets were statistically different from the controls ($p < 0.001$ to < 0.05). When the workers were placed into three exposure groups (0, < 25 ppm, and > 25 ppm styrene), dose-response relationships were observed for prevalences of abnormal responses for four lymphocyte subsets and, in the case of two subsets, abnormal responses were observed in the group exposed to < 25 ppm styrene. Natural killer cell activity (a lymphocyte function), measured in a different group of workers in the same study, was decreased compared to unexposed worker controls. The median exposure, given in terms of urinary metabolites, was calculated as 21 ppm based on the data of Guillemin *et al.* (1982). The data show that exposure of these workers to air styrene levels below 50 ppm, and probably at levels near 25 ppm, resulted in alterations of the immune system.

Governa *et al.* (1994) observed reduced chemotactic responses of polymorphonuclear lymphocytes (PMNs) obtained from 21 styrene-exposed workers. However, the lack of exposure data prevents a quantitative assessment. In the same study, 0.1 - 0.6 mM styrene inhibited the chemotaxis of isolated healthy PMNs.

V. Effects of Animal Exposure

In a subchronic study, carried out under the auspices of NTP (NTP, 1992), mice and rats were exposed by inhalation to styrene vapors to establish a maximum tolerated dose for chronic studies. Mice were exposed to 0, 62.5, 125, 250, or 500 ppm styrene (6 hr/d, 5 d/wk, 13 wks). Among males deaths occurred in the 250 ppm group. Body weights among all exposed mice were lower than controls, and the difference was about 9 percent. Lung, olfactory epithelial, and forestomach lesions were observed in females and males. In females, degeneration of the adrenal gland cortex was observed. An effect not discussed in the chairperson's report, but recorded in the original laboratory report, was an increased estrous cycle length among the female mice at all styrene doses. A LOAEL of 62.5 ppm is indicated by the olfactory epithelial, forestomach and respiratory tract lesions in mice of both sexes and for lesions in the adrenal cortex in the female mice. Rats were exposed to 0, 125, 250, 500, 1000, or 1500 ppm styrene (6 hr/d, 5 d/wk, 13 wks). No deaths occurred, but reduced body weights were observed at the two highest doses. Lesions of the respiratory tract were observed at all dose levels. A LOAEL of 125 ppm is therefore indicated for the rats.

Rats were exposed by ingestion for 2-years to styrene in drinking water (0, 125, and 250 ppm). (The water solubility of styrene is 310 ppm.) The only effect was a styrene-related reduction in water consumption (Beliles *et al.*, 1985).

Kishi *et al.* (1995) carried out a developmental study on rat pups born to dams exposed by inhalation to styrene (0, 50, 300 ppm; 6-hr/d; gestation days 7-21). Although the small number of litters (n=2) at the 50 ppm dose prevented detailed statistical analysis, the data suggest that exposure of the dams to 50 ppm styrene resulted in deficits and delays in some motor and coordination abilities among the pups. Pups born to dams exposed to 300 ppm exhibited statistically significant increases in spontaneous activity and in the delay of some neurobehavioral functions. Many of the effects became diminished as the pups aged. Measurements of reproductive toxicity (maternal weight gain, length of gestation, number of live

births) did not change. Postnatal body weights were lower among the styrene-exposed pups, but the differences became less as the pups aged to 125-days.

A follow-up developmental study by the same research group investigated neurochemical levels in rat pups born to dams exposed by inhalation to styrene (0, 50, 300 ppm; 6 hr/day on gestation days 6-20) (Katakura *et al*., 1999). Cerebrum weights of day 0 pups were significantly lower when compared to cerebrum weights of *ad libitum* fed animals, but not pair-fed animals. At the highest dose, occasional reductions in neuroamines, i.e. 5-hydroxytryptamine, homovanillic acid, and 5-hydroxyindoleacetic acid, were seen in various parts of the brains of rat pups compared to one or both control groups on day 0 and day 21. No reproductive or histopathological changes were seen.

Rosengren and Haglid (1989) investigated whether long term inhalation exposure (three months) to styrene (90 and 320 ppm) could induce long lasting astroglial alterations in Sprague Dawley rats, traceable four months after exposure ceased. Styrene exposure at 320 ppm induced the alterations as shown by raised concentrations of the glial cell marker, glial fibrillary acidic protein (GFA), in the sensory motor cortex and in the hippocampus. GFA is the structural protein of the astroglial filaments. These filaments form after damage to the central nervous system from any cause. The authors concluded that exposure to styrene at moderate exposure levels induces regional, long lasting astroglial reactions that serve as an indicator of solvent induced brain damage.

Mice, exposed acutely (14 days) by inhalation to 125 - 500 ppm styrene, exhibited decreased spleen / body weight, splenic hypocellularity, altered lymphocyte proportions among subsets, and increased proliferative response to mitogens (Corsini *et al.*, 1994). Mice and rats, exposed by gavage to high levels of styrene (18, 27, 45 mg/kg - mouse; 118, 177, 294 mg/kg - rat) for 5 days/week for 4 weeks, exhibited decreased resistance to encephalomyocarditis virus, *Plasmodium berghie* (a malaria parasite), and *Nippostrongylus braseleinisi* (a parasitic worm) (Dogra *et al.*, 1992).

Groups of 70 male and 70 female Charles River CD (Sprague-Dawley-derived) rats were exposed whole body to styrene vapor at 0, 50, 200, 500, or 1000 ppm 6 h/day 5 days/week for 104 weeks (Cruzan *et al*., 1998). A battery of hematologic and clinical pathology examinations was conducted at 13, 26, 52, 78, and 104 weeks. Nine or 10 rats per sex per group were necropsied after 52 weeks of exposure and the remaining survivors were necropsied after 104 weeks. Control and high-exposure rats received a complete histopathologic examination, while target organs, gross lesions, and all masses were examined in the other 3 groups. Styrene had no effect on survival in males, but females exposed to 500 or 1000 ppm had a dose-related increasein survival. Levels of styrene in the blood at the end of a 6-h exposure during week 95 were proportional to exposure. Levels of styrene oxide in the blood of rats exposed to 200 ppm or greater styrene were proportional to styrene exposure concentration. The authors found no changes of toxicologic significance in hematology, clinical chemistry, urinalysis, or organ weights. Styrene-related non-neoplastic histopathologic changes were confined to the olfactory epithelium of the nasal mucosa. (The authors also found no evidence of cancer induction.)

Groups of 70 male and 70 female CD-1 mice were exposed in whole body inhalation chambers to styene vapor concentrations of 0, 20, 40, 80, and 160 ppm 6 hrs/day, 5 days/week, over a period of up to 2 years (Huntingdon Life Sciences, 1998). Ten mice per sex per group were

necropsied after 52 and 78 weeks of exposure, and the remaining survivors necropsied after 104 weeks. Due to increased mortality in female control mice, terminal sacrifice for this group occurred at 98 weeks. Two female mice exposed to 160 ppm styrene died during or immediately following the first week of exposure. Histopathology revealed liver necrosis that was a likely contributor to the deaths. Reduced body weight gain and increased food consumption were observed in male mice at the two highest exposure levels and in female mice at the highest exposure level. Both styrene monomer and styrene oxide in blood increased with exposure concentration. No changes of toxicologic significance in hematology, ophthalmology, clinical chemistry, urinalysis, or organ weights were noted. Styrene-related non-neoplastic histopathologic changes were seen in the lungs (bronchiolar-alveolar hyperplasia) and nasal olfactory epithelium (respiratory metaplasia, degeneration or necrosis, and changes to the underlying Bowman's glands) from all exposure groups. The nasal lesions showed progression with time. Focal loss of bone from the turbinate was also seen more frequently as the study progressed. In addition, atrophy of the olfactory nerve fibers was present in mice at the three highest exposure concentrations.

VI. Derivation of the Chronic Reference Exposure Level (BMC Approach)

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Study	Mutti *et al.* (1984b)
Study populations	Human (occupational)
Exposure method	Inhalation
Critical effects	Central nervous system
LOAEL	15 ppm
NOAEL	Not established
BMC_{05}	1.7 ppm
Exposure continuity	8 hr/d (10 m^3 per 20 m^3 day), 5 d/wk
Exposure duration	8.6 years (average years at work)
Average occupational exposure	0.61 ppm (1.7 x 10/20 x 5/7)
Human equivalent concentration	0.61 ppm
LOAEL uncertainty factor	Not needed in the BMC approach
Subchronic uncertainty factor	1 (average exposure 12.3% of lifetime)
Interspecies uncertainty factor	1
Intraspecies uncertainty factor	3
Cumulative uncertainty factor	3
Inhalation reference exposure level	0.2 ppm (200 ppb; 0.9 mg/m^3; 900 $\mu g/m^3$)

The most relevant chronic noncancer effect due to styrene exposure is neurotoxicity. The Mutti *et al*. (1984b) occupational study presented convincing dose-response information and was well designed and executed in terms of experimental protocol and statistical evaluation, which included tests for false positive and false negative responses. While not all confounders could be ruled out (e.g., compensatory mechanisms, biorhythms, workers who leave because of styrene related illness), careful attention was paid to include eligibility criteria for the control group that correct for confounders unique for this population (e.g., limited ethanol intake, a control work-force not exposed to neurotoxic substances, and a test to allow a match for general intelligence). The use of urinary metabolites to measure exposure dose is based on the observation that the

next-morning urinary MA+PGA is directly related to the air level of styrene. The Guillemin *et al.* (1982) study provides the basis for the conversion of urinary MA+PGA levels to styrene exposure levels used by Mutti *et al.* (1984b).

The quantal dose-response data by Mutti et al. (1984b) is applicable for use in a benchmark concentration (BMC) approach. The quantal grouping of the number of subjects that performed abnormally in $\geq$3 tests based on their urinary metabolite concentrations was chosen for a BMC analysis (see Table 1). Basing the BMC on abnormal responses to >3 tests reduces the complexity of multiple test comparisons and the potential for inappropriate comparison of different neuropsychological tests between control and exposure groups for statistical purposes. Also, the potential for false positive responses is reduced due to the zero background level of abnormal responses in the control group when the criteria are >3 abnormal tests. Using a log-normal probit analysis (Tox-Risk, version 3.5; ICF-Kaiser Inc., Ruston, LA) with the data (emphasized in bold typeface) in Table 1 (above) the maximum likelihood estimate (MLE) for a 5% response was 4.0 ppm. The resulting 95% lower confidence limit at the MLE provided a BMC_{05} of 1.7 ppm. A BMC_{05} is considered to be similar to a NOAEL in estimating a concentration associated with a low level of risk. Following adjustment for exposure continuity (10 m^3 per 20 m^3 day for 5 d/wk) and application of an UF of 3 to account for human intraspecies variability, a REL of 0.2 ppm (0.9 mg/m^3) was attained. For exposure data that utilizes healthy human subjects, the resulting BMC represents a less than 10% incidence in the general population. When combined with an UF of 3, as carried out above, the resulting REL will be protective of the vast majority of individuals.

This analysis of the quantal data is supported by recognizing that, in a population of 50 subjects, individual test-specific effects that occur at low doses may not have been observed. If the criterion for abnormality is expressed in terms of CNS dysfunction, defined by all tests, the sensitivity of the testing procedure is increased and the low dose effects are more easily observed. The quantal data of Mutti *et al.* (1984b), i.e., the proportion of subjects responding abnormally to the tests, therefore provide a more sensitive approach to detecting low dose effects. Collasping a battery of test data to increase sensitivity may introduce the dilemma of multiple test comparisons, as noted above. However, OEHHA believes that a statistical method to correct for this, known as a Bonferroni correction, is unnecessary. The REL development is based on calculating a statistic of one effect of a complex of responses (or a syndrome) that results from CNS dysfunction, and not based on calculating a statistic for each test within the group of tests. The apparent global nature of the neurological syndrome resulting from long-term styrene exposure, in addition to basing the BMC on abnormal responses to >3 tests, should more than adequately address any concerns that may result from combining neurological test data.

Applying NOAEL/LOAEL methodology to the Mutti *et al*. (1984b) quantal data yields an exposure value similar to that attained with the BMC approach. The LOAEL of 15 ppm is adjusted to an equivalent continuous exposure of 5.36 ppm (15 ppm x 10/20 m^3 x 5/7 d/wk). Use of a LOAEL UF of 3 and an intraspecies UF of 10 resulted in an estimated REL of 0.2 ppm (0.8 mg/m^3).

The U.S. EPA (1996) calculated a reference concentration (RfC) of 0.3 ppm (1 mg/m^3), which is slightly higher than the OEHHA-derived chronic REL of 0.2 ppm (0.9 mg/m^3). The RfC for styrene is also based on the findings of Mutti *et al*. (1984b), but utilized the continuous data for

development of the RfC and used standard NOAEL methodology for the RfC derivation. U.S. EPA (1996) established a NOAEL for the lowest exposure group (<150 MA+PGA mmole/mole creatinine; equivalent to < 25 ppm styrene). However, OEHHA staff believe that the use of the continuous data to establish a NOAEL overlooks the advantages of using the BMC approach using the quantal data. These advantages are that the BMC_{05} reflects the shape of the dose-response curve and takes into account the number of subjects involved in the study. In addition, OEHHA staff evaluated the quantal data with the Fisher's Exact Test and determined the probabilities of abnormal responses among the exposed subjects based on the unexposed subjects whose responses were assumed to be normal. At the lowest exposure, the probability that the proportion of subjects responding abnormally to $\geq$1 and $\geq$3 tests was within the expected range was p = 0.005 and p = 0.04, respectively, indicating that neuropsychological deficits due to styrene occur in the low dose subgroup. Thus, the quantal data indicate that a NOAEL was not established in this study.

With regard to application of uncertainty factors, U.S. EPA (1996) applied a UF of 3 for intraspecies variability and a partial UF of 3 for lack of information on chronic studies because the critical study was considered intermediate, i.e., between subchronic and chronic duration (Foureman, 1994). OEHHA applied a UF of 1 because the mean exposure duration, 8.6 years, was greater than 12 percent of expected lifetime (8.6/70 = 12.3%). The U.S. EPA (1996) also included a modifying factor of 3 for database deficiencies. The criteria for use of modifying factors are not well specified by U.S. EPA. Such modifying factors were not used by OEHHA.

In addition to the OEHHA and the U.S. EPA hazard assessments, the Agency for Toxic Substances and Disease Registry (ATSDR) also calculated a chronic inhalation minimal risk level (MRL) for styrene (ATSDR, 1992). The calculation was based on the same Mutti *et al.* (1984b) worker study. ATSDR (1992) identified the lowest exposure group as a LOAEL and assigned an air styrene level of 25 ppm. To derive the MRL, ATSDR corrected the LOAEL for discontinuous exposure and applied uncertainty factors (UFs) for the use of a LOAEL and for intraspecies variability. The MRL was calculated as: 25 x (8/24 x 5/7) / 10 x 10 equal 0.06 ppm (ATSDR, 1992). The MRL was a factor of 3 different from the proposed REL.

For comparison, chronic exposure levels for styrene can be developed from chronic inhalation studies in rats (Cruzan *et al*., 1998) and mice (Huntingdon Life Sciences, 1998). The mice were more sensitive to the styrene vapors than were rats, and a LOAEL of 20 ppm was identified based on lesions in various organs in both sexes. The adjustment factor for discontinuous exposure is (6/24 x 5/7) = 0.18. The uncertainty factors are: 10 for intraspecies variability, 3 for interspecies sensitivity, and 10 for adjustment from a LOAEL to a NOAEL. The resultant exposure level is (20 ppm x 0.18) / 300 which equals 0.01 ppm or 10 ppb (40 $\mu g/m^3$). Besides the different toxic endpoints between the chronic mouse exposure study and human occupational studies, the well designed human study of Mutti *et al.* (1984b) is preferable for REL development because it does not introduce the uncertainties associated with interspecies extrapolations.

The NOAEL of 50 ppm from the chronic rat study of Cruzan *et al*. (1998) may be adjusted to an equivalent continuous exposure of 8.9 ppm. Use of an RGDR of 1, an interspecies UF of 3, and an intraspecies UF of 10 resulted in an estimated REL of 300 ppb (1300 $\mu g/m^3$) for styrene.

VII. Data Strengths and Limitations for Development of the REL

The strengths of the REL for styrene include the excellent database available on styrene effects and the availability of a suitable human study for use as the key study. Limitations include the lack of direct exposure data and selection bias. Although a NOAEL was not observed in the key study, the BMC_{05} is considered to be similar to a NOAEL in estimating a concentration associated with a low level of risk.

Use of urinary metabolite concentrations to indirectly determine styrene exposure, while an accepted approach, still introduces another level of uncertainty in the hazard assessment. In addition, potential absorption of styrene via dermal exposure in the reinforced plastics industry has not been addressed and may overestimate the air concentration determined by urinary metabolite levels. However, unlike air levels, the presence of urinary metabolites of styrene gives an unequivocal indication that an individual has been exposed to styrene. At the present time, a system does not exist to obtain direct exposure information, although a recent report suggests a methodology is being developed (Jensen *et al.*, 1995).

A potential bias in the key study was the finding that general intelligence, as measured by the vocabulary test, appeared to be negatively correlated with both age and exposure intensity. This finding suggests that age may also be a factor in poor neuropsychological test scores of highly exposed subgroups. Another source of uncertainty is that the reinforced plastics industry, from which the workers in the Mutti *et al.* (1984) study were taken, is characterized by a large turnover of highly exposed workers (Wong, 1990; Kogevinas *et al.*, 1993). This possible selection bias may result in more sensitive workers leaving employment while more tolerant workers remain.

VIII. References

Ashley DL, Bonin MA, Cardinali JM, McCraw JM, and Wooten JV. 1994. Blood concentrations of volatile organic compounds in a nonoccupationally exposed US population and in groups with suspected exposure. Clin. Chem. 40:1401-1404.

ATSDR. 1992. Agency for Toxic Substances and Disease Registry. Toxicological Profile for Styrene. Atlanta, GA: U.S. Department of Health and Human Services, Public Health Service, ATSDR.

Bardodej Z, and Bardodejova E. 1970. Biotransformation of ethyl benzene, styrene, and alpha-methylstyrene in man. Am. Ind. Hyg. Assoc. J. 31:206-209.

Behari M, Choudhary C, Roy S, and Maheshwari MC. 1986. Styrene-induced peripheral neuropathy. A case report. Eur. Neurol. 25:424-427.

Belvedere G, Tursi F, and Vainio F. 1983. Non-microsomal activation of styrene to styrene oxide. In: Extrahepatic Drug Metabolism and Chemical Carcinogenesis. Rydstrom J, Montelius J, and Bengtsson M, eds. The Netherlands: Elsevier Science Publishers. pp. 193-200.

Beliles RP, Butala JH, Stack CR, and Makris S. 1985. Chronic toxicity and three-generation reproduction study of styrene monomer in the drinking water of rats. Fundam. Appl. Toxicol. 5:855-868.

Bergamaschi E, Smargiassi A, Mutti A, Franchini I, and Lucchini R. 1995. Immunological changes among workers occupationally exposed to styrene. Int. Arch. Occup. Environ. Health. 67:165-171.

Berode M, Droz PO, Boilllat MA, and Guillemin M. 1986. Effect of alcohol on the kinetics of styrene and its metabolites in volunteers and in workers. Appl. Ind. Hyg. 1:26-28.

Bhamre S, Anandatheerathavarada HK, Shankar SK, Boyd MR, and Ravindranath V. 1993. Purification of multiple forms of cytochrome P450 from a human brain and reconstitution of catalytic activities. Arch. Biochem. Biophys. 301:251-255.

Brown NA. 1991. Reproductive and developmental toxicity of styrene. Reprod. Toxicol. 5:3-29.

CARB. 1999a. California Air Resources Board. Toxics Air Quality Data. Substance Chooser. Styrene. Available online at http://www.arb.ca.gov/aqd/toxics.htm

CARB. 1999b. Air toxics emissions data collected in the Air Toxics Hot Spots Program CEIDARS Database as of January 29, 1999.

Campagna D, Mergler D, Huel G, Belanger S, Truchon G, Ostiguy C, and Drolet D. 1995. Visual dysfunction among styrene-exposed workers. Scand. J. Work Environ. Health. 21: 382-390.

Chakrabarti S, Duhr A-A, Sececal-Quevillon M, and Richer C-L. 1993. Dose-dependent genotoxic effects of styrene on human blood lymphocytes and the relationship to its oxidative and metabolic effects. Environ. Mol. Mutagen. 22:85-92.

Checkoway H, Echeverria D, Moon J-D, Heyer N, and Costa LG. 1994. Platelet monoamine oxidase B activity in workers exposed to styrene. Int. Arch. Occup. Health. 66:359-362.

Cherry N, and Gautrin D. 1990. Neurotoxic effects of styrene: further evidence. Br. J. Ind. Med. 47:29-37.

Chia S-E, Jeyaratnam J, Ong C-N, Ng T-P, and Lee H-S. 1994. Impairment of color vision among workers exposed to low concentrations of styrene. Am. J. Ind. Med. 26:481-488.

Corsini E, Luster MI, Morgan DL, Craig WA, and Rosenthal GJ. 1994. Styrene inhalation and immune function in mice. Inhal. Toxicol. 6:647-654.

Costa LG. 1996. Biomarker research in neurotoxicology: the role of mechanistic studies to bridge the gap between the laboratory and epidemiological investigations. Environ. Health Perspect. 104 (Suppl 1):55-67.

Crump KS, Howe R. Probit-A computer program to extrapolate quantile animal toxicological data to low doses. Ruston (LA): KS Crump & Company, Inc.; 1983.

Crump K. A new method for determining allowable daily intakes. Fundam Appl Toxicol 1984;4:860-866.

Cruzan G, Cushman JR, Andrews LS, Granville GC, Johnson KA, Hardy CJ, Coombs DW, Mullins PA, Brown WR. 1998. Chronic toxicity/oncogenicity study of styrene in CD rats by inhalation exposure for 104 weeks. Toxicol. Sci. 46(2):266-281

Csanady GA, Mendrala AL, Nolan RJ, and Filser JG. 1994. A physiologic pharmacokinetic model for styrene and styrene-7,8-oxide in mouse, rat, and man. Arch. Toxicol. 68:143-157.

Dean JA. 1985. Lange's Handbook of Chemistry. 13th ed. New York: McGraw-Hill Book Co.

Dogra RKS, Chandra K, Chandra S, Gupta S, Khanna L, Srivastava SN, Shukla LJ, Katiyar JC, and Shanker R. 1992. Host resistance assays as predictive models in styrene immunomodulation. Int. J. Immunopharmacol. 14:1003-1009.

Fallas C, Fallas J, Maslard P, and Dally S. 1992. Subclinical impairment of colour vision among workers exposed to styrene. Br. J. Ind. Med. 49:679-682.

Flodin U, Ekberg K, and Andersson L. Neuropsychiatric effects of low exposure to styrene. Br. J. Ind. Med. 46:805-808.

Fox AJ, and Collier PF. 1976. Low mortality rates in industrial cohort studies due to selection for work and survival in the industry. Brit. J. Prev. Soc. Med. 30:225-230.

Foureman GL. 1994. Rationale and derivation of the U.S. Environmental Protection Agency's inhalation reference concentration for styrene. Toxic Substances J. 13:283-302.

Gamberale F, Lisper HO, and Anshelm-Olson B. 1975. Effect of styrene gases on reaction time among workers in plastic boat industry. Arbete och Halsa 8:23 (Swedish; cited in World Health Organization, 1983).

Gobba F, and Cavelleri A. 1993. Kinetics of urinary excretion and effects on colour vision after exposure to styrene. IARC Scientific Publ. 127:79-88.

Gotell P, Axelson O, Lindelof B. 1972. Field studies on human styrene exposure. Work Environ. Health 9(2): 76-83 (Swedish; cited in World Health Organization, 1983).

Governa M, Valentino M, and Visona I. 1994. Chemotactic activity of human polymorphonuclear leukocytes and industrial xenobiotics: a brief review. Toxicology 91:165-177.

Guillemin MP, and Bauer D. 1979. Human exposure to styrene. II. Elimination kinetics of urinary mandelic and phenylglyoxylic acids after single experimental exposure. Int. Arch. Occup. Environ. Health. 44:249-263.

Guillemin M, Bauer D, Martin B, and Marazzi A. 1982. Human exposuire to styrene. IV. Industrial hygiene investigations and biological monitoring in the polyester industry. Int. Arch. Occup. Environ. Health. 51:139-150.

Guillemin MP, and Berode M. 1988. Biological monitoring of styrene: a review. Am. Ind. Hyg. Assoc. J. 49:497-505.

Hallier E, Goergens HW, Karels H, and Golka K. 1995. A note on individual differences in the urinary excretion of optical enantiomers of styrene metabolites and of styrene-derived mercapturic acids in humans. Arch. Toxicol. 69:300-305.

HSDB. 1999. Hazardous Substances Data Bank. Available online at http://sis.nlm.nih.gov

Hodgson AT, Wooley JD, and Daisey JM. 1993. Emissions of volatile organic compounds from new carpets measured in a large-scale environmental chamber. J. Air Waste Manage. Assoc. 43:316-324.

Huntingdon Life Sciences. 1998. Styrene: 104 week repeat dose inhalation combined toxicity/carcinogenicity study in mice, volume 1. Huntingdon, England, report issued May 28, 1998.

Jensen B, Murer AJL, Olsen E, and Christensen JM. 1995. Assessment of long-term styrene exposure: a comparative study of a logbook method and biological monitoring. Int. Arch. Occup. Environ. Health. 66:399-405.

Katakura Y, Kishi R, Ikeda T, and Miyake H. 1999. Effects of prenatal exposure to styrene on neurochemical levels in rat brain. Toxicol. Lett. 105:239-249.

Khanna VK, Husain R, and Seth PK. 1994. Effect of protein malnutrition on the neurobehavioral toxicity of styrene in young rats. J. Appl. Toxicol. 14:351-356.

Kishi R, Chen BQ, Katadura Y, Ikeda T, and Miyake H. 1995. Effect of prenatal exposure to styrene on the neurobehavioral development, activity, motor coordination, and learning behavior of rats. Neurotoxicol. Teratol. 17:121-130.

Kogevinas M, Ferro G, Saracci R, Andersen A, Biocca M, Coggon D, Gennaro V, Hutchings S, Kolstad H, Lundberg I, Lynge E, and Partanen T. 1993 Cancer mortality in an international cohort of workers exposed to styrene. IARC Scientific Publications. 127:289-300.

Kolstad HA, Bonde JPE, Spano M, Giwercman A, Zschiesche W, Kaae D, and Roeleveld D. 1999a. Sperm chromatin structure and semen quality following occupational styrene exposure. Scand. J. Work Environ. Health 25(suppl. 1):70-73.

Kolstad HA, Bisanti L, Roeleveld N, Bonde JPE, and Joffe M. 1999b. Time to pregnancy for men occupationally exposed to styrene in several European reinforced plastics companies. Scand. J. Work Environ. Health 25(suppl. 1): 66-69.

Kolstad HA, Juel K, Olsen J, and Lynge E. 1995. Exposure to styrene and chronic health effects: mortality and incidence of solid cancers in the Danish reinforced plastics industry. Occup. Environ. Med. 52:320-327.

Leibman KC. 1975. Metabolism and toxicity of styrene. Environ. Health Perspect. 11:115-119.

Lindbohm M-L. 1993. Effects of styrene on the reproductive health of women: a review. IARC Scientific Publ. 127:163-169.

Lindstrom K, Harkonen H, and Hernberg S. 1976. Disturbances in psychological functions of workers occupationally exposed to styrene. Scand. J. Work Environ. Health 3:129-139.

Mackay CJ, and Kelman GR. Choice reaction time in workers exposed to styrene vapour. Human Toxicol. 5:85-89.

Matikainen E, Forsman-Gronholm L, Pfaffli P, and Juntunen J. Neurotoxicity in workers exposed to styrene. 1993. IARC Scientific Publ. 127:153-161.

Miller VP, DePillis GD, Ferrer JC, Mauk AG, and deMontellano PRO. 1992. Monooxygenase activity of cytochrome c peroxidase. J. Biol. Chem. 267:8936-8942.

Moller C, Odkvist L, Larsby B, *et al*. 1990. Otoneurological findings in workers exposed to styrene. Scand. J. Work Environ. Health 16(3):189-194.

Murata K, Araki S, and Yokoyama K. 1991. Assessment of the peripheral, central, and autonomic nervous system function in styrene workers. Am. J. Ind. Med. 20:775-784.

Mutti A. 1993. Mechanisms and biomarkers of solvent-induced behavioral and neuroendocrine effects. In: Use of Biomarkers in Assessing Health and Environmental Impacts of Chemical Pollutants. Travis CC, ed., New York: Plenum Press. pp 183-199.

Mutti A, Falzoi M, Romanelli MC, Bocchi MC, Ferroni C, and Frandhini I. 1988. Brain dopamine as a target for solvent toxicity: effects of some monocyclic aromatic hydrocarbons. Toxicology 49:77-82.

Mutti A, Mazzucchi A, Rustichelli P, Frigeri G, Arfini G, and Franchini I. 1984b. Exposure-effect and exposure-response relationships between occupational exposure to styrene and neurophychological functions. Am. J. Ind. Med. 5:275-286.

Mutti A, Vescovi PP, Falzoi M, Arfini G, Valenti G, and Franchini I. 1984a. Neuroendocrine effects of styrene on occupationally exposed workers. Scand. J. Work Environ. Health 10:225-228.

Nakajima T, Elovaara E, Gonzalez FJ, Gelboin HV, Raunio H, Pilkonen O, Vainio H, and Aoyama T. 1994. Styrene metabolism by cDNA-expressed human hepatic and pulmonary cytochromes P450. Chem. Res. Toxicol. 7:891-896.

Newhook R, and Caldwell I. 1993. Exposure to styrene in the general Canadian population. IARC Scientific Publ. 127:27-33.

NTP. 1992. Pathology working group's chairperson's report. 13-Week Toxicity Study of Styrene (CO2200B) in F344 Rats and B6C3F1 Mice by Inhalation. Report dated 28 April 1992.

Perbellini L, Mozzo P, Turri PV, Zedde A, and Brugnone F. 1988. Biological exposure index of styrene suggested by a physiologico-mathematical model. Int. Arch. Occup. Environ. Health 60:187-193.

Rebert CS, and Hall TA. 1994. The neuroepidemiology of styrene: a critical review of representative literature. Crit. Rev. Toxicol. 24(Suppl 1):S57-S106.

Rosengren LE, Haglid KG. 1989. Long term neurotoxicity of styrene. A quantitative study of glial fibrillary acidic protein (GFA) and S-100. Br. J. Ind. Med. 46(5):316-320.

Savolainen H. 1977. Some aspects of the mechanisms by which industrial solvents produce neurotoxic effects. Chem.-Biol. Interact. 18:1-10.

Stewart, RD, Dodd HC, Baretta ED, and Schaffer AW. 1968. Human exposure to styrene vapor. Arch. Environ. Health. 16:656-662.

Treibig G, Schaller K-H, and Valentin H. 1985. Investigations on neurotoxicity of chemical substances at the workplace. VII. Longitudinal study with determination of nerve conduction velocities in persons occupationally exposed to styrene. Int. Arch. Occup. Environ. Health. 56:239-247.

Tursi F, Samaia M, Salmoma M, and Belvedere G. 1983. Styrene oxidation to styrene oxide in human erythrocytes catalyzed by oxyhemoglobin. Experientia. 39:593-594.

U.S. EPA. 1996. Integrated Risk Information System (IRIS). US Environmental Protection Agency, Washington, D.C. (styrene summary last revised 07/01/93,CD-ROM Version). Denver, CO: Microdex, Inc. (Edition expires 07/31/96).

Weast RC. 1979. CRC Handbook of Chemistry and Physics. Vol. 60. Weast RC, and Astle MJ, eds. Boca Raton, FL: CRC Press.

Wigaeus E, Lof A, Bjurstrom R, and Nordqvist MB. 1983. Exposure to styrene. Scand J. Work Environ. Health. 9:479-488.

WHO. 1983. Environmental Health Criteria 26: Styrene. Finland: World Health Organization.

Wilson HK, Robertson SM, Waldron HA, and Gompertz D. 1983. Effect of ethanol on the kinetics of mandelic acid excretion in volunteers exposed to styrene vapour. Br. J. Ind Med. 40:75-80.

Wong O. 1990. A cohort mortality study and a case-control study of workers potentially exposed to styrene in the reinforced plastics and composites industry. Br. J. Ind. Med. 47:753-762.

CHRONIC TOXICITY SUMMARY

SULFURIC ACID

(dithionic acid; pyrosulphuric acid)

CAS Registry Number: 7664-93-9

II. I. Chronic Toxicity Summary

Inhalation reference exposure level	**1 µg/m^3**
Critical effect(s)	Bronchiolar epithelial hyperplasia, and thickening of the bronchial walls in monkeys
Hazard index target(s)	Respiratory system

III. Physical and Chemical Properties (HSDB, 1995; CRC, 1994; CARB, 1997)

Description	Colorless liquid
Molecular formula	H_2SO_4
Molecular weight	98.1 g/mol
Density	1.84 g/cm^3 @ 15° C
Boiling point	330±0.5°C (100%)
Melting point	10.36°C (100%)
Vapor pressure	<0.001 torr @ 25° C; 1 torr @ 145.8° C
Solubility	Soluble in water
Conversion factor	Not applicable

III. Major Uses or Sources

Sulfuric acid is a strong acid used as an intermediate in the synthesis of linear alkylbenzene sulfonation surfactants used in dyes, in petroleum refining, for the nitration of explosives, in the manufacture of nitrocellulose, in caprolactam manufacturing, as the electrolyte in lead-acid batteries, and as a drying agent for chlorine and nitric acid. Sulfuric acid is formed in the atmosphere from sulfur dioxide, from sulfur trioxide, and from oleum (a combination of sulfur trioxide and sulfuric acid used industrially). The annual statewide industrial emissions from facilities reporting under the Air Toxics Hot Spots Act in California based on the most recent inventory were estimated to be 4460 pounds of sulfuric acid (CARB, 1999).

IV. Effects of Human Exposures

Workers in the lead battery industry showed etching and erosion of the teeth after 4 months exposure to an average concentration of 0.23 mg/m^3 H_2SO_4 (Gamble *et al.*, 1984). Dental erosion increased in a dose-dependent manner with longer duration of exposure.

A study of 33 storage battery plant workers exposed to H_2SO_4 concentrations as high as 35 mg/m^3 showed a greater group mean decrease in FEV_1 across the time of their work shift compared to workers who were not exposed to sulfuric acid (El-Saddik *et al.*, 1972). The salivary pH of the sulfuric acid exposed workers, a qualitative measure of acid exposure, was lower than the controls during the course of the work shift.

OEHHA recently reviewed the California Ambient Air Quality Standard (CAAQS) for sulfates (25 μg/m^3 for 24 hours) to see if it adequately protects children (OEHHA, 2000). The report was peer-reviewed by the Air Quality Advisory Committee. The report indicates that H+ itself may play a role in the effects seen in epidemiological studies of sulfate air pollution. Controlled acute inhalation studies in humans and laboratory animals of pH neutral or nearly neutral sulfate salts (e.g., ammonium sulfate) (Utell et al., 1983; Lippman et al., 1987; Schlesinger et al., 1990), even at relatively high concentrations, do not produce the effects reported from epidemiologic studies of sulfates (asthma exacerbation, bronchoconstriction, decrements in lung function) that might be expected from short-term excursions. The controlled exposure studies show that sulfate aerosols containing strong acids, such as sulfuric acid and, to a lesser extent, ammonium bisulfate, produce functional and structural changes in healthy subjects consistent with those observed in epidemiological studies. A working hypothesis is that H^+ is a causal factor for adverse human health effects (e.g., see Lippmann and Thurston, 1996) and that, among the commonly measured particulate matter (PM) indices, $SO_4^=$ is the best surrogate metric for H^+.

A large number of epidemiologic studies have been conducted showing that elevated levels of several air pollutants, including acid aerosols, sulfur and nitrogen oxides, and particulate sulfates are correlated with an increased prevalence of pulmonary disease (U.S. EPA, 1989; OEHHA 2000). Elevated sulfate levels (1.6 ppb or 6.6 μg/m^3) have been associated with statistically significant decrements in FVC and FEV_1 in a cohort of Canadian children (Stern *et al.*, 1989). Further analysis of these data led Bates and Sitzo (1989) to conclude that H_2SO_4 was the most likely cause for the pulmonary changes observed. Similarly, Ostro *et al.* (1989) reported a statistical association between asthma-related symptoms reported by 209 asthmatics and sulfate and acidity levels in ambient air in Denver. Delfino et al. (1997) found that ambient H+ was associated with emergency room visits by children for respiratory symptoms in a study in Montreal. Additionally, Damokosh et al. (1993) in a follow-up analysis of the 6-City study suggested associations between average H+ concentration and chronic bronchitic symptoms. The relative odds of bronchitic symptoms with the highest acid concentration (58 nmoles/m^3 H+)_ versus the lowest concentration (16 nmoles/m^3) was 2.4 (95% CI:1.9 to 3.2). Furthermore in a study of children in 24 U.S. and Canadian communities (Dockery et al., 1996) in which the analysis was adjusted for the effects of gender, age, parental asthma, parental education, and parental allergies, bronchitic symptoms were confirmed to be significantly associated with strongly acidic PM (OR= 1.66; 95% CI 1.11-2.48). It was also found that FVC and FEV_1 were lower in locales with high particle acidity (Raizenne et al., 1996). Gwynn et al. (2000) reported an association between both H+ and sulfate particles and respiratory hospital admissions and mortality in Buffalo, NY. Acidic sulfates may act to increase the toxicity of particles by enhancing the availability of metals present in the particles to generate reactive oxygen species in the respiratory epithelium. This may account for some of the effects seen in these epidemiological studies and makes it difficult to use these studies as a basis for a Reference Exposure Level for sulfuric acid. The relationship between the effect levels observed in these

studies and the proposed REL is discussed in the section below on the potential for differential impacts on children's health.

The occupational standard for sulfuric acid is based on a study in human subjects by Amdur *et al*. (1952). In their study, 22 healthy male subjects were exposed to 0, 0.35, 0.4, 0.5, 1, 2, or 5 mg/m^3 for 5-15 minutes. The odor, taste, and irritation threshold was 1 mg/m^3. Since the basis for this standard is an acute exposure, it is not useful in determining a chronic non-cancer REL for sulfuric acid. A review of chronic human exposures to sulfuric acid and resulting carcinogenicity outcomes can be found in IARC (1992). However, none of the studies in that review examined non-cancer endpoints.

Sulfuric acid and oleum (supersaturated anhydrous sulfuric acid with varying concentrations of free sulfur trioxide) are absorbed as salts of sulfate anion (SO_4^{2-}), and are excreted as organic sulfates, neutral sulfur, or neutral sulfur compounds such as sulfur-containing amino acids. The low systemic toxicity of these metabolites is likely of secondary importance to the irritation caused by the inhaled acid.

V. Effects of Animal Exposures

An exposure of 9 cynomolgus monkeys per group to H_2SO_4 concentrations of 0, 0.38, 0.48, 2.43, and 4.79 mg/m^3 continuously for 78 weeks resulted in dose-dependent adverse histological changes in lung and bronchiolar epithelial and parenchymal tissue in addition to a dose-dependent decrease in blood oxygenation (Alarie *et al.*, 1973). In the animals exposed to 0.38 mg/m^3, significant bronchiolar epithelial hyperplasia was observed in 5 of 9 animals; thickening of the bronchiolar walls was observed in 3 of 9 animals. A slight focal bronchial epithelial hyperplasia was present in 4 of the 9 animals. One animal died after 4 weeks exposure to 0.38 mg/m^3. Although signs of pulmonary edema and cardiac hypertrophy were found, the cause of death was not determined.

Respiratory system effects of H_2SO_4 exposure in monkeys (Alarie *et al*., 1973)

H_2SO_4 (g/m^3)	*Particle size MMD*	*Bronchiolar epithelial hyperplasia Incidence – severity*	*Thickening of walls of respiratory bronchioles Incidence – severity*	*Increase in thickness of alveolar walls Incidence – severity*
0		0/9	0/9	0/9
0.38	2.15	5/8 – slight	3/8 - slight	0/8
0.48	0.54	0/8	0/8	0/8
2.43	3.60	8/8 – moderate	8/8 – moderate	8/8 – moderate
4.79	0.73	8/8 – moderate to severe	8/8 – moderate to severe	0/8

Alarie *et al.* (1973) also exposed groups of 50 guinea pigs of each sex to 0, 0.08, or 0.1 mg/m^3 H_2SO_4 continuously for 52 weeks. The group exposed to 0.1 mg/m^3 also received larger sized particulates than the 0.08 mg/m^3 group (2.78 μm vs. 0.84 μm, respectively). No exposure related effects were observed in the animals exposed to 0.08 mg/m^3, whereas exposure of 0.1 mg/m^3 resulted in decreased body weights in the female guinea pigs. No other histological changes in any organs were observed at the end of the 52-week study.

Rabbits (4 per group) were exposed to 250 μg/m^3 H_2SO_4 1 hour/day, 5 days/week for 4, 8, or 12 months. They showed significantly increased bronchoconstriction upon acetylcholine challenge after 8 and 12 months exposure, compared with a control group of 4 animals that received no H_2SO_4 (Gearhart and Schlesinger, 1986, 1988). Mucociliary clearance was also impaired by H_2SO_4 exposure and did not improve 3 months after cessation of exposure. A decline in dynamic lung compliance was observed after 12 months exposure. There was no evidence of inflammatory cell infiltration in the lungs of the exposed animals.

In guinea pigs, significantly slower and irregular breathing patterns were noted when the animals had inhaled albumin followed by 30 minute exposures to H_2SO_4 at 1.91 mg/m^3 twice per week for 5 weeks (Kitabatake *et al.*, 1979). Similarly, when guinea pigs were exposed to 2.49 mg H_2SO_4/m^3 for 4 hours/day, 6 days/week for 4 weeks, *in vitro* lung histamine release was significantly enhanced following heterogeneous albumin inhalation, compared to control animals unexposed to albumin (Fujisawa *et al.*, 1986; Iguchi *et al.*, 1986). In guinea pigs, sulfuric acid caused significantly greater lung function changes when adsorbed on the surface of zinc oxide particles as compared with pure sulfuric acid (Amdur and Chen, 1989). An exposure to 24 μg/m^3 sulfuric acid, layered on zinc oxide, produced significant reductions in lung function when followed by a brief exposure to 0.15 ppm ozone (Chen *et al.*, 1991).

A chronic exposure of beagle dogs to an average concentration of 889 μg/m^3 H_2SO_4 for 21 hours/day over a 620 day period resulted in increased expiratory resistance, reduced carbon monoxide diffusing capacity, reduced total and residual lung volume, and decreased lung and heart weights (Lewis *et al.*, 1973).

In apparent contrast to the above studies, rats and guinea pigs exposed to H_2SO_4 at 10 mg/m^3 for 6 hours/day, 5 days/week for 6 months exhibited no adverse histologic changes in lung tissue. Lung function measurements were not reported in this study (Cavender *et al*., 1978).

Mice inhaled sulfuric acid mist at a concentration of 1.4 mg/m^3 in combination with a carbon particle mixture (1.5 mg/m^3) for 3 hours/day, 5 days/week for up to 20 weeks. The exposure resulted in significant alterations in specific antibody titer (decreased IgG, Ig_{2a}, IgM; increased IgG_{2b}), depression of primary splenic antibody response, and decreased resistance to respiratory infection as measured by mortality and survival time compared to controls (Fenters *et al.*, 1979).

There are no reliable studies indicating that sulfuric acid is a developmental or reproductive toxicant. In the absence of massive overexposure leading to maternal acidemia, H_2SO_4 will be neutralized in the maternal circulation and is unlikely to reach the fetus.

VI. Derivation of Chronic Reference Exposure Level

Study	Alarie *et al.*, 1973
Study population	Cynomolgus monkeys (5 males and 4 females per group or vice versa)
Exposure method	Continuous inhalation exposures (0, 380, 480, 2400, or 4800 μg/m^3) for 78 weeks
Critical effects	Significantly increased bronchial epithelial hyperplasia and bronchial thickening
LOAEL	380 μg/m^3
NOAEL	Not observed
Exposure continuity	The exposure was continuous during the experiment.
Exposure duration	78 weeks
Average experimental exposure	380 μg/m^3 for the LOAEL group
Human equivalent concentration	380 μg/m^3
LOAEL uncertainty factor	3 (slight effects)
Subchronic uncertainty factor	3
Interspecies uncertainty factor	3 (non-human primate)
Intraspecies uncertainty factor	10
Cumulative uncertainty factor	300
Reference exposure level	1 μg/m^3

The study by Alarie *et al.* (1973) identified a LOAEL for chronic exposure to sulfuric acid of 380 μg/m^3. The principal uncertainties of this study are the small sample size of the test groups and the absence of an observed NOAEL. A lower chronic LOAEL for bronchial reactivity is presented by Gearhart and Schlesinger (1986, 1988) for rabbits (250 μg/m^3). This study was not selected as the basis of the REL because Gearhart and Schlesinger used only a single concentration of sulfuric acid, exposed the animals only for 1 hour per day for 5 days/week, used only 4 animals per group, and measured effects over the course of up to 12 months. The predominant weakness in the rabbit study, however, was the extreme discontinuity of the exposures (1 hour/day, 5 days/week), which would have necessitated use of a very large continuity adjustment. For these reasons, in addition to obvious physiological and genetic similarity arguments, the study in monkeys by Alarie *et al.* (1973) was felt to be more appropriate as the basis for the chronic REL for sulfuric acid. Alarie *et al*. (1975) determined a NOAEL for sulfuric acid in monkeys of 0.1 mg/m^3. However, other particulate matter (fly ash) was also present during the exposure. The Alarie *et al*. (1973) report provides data from exposure to sulfuric acid alone.

A free-standing NOAEL for histological changes in 100 guinea pigs exposed continuously for 1 year to 0.08 mg/m^3 was reported by Alarie *et al.* (1973). Guinea pigs respond to high concentrations of sulfuric acid by occasional laryngeal spasms that appear similar to a human asthmatic attack (Silbaugh *et al.*, 1981; Amdur and Chen, 1989). As a result, guinea pigs are thought to be sensitive models for the acute effects of sulfuric acid. For chronic effects of sulfuric acid on the lung, monkeys are likely a suitable model due to their physiological and structural similarites to humans.

For comparison, a chronic REL based on the guinea pig free-standing NOAEL of 0.08 mg/m^3 in animals exposed continuously for one year (Alarie *et al*., 1973) would be 0.8 μg/m^3.

VII. Data Strengths and Limitations for Development of the REL

The major strength of the study on sulfuric acid is the use of health effects observations from continuous long-term exposures to a primate. The major weaknesses are the lack of adequate human health effects data and the lack of a NOAEL observation.

VIII. Potential for Differential Impacts on Children's Health

There are no reliable studies indicating that sulfuric acid is a developmental or reproductive toxicant. Children are likely to be at greater risk from long-term exposures because their bodies are growing, and their developmental processes, especially in the lung, may well be impacted by air pollution exposures. Elevated sulfate levels (1.6 ppb or 6.6 μg/m^3) have been associated with statistically significant decrements in FVC and FEV1 in a cohort of Canadian children (Stern et al., 1989). The chronic REL for sulfuric acid of 1 μg/m^3 is below the level associated with those decrements in pulmonary function. However, in a study of moderately to severe asthmatic children (ages 7-13) (Thurston et al., 1997), a sensitive subpopulation for sulfate effects, approximately 1 μg/m3 was the lowest level of ambient sulfate measured. The mean daily morning to afternoon peak airflow change, the use of beta-agonist medication, and the number of chest symptoms versus sulfate concentration in these children extrapolated linearly down to 1 μg/m3. Thurston et al. (1997) also examined earlier data from Ontario (Burnett et al., 1994) on respiratory admissions to hospitals, and concluded that the sulfate threshold of effects, if it exists, lies below 5 μg/m^3, perhaps at about 2 μg/m^3. It should be noted that the sulfate and hydrogen ion effects are difficult to disentangle from each other and from the effcts of other PM constituents. The chronic REL of 1 μg/m^3 appears to have a relatively low margin of safety with respect to the epidemiological studies, but these observations are consistent with the proposed REL of 1 μg/m^3 since asthmatic children appear to be the critically sensitive human population for exposure to sulfuric acid (or sulfate).

IX. References

Alarie Y, Busey WM, Krumm AA, Ulrich CE, and Va V. 1973. Long-term continuous exposure to sulfuric acid mist in cynomolgus monkeys and guinea pigs. Arch. Environ. Health 27:16-24.

Alarie YC, Krumm AA, Busey WM, Urich CE, and Kantz RJ. 1975. Long-term exposure to sulfur dioxide, sulfuric acid mist, fly ash, and their mixtures. Results of studies in monkeys and guinea pigs. Arch. Environ. Health 30(5):254-262.

Amdur MO, and Chen LC. 1989. Furnace-generated acid aerosols: Speciation and pulmonary effects. Environ. Health Perspect. 79:147-150.

Amdur MO, Silverman L, and Drunker P. 1952. Inhalation of sulfuric acid mist by human subjects. Arch. Ind. Hyg. Occup. Med. 6:305-313.

Bates DV, and Sitzo R. 1989. The Ontario air pollution study: Identification of the causative agent. Environ. Health Perspect. 79:69-72.

Burnett RT, Dales RE, Raizenne ME, Krewski D, Summers PW, Roberts GR, Raad-Young M, Dann T, and Brook J. 1994. Effects of low ambient levels of ozone and sulfates on the frequency of respiratory admissions to Ontario hospitals. Environ Res 65:172-194.

CARB. 1997. California Air Resources Board. Toxic Air Contaminant Identification List Summaries.

CARB. 2000. California Air Resources Board. California Emissions Inventory Development and Reporting System (CEIDARS). Data from Data Base Year 1998. February 12, 2000.

CRC. 1994. CRC Handbook of Chemistry and Physics, 75th edition. Lide DR, ed. Boca Raton, FL: CRC Press Inc.

Cavender FL, Singh B, and Cockrell BY. 1978. The effects in rats and guinea pigs from six months exposures to sulfuric acid mist, ozone, and their combination. J. Environ. Pathol. Toxicol. 2:485-492.

Chen LC, Miller PD, Lam HF, Guty J, and Amdur MO. 1991. Sulfuric acid-layered ultrafine particles potentiate ozone-induced airway injury. J. Toxicol. Environ. Health 34:337-352.

Damokosh AI, Spengler JD, Dockery DW, Ware JH, and Speizer FE. 1993. Effects of acidic particles on respiratory symptoms in 7 US communities. Am Rev Respir Dis 147:A632.

Delfino RJ, Murphy-Moulton AM, Burnett RT, Brook JR, and Becklake MR. 1997. Effects of air pollution on emergency room visits for respiratory illnesses in Montreal, Quebec. Am J Respir Crit Care Med 155:568-576.

Dockery DW, Cunningham J, Damokosh AI, Neas LM, Spengler JD, Koutrakis P, Ware JH, and Speizer FE 1993. Health effects of acid aerosols on North American children: respiratory symptoms. Environ Health Perspect 104:500-505.

El-Saddik YM, Osman HA, and El-Gazzar RM. 1972. Exposure to sulfuric acid in manufacturing of storage batteries. J. Occup. Med. 14:224-226.

Fenters JD, Bradof JN, Aranyi C, Ketels K, Ehrlich R, and Gardner DE. 1979. Health effects of long-term inhalation of sulfuric acid mist-carbon particle mixtures. Environ. Res. 19:244-257.

Fujisawa T, Iguchi K, and Uchida Y. 1986. Effects of exposure to sulfuric acid mist on the induction of experimental asthma in guinea pigs. Japan J. Allergol. 35(2):137-144.

Gamble J, Jones W, Hancock J, and Meckstroth R. 1984. Epidemiologic environmental study of lead acid battery workers. III. Chronic effects of sulfuric acid on the respiratory system and teeth. Environ. Res. 35:30-52.

Gearhart JM, and Schlesinger RB. 1986. Sulfuric acid-induced airway hyperresponsiveness. Fundam. Appl. Toxicol. 7:681-689.

Gearhart JM, and Schlesinger RB. 1988. Response of the tracheobronchial mucociliary clearance system to repeated irritant exposure: effect of sulfuric acid mist on function and structure. Exp. Lung Res. 14:587-605.

Gwynn RC, Burnett RT, and Thurston GD. 2000. A time-series analysis of acidic particulate matter and daily mortality and morbidity in the Buffalo, New York region. Environ Health Perspect. 108:125-133.

HSDB. 1995. Hazardous Substances Data Bank. National Library of Medicine, Bethesda, MD (TOMES® CD-ROM Version). Denver, CO: Micromedex, Inc. (Edition expires 7/31/96).

IARC. 1992. International Agency for Research on Cancer. IARC Monographs on the Evaluation of Carcinogenic Risks to Humans. Vol. 54. Occupational Exposure to Mists and Vapours from Strong Inorganic Acids and other Industrial Chemicals. Lyon, France: IARC, pp. 41-130

Iguchi K, Fujisawa T, and Uchida Y. 1986. Effects of exposure to sulfuric acid mist on the induction of experimental asthma in guinea pigs. Japan J. Allergol. 35(6):402-408.

Kitabatake M, Imai M, Kasama K, Kobayashi I, Tomita Y, and Yoshida K. 1979. Effects of air pollutants on the experimental induction of asthma attacks in guinea pigs: Sulfuric acid mist and mixture of the mist and sulfur dioxide. Mie Med. J. 29(1):29-37.

Lewis TR, Moorman WJ, Ludmann WF, and Campbell KI. 1973. Toxicity of long-term exposure to oxides of sulfur. Arch. Environ. Health 26:16-21.

Lippman M, Gearhart JM, Schlesinger RB. 1987. Basis for a particle size-selective TLV for sulfuric acid aerosols. Appl Ind Hyg 2:188-199.

Lippmann M, Thurston GD. 1996. Sulfate concentrations as an indicator of ambient particulate matter air pollution for health risk evaluations. J. Expo. Anal. Environ. Epidemiol. 6(2):123-146.

OEHHA. 2000. Office of Environmental Health Hazard Assessment. Staff report. Adequacy of California Ambient Air Quality Standards: Senate Bill No. 25 - Children's Environmental Health Protection.

Ostro B, Lipsett M, Wiener M, and Selner JC. 1989. A panel study of the effect of acid aerosols on asthmatics. J. Air Waste Management Assoc. 89-94.1:1-15.

Raizenne M, Neas LM, Damokosh AI, Dockery DW, Spengler JD, Koutrakis P, Ware JH, and Speizer FE. 1996. Health effects of acid aerosols on North American children: pulmonary function. Environ Health Perspect 104: 506-514.

Schlesinger RB, Chen LC, Finkelstein I, and Zelikoff JT. 1990. Comparative potency of inhaled acidic sulfates: speciation and the role of hydrogenion. Environ Res 52:210-224.

Silbaugh SA, Mauderly JL, Macken CA. 1981. Effects of sulfuric acid and nitrogen dioxide on airway responsiveness of the guinea pig. J. Toxicol. Environ. Health 8(1-2):31-45.

Stern B, Jones L, Raizenne M, Burnett R, Meranger JC, Franklin CA. 1989. Respiratory health effects associated with ambient sulfates and ozone in two rural Canadian communities. Environ. Res. 49(1):20-39.

Thurston GD, Lippmann M, Scott MB, Fine JM. 1997. Summertime haze air pollution and children with asthma. Am. J. Respir. Crit. Care Med. 155(2):654-660.

U.S. EPA. 1989. U.S.Environmental Protection Agency. An Acid Aerosols Issue Paper: Health Effects and Aerometrics. EPA/600/8-88/00SF. OHEA/ORD. Research Triangle Park, NC: U.S. EPA.

Utell MJ, Morrow PE, Speers DM, Darling J, Hyde RW. 1983. Airway responses to sulfate and sulfuric acid aerosols in asthmatics: an exposure-response relationship. Am Rev Resp Dis 128:444-450.

CHRONIC TOXICITY SUMMARY

TOLUENE

(Methyl benzene; methyl benzol; phenyl methane; toluol)

CAS Registry Number: 108-88-3

I. Chronic Toxicity Summary

Inhalation reference exposure level	**300 µg/m^3 (70 ppb)**
Critical effect(s)	Neurotoxic effects (decreased brain [subcortical limbic area] weight, altered dopamine receptor binding).
Hazard index target(s)	Nervous system; respiratory system; teratogenicity

II. Physical and Chemical Properties (HSDB (1999) except as noted)

Description	Colorless liquid
Molecular formula	C_7H_8
Molecular weight	92.13 g/mol
Density	0.8661 g/cm^3 @ 20°C
Boiling point	110.6 ° C (CRC, 1994)
Melting point	–94.9° C (CRC, 1994)
Vapor pressure	28.1 torr @ 25°C (U.S. EPA, 1984)
Solubility	miscible in most organic solvents
Conversion factor	1 ppm = 3.76 mg/m^3 @ 25°C

III. Major Uses or Sources

Toluene occurs naturally as a component of crude oil and is produced in petroleum refining and coke oven operations; toluene is a major aromatic constituent of gasoline (HSDB, 1999). It is used in household aerosols, nail polish, paints and paint thinners, lacquers, rust inhibitor, adhesives and solvent based cleaning agents. Toluene is also utilized in printing operations, leather tanning and chemical processes. Benzene and other polycyclic aromatic hydrocarbons are common contaminants of toluene. Toluene is considered a sentinel chemical for benzene in the context of air and water sample monitoring. In 1996, the latest year tabulated, the statewide mean outdoor monitored concentration of toluene was approximately 2.2 ppb. For 1998, annual statewide industrial emissions of toluene from facilities reporting under the requirements of the Air Toxics Hot Spots Act in California were estimated to be 5,176,626 pounds (CARB, 1999). Note that this estimate is for stationary sources, and does not include emissions from mobile sources.

IV. Effects of Human Exposures

Neurological Effects
Most studies reporting adverse effects due to chronic toluene exposures involve either toluene-containing solvent abuse or occupational exposure to toluene. Solvent abusers are generally exposed to higher levels of toluene than are workers. A continuum of neurotoxic effects ranging from frank brain damage to degraded performance on psychometric tests which roughly track exposure levels has been observed.

Solvent abusers
Chronic toluene abuse has been shown to cause permanent changes in brain structure (loss of grey and white matter differentiation; cerebral, cerebellar and brainstem atrophy) which correlated with brain dysfunction as measured by magnetic resonance imaging (MRI) and brainstem auditory evoked response (BAER) evaluations (Caldemeyer *et al.*, 1996; Filley *et al.*, 1990; Ikeda and Tsukagoshi, 1990; Rosenberg *et al.*, 1988a; Rosenberg *et al.*, 1988b; Yamanouchi *et al.*, 1995; reviewed by Agency for Toxic Substances and Disease Registry (ATSDR), 1999).

Eleven chronic solvent (spray lacquer; ≈ 60% toluene, 10% dichloromethane) abusers were examined using MRI and BAER tests (Rosenberg *et al.*, 1988b). Neurological abnormalities were seen in four of 11 subjects and included brainstem, cerebellar, cognitive and pyramidal findings. Brain MRIs were abnormal in three of 11 subjects and indicated the occurrence of diffuse cerebral, cerebellar, and brainstem atrophy and loss of differentiation between the gray and white matter throughout the CNS. BAERs were abnormal in five of 11 individuals. All three individuals with abnormal MRI scans also had abnormal neurological examinations and BAERs. However, two of five individuals with abnormal BAERs had normal neurological examinations and MRI scans. The authors suggested that BAERs may detect early CNS injury from toluene inhalation, even at a time when neurological examination and MRI scans are normal.

Two subjects of a group of 22 hospitalized solvent abusers (primarily abusing toluene-based solvents) demonstrated decreases in intelligence quotient (IQ) as measured by the comparison of tests administered before the commencement of solvent abuse with tests administered during hospitalization for long-term solvent abuse (Byrne *et al.*, 1991).

Filley *et al.* (1990) studied 14 chronic toluene abusers using MRI and neuropsychological evaluations. The neuropsychological testing indicated that three patients functioned normally, three were in a borderline range, and eight were impaired. Independent analyses of white matter changes on MRI demonstrated that the degree of white matter abnormality was strongly correlated ($p < 0.01$) with neuropsychological impairment. The authors concluded that dementia in toluene abuse appears to be related to the severity of cerebral white matter involvement.

Six chronic toluene abusers were examined using MRI by Caldemeyer *et al.* (1996). All patients examined demonstrated white matter atrophy and T2 hyperintensity (T2: “Spin-spin” relaxation time; a time constant that reflects the rate at which protons stop rotating in phase with each other because of the local magnetic fields of adjacent nuclei; OTA, 1984), and five of six demonstrated

T2 hypointensity of the basal ganglia and thalami. The authors noted a correlation between the severity of white matter degeneration and degree of neurological dysfunction. However, there was no correlation between the severity of imaged white matter changes and the presence of T2 hypointensity or duration of toluene abuse. Additionally, no definite clinical evidence of damage to the basal ganglia and thalami was found despite the MR imaging finding of T2 hypointensity..

Ungar *et al*. (1994) developed a physical bilayered model of dipalmitoylphosphatidylcholine (DPPC) and toluene, and subjected DPPC control and toluene-mixed bilayers to MRI. T1 (T1: "Spin-lattice" relaxation time; a time constant that reflects the rate at which excited protons exchange energy with the surrounding environment; OTA, 1984) and T2 were measured as a function of toluene and lipid concentrations. Measurements of the DPPC-toluene model indicated that toluene-containing lipid bilayers substantially shortened T2 and had little effect on T1. By comparison, DPPC alone had little effect on either T1 or T2. The authors believe that these results suggest that partitioning of toluene into the lipid membranes of cells in cerebral tissue may be responsible for the hypointensity of basal ganglia noted on T2-weighted MR images of brains of toluene abusers.

Occupational exposure

Solvent workers exposed to 42.8 ppm toluene (estimated as a time-weighted average) for an average duration of 6.8 years reported a significantly greater incidence of sore throat, dizziness and headache than controls; the sore throat and headache incidence demonstrated a rough dose-response (Yin *et al.*, 1987).

Orbaek and Nise (1989) examined the neurological effects of toluene on 30 rotogravure printers, 33-61 years of age (mean 50), employed at two Swedish printing shops for 4-43 years (median 29) in 1985. Mean exposure levels at the two printing shops were 43 and 157 mg/m^3 of toluene, respectively; however, before 1980 the exposure levels had exceeded 300 mg/m^3 in both shops. The authors noted that rotogravure printing provides an occupational setting with practically pure toluene exposure. Comparisons were made to a reference group of 72 men aged 27-69 (mean 47). The alcohol consumption of both the workers and referents was also determined (< 200 g/week or > 200 g/week). Neurological function in the workers and referents was evaluated using interviews and psychometric testing; the results from each of the two printing shops were pooled. The printers reported statistically significantly higher occurrences of fatigue (60%), recent short-term memory problems (60%), concentration difficulties (40%), mood lability (27%), and other neurasthenic symptoms. The printers also scored significantly worse than referents in a number of psychometric tests, including synonym, Benton revised visual retention and digit symbol tests, even after adjustment for age. For all comparisons, tests of interaction between the effects of toluene exposure and alcohol consumption were not statistically significant.

A battery of neurobehavioral tests was performed in 30 female workers exposed to toluene vapors in an electronic assembly plant (Foo *et al.*, 1990). The average number of years worked was 5.7 ± 3.2 for the exposed group and 2.5 ± 2.7 years for the controls. Study subjects did not smoke tobacco or drink alcohol, were not taking any medications, and had no prior history of central or peripheral nervous system illness or psychiatric disorders. The exposed group of workers inhaled a time-weighted average (TWA) of 88 ppm (330 mg/m^3) toluene while the

control workers inhaled 13 ppm (49 mg/m^3). A significant decrease in neurobehavioral performance was observed in the exposed workers in 6 out of 8 tests. Irritant effects were not examined, and concurrent exposures to other chemicals were not addressed. In this study, 88 ppm was considered a LOAEL for central nervous system effects. However, the workers designated by the authors to be controls did not comprise a true control group, since they were exposed to 13 ppm toluene. This may have resulted in an underestimation of the effects of exposure to 88 ppm toluene. Similar effects were noted in a follow-up study by Boey *et al.* (1997).

Abbate *et al.* (1993) evaluated alterations induced in the auditory nervous system by exposure to toluene in a group of rotogravure workers. A sample of 40 workers of normal hearing ability was selected from a group of 300 workers who were apparently in good health but were professionally exposed to toluene (12 – 14 years exposure, 97 ppm average exposure, exposure assessment not described). They were subjected to an adaptation test utilizing a BAER technique with 11 and 90 stimulus repetitions a second. The results were compared with an age and sex-matched control group not professionally exposed to solvents. A statistically significant alteration in the BAER results was noted in the toluene-exposed workers with both 11 and 90 stimuli repetitions. The authors suggested that these results can be explained as a toluene-induced effect on physiologic stimulus conduction mechanisms, even in the absence of any clinical sign of neuropathy. Furthermore, this effect could be observed in the responses of the entire auditory system, from peripheral receptors to brainstem nuclei.

A group of 49 printing-press workers occupationally exposed to toluene for approximately 21.6 years was studied by Vrca *et al.* (1997). Toluene exposure levels were determined from blood toluene and urinary hippuric acid levels, and were estimated to range from 40-60 ppm. No control group was used. Brain evoked auditory potential (BEAP; similar to BAER) and visual evoked potential (VEP) measurements were performed on a Monday morning after a nonworking weekend. There was a significant increase in the latencies of all the BEAP waves examined, except for P2 waves, as well as in the interpeak latency (IPL) P3-P4, while IPL P4-P5 decreased significantly with the length of exposure. No correlation was noted between the amplitude of BEAP waves and the length of exposure. The amplitude but not the latency of all the VEPs examined decreased significantly with the length of exposure.

The effects of acute and chronic toluene exposure on color vision were studied in a group of eight rotogravure printing workers (Muttray *et al.*, 1999). The workers had been employed as printers for an average of 9.8 years. The color vision acuity of the workers before and after an acute toluene exposure (28 – 41 minutes in duration, concentration 1115 – 1358 mg/m^3) was evaluated using the Farnsworth panel D-15 test, the Lanthony desaturated panel D-15 test, and the Standard Pseudoisochromatic Plates part 2. A control group of 8 unexposed workers was also tested. Acute toluene exposure had no effect on color vision. Print worker performance prior to acute toluene exposure (chronic effects) was similar to controls on the Farnsworth panel D-15 and Standard Pseudoisochromatic Plates part 2 tests. Print worker performance on the Lanthony desaturated panel D-15 test was worse than that of controls (median scores of 1.18 and 1.05 for exposed and controls (higher number indicates degraded performance), respectively, but not significantly ($p = 0.06$). The authors noted that the small number of subjects limited the statistical power of the study.

Zavalic *et al.* (1998) examined the effects of chronic occupational toluene exposure on color vision using a group of 45 exposed workers (mean toluene exposure concentration = 120 ppm) and 53 controls. Color vision was evaluated using the Lanthony desaturated panel D-15 test; test scores were age and alcohol consumption-adjusted. Color vision was significantly impaired in toluene-exposed workers ($p < 0.0001$) compared to controls. It was also observed that there was no significant difference between test scores on Monday morning (prework) and Wednesday morning. The authors stated that the effect of toluene on color vision can be chronic and that the possible recovery period is longer than 64 hours.

Hepatic Effects

Greenburg *et al.* (1942) reported liver enlargement in 32 of 106 (30.2%) painters employed in an aircraft factory compared to 7% in a control group. However, there was some exposure to other solvents (ethanol, ethyl acetate, butyl acetate) and paint ingredients such as zinc chromate.

Liver toxicity has been reported in toluene solvent abusers (Fornazzari *et al.*, 1983). Eight of 24 solvent abusers demonstrated abnormal results in three liver function tests; however, the tests used were not specified. The test parameters returned to normal after two weeks of toluene abstinence, suggesting that any liver damage caused by toluene abuse in those patients was not long lasting.

A cross-sectional study by Boewer *et al.* (1988) showed no significant correlation between toluene exposure and the levels of serum enzymes (serum aspartate aminotransferase (ASAT), alanine aminotransferase (ALAT), γ-glutamyltransferase (GGT)) considered to be indicators of hepatic damage. In another cross-sectional study of 289 printing workers exposed to less than 200 ppm for 8 hours/day, 8 workers had significantly elevated serum enzymes (ALT/AST ratio, mean = 1.61) potentially indicative of liver damage. In each case, liver biopsy indicated a mild pericentral fatty change (Guzelian *et al.*, 1988). However, the mean toluene exposure concentration was not reported (only an upper bound), and no control group was included in the study.

V. Effects of Animal Exposures

Neurotoxic Effects

Sprague-Dawley rats (15/sex/group) were exposed to 0, 100, or 1481 ppm toluene for 6 hours/day, 5 days/week for 26 weeks (API, 1981). Neurohistopathological examinations were conducted in 3-5 rats/sex/group at weeks 9, 18, and 27. No significant treatment-related effects were reported. The study usefulness was limited because there were no other neurohistopathological examinations or organ weight measurements conducted on the animals.

Forkman *et al.* (1991) studied the potential neurotoxicity of toluene inhalation exposure (3700 mg/m^3 (1000 ppm), 21 hours/day, 5 days/week for 4 weeks) in male Sprague-Dawley rats. The rats were either trained in behavior meant to be performance tested and then exposed to toluene, or exposed and then trained. The rats were then subjected to several behavioral tests, including an operant test with baseline performance and extinction, motor coordination, and exploratory

activity. All tests were performed from 11 to 35 days after the end of the exposure. Exposure of trained rats to toluene resulted in a significantly different overall test performance when compared to controls. Rats trained after toluene exposure also had test performances different from controls, but the difference was not statistically significant.

Rats exposed to toluene concentrations of 1000 ppm or 100 ppm, 6 h/day, 5 days/week, for 3 or 6 months, respectively, demonstrated statistically significant decreased motor function as measured by degraded performance (approximately 60% and 65% of control at 1000 and 100 ppm toluene, respectively) on a rotarod performance test and decreases in spontaneous motor activity (approximately 62% of control at 100 ppm toluene) (Korsak *et al*., 1992).

von Euler *et al.* (1993) studied the effects of subchronic toluene inhalation exposure (80 ppm, 4 weeks, 5 days/week, 6 hours/day) on spatial learning and memory, dopamine-mediated locomotor activity and dopamine D_2 agonist binding in rats. Spatial learning (postexposure days 3-6) and memory (postexposure day 14) were tested using a water maze. Spontaneous and apomorphine-induced locomotor activity was evaluated on postexposure day 17. Effects on binding parameters of the dopamine D2 agonist S(-)[N-propyl- ^{3}H(N)]-propylnorapomorphine ([H]NPA) were determined using membrane preparations of the neostriatum of the rat brain. Toluene exposure caused a statistically significant impairment in spatial learning and memory. Toluene also significantly increased apomorphine-induced locomotion and motility but not rearing. Spontaneous locomotion, motility and rearing were not affected by toluene. Toluene exposure significantly increased the B_{max} and K_D values for [^{3}H]NPA binding. These results indicate that subchronic toluene exposure of rats to toluene causes persistent deficits in spatial learning and memory, a persistent increase in dopamine-mediated locomotor activity and an increase in the number of dopamine D_2 receptors in the neostriatum.

Male rat exposure to toluene (0, 40, 80, 160 or 320 ppm, 4 weeks, 6 hours/day, 5 days/week) , followed by a postexposure period of 29-40 days, resulted in decreased brain wet weights of the caudate-putamen (trend test for dose-response significant at $p < 0.05$) and subcortical limbic areas (trend test for dose-response significant at $p < 0.01$; significantly less than controls ($p < 0.001$) at concentrations of 80 ppm and higher) (Hillefors-Berglund *et al.*, 1995). Toluene exposure also significantly altered dopamine receptor activity (trend test for dose-response) as indicated by decreased IC_{50} (inhibition constant) (significantly less than controls ($p < 0.05$) at 80 ppm), K_H (inhibition constant for high-affinity receptor sites), K_L (inhibition constant for low-affinity receptor sites), and R_H% (high-affinity receptor site specific binding) values for dopamine competitive inhibition of [^{3}H]raclopride-binding in the caudate-putamen. Toluene exposure did not significantly affect the wet weights of the whole brain, serum prolactin levels, the K_D (disassociation constant) or the B_{max} (maximal specific binding) values of [^{3}H]raclopride-binding in the caudate-putamen and the subcortical limbic area, or the effect of dopamine on IC_{50} values at [^{3}H]raclopride-binding sites in the subcortical limbic area. Exposure to xylene or styrene (80 and 40 ppm, respectively; 4 weeks, 6 h/day, 5 days/week) followed by a postexposure period of 26-32 days had no effect on the parameters described above. The authors concluded that long-term exposure to low concentrations of toluene (≥ 80 ppm), but not xylene (80 ppm) or styrene (40 ppm), leads to persistent increases in the affinity of dopamine D_2 agonist binding in the rat caudate-putamen. The authors also suggested that the enhancement of

apomorphine-induced locomotor activity seen after toluene exposure by von Euler *et al.* (1993) may be related to the increased D_2 agonist activity described above (IC_{50}, K_H, K_L values).

Respiratory Effects
A study of the chronic effects of toluene in rats (5-20 animals per group) exposed for 106 weeks to 0, 30, 100, or 300 ppm (0, 113, 375, or 1125 mg/m^3) toluene showed no treatment-related effects on histopathology of major organs, including the nasal turbinates (CIIT, 1980). In this study, the nasal histopathology examination sampling may have been inadequate to demonstrate the nasal lesions reported by the NTP (1990).

Rats (20 per group) exposed for 2 years to 0, 600, or 1200 ppm (0, 2261, or 4523 mg/m^3) toluene 6.5 hours/day, 5 days/week for 103 weeks were examined for hematological and histopathological effects in addition to gross observations of toxicity (NTP, 1990). Significant erosion of the olfactory epithelium was observed in male rats while degeneration of the respiratory and nasal epithelium was observed in both sexes at 600 ppm.

Mice were exposed chronically to 0, 120, 600, or 1200 ppm (0, 452, 2261, or 4523 mg/m^3) toluene 6.5 hours/day, 5 days/week, for 2 years (NTP, 1990). The only treatment-related effect was a significant increase in the number of animals with hyperplasia of the bronchial epithelium in the 1200 ppm exposure group.

Reproductive and Developmental Toxicity
Reproductive toxicity to maternal rats was observed during exposure to 1500 ppm toluene, 24 hours/day on days 9 to 14 of gestation (Hudak and Ungvary, 1978). Two dams out of 19 died during exposure. Fetuses from the 1500 ppm group showed increased incidence of sternebral alterations, extra ribs and missing tails. The same exposure on days 1 through 8 of gestation resulted in 5 deaths out of 14 dams. Fetuses in this regimen showed increased incidence of hydrocephaly and growth retardation compared to controls. A third regimen that exposed maternal rats to 1000 ppm on days 1 through 21 of gestation resulted in no maternal deaths or toxicity, and an increase in the incidence of skeletal variations in the fetuses. When exposed to 1500 ppm continuously, maternal mice died within 24 hours of exposure whereas exposure to 500 ppm had no apparent effect. Examination of the fetal mice showed significant growth retardation in the 500 ppm group.

A 2-generation study of the effects of 0, 100, 500, or 2000 ppm (0, 377, 1885, or 7538 mg/m^3) toluene in rats (males, 10-40 per group; females, 20-80 per group) was done by the American Petroleum Institute (API)(1985). Rats were exposed for 6 hours/day, 7 days/week for 80 days and a 15 day mating period. The mated females were then exposed to the same concentrations during days 1-20 of gestation and days 5-20 of lactation. After weaning, the F_1 pups were exposed 80 times to the appropriate exposure level and then randomly mated to members of the same exposure group. The F_1 generation showed significantly decreased body weight which persisted throughout lactation. No effects were observed on histopathology. No data were presented for the F_2 generation.

Da Silva *et al.* (1990) exposed rats and hamsters to 0 or 800 mg/m^3 toluene for 6 hours/day on gestation days 14-20 (rats), or days 6-11 (hamsters). Exposed rats demonstrated a significant

exposure-related decrease in birth weight compared with controls. In addition to low birth weight, the number of live pups was significantly lower in the 800 ppm group. No deficits in any parameter were noted in the hamsters. In this study, no neurobehavioral effects were noted in the offspring.

Hass *et al.* (1999) exposed rats to 0 or 1200 ppm toluene for 6 h per day from day 7 of pregnancy until day 18 postnatally. Developmental and neurobehavioral effects in the offspring were investigated using a test battery including assessment of functions similar to those in the proposed Organization for Economic Cooperation and Development (OECD) Testing Guidelines for Developmental Neurotoxicity Study (physical development, reflex development, motor function, motor activity, sensory function, and learning and memory). The exposure did not cause maternal toxicity or decreased offspring viability. However, lower birth weight, delayed development of reflexes, and increased motor activity in the open field was noted in the exposed offspring. The exposed female offspring had poorer scores on a Morris water maze test (they took longer to locate a hidden platform after platform relocation) at the age of 3.5 months indicating impaired cognitive function. The difference was not related to impaired swimming capabilities since swim speeds were similar to control values. The authors stated that exposure to 1200 ppm toluene during brain development caused long-lasting developmental neurotoxicity in rats.

Toluene has been listed under Proposition 65 as being known to the State of California to cause reproductive toxicity (OEHHA, 1999). Its NSRL is 7,000 micrograms per day.

VI. Derivation of Chronic Reference Exposure Level (REL)

Study	Hillefors-Berglund *et al*. (1995); supported by Orbaek and Nise (1989), Foo *et al.* (1990)
Study population	Male Sprague-Dawley rats
Exposure method	Inhalation
Critical effects	Decreased brain (subcortical limbic area) weight, altered dopamine receptor (caudate-putamen) binding
LOAEL	80 ppm
NOAEL	40 ppm
Exposure continuity	6 hours/day, 5 days/week
Exposure duration	4 weeks, followed by 29-40 days recovery
Average experimental exposure	7 ppm (40 × 6/24 hours × 5/7 days)
Human equivalent concentration	7 ppm (gas with systemic effects, based on RGDR = 1.0 using default assumption that $\lambda_a = \lambda_h$
Subchronic uncertainty factor	10
Interspecies uncertainty factor	1 (see below)
Intraspecies uncertainty factor	10
Cumulative uncertainty factor	100
Inhalation reference exposure level	0.07 ppm (70 ppb; 0.3 mg/m^3; 300 µg/m^3)

Supportive human study	Foo *et al.*, 1990
Study population	30 female workers in an electronic assembly plant
Exposure method	Occupational inhalation
Critical effects	Neurobehavioral deficits in 6 out of 8 tests
LOAEL	88 ppm
NOAEL	Not observed
Exposure continuity	10 m^3/day occupational inhalation rate, 5 days/week
Average occupational exposure	31.4 ppm (88 ppm x 10/20 x 5/7)
Exposure duration	5.7 $\pm$ 3.2 years (exposed group); 2.5 $\pm$ 2.7 years (controls)
LOAEL uncertainty factor	10
Subchronic uncertainty factor	3
Interspecies uncertainty factor	1
Intraspecies uncertainty factor	10
Cumulative uncertainty factor	300
Inhalation reference exposure level	0.1 ppm (100 ppb; 0.4 mg/m^3; 400 µg/m^3)

The critical animal study (Hillefors-Berglund *et al*., 1995) used to derive an REL for toluene describes adverse neurological effects in rats after a well characterized inhalation exposure to toluene. The study results contain both a LOAEL and a NOAEL. Decreased brain (subcortical limbic area) weight and altered dopamine receptor binding compared to controls were noted at the NOAEL, but the changes were not statistically significant; this suggests that if a threshold for

adverse neurological effects exists in this study, it would be at or below the observed NOAEL. The study LOAEL for altered dopamine receptor binding agrees qualitatively with results from similar studies (von Euler *et al.*, 1994). Additionally, toluene-induced neurotoxicity has been described in many studies by a variety of endpoints in both animals and humans (ATSDR, 1999). The adverse neurotoxic effects associated with toluene exposure in the rat study by Hillefors-Berglund *et al*. (1995), decreased brain (subcortical limbic area) weight and altered dopamine receptor binding, occur in areas of the rat brain that are structurally and functionally similar to brain areas (basal ganglia, thalami) of some human toluene abusers that demonstrate MRI alterations (T2 hypointensity). The altered MRI parameters may be the result of the partitioning of toluene into the lipid membranes of brain cells (Ungar *et al*., 1994). Table 1 lists several Reference Exposure Levels (RELs) calculated from the most sensitive animal and human neurotoxicity studies available. These RELs are also protective for other adverse endpoints, such as respiratory tract damage and teratogenicity.

Table 1: Reference Exposure Levels (RELs) from Selected Neurotoxicity Studies

Study	Duration	Effect	LOAEL (ppm)	LOAEL (ppm) (TWA)	NOAEL (ppm)	NOAEL (ppm) (TWA)	total UF[a]	REL (ppb)	REL (µg/m^3)
VonEuler *et al*. (1988)	4 weeks	rat: altered brain dopamine receptor binding	80	14.3			1000	14	54
Orbaek and Nise[b] (1989)	29 years	human: impairment on neuropsychometric tests	11.2 - 41	4 - 14.6			100	40 - 146	150 - 551
Foo (1990)	5.7 years	human: neurobehavioral tests	88	31.4			300	105	394
Korsak (1992)	6 months	rat: impaired motor function	100	17.9			100	179	671
Hillefors-Berglund (1995)	4 weeks	rat: decreased brain (subcortical limbic area) weight; altered brain dopamine receptor binding	80	14.3	40	7.1	100	71	271

LOAEL: Lowest Observable Effect Level; NOAEL: No Observable Effect Level
REL: Reference Exposure Levels; TWA: time-weighted average

a: Uncertainty Factors used to derive RELs

VonEuler *et al*. (1988)	LOAEL to NOAEL UF = 10, subchronic to chronic UF = 10, animal to human UF = 1, intraspecies variability = 10; total UF = 1000.
Orbaek and Nise (1989)	LOAEL to NOAEL UF = 10, intraspecies variability = 10; total UF = 100
Foo *et al*. (1990)	LOAEL to NOAEL UF = 10, subchronic to chronic UF = 3, intraspecies variability = 10; total UF = 300
Korsak *et al*. (1992)	LOAEL to NOAEL UF = 10, animal to human UF = 1, intraspecies variability = 10; total UF = 100.
Hillefors-Berglund *et al*. (1995)	subchronic to chronic UF = 10, animal to human UF = 1, intraspecies variability = 10; total UF = 100.

b: Pooled psychometric data from two printing plants with different toluene concentrations (11.2 and 41 ppm) were used to determine significant neurotoxic effects by Orbaek and Nise (1989). The range of RELs derived from that study lists the upper and lower bounds for risk associated with the pooled population exposures. ATSDR (1999) used the Orbaek and Nise (1989) study data, assuming an exposure concentration of 11.2 ppm, to derive a chronic inhalation minimal risk level (MRL).

If both human and animal adverse effect data on a chemical are available, OEHHA prefers to use the human data to develop a REL when possible. However, the study by Hillefors-Berglund *et al*. (1995) provides data (decreased brain [subcortical limbic area] weight and altered brain dopamine receptor binding) which are specific and sensitive measures of neurotoxicity that would not be obtainable in human studies. In contrast, the psychometric tests used to generate the neurotoxicity data in the human occupational exposure studies described above tend to be less sensitive and suffer from greater measurement uncertainty. Additionally, the Hillefors-Berglund *et al*. (1995) study has better exposure characterization than the human occupational exposure studies. Nonetheless, the human studies are useful in supporting the derivation of the REL for toluene. Ordinarily, an interspecies uncertainty factor of 3 would be applied, in addition to the human equivalent concentration calculation, to reflect the uncertainty associated with extrapolating from animals to humans. However, in this case the uncertainty in the interspecies extrapolation is reduced by the availability of human epidemiological data with generally consistent effect levels, after appropriate duration corrections. Based on comparison of the data in both animals and humans, it appears that a REL of 271 $\mu g/m^3$ (rounded to 300 $\mu g/m^3$ in the final derivation) would protect exposed humans from experiencing chronic neurotoxic effects.

VII. Data Strengths and Limitations for Development of the REL

The major strength of the REL for toluene is the use of an animal study with accurate exposure characterization and both LOAEL and NOAEL observations for an effect (neurotoxicity), supported by observations from other animal and human studies. A weakness is the uncertainty in predicting human health risk from animal adverse effect data. However, this is mitigated by the availability of human data showing effect levels that are, after appropriate corrections, broadly consistent with the animal data.

VII. References

Abbate C, Giorgianni C, Munao F, and Brecciaroli R. 1993. Neurotoxicity induced by exposure to toluene. An electrophysiologic study. Int. Arch. Occup. Environ. Health 64:389-92.

Agency for Toxic Substances and Disease Registry (ATSDR). 1999. Toxicological Profile for Toluene (Update). PB/95/100228/AS. U.S. Department of Health and Human Services, Public Health Service.

American Petroleum Institute (API). 1981. Twenty-six-week inhalation toxicity study of toluene in the rat. Conducted by Bio/dynamics Inc. and Institute of Neurotoxicity, Albert Einstein College of Medicine for API, Washington, DC.

American Petroleum Institute (API). 1985. Two-generation inhalation reproduction/fertility study on a petroleum-derived hydrocarbon. Doc. ID FYI-AX-0284-0294 IN. Microfiche No. 0294.

Boewer C, Enderlein G, Wollgast U, Nawka S, Palowski H, and Bleiber R. 1988. Epidemiological study on the hepatotoxicity of occupational toluene exposure. Int. Arch. Occup. Environ. Health 60:181-186.

Boey KW, Foo SC, and Jeyaratnam J. 1997. Effects of occupational exposure to toluene: a neuropsychological study on workers in Singapore. Ann. Acad. Med. Singapore 26:184-7.

Byrne A, Kirby B, Zibin T, and Ensminger S. 1991. Psychiatric and neurological effects of chronic solvent abuse. Can. J. Psychiatry 36:735-8.

Caldemeyer KS, Armstrong SW, George KK, Moran CC, and Pascuzzi RM. 1996. The spectrum of neuroimaging abnormalities in solvent abuse and their clinical correlation. J. Neuroimaging 6:167-73.

Chemical Industry Institute of Toxicology (CIIT). 1980. A twenty-four month inhalation toxicology study in Fischer-344 rats exposed to atmospheric toluene. Conducted by Industrial Bio-Test Laboratories, Inc., Decatur, IL, and Experimental Pathology Laboratories, Inc., Raleigh, NC, for CIIT, Research Triangle Park, NC. October 15.

CRC. 1994. CRC Handbook of Chemistry and Physics, 75th edition. Lide DR, ed. Boca Raton, FL: CRC Press Inc.

Da Silva VA, Malheiros LR, and Bueno FMR. 1990. Effects of toluene exposure during gestation on neurobehavioral development of rats and hamsters. Brazil J. Med. Biol. Res. 23:533-537.

Filley CM, Heaton RK, and Rosenberg NL. 1990. White matter dementia in chronic toluene abuse. Neurology 40:532-4.

Foo SC, Jeyaratnam, J, and Koh D. 1990. Chronic neurobehavioral effects of toluene. Br. J. Ind. Med. 47(7):480-484.

Forkman BA, Ljungberg T, Johnson AC, Nylen P, Stahle L, Hoglund G, and Ungerstedt U. 1991. Long-term effects of toluene inhalation on rat behavior. Neurotoxicol. Teratol. 13:475-81.

Fornazzari L, Wilkinson DA, Kapur BM, and Carlen PL. 1983. Cerebellar cortical and functional impairment in toluene abusers. Acta Neurol. Scand. 67:319-329.

Greenburg L, Mayers MR, Heiman H, and Moskowitz S. 1942. The effects of exposure to toluene in industry. JAMA 118:573-578.

Guzelian P, Mills S, and Fallon HJ. 1988. Liver structure and function in print workers exposed to toluene. J. Occup. Med. 30(10):791-796.

Hass U, Lund SP, Hougaard KS, and Simonsen L. 1999. Developmental neurotoxicity after toluene inhalation exposure in rats. Neurotoxicol. Teratol. 21:349-57.

Hazardous Substances Data Bank (HSDB). 1999. National Library of Medicine, Bethesda, MD (Internet version).

Hillefors-Berglund M, Liu Y, and von Euler G. 1995. Persistent, specific and dose-dependent effects of toluene exposure on dopamine D2 agonist binding in the rat caudate-putamen. Toxicology 100:185-94.

Hudak A, and Ungvary G. 1978. Embryotoxic effects of benzene and its methyl derivatives: Toluene, xylene. Toxicology 11:55-63.

Ikeda M and Tsukagoshi H. 1990. Encephalopathy due to toluene sniffing. Report of a case with magnetic resonance imaging. Eur. Neurol. 30:347-9.

Muttray A, Wolters V, Jung D, and Konietzko J. 1999. Effects of high doses of toluene on color vision. Neurotoxicol. Teratol. 21:41-5.

NTP. 1990. National Toxicology Program. Toxicology and carcinogenesis studies of toluene (CAS No. 108-88-3) in F344/N rats and B6C3F$_1$ mice (inhalation studies). NTP-TR-371.

Office of Environmental Health Hazard Assessment (OEHHA). 1999. Safe Drinking Water And Toxic Enforcement Act Of 1986. Chemicals Known To The State To Cause Cancer Or Reproductive Toxicity. Reproductive and Cancer Hazard Assessment Section, Oakland, CA.

Office of Technology Assessment (OTA). 1984. Nuclear Magnetic Resonance Imaging Technology. A Clinical, Industrial and Policy Analysis. Prepared by Steinberg EP and Cohen AB. Health Technology Case Study 27. Congress of the United States, Washington DC.

Orbaek P and Nise G. 1989. Neurasthenic complaints and psychometric function of toluene-exposed rotogravure printers. Am. J. Ind. Med. 16:67-77.

Rosenberg NL, Kleinschmidt-DeMasters BK, Davis KA, Dreisbach JN, Hormes JT and Filley CM. 1988. Toluene abuse causes diffuse central nervous system white matter changes. Ann. Neurol. 23:611-4.

Rosenberg NL, Spitz MC, Filley CM, Davis KA and Schaumburg HH. 1988. Central nervous system effects of chronic toluene abuse--clinical, brainstem evoked response and magnetic resonance imaging studies. Neurotoxicol. Teratol. 10:489-95.

Unger E, Alexander A, Fritz T, Rosenberg N and Dreisbach J. 1994. Toluene abuse: physical basis for hypointensity of the basal ganglia on T2-weighted MR images. Radiology 193:473-6.

U.S. Environmental Protection Agency (U.S. EPA). 1984. Health Effects Assessment for Toluene. EPA/540/1-86/033. Cincinnati, OH: U.S. EPA.

U.S. Environmental Protection Agency (U.S. EPA). 1994. Integrated Risk Information System (IRIS) database. Reference concentration (RfC) for toluene.

von Euler G, Ogren SO, Eneroth P, Fuxe K, and Gustafsson JA. 1994. Persistent effects of 80 ppm toluene on dopamine-regulated locomotor activity and prolactin secretion in the male rat. Neurotoxicology 15:621-4.

von Euler G, Ogren SO, Li XM, Fuxe K, and Gustafsson JA. 1993. Persistent effects of subchronic toluene exposure on spatial learning and memory, dopamine-mediated locomotor activity and dopamine D2 agonist binding in the rat. Toxicology 77:223-32.

Vrca A, Bozicevic D, Bozikov V, Fuchs R, and Malinar M. 1997. Brain stem evoked potentials and visual evoked potentials in relation to the length of occupational exposure to low levels of toluene. Acta Med. Croatica 51:215-9.

Yamanouchi N, Okada S, Kodama K, Hirai S, Sekine H, Murakami A, Komatsu N, Sakamoto T, and Sato T. 1995. White matter changes caused by chronic solvent abuse. Am. J. Neuroradiol. 16:1643-9.

Yin S, Li G, Hu Y, Zhang XM, Jin C, Inoue O, Seiji K, Kasahara M, Nakatsuka H, and Ikeda M. 1987. Symptoms and signs of workers exposed to benzene, toluene, or the combination. Ind. Health. 25(3):113-130.

Zavalic M, Mandic Z, Turk R, Bogadi-Sare A, Plavec D, Gomzi M, and Skender LJ. 1998. Assessment of colour vision impairment in male workers exposed to toluene generally above occupational exposure limits. Occup. Med. (Lond.) 48:175-80.

CHRONIC TOXICITY SUMMARY

2,4- and 2,6-TOLUENE DIISOCYANATE

(2,4- and 2,6-TDI; 2,4- and 2,6-diisocyanato-1-methylbenzene; 2,4- and 2,6-diisocyanatoluene)

CAS Registry Number: 584-84-9 or 26471-62-5 (mixture)

I. Chronic Toxicity Summary

Inhalation reference exposure level	**0.07 µg/m^3** (0.01 ppb)
Critical effect(s)	Decreased lung function in occupationally exposed workers
Hazard index target(s)	Respiratory system

II. Chemical Property Summary (HSDB, 1995; CRC, 1994)

Description	Colorless to pale yellow liquid
Molecular formula	$C_9H_6N_2O_2$
Molecular weight	174.15 g/mol
Boiling point	2,4-TDI: 251°C
Melting point	2,4-TDI: 20.5°C 2,6-TDI: 18.3°C
Vapor pressure	2,4-TDI: 0.008 torr @ 20°C
Solubility	Miscible with ether, acetone, benzene, carbon tetrachloride, chlorobenzene, diglycol monomethyl ether, kerosene, olive oil, alcohol; soluble in ethyl acetate
Conversion factor	7.1 µg/m^3 per ppb at 25ºC

III. Major Uses and Sources

Commercial toluene diisocyanate is comprised of approximately 80% 2,4-TDI and 20% 2,6-TDI. TDI is used in the manufacture of polyurethane foams, elastomers, and coatings (HSDB, 1995; Howard, 1989). It is also used in the manufacture of floor and wood finishes, lacquers, foam plastics, polyurethane foam coated fabrics, and insulation materials (HSDB, 1995; Howard, 1989; Duncan *et al.*, 1962). Emissions of TDI to the atmosphere can occur during production, handling, and processing of polyurethane foam (Howard, 1989) and coatings. The annual statewide industrial emissions from facilities reporting under the Air Toxics Hot Spots Act in California based on the most recent inventory were estimated to be 13,223 pounds of toluene diisocyanates, 35,663 pounds of toluene-2,4-diisocyanate, and 754 pounds of toluene-2,6-diisocyanate (CARB, 1999).

IV. Effects of Human Exposures

Diem *et al.* (1982) conducted a prospective study beginning in 1973 of 277 male workers involved in the production of TDI. The study examined pulmonary function, with nine examinations conducted over a five year period. A large group of workers (168) with no previously reported TDI exposure was examined 6 months prior to TDI production in the plant to provide baseline pulmonary function measurements. Personal sampling by continuous tape monitors provided exposure levels, but was not used until 2 years after the study was initiated. Sampling information resulted in a division of the workers into two groups: those exposed to levels below 68.2 ppb-months (which reflects the level of exposure of a worker for the entire 5 year duration in the low-exposure area (geometric mean = 1.1 ppb)) and those above this level. The arithmetic mean exposure level for the non-smokers was 1.9 ppb TDI in the high-exposure group and 0.9 ppb TDI in the low-exposure group (calculated by Hughes, 1993). The higher exposure group was further limited to those individuals who showed a normal FEV_1 to height ratio. Data were analyzed by the maximum likelihood weighted regression approach (Diem and Liukkonen, 1988). Both FEV_1 and forced expiratory flow (25-75%) [FEF (25-75%)] among workers who never smoked were found to be significantly reduced in the high-exposure group (n = 21) compared to the low-exposure group (n = 35). Categorizing workers based on time spent at exposure levels above 20 ppb demonstrated a significant difference in FEV_1 and FEF(25-75%) and this effect was also observed among current smokers. Among low-exposure workers, a smoking effect was observed, with smokers showing a significant decline in FEV_1.

A similar longitudinal study of lung function was conducted among workers exposed to TDI during the course of polyurethane foam production (Jones *et al.*, 1992). Participants (181 males and 46 females) were required to have 3 or more spirometric examinations over the 5 year study period. Exposure of males was evaluated by personal monitors and resulted in arithmetic mean low exposure levels of 0.3, 0.4, and 0.4 ppb TDI for never-smokers, ex-smokers, and current smokers, respectively. Among workers with high-level exposure, mean TDI levels were reported to be 1.3, 1.2, and 1.2 ppb for never-, ex-, and current smokers, respectively. Stepwise multiple linear regression methods (excluding asthmatics) were used in evaluating the data (Diem and Liukkonen, 1988). No relationship between TDI exposure and change in lung function was observed, although the prevalence of chronic bronchitis was significantly associated with exposure.

A longitudinal study of 780 workers exposed to TDI in the production of polyurethane foam was also conducted (Bugler *et al.*, 1991; unpublished). Exposure levels were established using continuous-tape personal monitoring devices. The mean exposure level was 1.2 ± 1.1 (SD) ppb TDI among 521 workers and 0.3 ± 0.18 ppb TDI in the control group. Another control group who handled cold urethane products had an 8 hour time-weighted average exposure of 0.6 ppb TDI. No significant longitudinal changes in FEV_1 were found after regression analysis, although FEV_1 decline was high among the control group. Exposure levels among the different groups were close, limiting the power of the study to detect changes. Approximately 3% of the 780 workers showed signs of TDI sensitization and, of these, over 80% were in the group exposed to 1.2 ppb.

Meta-analysis of the three data sets (Jones *et al.*, 1992; Bugler *et al.*, 1991; Diem *et al.*, 1982) showed that the difference in significance among the findings of each of the studies could have been due to chance. The change in the probability density for the decline in FEV_1 shifted in the same direction for all data sets and the smoker/non-smoker slope difference became less meaningful with the data set combination (Hasselblad, 1993).

Another toxicological area of concern with exposure to TDI is the development of sensitization, resulting in a well-documented condition known as "isocyanate asthma" of either immediate or delayed-type onset (Moscato *et al.*, 1991). The level of exposure required to either develop or trigger a sensitization reaction is not well documented, however. Weaknesses of studies showing pulmonary effects of TDI exposure include use of area sampling vs. breathing-zone measurement of exposure, poor statement of criteria for evaluating hypersensitivity, and the presence of other compounds in the environment which may influence lung function.

V. Effects of Animal Exposures

Mice were exposed to TDI concentrations ranging from 0.007 to 1.18 ppm for 3 hours/day for 5 days consecutively (Sangha and Alarie, 1979); decreased respiratory rate was observed in groups exposed to levels higher than 0.023 ppm TDI. Groups of four mice were also exposed to 0.031 and 0.250 ppm TDI for 3 hours/day for 3 days. Lesions of the external nares and respiratory epithelium were observed in the high dose group.

Female guinea pigs were exposed to 0.12, 0.36, 0.61, 0.96, and 10.00 ppm TDI (head-only) for 3 hours/day for 5 consecutive days (short protocol) or to 0.02 ppm TDI (whole body) plus controls for 6 hours/day, 5 days/week for 70 days (long protocol). The animals showed decreased respiration rate two hours into exposure at levels above 0.12 ppm TDI and had a cytophilic antibody response at 0.96 ppm and above (Karol, 1983). All animals exposed to 10 ppm died. Dermal sensitivity was evident among animals in the short protocol down to 0.12 ppm TDI. No antibody response or dermal sensitivity developed in the animals exposed to 0.02 ppm TDI in the long protocol.

Similarly, guinea pigs (8 females) were exposed head only to 1.40 ppm TDI for 3 hours/day for 4 days (no control group). In a second exposure regimen, animals (n = 24) were exposed to 0.02 ppm TDI for 6 hours/day, 4 days/week for 70 days (whole body) including a control group (n = 8) exposed to room air in a similar manner (Wong *et al.*, 1985). Half the animals (4/8) exposed to 1.40 ppm TDI showed pulmonary hypersensitivity (measured on days 37 and 38) and all developed TDI-specific IgE antibodies, whereas none of the animals in the 0.02 ppm TDI group showed either of these effects. Histopathological effects in the 1.40 ppm TDI group included interstitial inflammation, pleural thickening, and peripheral lymphoid hyperplasia. Interstitial inflammation was noted in 2/24 animals exposed to 0.02 ppm TDI.

SD rats and CD-1 mice were exposed to 0.05 or 0.15 ppm TDI for 6 hours/day, 5 days/week for 2 years (Loeser, 1983; nasal histopathology reported by Owen, 1984). Among female rats at both dose levels and male rats at the high dose level, histopathological effects observed included necrotic rhinitis, metaplasia, and inflammation of the respiratory epithelium. Female animals

showed dose-dependent increases in incidence and severity of this effect. Similar lesions were reported in mice, although they were not well characterized.

Reproductive toxicity of TDI was evaluated in a two-generation study conducted in rats (Tyl and Neeper-Bradley, 1989). Weanling rats (28/sex/dose) were exposed to 0, 0.020, 0.079, and 0.290 ppm TDI for 6 hours/day, 5 days/week, for 10 weeks, at which time the animals were randomly mated. Exposure of the females continued through gestation (excepting gestational day 20 through the fourth day postpartum), and exposure of the males continued only until the delivery of the F_1 generation. Weanlings in the F_1 generation were exposed in a manner similar to the parental (P_0) generation and bred after weaning to produce the F_2 generation. Body weights were significantly reduced among animals of both sexes in the highest dose group and weight gain was reduced among males in the highest dose group. Effects on the respiratory system in the P_0 generation animals included rhinitis of the epithelium in the two highest dose groups of both male and female animals. Hyperplasia of the respiratory epithelium was also increased in the high dose groups of both sexes among P_0 animals. Among males in the F_1 generation, the incidence of rhinitis was significantly increased at all exposure levels and the incidence of submucosal lymphoid infiltrates of the larynx and trachea was increased in the highest dose group. F_2 generation animals showed reduced pup weight and weight gain during the lactation period in the two highest dose groups.

Developmental toxicity of TDI was evaluated by exposing pregnant Sprague-Dawley rats (25/group) for 6 hours/day on gestational days 6-15 to 0, 0.021, 0.120, or 0.48 ppm TDI (Tyl, 1988). Reduced maternal body weight, decreased food consumption, and rales occurred among the dams in the 0.48 ppm TDI dose group. A significant fetal effect, a statistically significant increase in a specific skeletal malformation, was reported in the highest dose group.

VI. Derivation of the Chronic Reference Exposure Level

Study	Diem *et al.*, 1982
Study population	Human TDI production workers (n = 168)
Exposure method	Occupational inhalation exposure
Critical effects	Decreased lung function
LOAEL	0.014 mg/m^3 (1.9 ppb)
NOAEL	0.006 mg/m^3 (0.9 ppb) (non-smokers)
Exposure continuity	8 h/day (10 m^3/day occupational exposure), 5 d/wk
Exposure duration	5 years
Average occupational exposure	0.002 mg/m^3 for NOAEL group (0.006 x 10/20 x 5/7)
Human equivalent concentration	0.002 mg/m^3 for NOAEL group
LOAEL uncertainty factor	1
Subchronic uncertainty factor	3
Interspecies uncertainty factor	1
Intraspecies uncertainty factor	10
Cumulative uncertainty factor	30
Inhalation reference exposure level	0.00007 mg/m^3 (0.07 µg/m^3; 0.01 ppb)

The chronic REL is equivalent to the U.S. EPA RfC. OEHHA agreed with the U.S. EPA analysis and the selection of Diem *et al*. (1982) as the most appropriate study to use for the REL. The rationale for selection of this study is as follows. This study presented evidence of a decline in lung function, as indicated by decrements in FEV_1, among workers involved in TDI production. Other factors supporting its quality include:

(1) the absence of other confounding compounds in the work environment,
(2) the establishment of baseline lung function prior to exposure to TDI,
(3) a "parallel internal comparison" of study groups for lung function,
(4) an appropriate statistical analysis which took into account interindividual variability,
(5) breathing zone measurement of TDI (although commenced 2 years into the study), and
(6) a smoking effect on lung function.

VII. Data Strengths and Limitations for Development of the REL

The major strengths of the chronic REL for TDI are the use of human exposure data from workers exposed over a period of years and the observation of a NOAEL. The major weaknesses are the uncertainty in estimating exposure, the potential variability in exposure concentration, and the limited nature of the study that focused on lung effects.

VIII. References

Bugler J, Clark RL, Hill ID, and McDermott M. 1991. The acute and long-term respiratory effects of aromatic di-isocyanates. A five year longitudinal study of polyurethane foam workers. Study sponsored by the British Rubber Manufacturers Association, the International Isocyanate Institute, and the Health and Safety Executive, UK.

CARB. 2000. California Air Resources Board. California Emissions Inventory Development and Reporting System (CEIDARS). Data from Data Base Year 1998. February 12, 2000.

CRC. 1994. CRC Handbook of Chemistry and Physics, 75th edition. Lide DR, ed. Boca Raton, FL: CRC Press Inc.

Diem JE, Jones RN, Hendrick DJ, Glindmeyer HW, Dharmarajan V, Butcher BT, Salvaggio JE, and Weill H. 1982. Five-year longitudinal study of workers employed in a new toluene diisocyanate manufacturing plant. Am. Rev. Respir. Dis. 126:420-428.

Diem JE, and Liukkonen JR. 1988. A comparative study of three methods for analyzing longitudinal pulmonary function data. Stat. Med. 7:19-28.

Duncan B, Scheel LD, Fairchild EJ, Killens R, and Graham S. 1962. Toluene diisocyanate inhalation toxicity: pathology and mortality. Am. Ind. Hyg. Assoc. J. 23:447-456.

Hasselblad V. 1993. Analysis of studies of workers exposed to toluene diisocyanate. Report prepared for U.S. EPA, Environmental Criteria and Assessment Office, Research Triangle Park, NC.

Howard PH. (ed) 1989. Handbook of environmental fate and exposure data for organic chemicals. Vol. I. Large production and priority pollutants. Chelsea, MI: Lewis Publishers, Inc.

HSDB. 1995. Hazardous Substances Data Bank. National Library of Medicine, Bethesda, MD (TOMES® CD-ROM Version). Denver, CO: Micromedex, Inc. (Edition expires 7/31/96).

Hughes J. April and November, 1993. Memoranda from Janet Hughes, Tulane Medical Center, to Mark Greenburg, U.S. EPA.

Jones RN, Rando RJ, Glindmeyer, Foster TA, Hughes JM, O'Neil CE, and Weill H. 1992. Abnormal lung function in polyurethane foam producers: weak relationship to toluene diisocyanate exposures. Am. Rev. Respir. Dis. 148:671-677.

Karol MH. 1983. Concentration-dependent immunologic response to toluene diisocyanate (TDI) following inhalation exposure. Toxicol. Appl. Pharmacol. 68:229-241.

Loeser E. 1983. Long-term toxicity and carcinogenicity studies with 2,4-/2,6-toluene diisocyanate (80/20) in rats and mice. Toxicol. Lett. 15:71-81.

Moscato G, Dellabianca A, Vinci G, Candura SM, and Bossi MC. 1991. Toluene diisocyanate-induced asthma: clinical findings and bronchial responsiveness in 113 exposed subjects with work-related respiratory symptoms. J. Occup. Med. 33:720-725.

Owen PE. 1984. The toxicity and carcinogenicity to rat of toluene diisocyanate vapour administered by inhalation for a period of 113 weeks. Addendum report. Volume 2. Hazelton Laboratories Europe.

Sangha GK, and Alarie Y. 1979. Sensory irritation by toluene diisocyanate in single and repeated exposures. Toxicol. Appl. Pharmacol. 50:533-547.

Tyl RW. 1988. Developmental toxicity study of inhaled toluene diisocyanate vapor in CD (Sprague-Dawley) rats. Union Carbide, Bushy Run Research Center. Revised project report 50-592.

Tyl RW, and Neeper-Bradley TL. 1989. Two-generation reproduction study of inhaled toluene diisocyanate in CD (Sprague-Dawley) rats. Union Carbide, Bushy Run Research Center. Project No. 86-33-90704.

U.S. EPA. 1995. United States Environmental Protection Agency. Documentation of the reference concentration for chronic inhalation exposure (RfC) for 2,4-/2,6-toluene diisocyanate mixture. Integrated Risk Information System (IRIS on-line). Washington, DC: U.S. EPA. Available online at http://www.epa.gov/ngispgm3/iris

Wong KL, Karol MH, and Alarie Y. 1985. Use of repeated CO_2 challenges to evaluate the pulmonary performance of guinea pigs exposed to toluene diisocyanate. J. Toxicol. Environ. Health 15:137-148.

CHRONIC TOXICITY SUMMARY

TRICHLOROETHYLENE

(trichloroethylene; 1,1-2-trichloroethylene, 1,1-dichloro-2-chloroethylene, acetylene trichloride, and ethylene trichloride))

CAS Registry Number: 79-01-6

I. Chronic Toxicity Summary

Inhalation reference exposure level	**600 µg/m^3 (100 ppb)**
Critical effect(s)	Neurotoxicological effects (drowsiness, fatigue, headache) and eye irritation in workers.
Hazard index target(s)	Nervous system; eyes

II. Physical and Chemical Properties (Fan, 1988; CRC, 1994)

Description	Colorless liquid/vapor; sweetish, chloroform-like odor
Molecular formula	C_2HCl_3
Molecular weight	131.4
Density	1.47 g/cm^3 @ 20°C
Boiling point	87.2 °C
Melting point	–84.7°C
Vapor pressure	77 torr @ 25°C
Vapor density	4.5 (air = 1)
Solubility	Soluble in alcohol, ethers, petroleum distillates and other halogenated solvents
Conversion factor	1 ppm = 5.37 mg/m^3 @ 25° C

III. Major Uses or Sources

Trichloroethylene was once used as an extractant in food processing and has been used as an anesthetic and analgesic for medical purposes (Waters *et al.* 1977). Currently, it is widely used as a solvent in the industrial degreasing of metals, with secondary solvent uses in adhesive paint and polyvinyl chloride production (U.S. EPA, 1985). Trichloroethylene is used as a solvent in the textile industry, as a solvent for adhesives and lubricants, and as a low-temperature heat transfer fluid (IARC, 1979). Trichloroethylene is also implemented in the manufacturing of pesticides and other chemicals (Feldman, 1979). In 1996, the latest year tabulated, the statewide mean outdoor monitored concentration of trichloroethylene was approximately 0.035 ppb (CARB, 1999a). The annual statewide emissions from facilities reporting under the Air Toxics Hot Spots Act in California based on the most recent inventory were estimated to be 176,908 pounds (CARB, 1999b)

IV. Effects of Human Exposure

An occupational study of trichloroethylene (TCE) vapor emissions in a pump room was conducted by Vandervort and Polnkoff (1973). Workers were an average age of 40 and had been employed for an average of 8 years. For 11-day shift workers, individual 8 hour time weighted average (TWA) TCE exposure concentrations were extrapolated from two area samples; these averages ranged from 170-420 mg/m^3 (32-78 ppm). Nineteen workers (including the 11 workers whose work areas were sampled) completed a questionnaire and reported the following symptoms: 73% eye irritation, 70% drowsiness, 58% heart palpitations, 58% cough, 53% weakness and 52% dizziness. About half of the 19 exposed workers reported that consumption of small amounts of alcohol outside of work resulted in changes of skin color and severe intoxication. One worker of the 19 reported no adverse effects from the occupational exposure. Nine control workers experienced none of the above symptoms. Urine samples from the 19 exposed and 9 unexposed workers were collected before and after the work shift and examined for the TCE metabolites trichloroacetic acid (TCA) and trichloroethanol (TRI). TRI levels ranged from 4-260 mg/l and TCA levels ranged from 4-197 mg/l. Results of the urine assays showed a range of TCE metabolite concentrations and, therefore, confirmed that the workers were exposed to a variety of concentrations in their environments.

Nomiyama *et al.* (1977) examined 36 trichloroethylene workers, of which 9 males and 12 females were occupationally exposed to a constant concentration of trichloroethylene (TCE) and 18 males were exposed to variable concentrations (duration of exposure unspecified). The control group consisted of 6 males and 10 females who were of similar educational, sociologic and economic status to the trichloroethylene workers. Researchers used urinary excretion of TCE metabolites as an indicator of the level of TCE exposure in the working environment; total excreted trichloro-compounds of 100 mg in 4 hours corresponded to 100 ppm TCE present in the working environment (Bardodej, 1958; Medek, 1958). Of the 36 exposed workers, 5 were exposed to 0-25 ppm; 14 were exposed to 25-50 ppm; 6 were exposed to 50-100 ppm; 8 were exposed to 100-150 ppm; and 3 were exposed to 150-200 ppm TCE. In the low exposure group, workers experienced mucous membrane irritation in the eyes, nose and throat, in addition to drowsiness, fatigue and headache. These symptoms were persistent through the higher concentration exposures with an increase in eye irritation, headache, fatigue, and nasal obstruction above 100 ppm TCE. Increases in rhinorrhea and drowsiness were seen above 50 ppm TCE exposure.

Kimmerle and Eben (1973) exposed 4 human subjects (3 males and 1 female) to a subacute regimen of 48 $\pm$ 3 ppm trichloroethylene (TCE) for 4 hours a day over a period of 5 days. Levels of TCE and the metabolites trichlororethanol (TRI) and trichloroacetic acid (TCA) were determined. Trichloroethanol-blood levels were elevated immediately after exposure, and detection of trichloroethanol occurred up to 7 days after the last exposure. TCE-blood concentration increased slightly over the 5 days. Levels of urinary excreted trichloroethanol, as well as the TCA-concentration, increased throughout the study, with the female showing a significantly higher excretion of TCA. Levels of TCA were detected up to 12 days after the final exposure.

Okawa and Bodner (1973) studied the occupational exposure of 24 electrical plant workers to trichloroethylene (TCE). The plant worker group consisted of 22 males and 2 females ranging in age from 21-52 years old. Environmental samples of TCE were collected over three days and yielded varying concentrations of TCE related to the task performed in certain areas (duration of exposure unspecified). Spray booth operators were exposed to an average of 25.3 ppm TCE (13-40 ppm range) in addition to averages of 15.2 ppm n-propyl acetate (NPA) and 6 ppm toluene (TOL). Workers involved in washing board units were exposed to an average value of 39 ppm (6-82 ppm range) TCE. Although the workers wore respiratory protection during the washing procedure, the overall average of airborne TCE in this area was 48.3 ppm. In the testing area of the plant, researchers report that the amounts of toluene and n-propyl acetate were insignificant. Here, TCE levels were an average of 24.4 ppm (range = 8-44 ppm). The solder machine operators were exposed to an average of 44.0 ppm TCE (range = 23-87 ppm) with no NPA or TOL present. During the cleaning of the soldering machines, TCE levels rose to an average of 70.5 ppm (range = 30-106 ppm). Concentrations were only at these elevated levels for 20-30 minutes a day. Researchers note that although other agents were used in the work area, TCE was the only chemical found in significant amounts throughout the work area and that the levels of NPA and TOL were insignificant. An analysis of urinary TCE metabolites indicated that the workers were exposed to a time weighted average concentration of <50 ppm TCE. Three of the 24 workers reported that they were unaffected by their working conditions, but the most prominent complaints consisted of 70.8% workers experiencing nausea, 54.2% headache, 33.3% dizziness, 25.0% fatigue, 25% nose and throat irritation, and 20.8% eye irritation. Workers reported that these symptoms were alleviated hours after leaving the work environment. Researchers collected 8 hour urine samples from 20 of the workers and from 9 controls and analyzed them for TCE metabolites. Results of urinary analysis showed that the controls had exposure to an unspecified amount of TCE. TCA levels in exposed workers were elevated from that of the controls and correlated to the different exposures in specific work areas.

Phoon *et al.* (1984) reported on 5 cases of Stevens-Johnson syndrome (erythema multiforme major) with liver involvement which followed exposure to TCE. In two cases, reactions to the exposure began with a fever followed by an itchy rash on the face spreading over the body. Lesions were observed on the face, arms and in the mouth. Liver function tests were abnormal. One of the two developed jaundice with hepatomegaly. Case #3 developed a similar reaction after 5 weeks of exposure to 216-912 mg/m^3 TCE (40-170 ppm) as did case #5 after two weeks of exposure to 370 mg/m^3 TCE (69 ppm). Case #4 involved a 39 year old man exposed to <50 mg/m^3 TCE (< 9.3 ppm) for three weeks who developed the characteristic rash, lesions and jaundice with slight hepatomegaly. Upon returning to work over the next three weeks, he developed generalized erythrodermia and facial oedema, hepatosplenomegaly and liver failure with septicemia from which he died 14 days later.

Stewart *et al.* (1974) studied the effects of subacute trichloroethylene (TCE) exposure in combination with alcohol consumption. Seven men exposed to 200 ppm TCE ingested 1 quart of beer or 90 ml of 100-proof vodka and developed red blotches on their faces 30-40 minutes later. These lesions enlarged with time until they reached a peak intensity, whereupon they faded. One subject experienced facial flush with the consumption of alcohol for three weeks after the last TCE exposure, while another showed flushing six weeks after the last exposure.

V. Effects of Animal Exposure

Kjellstrand *et al.* (1983) studied the effects of both intermittent and continuous exposures of various concentrations of trichloroethylene on male and female mice over a period of 30 days. The concentrations used range from 37-3600 ppm, and 7 of the 14 groups were continuously or intermittently exposed to lower concentrations of 37, 75, 150, 225 and 300 ppm TCE. Continuous exposure studies were conducted over a period of 30 days for exposure groups of 37, 75, 150 and 300 ppm TCE. All groups consisted of 10 males and 10 females (except the 37 ppm group, consisting of 20 males and 20 females) and were compared to identical groups of air-exposed controls. Liver weights increased in a non-linear fashion as the concentration level of TCE increased. All groups exhibited statistically significant increases in liver weights as compared to the controls. In both the 37 and the 75 ppm groups, the increase in females was less than in males. No increase in spleen weight was detected at either the 37 or 75 ppm exposure level. At the 37 ppm level, a slight increase in plasma butyrylcholinesterase (BuChE) activity (not statistically significant) was also detected. A significant increase in kidney weight was seen in the male 75 ppm group and was more pronounced with increasing concentration. Male mice in the 75 ppm group also showed statistically significant increases in BuChE activity. In the 150 ppm group, male and female liver weight increases were statistically significant and of equal magnitude. A statistically significant increase was seen in the BuChE activity of the 150 ppm male mice. It was not until female mice were exposed to 300 ppm, that they showed slight increase in BuChE activity, while the males increased 3.5 times the controls. Liver weight increases for the 300 ppm group were close to the maximum with females showing greater increase than the males. Ten male and 10 female mice were continuously exposed to 150 ppm TCE for 30 days, but then allowed a 120 day rehabilitation period. Following rehabilitation, liver weights returned to levels comparable to the controls. The elevated BuChE activity returned to a normal level. No significant effects were seen after the period of rehabilitation. A continuous study was performed on 10 male and 10 female mice for 120 days at an exposure level of 150 ppm TCE. No further increase in liver weight occurred beyond the level reached in the 30 day study. Body weight gain was slightly decreased, and the same level of BuChE activity was seen as in the 30 day exposure. The intermittent study consisted of 30 days exposure to 225 ppm TCE for 16 hours a day, 7 days a week. A significant increase was seen in the BuChE activity of male mice, while females did not exhibit an increase in BuChE activity. Both males and females showed statistically significant increases in liver weight. Kidney weight increased in the same manner as in the continuous exposures. The authors noted that "extrapolation of the concentration-effect curve suggests that both liver weight and BuChE activities are influenced at still lower concentration."

Briving *et al.* (1986) examined neurotoxicity as a result of chronic trichloroethylene (TCE) inhalation exposure. Two groups of gerbils (6 in each group) were exposed to 50 or 150 ppm TCE for a period of 12 months. Two equivalent groups were used as controls. Two areas of the brain were specifically observed, the hippocampus and the posterior part of the cerebellar vermis. These discrete brain areas were previously shown to be sensitive towards chlorinated aliphatic solvents (Haglid *et al.*, 1981). Following exposure, gerbils were decapitated and measurements were made of total free tissue amino acids as well as high-affinity uptake and release of ^{3}H-aminobutyric acid (GABA) and ^{14}C-glutamate. A significant increase in

glutathione was seen in the hippocampus of the 150 ppm gerbils, but amino acid levels were not significantly affected. In the posterior part of the cerebellar vermis, glutamate and GABA accumulation levels increased in a dose-dependent manner, with significant increases seen at both 50 and 150 ppm TCE. Evaluation of the hippocampus revealed no significant changes. The authors suggest that the stimulation of transport functions for GABA and glutamate may be triggered by the presence of the TCE metabolite, trichloroethanol. Therefore, the levels of GABA and glutamate are indicative of the amount of trichloroethanol from TCE in the brain.

Kligerman *et al.* (1994) exposed 20 male CD rats to 0, 5, 50, or 500 ppm trichloroethylene (TCE) for 6 hours a day, over a period of 4 days. Groups at each concentration consisted of 5 rats. One of the cytogenetic effects measured was peripheral blood lymphocytes (PBLs), abnormal with regard to sister chromatid exchanges. Also analyzed, were the cell cycle, bone marrow micronuclei in polychromatic erythrocytes (MN-PCEs/1000) and micronuclei in cytochalasin B-blocked binucleated cells (MN-BN/1000). The 5 ppm and 500 ppm exposure groups showed a decrease (not statistically significant) in cell cycle. In addition, the 50 ppm group exhibited a statistically significant decrease in cell cycle. For all concentrations, there was an overall increase in the PCE percentage. The number of PCEs with micronuclei also rose with the increasing concentrations of 50 ppm and 500 ppm TCE (not statistically significant due to high control values). The researchers conclude that the resulting increase of MN in exposed rats is indicative of aneuploidy induction as opposed to chromosomal breakage, and that the lack of chromosome aberrations corresponds to spindle effects such as aneuploid induction. Concurrent results of increased levels of leukocyte aneuploidy were also found by Konietzko *et al.* (1978) in degreasing workers occupationally exposed to TCE.

Haglid *et al.* (1981) continuously exposed gerbils to 60 ppm or 320 ppm trichloroethylene (TCE) for 3 months. Following the exposure period, gerbils were maintained for 4 months in TCE-free conditions in order to observe any restoration of neuronal function. Both of the exposed groups as well as the control group consisted of six pairs of males and females. Brain samples were collected from the gerbils after the 4 month non-exposure period and used for determination of DNA and proteins. In order to determine areas of the brain that were sensitive to TCE, researchers examined biochemical and morphological changes in the hippocampus, the posterior part of the cerebellar vermis, and the brain stem. In addition to the biochemical tests, the cerebellum, brain stem, and cerebrum of two gerbils from each group, including the control, were used for neuropathological examination. Brain tissue from 2 gerbils in the control group and the 320 ppm group were examined under the electron microscope. No difference was seen in the body and brain weights of the exposed gerbils compared with controls. A slight but significant increase in soluble proteins was detected in the frontal cerebral cortex of the 60 ppm group, and a more significant elevation was seen in the visual cerebral cortex of both the 60 ppm and 320 ppm groups. In the 60 ppm group, a slight but significant decrease was seen in the soluble proteins of the sensory-motor cortex. Both groups exhibited significant decreases in levels of soluble proteins in the hippocampus, the brain stem, and in the posterior part of the cerebellar vermis. Soluble protein levels in the cerebellar hemisphere and anterior part of the vermis of gerbils in both exposed groups did not differ from the controls. The 320 ppm group showed significantly increased DNA levels in the posterior part of the sensory motor cortex and cerebellar vermis. The glial cytoplasmic protein (S 100 fraction) level of the 60 ppm group was decreased in the frontal and visual cerebral cortex, but increased in the posterior part of the

cerebellar hemisphere and the sensory-motor cortex. However, only a slight decrease of S 100 protein was observed in the visual cerebral cortex of 320 ppm exposed gerbils. The most notable S 100 increase occurred in the hippocampus, brain stem and the posterior part of the cerebellar vermis, indicating that either the glial cells were directly affected or that damage to surrounding neuronal cells caused an indirect response. There was an increase in DNA in the posterior part of the cerebellar vermis in the exposed gerbils, suggesting that TCE induced astroglial cell mitosis. Light microscopy revealed shrinkage of cell bodies and axon swelling occurred in various parts of the brain. The electron microscopy performed on control and 320 ppm brain tissues revealed increased levels of filament bundles in the cytoplasm of some Purkinje and Golgi cell perikarya, lysosomes, myelin bodies and lipid containing lysosomal structures in the exposed gerbils. Unique arrangements of filament bundles were seen in Purkinje and Golgi cell dendrites of the exposed group. A significant decrease in the number of microtubules was observed as well as a decrease in the number of synaptic vesicles in the granular layer. Also, the granular layer had decreased maximal nerve cell surface area. Nerve cells were affected by the exposure as several types were reduced in size with fewer organelles and more lysosomes and myelin bodies. Many axons and dendrites had reduced numbers of microtubles, and there were filament bundles observed that were not present in the controls. Lysosomal structures were increased in the synaptic terminals.

Kimmerle and Eben (1973) performed a subchronic study on 20 male rats for a period of 14 weeks. Rats were exposed to a mean concentration of 55.0 $\pm$4 ppm trichloroethylene (TCE) for 8 hours a day, 5 days a week. The control group consisted of 20 rats who in similar inhalation chambers under similar conditions to that of the exposed rats. Ten exposed rats were analyzed for TCE metabolite excretion on a daily basis. Blood levels of trichloroacetic acid (TCA), trichloroethanol (TRI) and chloral hydrate (CH) were measured during the 2nd, 3rd, 4th, 6th, 9th and 14th weeks. Weekly measurements of body weights were recorded. Macroscopic examinations were performed on the thyroid gland, heart, lungs, liver, kidneys, testes and adrenal glands. Hematological evaluations, liver function tests, and renal function tests were also conducted following exposure. Urinary levels of TRI varied individually among the rats, but a continuous increase in TRI was observed through the 10th week. TCA levels remained fairly constant throughout the duration of the experiment. TCE was not detectable in the blood or the tissues of exposed rats. Although liver and renal function tests did not reveal abnormalities, there was an increase in the liver weights of the exposed rats. The weights of the other organs examined were similar to the controls.

Norpoth *et al.* (1974) observed an increase in liver cytochrome P_{450} activity in 9 rats exposed to 50 ppm trichloroethylene for 28 days, compared with 9 control rats.

VI. Derivation of Chronic Reference Exposure Level

Study	Vandervort and Polnkoff (1973)
Study population	19 workersand 9 controls
Exposure method	Discontinuous occupational inhalation exposure
Critical effects	Drowsiness, fatigue, headache, and eye irritation
LOAEL	32 ppm (170 mg/m^3) in the heavy assembly area
NOAEL	Not observed
Exposure continuity	8 hours a day (10 m^3/day occupational inhalaton rate), 5 days a week
Exposure duration	8 years
Average occupational exposure	11.4 ppm for LOAEL group (32 x 10/20 x 5/7)
Human equivalent concentration	11.4 ppm for LOAEL group
LOAEL uncertainty factor	10
Subchronic uncertainty factor	1
Interspecies uncertainty factor	1
Intraspecies uncertainty factor	10
Cumulative uncertainty factor	100
Inhalation reference exposure level	0.1 ppm (100 ppb; 0.6 mg/m^3; 600 µg/m^3)

The Vandervort and Polnkoff (1973) study accounted for 8 years of human occupational exposure to TCE vapors. Sensitive, non-specific neurotoxicological endpoints were exhibited by a majority of those workers exposed. Although the time-weighted averages (TWAs) included a wide range of concentrations, the TWA of 32 ppm (170 mg/m^3) was shown to contribute to the high incidence (52 - 73%) of adverse effects experienced by the workers. Many of the symptoms reported by the workers may have been due to short-term fluctuations in the concentrations in the workplace. The symptoms were not reported separately for the various TWAs, therefore, the lowest TWA (32 ppm) was chosen as a LOAEL. Uncertainty includes the small number of workers studied, the limited extent of the effects mentioned, and the lack of a NOAEL. Strengths include the use of human data, the demonstration of a dose-response relationship, and exposure estimates correlated with urinary excretion measurements.

This study was the best chronic account of the non-carcinogenic effects of TCE on humans, but several other studies show similar results. Nomiyama *et al.* (1977) found similar endpoints of drowsiness, fatigue and eye irritation in 36 workers occupationally exposed to trichloroethylene. Okawa *et al.* (1973) also saw non-specific neurological endpoints in 24 electrical plant workers who were similarly exposed to TCE.

For comparison with the proposed REL of 100 ppb based on human studies, the LOAEL of 50 ppm trichloroethylene obtained by Briving *et al*. (1986) in gerbils exposed continuously for 12 months was used to estimate a REL based on animal data. Use of a LOAEL UF of 3, a subchronic UF of 1, an interspecies UF of 10, and an intraspecies UF of 10 resulted in an estimated REL of 200 ppb for trichloroethylene.

VII. Data Strengths and Limitations for Development of the REL

The strengths of the inhalation REL for trichloroethylene include the use of human exposure data from workers exposed over a period of years. Major areas of uncertainty are the lack of reproductive and developmental toxicity studies and the lack of observation of a NOAEL.

VIII. References

Bardodej Z, and Krivucova M. 1958. Determination of trichloroethyl alcohol (urochloralic acid) as an exposition test on workers with trichloroethylene (in Czech.). Cs. Hyg. 3:268-272. [as cited by Nomiyama and Nomiyama, 1977]

Briving C, Jacobson I, Hamberger A, Kjellstrand P, Haglid K, and Rosengren L. 1986. Chronic effects of perchloroethylene and trichloroethylene on the gerbil brain amino acids and glutathione. Neurotoxicology 7(1):101-108.

CARB. 1999a. California Air Resources Board. Toxics Air Quality Data. Substance Chooser. Trichloroethylene. Available online at http:/www.arb.ca.gov/aqd/toxics.htm

CARB. 1999b. Air toxics emissions data collected in the Air Toxics Hot Spots Program CEIDARS Database as of January 29, 1999.

CRC. 1994. CRC Handbook of Chemistry and Physics, 75th edition. Lide DR, ed. Boca Raton, FL: CRC Press Inc.

Fan A. 1988. Trichloroethylene: Water contamination and health risk assessment. Rev. Environ. Contam. Toxicol. 101:55-92.

Feldman R. 1979. Intoxications of the nervous system. Handbook of Clinical Neurology 36:457-464. [as cited by Juntunen 1986]

Haglid K, Briving C, Hansson H, Rosengren L, Kjellstrand, Stavron D, Swedin U, and Wronski A. 1981. Trichloroethylene: Long-lasting changes in the brain after rehabilitation. Neurotoxicology 2:659-673.

IARC. 1979. Trichloroethylene. IARC Monographs on the Evaluation of the Carcinogenic Risk of Chemicals to Humans. Vol. 20. Lyon:IARC, pp. 545-572.

Juntunen J. 1986. Occupational toxicology of trichloroethylene with special reference to neurotoxicity. New Concepts and Developments in Toxicology. PL Chambers, P Gehring, and F Sakai, eds. Amsterdam: Elsevier. pp. 189-200.

Kimmerle G, and Eben A. 1973. Metabolism, excretion and toxicology of trichloroethylene after inhalation: 1. Experimental exposure on rats. Arch. Toxicol 30:115-126.

Kimmerle G, and Eben A. 1973. Metabolism, excretion and toxicology of trichloroethylene after inhalation: 2. Experimental human exposures. Arch. Toxicol. 30:127-138.

Kjellstrand P, Holmquist B, Alm P, Kanje M, Romare S, Jonsson I, Mansson L, and Bjerkemo M. 1983. Trichloroethylene: Further studies of the effects on body and organ weights and plasma butyrylcholinesterase activity in mice. Acta. Pharmacol. Toxicol. 53:375-384.

Kligerman A, Bryant M, Doerr C, Erexson G, Evansky P, Kwanyuen P, and McGee J. 1994. Inhalation studies of the genotoxicity of trichloroethylene to rodents. Mutat. Res. 322:87-96.

Konietzko H, Haberlandt W, Heilbronner H, Reill G, and Weichardt H. 1978. Cytogenetische Untersuchungen an Trichlorathylen-Arbeitern. Arch. Toxicol. 40:201-206.

Medek V. 1958. The relation between trichloroethanol and trichloroacetic acid in the urine and trichloroethylene in the atmosphere. Pracov. Lek. 10:135-138. [as cited by Nomiyama and Nomiyama, 1977]

Nomiyama K, and Nomiyama H. 1977. Dose-response relationship for trichloroethylene in man. Int. Arch. Occup. Environ. Health 39:237-248.

Norpoth K, Witting U, and Springorum M. 1974. Induction of microsomal enzymes in the rat liver by inhalation of hydrocarbon solvents. Int. Arch. Arbeitsmed 33:315-321.

Okawa M, and Bodner A.1973. NIOSH: Health hazard evaluation/toxicity determination. Western Electric Company, Inc. report 72-74.

Phoon W, Chan M, Rajan V, Tan K, Thirumoorthy T, and Goh C. Stevens-Johnson syndrome associated with occupational exposure to trichloroethylene. Contact Dermatitis 10:270-276.

Stewart R, Hake C, and Peterson J. 1974. “Degreasers’ Flush;” Dermal response to trichloroethylene and ethanol. Arch. Environ. Health 29:1-5.

U.S. EPA. 1985. U.S. Environmental Protection Agency. Assessment of trichloroethylene as a potentially toxic air pollutant; proposed rule. Federal Register; Part IV 50(246):52422-52425.

Vandervort R, and Polnkoff P. 1973. NIOSH: Health hazard evaluation/toxicity determination. Dunham-Bush, Inc. report 72-34.

Waters E, Gerstner H, and Huff H. 1977. Trichloroethylene. I. An overview. J. Toxicol. Environ. Health 2:271-307. [as cited by Fan, 1988]

CHRONIC TOXICITY SUMMARY

TRIETHYLAMINE

(diethylaminoethane; ethanamine; N,N-diethylethanamine)

CAS Registry Number: 121-44-8

I. Chronic Toxicity Summary

Inhalation reference exposure level	**200 μg/m^3** (40 ppb)
Critical effect(s)	Eye effects in rats and humans
Hazard index target(s)	Eyes

II. Physical and Chemical Properties (Nelson and Bull, 1990, except as noted)

Description	Colorless, volatile liquid
Molecular formula	$C_6H_{15}N$
Molecular weight	101.9 g/mol
Density	0.726 g/cm^3 @ 25°C
Boiling point	89.3°C
Melting point	–114.7°C (CRC, 1994)
Vapor pressure	400 torr @ 31.5°C
Odor threshold	480 ppb (Amoore and Hautala, 1983)
Solubility	soluble in acetone, benzene and chloroform
Conversion factor	1 ppm = 4.14 mg/m^3 25°C

III. Major Uses or Sources

Triethylamine (TEA) is primarily used as a cross-linking catalyst in the production of polyurethane foam used in the manufacture of cores for metal castings (Albrecht and Stephenson, 1988). Triethylamine is also used as a catalyst for epoxy resins, and as a corrosion inhibitor for polymers (Nelson and Bull, 1990). TEA is one of the amines emitted from cattle feedlots (Mosier *et al*., 1973). In the gas phase TEA can react with nitric acid to form amine nitrates that become part of atmospheric particulates. The annual statewide industrial emissions from facilities reporting under the Air Toxics Hot Spots Act in California based on the most recent inventory were estimated to be 4152 pounds of triethylamine (CARB, 2000).

IV. Effects of Human Exposures

Acute, high level triethylamine exposures (20 mg/m^3 (4.8 ppm) for 8 hours) resulted in reversible ocular effects that included corneal swelling and halo vision in 4 out of 5 volunteer subjects (Akesson *et al.*, 1988). Jarvinen *et al*. (1999) reported exposure chamber studies of ocular

responses to TEA in volunteer subjects, who were industrial workers exposed to TEA during the course of their normal jobs (but had good general and ocular health). Four people were exposed for 4 hours to 3.0, 6.5, or 40.6 mg/m^3 triethylamine. Corneal thickness was measured by ultrasonography, clinical observations were recorded using ocular microscopy, and statistical analysis of the size, density and distribution of corneal endothelial cells was performed by automated analysis of photographs. Visual acuity and contrast sensitivity were evaluated using test charts. After exposure to 40.6 mg/m^3 there was a marked edema in the corneal epithelium, and subepithelial microcysts. However, corneal thickness increased only minimally. Vision was blurred in all subjects and visual acuity and contrast sensitivity decreased in three of the four. After exposure to 6.5 mg/m^3 two subjects experienced symptoms (e.g., blurred vision), and contrast sensitivity decreased in three of the four. There were no symptoms or decreases in contrast sensitivity after exposure to 3.0 mg/m^3 triethylamine for 4 hours.

A medical examination of 19 workers exposed at a polyurethane foam production plant to a time-weighted average concentration of 13 mg/m^3 (3.1 ppm) TEA showed reversible corneal edema in 5 workers (Akesson *et al.*, 1986). Peak concentrations were up to twice the time-weighted average level. A questionnaire on self-reported symptoms of visual disturbances revealed repeated occurrences of temporary eye irritation and "foggy vision." Small quantities of dimethylethanolamine, toluene diisocyanate, and methylene diphenyl isocyanate were also present in the workplace atmosphere.

Jarvinen and Hyvärinen (1997) reported loss of visual acuity and contrast sensitivity in 41 foundry workers (core makers) exposed to TEA. Concentrations of TEA were reported to have a mean of 46 mg/m^3 and a maximum of 486 mg/m^3, but were highly variable with numerous large excursions above a background of about 20 mg/m^3 during a two-hour period of continuous monitoring. It is therefore difficult to determine an effect level for the observed symptoms. Jarvinen (1998) also reported that cold box core makers exposed to TEA had a somewhat increased incidence of mild headaches.

V. Effects of Animal Exposures

Lynch *et al.* (1990) exposed male and female Fischer 344 rats to triethylamine at concentrations of 0, 25, or 247 ppm (0, 103.4, or 1022.2 mg/m^3) for 6 hours/day, 5 days/week. Groups of rats were necropsied at approximately 30, 60, and 120 days of exposure. The last corresponds to an elapsed time of 28 weeks. Endpoints examined included gross and histopathological examination of all major organs, including the lungs, nasal passages, and eyes. Clinical enzyme and nitrogen levels (BUN, ALT, AST, CPK, and creatinine), and hematological values (hemoglobin, RBC count) were also measured. No gross or histological effects in any organ were observed in any group. Clinical and hematological parameters were unchanged with exposure. However, all rats exposed to 247 ppm TEA manifested irritation. "At 247 ppm TEA the rats kept their eyes closed and noses buried in their fur during the entire exposure period." Thus 247 ppm is a LOAEL and 25 ppm is a NOAEL for eye and nose irritation in the rat.

In a short-term study by the same authors for Virginia Chemicals (1987), necrotizing inflammation of the nasal cavity, metaplasia of the trachea, and thymic atrophy were observed

after exposure to 1000 ppm (4140 mg/m^3) triethylamine 6 hours per day for 10 days. Two of five males and one of five females died from pulmonary edema after the seventh day. Thymic atrophy was noted in 7 out of 10 animals, and all animals exhibited necrotizing inflammation in the nasal epithelium.

Rabbits (6-12 per group), exposed to 48 or 100 ppm (199 or 414 mg/m^3) triethylamine for 7 hours/day, 5 days/week, for 6 weeks, showed concentration-dependent pathology in the eyes, lungs, liver, kidney, and heart (Brieger and Hodes, 1951). The eyes showed multiple punctate erosions of the corneal epithelium, and corneal edema at 48 ppm. Lung lesions included thickening of vascular walls; liver lesions included parenchymal degeneration. Overall the lesions in the 48 ppm group were less severe than those seen in the 100 ppm group. No control animals were included in this study, nor were the incidences of histologic effects among the exposed animals reported. All animals did survive the exposures. The lesser effects at 48 ppm in the rabbit (compared to those at 100 ppm) are consistent with the findings of Lynch *et al*. (1990) where 25 ppm was a NOAEL in the rat for eye and nose irritation.

A chronic 3-generation reproductive study in rats (10/sex/group) was inconclusive due to excessive mortality in controls (Davison *et al.*, 1965). In this study, rats were exposed to 0, 2, or 200 ppm triethylamine. The third generation of the 200 ppm group was changed to 500 ppm since no effects were noted in the 200 ppm group. Exposure of this group to 500 ppm resulted in decreased body weight and decreased water consumption.

VI. Derivation of Reference Exposure Level

Study	Lynch *et al.*, 1990; Brieger and Hodes, 1951
Study population	Rats; rabbits
Exposure method	Discontinuous whole-body inhalation
Critical effects	Eye irritation; lung and liver toxicity
LOAEL	48 ppm (Brieger and Hodes, 1951)
NOAEL	25 ppm (Lynch *et al.*, 1990)
Exposure continuity	6 or 7 hours/day, 5 days/week
Exposure duration	28 weeks; 6 weeks
Average experimental exposure	4.46 ppm for NOAEL group (25 x 6/24 x 5/7)
Human equivalent concentration	4.46 ppm (18.5 mg/m^3) for NOAEL group
LOAEL uncertainty facto	1
Subchronic uncertainty factor	1 (NOAEL is based on a 28 wk study in rats)
Interspecies uncertainty factor	10
Intraspecies uncertainty factor	10
Cumulative uncertainty factor	100
Reference exposure level	0.04 ppm (40 ppb; 200 μg/m^3)

The U.S. EPA (1995) based its Reference Concentration (RfC) of 7 μg/m^3 (2 ppb) for triethylamine on Lynch *et al*. (1990) but included a Modifying Factor (MF) of 10 for "database deficiencies" - "lack of developmental and reproductive effects, and of appropriate data in a second species." The criteria for use of modifying factors are not well specified by U.S. EPA.

Such modifying factors were not used by OEHHA. In addition OEHHA applied a subchronic UF of 1 since 24 male and 24 female rats in the NOAEL group were exposed to 25 ppm TEA for 28 weeks, while USEPA used a subchronic UF of 10. U.S. EPA considered 247 ppm to be a NOAEL. However, the Lynch *et al*. (1990) study indicates that the animals closed their eyes and buried their noses in their fur, likely to prevent the irritant effects of TEA on their eyes and respiratory tract. Thus adverse effects occurred at 247 ppm, although they could be considered repeated acute effects. Brieger and Hodes (1951) observed adverse effects in the eyes, lungs, and livers of rabbits after six weeks of discontinuous exposure to TEA. Thus 48 ppm is a LOAEL in this study.

For comparison, the five affected workers studied by Akesson *et al*. (1986) showed symptoms at 12-13 mg/m^3 TEA, which is equivalent to 4.5 mg/m^3 continuous exposure. (Other tasks were at 4-5 mg/m^3 TEA or 1.6 mg/m^3 continuous exposure.) Selection of a LOAEL UF of 3 (26% incidence of a reversible effect), a subchronic UF of 1 since the workers had been employed for 9.7 years (range = 4-11), and an intraspecies UF of 10 results in an estimated REL of 200 μg/m^3 based on human data. These workers experienced some short-term peak exposures to TEA and were also exposed to dimethylethanolamine (<0.1 mg/m^3), toluene diisocyanate, and methylene diphenyl isocyanate.

VII. Data Strengths and Limitations for Development of the REL

The major strengths of the triethylamine REL are the observation of a NOAEL in a controlled exposure experiment and finding of the same adverse effect, eye irritation, in humans and animals. The major weaknesses are the minimal amount of adequate human health effects information, the lack of dose-response data in a single experiment, and the lack of long-term exposure data.

VIII. Potential for Differential Impacts on Children's Health

There is no direct evidence in the literature to quantify a differential effect of TEA in infants and children. However, it is a respiratory irritant and thus has the potential to exacerbate asthma. In addition, other alkylamines are known to be associated with occupational asthma (Bernstein *et al*., 1999). There is some concern that TEA could have a similar effect.

IX. References

Akesson B, Bengtsson M, and Floren I. 1986. Visual disturbances by industrial triethylamine exposure. Int. Arch. Occup. Environ. Health 57:297-302.

Akesson B, Vinge E, and Skerfving S. 1988. Pharmacokinetics of triethylamine and triethylamine-N-oxide in man. Toxicol. Appl. Pharmacol. 100:529-538.

Albrecht WN, and Stephenson RL. 1988. Health hazards of tertiary amine catalysts. Scand. J. Work Environ. Health 14:209-219.

Amoore JE, and Hautala E. 1983. Odor as an aid to chemical safety: Odor thresholds compared with threshold limit values and volatilities for 214 chemicals in air and water dilution. J. Appl. Toxicol. 3(6):272-290.

Bernstein IL, Chan-Yeung M, Malo J-L, Bernstein DI (eds.). 1999. Asthma in the Workplace. 2nd ed. New York: Marcel Dekker, pp. 524-525.

Brieger H, and Hodes WA. 1951. Toxic effects of exposure to vapors of aliphatic amines. AMA Arch. Ind. Hyg. Occup. Med. 3(3):287-291.

CARB. 2000. California Air Resources Board. California Emissions Inventory Development and Reporting System (CEIDARS). Data from Data Base Year 1998. February 12, 2000.

CRC. 1994. CRC Handbook of Chemistry and Physics, 75th edition. Lide DR, ed. Boca Raton, FL: CRC Press Inc.

Davison RR, Hood DW, and McMullen B. 1965. Toxicity of triethylamine to albino rats. Prepared for the Office of Saline Water through Texas A&M University (final report reference 65-4F; Office of Saline Water Contract No. 14-01-0001-282). OTS # 303940. Doc. # 86870000536. Fiche # 0513614. [as cited in: U.S.EPA's Integrated Risk Information System (IRIS) database. 1995.]

Jarvinen P. 1998. Headache and blood pressure among triethylamine-exposed foundry workers. Occup. Med. (Lond.). 48(2):113-117.

Jarvinen P, Engstrom K, Riihimaki V, Ruusuvaara P, and Setala K. 1999. Effects of experimental exposure to triethylamine on vision and the eye. Occup. Environ. Med. 56(1):1-5.

Jarvinen P, and Hyvarinen L. 1997. Contrast sensitivity measurement in evaluations of visual symptoms caused by exposure to triethylamine. Occup. Environ. Med. 54(7):483-6.

Lynch DW, Moorman TR, Lewis P, Stober P, Hamlin R, and Scheuler RL. 1990. Subchronic inhalation of triethylamine vapor in Fischer-344 rats: Organ system toxicity. Toxicol. Ind. Health. 6(3/4):403-414.

Mosier AR, Andre CE, Viets FG Jr. 1973. Identification of aliphatic amines volatilized from cattle feedyard. Environ. Sci. Technol. 7:642-644.

Nelson MA, and Bull RJ. 1990. Triethylamine. In: Ethel Browning's Toxicity and Metabolism of Industrial Solvents. Vol. 2. Buhler DR, and Reed DJ (eds). Amsterdam: Elsevier, pp. 129-133.

U.S.EPA. 1995. U.S. Environmental Protection Agency. Integrated Risk Information System (IRIS) database. Reference concentration for chronic inhalation exposure (RfC) for triethylamine.

Virginia Chemicals. 1987. Pathologic findings in Fischer 344 rats exposed by inhalation to allylamine, ethylamine, diethylamine, and triethylamine with cover letter dated 042484. OTS # 308080. Doc # 86-870000813. Fiche # 0515251.

CHRONIC TOXICITY SUMMARY

VINYL ACETATE

(1-acetoxyethylene; acetic acid, vinyl ester; acetic acid, ethenyl ester; VAC; vinyl A monomer; ethenyl ethanoate)

CAS Registry Number: 108-05-4

I. Chronic Toxicity Summary

Inhalation reference exposure level	**200 µg/m^3** (50 ppb)
Critical effect(s)	Nasal epithelial lesions in rats and mice
Hazard index target(s)	Respiratory system

II. Physical and Chemical Properties (HSDB, 1994)

Description	Colorless liquid
Molecular formula	$C_4H_6O_2$
Molecular weight	86.09 g/mol
Density	0.932 g/cm^3 @ 20°C
Boiling point	72.7° C
Melting point	–93.2°C
Vapor pressure	115 torr @ 25°C
Solubility	Slightly soluble in water, soluble in ethane, acetone, chloroform; >10% soluble in ethanol and benzene
Conversion factor	1 ppm = 3.52 mg/m^3 @ 25°C

III. Major Uses and Sources

The major use of vinyl acetate monomer is in the manufacture of polyvinyl and vinyl acetate copolymers, which are used in water-based paints, adhesives, paper coatings, and applications not requiring service at extreme temperatures (HSDB, 1994). It is also used in safety glass interlayers and in hair sprays (HSDB, 1994). In the atmosphere vinyl acetate breakdown can result in formation of acetaldehyde. The annual statewide industrial emissions from facilities reporting under the Air Toxics Hot Spots Act in California based on the most recent inventory were estimated to be 3855 pounds of vinyl acetate (CARB, 2000).

IV. Effects of Human Exposures

Deese and Joyner (1969) conducted an occupational study of 21 chemical workers with a mean length of employment of 15.2 years and exposed to a time-weighted average of 8.6 ppm (30.3 mg/m^3) VA. No adverse effects were noted following chest x-ray, electrocardiogram, blood

chemistry, and urinalysis. The control group (sample size unspecified) consisted of workers in units not exposed to VA. Deese and Joyner (1969) also showed intolerable eye irritation in 3 out of 3 subjects exposed for an unspecified extended period of time to 21.6 ppm (76 mg/m^3) VA. Upper respiratory irritation was also experienced by a majority of 5 subjects. Odor was detected at 0.4 ppm (1.4 mg/m^3) in 3 out of 3 subjects.

V. Effects of Animal Exposures

A 104-week inhalation study in rats and mice (90/sex/group) was conducted using concentrations of 0, 50, 200, or 600 ppm (0, 176, 704, or 2113 mg/m^3) vinyl acetate (VA) (Owen, 1988). The study was later published by Bogdanffy *et al.* (1994). Exposures were for 6 hours/day, 5 days/week. Histology was performed on all major organs. There was no mortality resulting from these exposures. A close examination of the effects of VA on the lung and nasal passages showed significant lesions in the nasal cavity, bronchi, and lungs of rats exposed to 600 ppm VA. Lesions included olfactory epithelial metaplasia/atrophy (see table below) and nest-like epithelial folds in the nasal cavity, exfoliation of bronchial epithelium, fibrous intraluminal projections in the bronchi, and pigmented histiocyte accumulation in the lungs. Body weight gain of rats was significantly decreased in the 600 ppm VA group. Rats treated with 200 ppm VA showed some evidence of epithelial atrophy and metaplasia in the nasal cavity. No effects were observed in the rats exposed to 50 ppm VA.

Number of male rats with olfactory epithelial atrophy (Bogdanffy *et al*. 1994)

VA (ppm)	N in group	Very slight	Slight	Moderate	Severe
0	58	0	0	0	0
50	59	1	2	0	0
200	60	4	47***	2	0
600	60	0	7*	33***	10***

* $p<0.05$; ***$p<0.001$ by Fisher's pair-wise test compared to control group

Mice also exhibited significant histological lesions in the respiratory tract following exposure to 200 ppm VA or greater. The lesions included atrophy of the olfactory epithelium and submucosal gland. At 600 ppm, hyperplasia of the trachea was observed, in addition to exfoliation/flattening of the bronchial epithelium and decreased body weight gain. Relative brain and kidney weights were increased in the 600 ppm group at the end of the study, and absolute liver, heart and kidney weights were also significantly elevated. No adverse effects were observed in the 50 ppm group.

A 13-week study on the effects of VA in mice was conducted by Owen (1980a). Mice (10/sex/concentration) were exposed to 0, 50, 200, or 1000 ppm (0, 176, 704, or 3520 mg/m^3) VA for 6 hours/day, 5 days/week for 13 weeks. A concentration-dependent increase in the incidence of diffuse rhinitis, beginning at the 200 ppm concentration, was detected using histopathological examination. Focal pneumonitis was observed in the 1000 ppm treatment group. No adverse effects were seen in the 50 ppm treatment group. An identical study in rats was also conducted by Owen (1980b). In this study, body weight gain was significantly reduced

in male and female rats exposed to 1000 ppm VA. An increase in the incidence of mild histiocytic alveolitis was observed in the 1000 ppm group.

Irvine (1980) conducted a study on the developmental toxicity of VA in rats. Groups of 24 pregnant female rats were exposed to 0, 52, 198, or 1004 ppm (0, 182, 696, or 3533 mg/m^3) VA for 6 hours/day on days 6-15 of gestation. Significant maternal toxicity, as measured by reduced weight gain from day 10 through day 15, was observed in animals exposed to 1004 ppm. Fetotoxicity, as measured by reduced crown-rump length, reduced body weight, and increased incidence of ossification defects in the sternebrae and occipital regions, was observed in the 1004 ppm group. No maternal or fetal effects were seen at the lower two VA treatments.

In another developmental toxicity study, groups of 23-24 Crl:CD(SD)BR rats were given 0, 200, 1000, or 5000 ppm VA in drinking water or exposed 6 hr/day to 0, 50, 200, or 1000 ppm VA on gestation days 6-15 of gestation. The authors (Hurtt *et al*., 1995) estimated that the doses by both routes were approximately 0, 25, 100, or 500 mg/kg/day. VA in the drinking water produced no evidence of maternal or developmental toxicity at any dose. In the inhalation study, maternal toxicity was indicated by a reduction in weight gain of dams exposed to 1000 ppm. Fetal toxicity was evident by a significant decrease in mean fetal weight and mean crown-rump length in fetuses from the 1000-ppm group and by a significant increase in the incidence of minor skeletal alterations (especially delayed ossification) in fetuses from dams exposed to 1000 ppm VA. These results indicated to the authors that VA is not uniquely toxic to the conceptus. The NOAEL was greater than 5000 ppm via the drinking water and 200 ppm by the inhalation route.

VI. Derivation of Chronic Reference Exposure Level

Study	Bogdanffy *et al*., 1994
Study population	Male and female Sprague-Dawley rats and CD-1 mice (90/sex/group)
Exposure method	Discontinuous inhalation exposures (0, 50, 200, or 600 ppm) over 104 weeks
Critical effects	Histological lesions of the nasal epithelium
LOAEL	200 ppm
NOAEL	50 ppm
Exposure continuity	6 hours/day, 5 days/week
Exposure duration	104 weeks
Average experimental exposure	8.9 ppm for NOAEL group (50 x 6/24 x 5/7)
Human Equivalent Concentration (HEC)	1.4 ppm for NOAEL group (RGDR = 0.15 based on a gas with respiratory effects in both rats and mice)
LOAEL uncertainty factor	1
Subchronic uncertainty factor	1
Interspecies uncertainty factor	3
Intraspecies uncertainty factor	10
Cumulative uncertainty factor	30
Inhalation reference exposure level	0.05 ppm (50 ppb, 0.2 mg/m^3, 200 μg/m^3)

The chronic REL is the U.S. EPA RfC (U.S. EPA, 1995) for vinyl acetate. Acetaldehyde, a hydrolysis product of vinyl acetate, was present in the Owen (1988) study at a concentration of 49 ppm (89 mg/m^3). The duration-adjusted concentration for acetaldehyde was 16 mg/m^3, whereas the NOAEL for histological lesions in rats by Appleman *et al.* (1982) was 48.75 mg/m^3 acetaldehyde. Therefore, the concentration of acetaldehyde was not considered to account for significant irritation in the Owen (1988) study. OEHHA accepted the U.S. EPA analysis.

For comparison, Irvine (1980) obtained a NOAEL of 198 ppm for fetotoxicity in rats exposed 6 hours/day on days 6-15 of gestation. This is equivalent to 50 ppm continuous exposure during development. Multiplying by an RGDR of 1 and dividing by a total UF of 30 (3 for interspecies and 10 for intraspecies) results in a REL estimate based on fetotoxicity of 1.7 ppm. The results of Hurtt *et al*. (1995) also yield an estimate of 1.7 ppm.

VII. Data Strengths and Limitations for Development of the REL

The strengths of the inhalation REL for vinyl acetate include the availability of controlled exposure lifetime inhalation studies in multiple species at multiple exposure concentrations and with adequate histopathological analysis, and the observation of a NOAEL. The major area of uncertainty is the lack of adequate human exposure data.

VIII. Potential for Differential Impacts on Children's Health

Since the chronic REL (0.05 ppm) is lower than the comparison estimate based on developmental effects (1.7 ppm), the REL is likely to be protective of children. However, there is no direct evidence in the literature to quantify a differential effect of vinyl acetate in infants and children relative to adults.

IX. References

Appleman LM, Woutersen RA, and Feron VJ. 1982. Inhalation toxicity of acetaldehyde in rats. I. Acute and subacute studies. Toxicology 23:293-307.

Beems RB. 1988. Report No. V 88.133: Histopathology of the respiratory tract of mice used in a 104-week inhalation study with vinyl acetate. (TNO-CIVO Institutes, April 1988).

Bogdanffy MS, Dreef-van Der Meulen HC, Beems RB, Feron VJ, Cascier TC, Tyler TR, Vinegar MB, and Rickard RW. 1994. Chronic toxicity and oncogenicity inhalation study with vinyl acetate in the rat and mouse. Fundam. Appl. Toxicol. 23:215-229.

CARB. 2000. California Air Resources Board. California Emissions Inventory Development and Reporting System (CEIDARS). Data from Data Base Year 1998. February 12, 2000.

Deese DE, and Joyner RE. 1969. Vinyl acetate: A study of chronic human exposure. Am. Ind. Hyg. Assoc. J. 30:449-457.

Dreef-van der Meulen HC. 1988. Report No. V 88.033/270836: Histopathology of the respiratory tract of rats used in a 104 week inhalation study with vinyl acetate: Revised version. (TNO-CIVO Institutes, October 1988).

HSDB. 1994. Hazardous Substances Data Bank. National Library of Medicine, Bethesda, MD (TOMES® CD-ROM version). Denver, CO: Micromedex, Inc. (Edition expires 11/31/94).

Irvine LFH. 1980. Vinyl acetate oral and inhalation teratology studies in the rat. Report prepared by Hazelton Laboratories Europe Ltd., Harrogate, England for the Society of the Plastics Industry, Inc., New York. Report No. 2195-51/6&7.

Owen PE. 1980a. Vinyl acetate: 3 month inhalation toxicity study in the mouse. Report prepared by Hazelton Laboratories Europe Ltd., Harrogate, England for the Society of the Plastics Industry, Inc., New York. Report No. 2303-51/5.

Owen PE. 1980b. Vinyl acetate: 3 month inhalation toxicity study in the rat. Report prepared by Hazelton Laboratories Europe Ltd., Harrogate, England for the Society of the Plastics Industry, Inc., New York. Report No. 2286-51/5.

Owen PE. 1988. Vinyl acetate: 104 week inhalation combined chronic toxicity and carcinogenicity study in the rat and mouse. Report prepared by Hazelton Laboratories Europe Ltd., North Yorkshire, England, for the Society of the Plastics Industry, Inc., NY. Report No. 4661-51/17a. December 1987. [as cited in U.S. EPA, 1995.]

U.S.EPA. 1995. U.S. Environmental Protection Agency. Integrated Risk Information System (IRIS) database. Reference concentration (RfC) for vinyl acetate.

CHRONIC TOXICITY SUMMARY

XYLENES

(Xylol or commercial xylenes (mixture of 60-70% m- and remaining percentage is mix of o- and p- xylenes), technical grade xylenes or mixed xylenes (20% o-xylene, 40% m-xylene, 20% p-xylene, 20% ethyl benzene, and traces of toluene and C9 aromatics), o-xylene (1,2-dimethylbenzene or 2-xylene), m-xylene (1,3-dimethylbenzene or 3-xylene), p-xylene (1,4-dimethylbenzene or 4-xylene), also noted as methyltoluene, benzene-dimethyl, dimethylbenzene)

CAS Registry Numbers.: 1330-20-7 (technical mixture of o-, p-, and m-xylene); 95-47-6 (o-xylene); 108-38-3 (m-xylene); 106-42-3 (p-xylene)

I. Chronic Toxicity Summary

Inhalation reference exposure level	**700 µg/m^3 (200 ppb)** (for technical or mixed xylenes or sum of individual isomers of xylene)
Critical effect(s)	CNS effects in humans; irritation of the eyes, nose, and throat
Hazard index target(s)	Nervous system; respiratory system

II. Physical and Chemical Properties (ATSDR, 1995; HSDB, 1995; CRC, 1994)

Description	Colorless liquid
Molecular formula	C_8H_{10}
Molecular weight	106.16 g/mol
Density	0.864 g/cm^3 @ 20°C(technical mixture); 0.881 (o-); 0.860 (m-); 0.861 (p-)
Boiling point	137-140°C @ 760 TorrHg (technical mixture); 144.5 °C (o-); 139.1°C (m-); 138.3 °C (p-)
Melting point	–25.2 °C (o-); –47.8°C (m-); +13.2 °C (p-)
Vapor pressure	6.6 torr (o-); 8.39 torr (m-); 8.87 torr (p-) all @ 25°C.
Solubility	Practically insoluble in water; miscible with absolute alcohol, ether and many other organic solvents
Conversion factor	1 ppb = 4.34 µg/m^3

III. Major Uses or Sources

Mixtures of o-, p-, and m-xylenes are extensively used in the chemical industry as solvents for products including paints, inks, dyes, adhesives, pharmaceuticals, and detergents (HSDB, 1995). In the petroleum industry xylenes are used as antiknock agents in gasoline, and as an intermediate in synthetic reactions. Of the three isomers, p-xylene is produced in the highest

quantities in the U.S. for use in the synthesis of phthalic, isophthalic, and terephthalic acid used in manufacture of plastics and polymer fibers including mylar and dacron. In 1996, the latest year tabulated, the statewide mean outdoor monitored concentration of meta/para-xylene was approximately 1 ppb (CARB, 1999a). The annual statewide emissions from facilities reporting under the Air Toxics Hot Spots Act in California based on the most recent inventory were estimated to be 3,568,318 pounds of xylenes (CARB, 1999b). Also reported were speciated emissions of p-xylene - 51,203 pounds, of o-xylene - 34,573 pounds, and of m-xylene - 30,440 pounds. (Xylenes are also present in motor vehicle exhaust.)

IV. Effects of Human Exposure

Information on the toxicity of xylenes to humans is almost exclusively limited to case reports of acute exposures and studies of occupational exposures in which persons often inhaled a mixture of hydrocarbon solvents 8 hours per day, 5-6 days per week. These studies often have incomplete information on the airborne concentrations of xylene and other hydrocarbons. One study examining chronic effects in humans from inhalation of predominantly mixed xylenes was identified (Uchida *et al.*, 1993) and one 4-week controlled exposure study examining the effects of p-xylene exclusively was identified (Hake *et al.*, 1981). No studies examining the chronic effects of oral or dermal xylene exposure in humans were identified.

Pharmacokinetic studies have documented the absorption of xylene in humans through inhalation, oral, and dermal routes of exposure. Approximately 60% of inspired xylene is retained systemically (Sedivec and Flek, 1979). The majority of ingested xylene (~90%) is absorbed into the systemic circulation (ATSDR, 1995). Xylene is also absorbed dermally; the rate of absorption of xylene vapor is estimated as 0.1-0.2% of that by inhalation (Riihimaki and Pfaffli, 1978). Loizou *et al*. (1999) exposed human volunteers to 50 ppm *m*-xylene for 4 hours and determined that the dermal route of exposure contributed 1.8% of the total body burden. Measurement of the rate of absorption through direct contact with the skin produced variable results ranging from 2 $\mu g/cm^2/min$ (Engstrom *et al.*, 1977) to 75-160 $\mu g/cm^2/min$ (Dutkiewicz and Tyras, 1968).

Xylene exposure has been associated with effects in a number of organ systems including the lungs, skin and eyes; neurological system; heart and gastrointestinal system; kidney; and possibly the reproductive system.

Pulmonary effects have been documented in occupational exposures to undetermined concentrations of mixed xylenes (and other solvents) and include labored breathing and impaired pulmonary function (Hipolito 1980; Roberts *et al.*, 1988). High levels of xylene exposure for short periods are associated with irritation of the skin, eyes, nose and throat (ATSDR, 1995). Chronic exposure to xylenes has been associated with eye and nasal irritation (Uchida *et al.*, 1993).

The central nervous system is affected by both short term and long term exposure to high concentrations of xylene with: 100-200 ppm associated with nausea and headache; 200-500 ppm with dizziness, irritability, weakness, vomiting, and slowed reaction time; 800-10,000 ppm with

lack of muscle coordination, giddiness, confusion, ringing in the ears, and changes in sense of balance; and >10,000 ppm with loss of consciousness (HESIS, 1986). Other documented, neurological effects include impaired short term memory, impaired reaction time, performance decrements in numerical ability, and impaired equilibrium (dizziness) and balance (Carpenter *et al.*, 1975; Dudek *et al.*, 1990; Gamberale *et al.*, 1978; Riihimaki and Savolainen, 1980; Savolainen and Linnavuo, 1979; Savolainen and Riihimaki 1981; Savolainen *et al.*, 1979; 1984; 1985).

Chronic exposure to xylenes (with other hydrocarbons) has been associated with cardiovascular and gastrointestinal effects. Heart palpitations, chest pain, and abnormal electrocardiogram were noted (Hipolito, 1980; Kilburn *et al.*, 1985) as were effects on the gastrointestinal system producing nausea, vomiting and gastric discomfort in exposed workers (Goldie, 1960; Hipolito, 1980; Uchida *et al.*, 1993; Klaucke *et al.*, 1982; Nersesian *et al.*, 1985).

Results of studies of renal effects of xylene are mixed and come from case reports and occupational studies where multiple chemical exposures are common. The effects from subchronic exposure documented by Hake *et al.* (1981) and from chronic exposure documented by Uchida *et al.* (1993) did not include renal effects. However, Morley *et al.* (1970) found increased BUN and decreased creatinine clearance; Martinez *et al.* (1989) found distal renal tubular acidemia; Franchini *et al.* (1983) found increased levels of urinary β-glucuronidase; and Askergren (1981, 1982) found increased urinary excretion of albumin, erythrocytes, and leukocytes.

Reproductive effects were documented by Taskinen *et al.* (1994) who found increased incidence of spontaneous abortions in 37 pathology and histology workers exposed to xylene and formaldehyde in the work place. The multiple chemical exposures and the small number of subjects in this study limit the conclusions that can be drawn as to reproductive effects of xylene in humans.

No hematological effects have been identified in studies where exposure was to xylene only. Previous studies identifying hematological effects included known or suspected exposure to benzene (ATSDR, 1995; ECETOC, 1986). One series of case reports identified lowered white cell counts in two women with chronic occupational exposure to xylene (Hipolito, 1980; Moszczynsky and Lisiewicz, 1983; 1984), although they may also have had multiple chemical exposures.

Groups of male volunteers (1 to 4 subjects/group) were exposed to p-xylene in a controlled-environment chamber for 7.5, 3, or 1 hr/day, 5 days/week for 4-weeks (Hake *et al*., 1981). The p-xylene concentration was changed on a weekly basis starting at 100 ppm the first week, followed by 20 ppm, 150 ppm, and 100 ppm (average, with a range of 50 to 150 ppm) over subsequent weeks. In addition, groups of female volunteers (2 or 3/group) were exposed to 100 ppm p-xylene for 7.5, 3, or 1 hr/day for 5 days. The volunteers acted as their own controls, with exposure to 0 ppm p-xylene occurring for two days (males) or one day (females) the week before and the week after the xylene exposures. No serious subjective or objective health responses, including neurological tests, cognitive tests and cardiopulmonary function tests were observed. Odor was noted, but the intensity decreased usually within the first hour of exposure. The

authors concluded that p-xylene may have a weak irritating effect on the soft tissues starting at 100 ppm, but overall, the small sample size and high variability among the volunteers made all results difficult to interpret.

The Uchida *et al.* (1993) study included a relatively large number of workers studied, exposure for an average of 7 years to xylenes predominately and a comprehensive set of medical examinations to document potential effects. A survey of 994 Chinese workers involved in the production of rubber boots, plastic coated wire and printing processes employing xylene solvents was carried out. The survey consisted of fitting individual workers with diffusive samplers for an 8 hour shift. At the end of the 8 hour shift the samplers were recovered for analysis of solvent exposure, and urine samples were collected for analysis of xylene metabolites. The following day workers answered a questionnaire concerning subjective symptoms, and blood and urine were collected for analysis. Out of this group of xylene-exposed workers, 175 individuals (107 men and 68 women) were selected for further study and analysis based on completion of their health examinations and on results from diffusive samplers showing that xylene constituted 70% or more of that individual's exposure to solvents in the workplace. The control population consisted of 241 (116 men and 125 women) unexposed workers from the same factories or other factories in the same region, of similar age distribution, of similar time in this occupation (average of 7 years), and having a similar distribution of alcohol consumption and cigarette usage. The xylene-exposed and unexposed groups were given health examinations which evaluated hematology (red, white, and platelet cell counts, and hemoglobin concentration), serum biochemistry (albumin concentration, total bilirubin concentration, aspartate aminotransferase, alanine aminotransferase, gamma-glutamyl transferase, alkaline phosphatase, leucine aminopeptidase, lactate dehydrogenase, amylase, blood urea nitrogen, creatinine), and subjective symptoms (survey of symptoms occurring during work and in the previous three months).

Results of analysis of the diffusive samplers showed that workers were exposed to a geometric mean of 14.2 ± 2.6 ppm xylene (arithmetic mean of 21.3 ± 21.6 ppm). This was broken down into geometric means of 1.2 ppm o-xylene, 7.3 ppm m-xylene, 3.8 ppm p-xylene, 3.4 ppm ethyl benzene, and 1.2 ppm toluene. N-Hexane was rarely present and no benzene was detected. Analysis of data from the health examinations found no statistically significant difference ($p<0.10$) between hematology and serum biochemistry values for xylene-exposed and unexposed populations. The frequency of an elevated ratio of aspartate aminotransferase to alanine transferase and of elevated ratio of alkaline phosphatase to leucine aminopeptidase was significantly ($p<0.01$) higher in exposed men than in the control population of men. Results of the survey of subjective symptoms found differences in symptoms occurring during work and during a similar analysis over the preceding three month period, apparently related to effects on the central nervous system and to local effects on the eyes, nose and throat. The frequency of five symptoms experienced during work was significantly ($p<0.01$) elevated in either xylene-exposed men or women including: dimmed vision, unusual taste, dizziness, heavy feeling in the head, and headache. The frequency of four symptoms experienced during work were significantly ($p<0.01$) elevated in both men and women including irritation in the eyes, nasal irritation, sore throat, and floating sensation. Ten subjective symptoms occurring in the previous three months were significantly ($p<0.01$) elevated in exposed men and women including nausea, nightmare, anxiety, forgetfulness, inability to concentrate, fainting after suddenly standing up,

poor appetite, reduced grasping power, reduced muscle power in the extremities, and rough skin. Dose dependency appeared to exist for 3 subjective symptoms noted during work: irritation in the eyes, sore throat, floating sensation, and for one symptom occurring in the last three months, poor appetite.

V. Effects of Animal Exposure

A limited number of chronic toxicity studies are available for xylene including two inhalation studies with o-xylene (Tatrai *et al.*, 1981; Jenkins *et al.*, 1970) and one oral chronic study with mixed xylenes (NTP, 1986). No chronic dermal studies could be identified. A spectrum of adverse effects has been documented in shorter term studies which potentially could occur with chronic exposure. These studies are presented here along with a brief description of the three chronic studies identified. Xylene affects a number of organ systems including the pulmonary system, the cardiovascular system, the gastrointestinal system, the hepatic system, the renal system, the dermis, and the eye, and it has numerous neurological effects and developmental effects.

Animal data are consistent with human data in documenting respiratory effects from xylene exposure. Acute and subacute exposures in mice, rats, and guinea pigs have been associated with decreased metabolic capacity of the lungs; decreased respiratory rate; labored breathing; irritation of the respiratory tract; pulmonary edema; and pulmonary inflammation (Carpenter *et al.*, 1975; De Ceaurriz *et al.*, 1981; Elovaara *et al.*, 1987; 1989; Furnas and Hine, 1958; Korsak *et al.*, 1988; 1990; Patel *et al.*, 1978; Silverman and Schatz, 1991; Toftgard and Nilsen, 1982).

Limited evidence is available in animal studies for cardiovascular effects resulting from xylene exposure. Morvai *et al.* (1976; 1987) conducted two studies. The first study observed rats following acute and intermediate duration inhalation exposure to very high (unspecified) levels of xylene and recorded ventricular repolarization disturbances, atrial fibrillation, arrhythmias, occasional cardiac arrest and changes in electrocardiogram (Morvai *et al.*, 1976). In a subsequent study morphological changes in coronary microvessels were seen in rats exposed to 230 ppm xylene (isomer composition unspecified) (Morvai *et al.*, 1987). However the chronic toxicity studies conducted by the National Toxicology Program (NTP, 1986) and by Jenkins *et al.* (1970), as well as other shorter term studies (Carpenter *et al.*, 1975; Wolfe, 1988), have not identified histopathological lesions of the heart.

Studies identifying adverse gastrointestinal effects, hematological effects, or musculoskeletal effects in animals were not identified. Studies reporting no hematological effects include Carpenter *et al.* (1975) (rats exposed to 810 ppm of mixed xylenes for 10 weeks, 5 days/week, 6 hours/day and dogs exposed for 13 weeks to 810 ppm mixed xylenes, 5 days/week, 6 hours/day) and Jenkins *et al.* (1970) (rats, guinea pigs and dogs exposed for 6 weeks to 780 ppm o-xylene, 5 days/week, 8 hours per day). Carpenter *et al.* (1975) and the NTP (1986) reported no effects on the musculoskeletal system.

Hepatic effects have been documented after acute exposure to high concentrations of xylene (2,000 ppm) or subacute exposure to lower concentrations (345-800 ppm) of mixed xylene or individual isomers. These effects include increased cytochrome P-450 and b5 content, increased hepatic weight, increased liver to body weight ratios, decreased hepatic glycogen, proliferation

of endoplasmic reticulum, changes in distribution of hepatocellular nuclei, and liver degeneration (Bowers *et al.*, 1982; Condie *et al.*, 1988; Elovaara, 1982; Elovaara *et al.*, 1980; Muralidhara and Krishnakumari 1980; Patel *et al.*, 1979; Pyykko 1980; Tatrai and Ungvary, 1980; Tatrai *et al.*, 1981; Toftgard and Nilsen, 1981; 1982; Toftgard *et al.*, 1981; Ungvary *et al.*, 1980).

Renal effects have been identified in studies with rats, guinea pigs, dogs, and monkeys exposed to 50-2,000 ppm of xylenes. These effects include increased cytochrome P-450 content and increased kidney to body weight ratios (Condie *et al.*, 1988; Elovaara 1982; Toftgard and Nilsen, 1982). Condie *et al.* (1988) also noted tubular dilation, atrophy, and increased hyaline droplets in the kidney of Sprague-Dawley rats administered 150 mg/kg/day orally of mixed xylenes. This response is consistent with early nephropathy.

Xylene has been found to affect the dermis and eyes of animals. Hine and Zuidema (1970) found skin erythema and edema, epidermal thickening, and eschar formation in response to xylene exposure. Direct instillation of xylenes into the eyes of rabbits produces eye irritation (Hine and Zuidema, 1970; Smyth *et al.*, 1962)

Numerous neurological effects have been documented in response to acute and subchronic xylene exposures ranging from 100 to 2,000 ppm. This is consistent with effects on neurofunction documented in humans. These effects include narcosis, prostration, incoordination, tremors, muscular spasms, labored respiration, behavioral changes, hyperactivity, elevated auditory thresholds, hearing loss, and changes in brain biochemistry (Andersson *et al.*, 1981; Carpenter *et al.*, 1975; De Ceaurriz *et al.*, 1983; Furnas and Hine, 1958; Ghosh *et al.*, 1987; Gralewicz *et al*., 1995; Kyrklund *et al.*, 1987; Molnar *et al.*, 1986; NTP, 1986; Pryor *et al.*, 1987; Rank 1985; Rosengren *et al.*, 1986; Savolainen and Seppalainen, 1979; Savolainen *et al.*, 1978; 1979a; Wimolwattanapun *et al.*, 1987).

Developmental effects have been documented in pregnant animals exposed to xylenes. ATSDR (1995) concluded that the body of information available for developmental effects is consistent with the hypothesis that xylene is fetotoxic and many of the fetotoxic responses are secondary to maternal toxicity. However, the ATSDR also observed that there was a large variation in the concentrations of xylene producing developmental effects and of those producing no developmental effects. The ATSDR thought that these differences were influenced by a number of factors (strain and species of animal, purity of xylene, method of exposure, exposure pattern and duration, etc.). The two most common test species have been the rat and the mouse.

With respect to rats, Mirkova *et al*. (1983) exposed groups of pregnant rats (unspecified strain of white rats) to clean air or 2.3, 12, or 120 ppm of xylene (unspecified composition) for 6 h/day on days 1-21 of gestation. They reported increased postimplantation losses and fetotoxicity (reduced fetal weights) as well as a statistically increased incidence of visceral abnormalities (including ossification defects in bones of the skull) at xylene air concentrations of 12 ppm and above. The ATSDR has suggested that the Mirkova *et al*. (1983) study results may have been influenced by poor animal husbandry as indicated by the low conception rates and the high incidence of fetal hemorrhages seen in the controls. Hass and Jakobsen (1993) attempted to replicate the findings of Mirkova *et al*. (1983). Hass and Jakobsen (1993) exposed groups of 36 pregnant Wistar rats to clean air or 200 ppm of xylene for 6 h/day on days 4-20 of gestation.

Unlike Mirakova *et al*. (1983), there was no sign of maternal toxicity and no decrease in fetal weights and no increase in soft-tissue or skeletal malformations. A large increase in the incidence of delayed ossification of the *os maxillare* of the skull, however, was observed (53% of experimental fetuses as opposed to 2% of the controls). Potential neurological/muscular changes measured as performance on a rotorod were also noted upon testing of 2-day-old rat pups.

Ungvary *et al*. (1985) exposed CFY rats by inhalation to air concentrations of xylene (60 ppm, 440 ppm, 800 ppm) for 24 h/day on days 7-15 of gestation. Maternal toxicity was described as moderate and dose-dependent. They observed weight retarded fetuses at all air concentrations. However, there was no increase in malformations, and an increase in minor anomalies and resorbed fetuses occurred only at the highest concentration. In a separate study investigating the interactions between solvents and other agents, Ungvary (1985) exposed CFY rats to either 140 ppm or 440 ppm of xylene on days 10-13 of gestation and also reported increases for either condition in weight retarded and skeletal retarded fetuses without any increase in malformations. Hudak and Ungvary (1978) had earlier examined the effect of 230 ppm xylene (24 h/day, days 9-14 of pregnancy) in the CFY rat and reported effects on skeletal development (e.g., fused sternebrae). In contrast to the other Ungvary findings, no effect on fetal weight was observed. Bio/dynamics (1983) conducted an inhalation exposure study in the rat (CrL-CD (SD) BR strain). Rats were exposed 6 h/day during premating, mating, gestation and lactation. Exposure concentrations were 0, 60, 250, and 500 ppm. Most measures for adverse effects on fetal development were not significantly increased. Mean fetal weights at the highest exposure level were lower than controls, but this difference was significant only for the female fetuses. These depressed weights were, however, still significant on day 21 of lactation. Other adverse effects (such as increased soft tissue and skeletal abnormalities, increased fetal resorptions) were not increased significantly at any of the test concentrations.

Ungvary *et al*. (1980a) tested by inhalation the individual ortho, meta, and para isomers of xylene in the CFY rat. Pregnant rats were exposed 24 h/day on days 7 –14 of pregnancy to 35, 350, or 700 ppm of each isomer. An increased incidence of weight retarded fetuses was observed for each isomer at the 700 ppm level, and for the ortho isomer at the 350 ppm level. Post implantation losses were increased only at the 700 ppm level in the para-xylene exposed group. Skeletal anomalies were increased only at the 700 ppm level for the meta and para isomers of xylene. Rosen *et al*. (1986) evaluated the effects of prenatal exposure to para-xylene in the rat. They exposed pregnant Sprague-Dawley rats by inhalation to either 800 ppm or 1600 ppm of p-xylene from days 7-16 of gestation. Despite the high concentrations, no effects were seen on litter size or weight at birth or on the subsequent growth rates of the pups.

Hass *et al.* (1995) examined postnatal development and neurobehavioral effects in rats following prenatal exposure to 0 or 500 ppm technical xylene 6 hr/day on gestation days 7-20 of pregnancy. Xylene exposure caused no signs of maternal toxicity and no difference in the number of live or dead fetuses. The mean birth weight in exposed litters was about 5% lower compared to control litters but the difference was not statistically significant. Body weights were similar between groups during the preweaning and postweaning period but lower absolute brain weights were observed in exposed animals. Exposed offspring showed a delay in the ontogeny of the air righting reflex and exhibited impaired performance in behavioral tests for neuromotor

abilities (Rotorod) and for learning and memory (Morris water maze). In a follow-up study under the same exposure conditions, exposed offspring exhibited impaired performances in the Morris water maze at 16, 28, and 55 weeks of age, although the difference was not statistically significant at 55 weeks (Hass *et al*., 1997). These data indicate that xylene exposure during development may cause long-lasting deficits on learning and memory in offspring.

With respect to mice, Ungvary *et al*. (1985) exposed CFLP mice by inhalation to air concentrations of xylene (120 ppm, 230 ppm) for 24 h/day on days 7-15 of gestation. In the mouse, they observed increased incidences of weight-retarded fetuses and increased skeletal retarded fetuses at 230 ppm. Shigeta *et al*. (1983) exposed pregnant ICR mice to approximately 0, 120, 230, 460, and 920 ppm of xylene in an exposure chamber for 6 h/day on days 6-12 of gestation. Shigeta *et al.* (1983) reported significant decreases in fetal weight in the 460 ppm and 920 ppm dose groups only. There was no difference in the number of live or dead fetuses. Decreased weight gains and delayed development of body hair and teeth were observed at the 920 ppm exposure level. Dose-response relations were reported for delayed ossification of the sternebrae. Marks *et al*. (1982) noted that 2060 mg/kg/day of mixed xylene administered orally is associated with cleft palate and decreased fetal weight in the mouse.

Ungvary *et al*. (1985) also tested the individual ortho, meta, and para isomers of xylene at 120 ppm in the CFLP mouse. Each isomer of xylene also increased the incidence of weight-retarded fetuses and skeletal retarded fetuses at 120 ppm. There was no increase in malformations.

Of the three chronic studies available (Tatrai *et al.*, 1981; Jenkins *et al.*, 1970; NTP 1986) none comprehensively examined systemic effects. The study by Tatrai *et al.* (1981) exposed rats for one year, 7 days/week, 8 hours per day to 1096 ppm o-xylene. This exposure was a LOAEL for body weight gain in males and a NOAEL for hepatic effects in male rats. Jenkins *et al.* (1970) exposed rats, guinea pigs, squirrel monkeys, and beagle dogs for 90-127 days continuously to 78 ppm of o-xylene. The study examined body weight gain; hematological parameters including white cell counts, red blood cell counts, and hematocrit; serum biochemistry including bromosulfophthalein retention, blood urea nitrogen, alanine aminotransferase, aspartate aminotransferase, alkaline phosphatase, and creatinine and liver function including alkaline phosphatase, tyrosine aminotransferase, and total lipids. No effects were observed in any of the parameters examined in this study. This study found a NOAEL for all effects examined of 78 ppm o-xylene. The NTP (1986) study administered 0, 250, or 500 mg/kg/day doses of mixed xylene in corn oil by gavage 5 days/week for 103 weeks to groups of F344/N rats of both sexes, 50 animals per group. B6C3F1 mice were treated in a similar manner but given 0, 500 or 1000 mg/kg/day of mixed xylenes in corn oil by gavage. A complete histopathological examination of all tissues was made as well as determination of body weight gain. Based on histopathology of all organ systems, a NOAEL of 500 mg/kg/day was observed for rats and a NOAEL of 1000 mg/kg/day was observed for mice.

VI. Derivation of Chronic Reference Exposure Level (REL)

Study	Uchida *et al.* (1993)
Study population	175 xylene-exposed factory workers and control population of 241 factory workers
Exposure method	Inhalation
Critical Effects	Dose related increase in the prevalence of eye irritation, sore throat, floating sensation, and poor appetite.
LOAEL	14.2 ppm (geometric mean of exposure concentrations)
NOAEL	Not applicable
Exposure continuity	8 hr/d (10 m^3/day occupational inhalation rate), 5 d/wk
Exposure duration	Occupational exposure for an average of 7 years
Average occupational exposure	5.1 ppm for LOAEL group (14.2 x 10/20 x 5/7)
Human equivalent concentration	5.1 ppm for LOAEL group
LOAEL uncertainty factor	3
Subchronic uncertainty factor	1
Interspecies uncertainty factor	1
Intraspecies uncertainty factor	10
Cumulative uncertainty factor	30
Inhalation reference exposure level	0.2 ppm (200 ppb; 0.7 mg/m^3; 700 µg/m^3) for mixed xylenes or for total of individual isomers

A number of issues are important in considering the uncertainty associated with this REL. For ATSDR (1995) the animal and human toxicity data suggest that mixed xylenes and the different xylene isomers produce similar effects, although different isomers are not equal in potency for producing a given effect. Therefore exposure of workers to a mix of xylenes in the Uchida *et al.* (1993) study would be expected to generate a similar spectrum of responses as exposure to single isomers, however the intensity of particular effects could be different. The use of a neurological endpoint for derivation of a REL is supported by the large number of inhalation and oral studies associating neurological effects with xylene exposure. ATSDR (1995) indicates that neurological effects are a sensitive endpoint. The observation that floating sensation is apparently related to dose further supports the concept that this subjective symptom related to neurological effects was due to xylene exposure.

A UF of 3, rather than 10, was applied for the LOAEL to NOAEL extrapolation due to the generally mild adverse effects observed and the principally low incidence (<50%) of the effects. A factor of 1 was used for subchronic uncertainty. Although the average occupational exposure was only 7 years, there were 176 xylene-exposed workers of average age 29.7±9.0 years (arithmetic mean ±SD) for whom, according to the report, there had been essentially no change in workplace in their working life. Thus, many workers would likely have been exposed for more than 8.4 years, the cut-off point for chronic human exposure. Another issue is the use of diffusive samplers in the Uchida *et al.* (1993) study. These samplers provide a time weighted

average concentration of hydrocarbon and cannot indicate the maximum concentrations a worker is exposed to. It is unknown whether peak concentrations alter the response to xylenes in humans.

For comparison with the proposed REL of 200 ppb based on human studies, (1) the free-standing NOAEL of 78 ppm o-xylene obtained by Jenkins *et al*. (1970) in rats and guinea pigs continuously exposed for 90 days was used to estimate a REL based on animal data. Use of an RGDR of 1, a subchronic UF of 3, an interspecies UF of 3, and an intraspecies UF of 10 result in a REL of 800 ppb for o-xylene for systemic effects. (2) Tatrai *et al*. (1981) found a free standing LOAEL of 1096 ppm o-xylene for body weight gain in male rats exposed every day for 8 hours. Time adjustment to continuous exposure and use of an RGDR of 1, a LOAEL UF of 3 for a mild effect, an interspecies UF of 3, and an intraspecies UF of 10 result in a REL of 4000 ppb. (3) Ungvary *et al*. (1985) exposed mice by inhalation continuously to 120 ppm or 230 ppm xylene for 24 h/day on days 7-15 of gestation. The LOAEL was 230 ppm and the NOAEL was 120 ppm. No time adjustment is needed. Use of an RGDR of 1, a subchronic UF of 1, an interspecies UF of 3, and an intraspecies UF of 10 results in a REL of 4000 ppb for xylene for developmental effects.

VII. Data Strengths and Limitations for Development of the REL

The strengths of the inhalation REL for xylene include the use of human exposure data from 175 workers exposed over a period of years. Major areas of uncertainty are the uncertainty in estimating exposure, the potential variability in exposure concentration, and the lack of observation of a NOAEL in the key study.

VIII. References

ATSDR. 1995. Agency for Toxic Substances and Disease Registry. Toxicological Profile for Xylenes (Update). Atlanta, GA: ATSDR. U.S. Printing Office 1995-639-298.

Andersson K, Fuxe K, Nilsen OG, Toftgard R, Eneroth P, Gustafsson JA. 1981. Production of discrete changes in dopamine and noradrenaline levels and turnover in various parts of the rat brain following exposure to xylene, ortho-, meta-, and para-xylene, and ethylbenzene. Toxicol. Appl. Pharmacol. 60:535-548.

Askergren A. 1981. Studies on kidney function in subjects exposed to organic solvents: III. Excretion of cells in the urine. Acta Med. Scand. 210:103-106.

Askergren A. 1982. Organic solvents and kidney function. In: Mehlman MA, ed. Advances in Modern Environmental Toxicology. Vol. 2. Princeton Junction, NJ: Senate Press. Pp. 157-172.

Bio/dynamics Inc. 1983. Parental and fetal reproduction inhalation toxicity study in rats with mixed xylenes. Prepared for the American Petroleum Institute, Health and Environmental Services Department. HESD Publ. No. 31-31481.

Bowers DJ, Cannon MS, and Jones DH. 1982. Ultrastructural changes in livers of young and aging rats exposed to methylated benzenes. Am. J. Vet. Res. 43:679-683.

CARB. 1999a. California Air Resources Board. Toxics Air Quality Data. Substance Chooser. Meta/para-Xylene. Available online at http:/www.arb.ca.gov/aqd/toxics.htm

CARB. 1999b. Air toxics emissions data collected in the Air Toxics Hot Spots Program CEIDARS Database as of January 29, 1999.

Carpenter CP, Kinkead ER, Geary DJ Jr, Sullivan LJ, King JM. 1975. Petroleum hydrocarbon toxicity studies: V. Animal and human response to vapors of mixed xylenes. Toxicol. Appl. Pharmacol. 33:543-558.

CRC. 1994. CRC Handbook of Chemistry and Physics, 75th edition. Lide DR, ed. Boca Raton, FL: CRC Press Inc.

Condie LW, Hill JR, Borzelleca JF. 1988. Oral toxicology studies with xylene isomers and mixed xylenes. Drug Chem. Toxicol. 11. 329-354.

De Ceaurriz JC, Micillino JC, Bonnet P, Guenier JP. 1981. Sensory irritation caused by various industrial airborne chemicals. Toxicol. Lett. 9:137-143.

De Ceaurriz J, Desiles JP, Bonnet P. Marignac B, Muller J, Guenier JP. 1983. Concentration-dependent behavioral changes in mice following short-term inhalation exposure to various industrial solvents. Toxicol. Appl. Pharmacol. 67:383-389.

Dudek B, Gralewicz K, Jakubowski M, Kostrzewski P, Sokal J. 1990. Neurobehavioral effects of experimental exposure to toluene, xylene and their mixture. Pol. J. Occup. Med. 3:109-116.

Dutkiewicz T, and Tyras H. 1968. Skin absorption of toluene, styrene, and xylene by man. Br. J. Ind. Med. 25:243.

ECETOC. 1986. European Chemical Industry Ecology and Toxicology Centre. Joint assessment of commodity chemicals: No 6. Xylenes. Brussels, Belgium: ECETOC.

Elovaara E. 1982. Dose-related effects of m-xylene inhalation on the xenobiotic metabolism of the rat. Xenobiotica 12:345-352.

Elovaara E, Collan Y, Pfaffli P, Vainio H. 1980. The combined toxicity of technical grade xylene and ethanol in the rat. Xenobiotica 10:435-445.

Elovaara E, Zitting A, Nickels J, Aitio A. 1987. m-Xylene inhalation destroys cytochrome P-450 in rat lung at low exposure. Arch. Toxicol. 61:21-26.

Elovaara E, Engstrom K, Hayri L, Hase T, Aitio A. 1989. Metabolism of antipyrine and m-xylene in rats after prolonged pretreatment with xylene alone or xylene with ethanol, phenobarbital, or 3-methylcholanthrene. Xenobiotica 19:945-960.

Engstrom K, Husman K, and Riihimaki V. 1977. Percutaneous absorption of m-xylene in man. Int. Arch. Occup. Environ. Health 39:181-189.

Franchini I, Cavatorta A, Falzoi M, Lucertini S, Mutti A. 1983. Early indicators of renal damage in workers exposed to organic solvents. Int. Arch. Occup. Environ. Health 52:1-9.

Furnas DW, and Hine CH. 1958. Neurotoxicity of some selected hydrocarbons. Arch. Ind. Health 18:9-15.

Gamberale F, Annwall G, and Hultengren M. 1978. Exposure to xylene and ethylbenzene: III. Effects on central nervous functions. Scand. J. Work Environ. Health 4:204-211.

Ghosh TK, Copeland RJ, Parui RN, Mookherjee S, Pradhan SN. 1987. Effects of xylene inhalation on fixed-ratio responding in rats. Pharmacol. Biochem. Behav. 27:653-657.

Goldie I. 1960. Can xylene (xylol) provoke convulsive seizures? Ind. Med. Surg. 29:33-35.

Gralewicz S, Wiaderna D, and Tomas T. 1995. Development of spontaneous, age-related nonconvulsive seizure electrocortical activity and radial-maze learning after exposure to m-xylene in rats. Int. J. Occup. Med. Environ. Health 8:347-360.

Hake CLR, Stewart RD, Wu A, *et al.* 1981. p-Xylene: Development of a biological standard for the industrial worker. Report to the National Institute for Occupational Safety and Health, Cincinnati, OH, by the Medical College of Wisconsin, Inc., Milwaukee, WI. PB82-152844.

Hass U, and Jakobsen BM. 1993. Prenatal toxicity of xylene inhalation in the rat: A teratogenicity and postnatal study. Pharmacol. Toxicol. 73:20-23.

Hass U, Lund SP, Simonsen L, and Fries AS. 1995. Effects of prenatal exposure to xylene on postnatal development and behavior in rats. Neurotoxicol. Teratol. 17(3):341-349.

Hass U, Lund SP, and Simonsen L. 1997. Long-lasting neurobehavioral effects of prenatal exposure to xylene in rats. Neurotoxicol. 18(2):547-552.

HESIS. 1986. Hazard Evaluation System and Information Service, Fact Sheet #7, Xylene. State of California, Department of Health Services, Department of Industrial Relations, CAL/OSHA. 2151 Berkeley Way, Berkeley CA 94704.

Hine CH, and Zuidema HH. 1970. The toxicological properties of hydrocarbon solvents. Ind. Med. 39:39-44.

Hipolito RN. 1980. Xylene poisoning in laboratory workers: Case reports and discussion. Lab. Med. 11:593-595.

HSDB. 1995. Hazardous Substances Data Bank. On-line data base. Xylenes. Micromedex, Inc. Vol. 25.

Hudak A, and Ungvary G. 1978. Embryotoxic effects of benzene and its methyl derivatives: Toluene, xylene. Toxicology 11:55-63.

Jenkins LJ, Jones RA, and Siegel J. 1970. Long-term inhalation screening studies of benzene, toluene, o-xylene, and cumene on experimental animals. Toxicol. Appl. Pharmacol. 16:818-823.

Kilburn KH, Seidman BC, and Warshaw R. 1985. Neurobehavioral and respiratory symptoms of formaldehyde and xylene exposure in histology technicians. Arch. Environ. Health 40:229-233.

Klaucke DN, Johansen M, and Vogt RL. 1982. An outbreak of xylene intoxication in a hospital. Am. J. Ind. Med. 3:173-178.

Korsak Z, Sokal JA, Dedyk A, Tomas T, Jedrychowski R. 1988. Toxic effects of combined exposure to toluene and xylene in animals: I. Acute inhalation study. Pol. J. Occup. Med. 1:45-50.

Korsak Z, Sokal JA, Wasiela T, Swiercz R. 1990. Toxic effects of acute exposure to particular xylene isomers in animals. Pol. J. Occup. Med. 3:221-226.

Kyrklund T, Kjellstrand P, and Haglid K. 1987. Brain lipid changes in rats exposed to xylene and toluene. Toxicology 45:123-133.

Loizou GD, Jones K, Akrill P, Dyne D, and Cocker J. 1999. Estimation of the dermal absorption of *m*-xylene vapor in humans using breath sampling and physiologically based pharmacokinetic analysis. Toxicol. Sci. 48:170-179.

Marks TA, Ledoux TA, and Moore JA. 1982. Teratogenicity of a commercial xylene mixture in the mouse. J. Toxicol. Environ. Health 9:97-105.

Martinez JS, Sala JJG, Vea AM, *et al.* 1989. Renal tubular acidosis with an elevated anion gap in a ‘glue sniffer’: Letter to editor. Human Toxicol. 8:139-140.

Mirkova E, Hinkova L, Vassileva L, *et al.* 1979. Xylene neurotoxicity in pregnant rats and fetuses. Activ. Nerv. Suppl. (Praha) 21:265-268.

Mirkova E, Zaikov C, Antov G, Mikhailova A, Khinkova L, Benchev I. 1983. Prenatal toxicity of xylene. J. Hyg. Epidemiol. Microbiol. Immunol. 27:337-343.

Molnar J, Paksy KA, and Naray M. 1986. Changes in the rat's motor behavior during 4-hr inhalation exposure to prenarcotic concentrations of benzene and its derivatives. Acta Physiol. Hung. 67:349-354.

Morley R, Eccleston DW, Douglas CP, Greville WE, Scott DJ, Anderson J. 1970. Xylene poisoning: A report on one fatal case and two cases of recovery after prolonged unconsciousness. Br. Med. J. 3:442-443.

Morvai V, Hudak A, Ungvary G, and Varga B. 1976. ECG changes in benzene, toluene and xylene poisoned rats. Acta Med. Acad. Sci. Hung. 33:275-286.

Morvai V, Ungvary G, Herrmann HJ, and Kuhne C. 1987. Effects of quantitative undernourishment, ethanol and xylene on coronary microvessels of rats. Acta Morphol. Hung. 35: 199-206.

Moszczynski P, and Lisiewicz J. 1983. Occupational exposure to benzene, toluene and xylene and the T lymphocyte functions. J. Clin. Hematol. Oncol. 13:37-41.

Moszczynski P, and Lisiewicz J. 1984. Occupational exposure to benzene, toluene and xylene and the T lymphocyte functions. Haematologia 17:449-453.

Muralidhara, and Krishnakumari MK. 1980. Mammalian toxicity of aromex and xylene used in pesticidal formulations. Indian J. Exp. Biol. 18:1148-1151.

Nersesian W, Booth H, Hoxie D, Hinckley W, Shehata T. 1985. Illness in office attributed to xylene [Letter]. Occup. Health Saf. 54:88.

NTP. 1986. National Toxicology Program technical report on the toxicology and carcinogenesis studies of xylenes (mixed) (60% m-xylene, 14% p-xylene, 9 % o-xylene, and 17% ethylbenzene) (CAS No. 1330-20-7) in F344/N rats and B6C3F1 mice, gavage studies. Research Triangle Park, NC: U.S. Department of Health and Human Services, Public Health Service, National Institutes of Health, National Toxicology Program. NTP TR 327. NIH Publication No. 87-2583.

Patel JM, Harper C, and Drew RT. 1978. The biotransformation of p-xylene to a toxic aldehyde. Drug Metab. Dispos. 6:368-374.

Patel JM, Harper C, Gupta BN, and Drew RT. 1979. Changes in serum enzymes after inhalation exposure of p-xylene. Bull. Environ. Contam. Toxicol. 21:17-24.

Pryor GT, Rebert CS, and Howd RA. 1987. Hearing loss in rats caused by inhalation of mixed xylene and styrene. J. Appl. Toxicol. 7:55-61.

Pyykko K. 1980. Effects of methylbenzenes on microsomal enzymes in rat liver, kidney and lung. Biochim. Biophys. Acta 633:1-9.

Rank J. 1985. Xylene induced feeding and drinking behavior and central adrenergic receptor binding. Neurobehav. Toxicol. Teratol. 7:421-426.

Riihimaki V, and Pfaffli P. 1978. Percutaneous absorption of solvent vapors in man. Scand. J. Work Environ. Health 4:73-85.

Riihimaki V, and Savolainen K. 1980. Human exposure to m-xylene: Kinetics and acute effects on the central nervous system. Ann. Occup. Hyg. 23:411-422.

Roberts FP, Lucas EG, Marsden CD, and Trauer T. 1988. Near-pure xylene causing reversible neuropsychiatric disturbance [Letter]. Lancet ii:273.

Rosen MB, Crofton KM, Chernoff, N. 1986. Postnatal evaluation of prenatal exposure to p-xylene in the rat. Toxicol. Lett. 34:223-229.

Rosengren LE, Kjellstrand P, Aurell A, and Haglid KG. 1986. Irreversible effects of xylene on the brain after long term exposure: A quantitative study of DNA and the glial cell marker proteins S-100 and GFA. Neurotoxicology 7:121-136.

Savolainen H, Vainio H, Helojoki M, and Elovaara E. 1978. Biochemical and toxicological effects of short-term, intermittent xylene inhalation exposure and combined ethanol intake. Arch. Toxicol. 41:195-205.

Savolainen K, and Linnavuo M. 1979. Effects of m-xylene on human equilibrium measured with a quantitative method. Acta Pharmacol. Toxicol. 44:315-318.

Savolainen H, and Seppalainen AM. 1979. Biochemical and physiological effects of organic solvents on rat axon membranes isolated by a new technique. Neurotoxicology 1:467-477.

Savolainen H, Riihimaki V, and Linnoila M. 1979. Effects of short-term xylene exposure on psychophysiological functions in man. Int. Arch. Occup. Environ. Health 44:201-211.

Savolainen H, Pfaffli P, Helojoki M, and Tengen M. 1979a. Neurochemical and behavioral effects of long-term intermittent inhalation. Acta Pharmacol. Toxico1. 44:200-207.

Savolainen K, and Riihimaki V. 1981. An early sign of xylene effect on human equilibrium. Acta Pharmacol. Toxicol. 48:279-283.

Savolainen K, Kekoni J, Riihimaki V, and Laine A. 1984. Immediate effects of m-xylene on the human central nervous system. Arch. Toxicol. Suppl 7:412-417.

Savolainen K, Riihimaki V, Luukkonen R, and Muona O. 1985. Changes in the sense of balance correlate with concentrations of m-xylene. Pr. J. Ind. Med. 42:765-769.

Sedivec V, and Flek J. 1976. The absorption, metabolism, and excretion of xylenes in man. Int. Arch. Occup. Environ. Health 37:205-217.

Shigeta S, Aikawa H, Misawa T, Suzuki K. 1983. Fetotoxicity of inhaled xylene in mice. Teratology 28:22A.

Silverman DM, and Schatz RA. 1991. Pulmonary microsomal alterations following short-term low-level inhalation of para-xylene in rats. Toxicology 65:271-281.

Smyth HJ, Carpenter CP, Weil CS, Pozzani UC, Striegel JA. 1962. Range-finding toxicity data: List VI. Am. Ind. Hyg. Assoc. J. 23:95-107.

Taskinen H, Kyyronen P, Hemminki K, Hoikkala M, Lajunen K, Lindbohm ML. 1994. Laboratory work and pregnancy outcome. J. Occup. Med. 36(3):311-319.

Tatrai E, and Ungvary G. 1980. Changes induced by o-xylene inhalations in the rat liver. Acta Med. Acad. Sci. Hung. 37:211-216.

Tatrai E, Ungvary G, Cseh IR, *et al.* 1981. The effects of long-term inhalation of ortho-xylene on the liver. Ind. Environ. Xenobiotica. Proceedings of International Conference, Prague, Czechoslovakia, May 27-30, 1980. New York, NY: Springer-Verlag, pp. 293-300.

Toftgard R, and Nilsen OG. 1981. Induction of cytochrome P-450 in rat liver after inhalation of aromatic organic solvents. In: Ind. Environ. Xenobiotics, Proceedings of International Conference, Prague, Czechoslovakia, May 27-30, 1980. New York, NY: Springer-Verlag, pp. 307-317.

Toftgard R, Nilsen OG, and Gustafsson J-A. 1981. Changes in rat liver microsomal cytochrome P-450 and enzymatic activities after the inhalation of n-hexane, xylene, methyl ethyl ketone and methylchloroform for four weeks. Scand. J. Work Environ. Health 7:31-37.

Toftgard R, and Nilsen OG. 1982. Effects of xylene and xylene isomers on cytochrome P-450 and *in vitro* enzymatic activities in rat liver, kidney, and lung. Toxicology 23:197-212.

Uchida Y, Nakatsuka H, Ukai H, Watanabe T, Liu YT, Huang MY *et al.* 1993. Symptoms and signs in workers exposed predominantly to xylenes. Int. Arch. Occup. Environ. Health 64:597-605.

Ungvary G, Cseh J, Manyai S. Molnar A, Szeberenyi S, and Tatrai E. 1980. Enzyme induction by o-xylene inhalation. Acta Med. Acad. Sci. Hung. 37:115-120.

Ungvary G, Tatrai E, Hudak A, Barcza G, Lorincz M. 1980a. Studies on the embryotoxic effects of ortho-, meta- and para-xylene. Toxicology 18.61-74.

Ungvary G, Varga B, Horvath E, Tatrai E, Folly G. 1981. Study on the role of maternal sex steroid production and metabolism in the embryotoxicity of para-xylene. Toxicology 19:263-268.

Ungvary, G. and Tatrai, E. 1985. On the embryotoxic effects of benzene and its alkyl derivatives in mice, rats, and rabbits. Arch. Toxicol. Suppl. 8:425-430.

Ungvary, G. 1985. The possible contibution of industrial chemicals (organic solvents) to the incidence of congenital defects caused by teratogenic drugs and consumer goods – an experimental study. In: Prevention of Physical and Mental Congenital Defects, Part B: Epidemiology, Early Detection and Therapy, and Environmental Factors. Prog. Clin. Biol. Res. 160:295-300.

Wimolwattanapun S, Ghosh TK, Mookherjee S, Copeland RL Jr, Pradhan SN. 1987. Effect of inhalation of xylene on intracranial self-stimulation behavior in rat. Neuropharmacology 26: 1629-1632.

Wolfe GW. 1988. Subchronic toxicity study in rats with m-xylene. Report by Hazleton Laboratories America, Inc., Rockville MD. Sponsored by Dynamac Corporation, Rockville, MD.

Response to Comments on the October 1997 Draft of the

Air Toxics Hot Spots Risk Assessment Guidelines Part III: Determination of Noncancer Chronic Reference Exposure Levels

Responses to Comments on the Methodology and the First Forty Chemicals

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Allied Signal - Naphthalene

Allied Signal Chemicals of Morristown, New Jersey, submitted comments on December 15, 1997 regarding the draft chronic reference exposure level for **naphthalene** presented in the OEHHA Technical Support Document for the Determination of Noncancer Chronic Reference Exposure Levels. The proposed chronic REL was based on a 2-year NTP study (1992) with mice. Olfactory epithelial metaplasia and respiratory epithelial hyperplasia were noted in most (>95%) exposed animals at the lowest concentration (10 ppm) tested but not in any of the control animals. Adjustment for discontinuous exposure and a cumulative 1000-fold uncertainty factor for interspecies differences, intraspecies variability, and lack of a NOAEL resulted in a proposed REL of 9 $\mu g/m^3$ (2 ppb).

Comment 1. In general, additional details of the study should be provided, such as species used, methods of administration, and all results (not just selected findings).

Response. The presentation of research findings has been reviewed and additional details have been added where warranted.

Comment 2. In addition, the results of an unpublished 13-week inhalation study in rats (Coombs, D.W., Kiernan, P.C., Hardy, C.J., Crook, D., Lewis, D.J., and Gopinath, C. Naphthalene: 13-Week Inhalation Study in Rats, Huntingdon Research Centre Ltd., England, Report No. LDA 2/930704, April 28, 1993) is not mentioned.

Response. The document cited has been requested from the study authors and, if a copy of this unpublished study is obtained, a review will be added to the naphthalene section of the OEHHA document.

Comment 3. The summary of the chronic inhalation bioassay in $B6C3F_1$ mice (NTP, 1992) should also include the results/conclusion for carcinogenicity, a primary objective of this study. The lesions mentioned in this summary should be identified as "nonneoplastic" lesions.

Response. The potential carcinogenicity of naphthalene was evaluated separately by OEHHA. OEHHA does not currently consider naphthalene to be a carcinogen, thus any lesions mentioned would be noncarcinogenic. The Technical Support Document focuses on noncancer endpoints as noted in the Introduction.

Comment 4. The data presented in Table 1 do not appear accurate, as the incidence for 0 ppm is for female mice and the incidences for 10 and 30 ppm are for male mice. It seems that the table should include consistent data for both sexes in order to be complete.

Response. OEHHA thanks the commentator for pointing out errors in the table. Corrections have been made.

Comment 5. The draft OEHHA document does not include results from an unpublished subchronic inhalation toxicity study in male and female Sprague-Dawley rats which was conducted by Huntingdon Research Centre Ltd., and then reviewed and found acceptable by the U.S. EPA (Coombs et al., 1993). Rats were exposed to naphthalene vapor for 13-weeks (snout only, 6 hours/day, 5 days/week) at nominal concentrations of 0, 2, 10 or 60 ppm. Compared to controls, treatment-related effects were observed in all groups. In the low-dose group (2 ppm or 0.01 mg/L), male and female rats had minimal degenerative changes and proliferative lesions in the nasal passages. In the mid-dose group (10 ppm or 0.052 mg/L), moderate degenerative and proliferative nasal passage lesions were produced, along with hypertrophy of the respiratory epithelium and decreased body weight gain and food consumption (males only). In the high-dose group (60 ppm or 0.315 mg/L), marked degenerative and proliferative nasal passage lesions were produced, as well as degenerate fibers in the spinal cord and sciatic nerve in one male, and decreased body weight gain and food consumption. Based on this information, the systemic NOEL for both sexes was estimated to be < 2 ppm (0.01 mg/L).

Response. As noted previously, OEHHA thanks the commentator for providing information about this unpublished study. OEHHA is attempting to obtain a copy for review. Based on the data summary provided by the commentator, the results obtained in this 13-week are consistent with those observed in the 104 week NTP bioassay.

Comment 6. The summary of the Shopp et al. study (1984) focuses only on a single finding for which the toxicological significance is not clear (dose-related inhibition of liver aryl hydrocarbon hydroxylase activity in both sexes). The summary should include results of all other parameters evaluated. At a daily dose level of up to 1/4 the LD50 (133 mg/kg) for 90 days, there was no treatment-related mortality, no significant effects on body weight, and no significant changes in organ weights, with the exception of reduced spleen weights in females at 133 mg/kg. Although an organ (spleen) associated with immune function showed decreased weight, there was no evidence of immunotoxicity in any treatment group of either sex. No treatment-related effects were seen in serum enzyme and electrolyte levels. A screen of the effects of the 90-day naphthalene treatment on various aspects of the liver drug metabolizing system indicated no alterations, with the exception of the specific dose-related inhibition of aryl hydrocarbon hydroxylase activity.

Response. Text has been added describing these additional findings of the study of Shopp and associates.

Comment 7. The summary of the Navarro et al. study (1991) does not mention the species used, the method of oral administration, or the number of days the animals were dosed. However, this information can be derived from the reference title. Pregnant female Sprague-Dawley CD rats were administered naphthalene (0, 50, 150, or 450 mg/kg/day) by gavage during gestational days 6-15. The adverse maternal effects observed at the low dose (50 mg/kg/day) consisted of transient clinical signs indicative of CNS depression. By the third day of dosing the dams

acquired a tolerance to the low dose and did not show maternal toxicity thereafter. However, the maternal toxicity (including CNS depression, reduced body weight, and altered food & water consumption) in dams receiving 150 and 450 mg/kg/day was greater and longer lasting.

Response. OEHHA thanks the commentator for this clarifying comment. Additional text has been added to the document.

Comment 8. The summary of the Harris et al, study (1979) does not indicate the method of administration of naphthalene or the species of animal used. Sprague-Dawley rats were administered naphthalene by ip injection.

Response. Clarifying details of this study have been added to the document.

Comment 9. The reference (U.S. EPA, 1986a or b) is confusing in the last two summaries (NZ white rabbit developmental study and rat dermal application study). The rabbit developmental study and the rat dermal application study are listed separately in the reference section as U.S. EPA, 1986 and U.S. EPA, 1986b, respectively.

Response. OEHHA thanks the commentator for noting this error; corrections have been made to the document.

Comment 10. It is our opinion that the use of an interspecies uncertainty factor of 10 and intraspecies uncertainty factor of 10 in the calculation of the REL for naphthalene (9 $\mu g/m^3$) is overly conservative and unrealistic based on the endpoint of upper respiratory system irritation. Rats and mice are far more susceptible to upper respiratory irritation than humans "based on (1) physiological differences in mode of breathing (obligate nasal for rat; oronasal for humans); (2) differences in overall geometry of the nasal passages, including the turbinate profiles; (3) the enormous difference in relative nasal cavity surface areas between rats and humans; (4) differences in the proportion of nasal cavity surface area covered by different epithelia; (5) differences in mucociliary clearance routes, especially in the anterior portion of the nasal cavity; and (6) differences in the inspiratory airflow routes. In rodents, almost 100% of a volatile chemical is absorbed or trapped in the tissues of the nasal passages.

Response. The data on naphthalene effects in the respiratory system suggest that the observed effects are <u>not</u> due to direct irritancy, but rather due to absorption and activation to a reactive metabolite. Thus interspecies comparisons of responses to direct acting irritants is not helpful. Naphthalene has low reactivity and low water solubility. Necrosis of olfactory epithelium (Plopper CG, Suverkropp C, Morin D, Nishio S, Buckpitt A, 1992, Relationship of cytochrome P-450 activity to Clara cell cytotoxicity. I. Histopathologic comparison of the respiratory tract of mice, rats and hamsters after parenteral administration of naphthalene, J Pharmacol Exp Ther 261(1):353-63) and of bronchial Clara cells (O'Brien et al., 1989, Tolerance to multiple doses of the pulmonary toxicant, naphthalene, Toxicol. Appl. Pharmacol. 99(3):487-500) have been noted

in rodents following intraperitoneal injection of naphthalene. Respiratory epithelial cells, as well as liver, appear to be major sites of activation of naphthalene to toxic intermediates.

Comment 11. For example, 10 ppm of naphthalene vapor, which is the NIOSH 10-hour TWA value recommended to protect humans from exposure to naphthalene vapor in the work place, produced an incidence of 96% and 100% nasal effects in male and female mice, respectively in the NTP study (1992). Furthermore, ACGIH recommends an 8-hour TLV TWA value of 10 ppm in view of the fact that irritation is experienced in humans at 15 ppm and that continued exposure at that concentration may result in fairly serious eye effects. (See American Conference Governmental Industrial Hygienists (ACGIH). Documentation of the Threshold Limit Values (TLVs) for Chemicals in the Work Environment, 5th Ed., 1986).

Response. Comparison of the REL with an occupational standard is not particularly informative. OEHHA agrees with the recommendations of USEPA and ACGIH itself that the occupational exposure limits such as NIOSH RELs and ACGIH TLVs are not an appropriate basis for the derivation of RELs to protect the general public, including sensitive subgroups, from exposures over a lifetime.

Comment 12. Taking into account the well documented interspecies differences with respect to nasal or upper respiratory irritation, an interspecies uncertainty factor of 3 is recommended as a more realistic uncertainty factor to use in the calculation of the REL. In fact one could easily justify an uncertainty factor of one.

Response. As noted previously, the data on naphthalene effects in the respiratory system suggest that the observed effects are <u>not</u> due to direct irritancy, but rather due to absorption and activation to a reactive metabolite. It is true that a default interspecies uncertainty factor of 3 was used by OEHHA for most chemicals for which a human equivalent concentration (HEC) was estimated. However, in the case of naphthalene, a factor of 3 was considered to be inadequate, because the major effects noted in human populations exposed to naphthalene, namely hemolytic anemia and cataracts, were not noted in animal studies. Thus available animal studies may underpredict human risks because of the relative insensitivity of rodents to these effects. Therefore a 10-fold interspecies uncertainty factor was used.

Comment 13. In addition, it is thought that the intraspecies uncertainty factor of 10 is much too conservative with materials that are irritants. The distribution from normal to sensitive populations is generally approximately 3-fold. (See Rotman, H.H., Fliegelman, M.J., Moore, T., Smith, R.G., Anglen, D.M., Kowarski, C.J. and Weg, J.G. Effects of low concentration of chlorine on pulmonary function in humans. J. Appl. Physiol. 54: 1120-1124, 1993).

Response: As noted in the previous response, since the mechanism of naphthalene respiratory toxicity differs from that for direct irritants, the variability in human responses to such irritants is not relevant to assessing the variability in human response to naphthalene exposure. In addition, the range of interindividual response may be broad for some irritants (e.g., formaldehyde).

Comment 14: In summary, a more practical REL of 0.022 ppm (~100 $\mu g/m^3$) is calculated when an uncertainty factor of 3 is used for both interspecies and intraspecies variability. Considering that only marginal effects were seen in a subchronic inhalation study at 2 ppm in rats, a sensitive species for nasal irritants, 0.022 ppm would clearly represent a safe level for chronic exposure to the general population.

Response: As described above, OEHHA can not support the changes suggested by the commentator in this case. Recently USEPA developed an inhalation reference concentration (RfC) for naphthalene. The USEPA RfC is in fact 3-fold *lower* than that proposed by OEHHA. USEPA used the same study, endpoint, and uncertainty factors as those proposed in 1997 by OEHHA. USEPA, however, added an additional 3-fold database uncertainty factor because of the lack of a two-generation reproductive toxicity study and chronic data for additional species.

Allied Signal – Allied Engineered Materials

Comments on the methodology used in the chronic REL TSD were made by Dr. George Rusch of Allied Signal – Allied Engineered Materials in a letter dated December 19, 1997.

Comment 1. I have reviewed the Technical Support Document dated October 1997, pages 1 - 46. In my judgement, it is well thought out and clearly presents the process to be used for the calculation of RELs. The consideration given to existing guidelines such as the RfDs, ADI and various occupational exposure guidance levels is well described as is the utilization of both toxicology and epidemiology studies. Again, in the area of risk assessment, the utilization of NOAELs and LOAELs are well described. I strongly support the use of variable uncertainty factors which take into consideration the type and severity of the effects observed and their relevance to man. Table 2 on page 21 is most informative. Utilization of all the considerations described in Section 3.2 - 3.6 on pages 22 -31 can lead to robust risk assessments. Table 7 presents a valuable, flexible approach in the calculation of uncertainty factors.

It is, however, important in reviewing the data on specific chemicals that uncertainty factors be carefully selected to most precisely estimate the true uncertainty. There can be a tendency, when looking at multiple uncertainties, to treat each in a conservative fashion such that the combined uncertainty factors is not reflective of the true uncertainty.

Response. The issue of treatment of multiple areas of uncertainty is an area of ongoing evaluation by both OEHHA and US EPA. Multiplying several uncertainty factors could yield unnecessarily conservative exposure guidelines. Thus US EPA has set the maximum uncertainty factor at 3,000 to partially offset such concerns. On the other hand, there are numerous areas of uncertainty not specifically addressed with conventional uncertainty factor approaches. Analysis of data on human variability, including genetic variability, by Dale Hattis and others indicate that human interindividual variability may be much greater than 10-fold for some chemicals. A factor of 10 for intraspecies variability will not be adequate for those chemicals. If an animal model is insensitive to a certain chemical, such as the rabbit model was to thalidomide, the standard interspecies uncertainty factor of 10 would also not protect humans. These uncertainties have been considered to potentially offset any over-conservatism arising from aggregation of multiple uncertainty factors. OEHHA's goal is to develop better data-based approaches in the future, but such methods are time-consuming and data-intensive.

Comment 2. It is also important, in the selection of key studies as the basis for the REL, that the most significant studies be given the greatest consideration. Again, there can be a tendency to select studies with low NOAELs over others that may be of higher quality and greater relevancy to the endpoint being evaluated. Focusing on key studies of good scientific quality will result in meaningful, valuable risk assessments. In contrast, risk assessments that use poor data and large or unsupported uncertainty factors, lack credibility and ultimately are of limited value for protecting the public.

Response. OEHHA agrees that human relevance and data quality are key issues. Human relevance was emphasized and human exposure data were used wherever possible. In numerous cases the key study did not involve the lowest exposure concentration for which adverse effects

have been claimed. In one case a chemical under review was dropped from the document because the scientific database was judged to be inadequate. Numerous other chemicals of concern to California air quality agencies were not included in the current document because of the poor quality of the scientific database. The need for large uncertainty factors can best be addressed by the development of better data. Most toxicity studies have been designed to acquire data for purposes other than health risk assessment and thus are not optimal for that purpose. But by the same token, failing to address potential health impacts because there are limitations in the scientific database would be imprudent public health policy.

Comment 3. My concern is based in part on a brief review of the "Proposed OEHHA Chronic Inhalation REL Summary" which lists RELs for many chemicals. Many of the actual values presented in this table appear to be very conservative. For example, the value of 100 $\mu g/m^3$ for ammonia, a normal biological metabolite, is well below the odor and irritation threshold; that for fluoride is an order of magnitude below the typical dose received by ingestion; and the level for dichlorodifluoromethane is one thousand times below the occupational exposure limit and over 50 thousand times below even marginal effect levels. I would suggest that those conducting the risk assessments review the guidance in the Technical Support Document and reconsider their calculations and approach where the values do not appear supported by the data. This will greatly increase the value of these guidance levels.

Response. The use of uncertainty factors was judicious and compares favorably with those used by US EPA in the derivation of Reference Concentrations (Table 9 of the Chronic Reference Exposure Level TSD). Occupational exposure limits (OELs) were by design not incorporated into this document. This is in accord with the developers of the OELs, who have cautioned against use of such values for protection of the general public. OEL values lack a consistent basis, are designed for healthy workers, and frequently approximate more closely a LOAEL rather than a NOAEL. In addition, frank toxic effects occur in some workers at some TLVs.

The ammonia RfC was adopted as the proposed chronic REL because the US EPA evaluation was considered adequate by OEHHA.

In the case of fluoride, differences in toxicity by route of exposure are known for several chemicals. For example, nickel, chromium VI, beryllium, and cadmium are much more toxic by the inhalation route than by the oral route.

In the case of dichlorodifluoromethane, OEHHA used the maximum uncertainty factor of 3,000 because staff based the REL on a LOAEL from an animal 90-day study (defined by USEPA as a “subchronic study” in rats and mice). To use different UFs we would have to change the default procedure in some way such as considering a guinea pig 90 day experiment to be chronic (and thus using a lower UF) or to have evidence that humans were not more sensitive to dichlorodifluoromethane than animals or that there was limited intraspecies variability to dichlorodifluoromethane. If we had the latter type of information, we would probably also have human data on which to base the chronic REL.

California Mining Association

Comments on the chronic REL for **hydrogen cyanide** were made by Denise M. Jones, Executive Director of the California Mining Association. The proposed chronic REL for HCN is the USEPA RfC of 3 $\mu g/m^3$.

Comment 1. Based on our review of this document, the California Mining Association strongly recommends against adoption of the proposed Chronic Reference Exposure Level (REL) for hydrogen cyanide (HCN). Several of CMA's member companies use dilute sodium cyanide solutions as an essential component of their ore extraction processes. Potential emission sources include heap leach pads, leaching tanks, solution retention ponds, carbon circuit tanks, and electrowining processes. The results of numerous studies investigating potential HCN emissions from the preceding sources are regulated by the Mine Safety and Health Administration (MSHA) at 10 ppm or less in the work place. The MSHA HCN exposure limit is based on peer-reviewed data and was ratified during a formal rulemaking process.

Response. The proposed chronic REL for HCN is the USEPA RfC of 3 $\mu g/m^3$, which has been in use since 1994. All USEPA Reference Concentrations (RfCs), available when the Technical Support Document (TSD) on chronic Reference Exposure Levels was drafted in October 1997, are being used as chronic RELs. RfCs are already used by the USEPA and by California's Department of Toxic Substances Control and were earlier incorporated by reference in Appendix F of the Emissions Inventory Criteria and Guidelines for the Air Toxics "Hot Spots" Program for use in screening risk assessments in the Hot Spots Program. These Guidelines were effective July 1, 1997. The Risk Assessment Advisory Committee (RAAC) recommended that CalEPA harmonize where possible with USEPA on risk assessment. Governor Wilson's Executive Order W-137-96 concerned the enhancement of consistency and uniformity in risk assessment between Cal EPA and USEPA. Use of RfCs as chronic RELs was one action that OEHHA took to address the RAAC recommendation and to implement the Executive Order. RfCs released after October 1997, including ones that are revisions of those in the October 1997 draft, will be evaluated for use in the Hot Spots program by reviewing the scientific basis of each RfC when it becomes available and by determining whether the scientific literature cited in the RfC is appropriate. Appropriate RfCs will be submitted yearly to the SRP for their review and possible endorsement.

The MSHA HCN level is for healthy workers exposed during a normal work-week. The chronic REL is for continuous ambient exposure for the entire population including sensitive individuals, such as infants, children, the elderly, and the respiratory impaired. The MSHA HCN value is not relevant for such exposure, other than to indicate that the chronic REL should be lower than it. OEHHA staff note that the ACGIH has a STEL (ceiling limit) of 5 ppm (5.5 mg/m^3) for HCN, somewhat lower than the MSHA value of 10 ppm.

Comment 2. OEHHA has proposed adoption of the U.S. EPA reference concentration (RFC) for HCN, as published in the IRIS database. Supporting documentation provided for the proposed HCN Chronic REL indicates that U.S. EPA's RFC is based on <u>only</u> one study, performed in Egypt and published in 1975. The Egyptian study evaluated 36 male electroplating

workers in three factories using a mixture of chemical compounds containing cyanide. Based on our review of available literature, and our members considerable experience in the evaluation of the potential health effects of chronic compounds used in the mining industry, the U.S. EPA adopted the HCN RFC without sufficient scientific study. The following summarizes our specific concerns regarding the Egyptian study and U.S. EPA's RFC. No NOAEL was identified. It is uncertain as to whether HCN exposure levels were accurately characterized. No information was supplied to evaluate potentially conflicting factors, such as food supply, lifestyle or working conditions. As a result of the preceding issues, it is not possible to objectively determine whether OEHHA's proposed Chronic HCN REL is more protective of human health than the existing HCN REL. In conclusion, based on our review of available data, no compelling scientific evidence has been supplied by U.S. EPA or OEHHA to justify adoption of the proposed HCN chronic REL. As a result, we strongly recommend against adoption of the proposed HCN chronic REL.

Response. As stated above the chronic REL is for ambient exposure of the general population. The comment points out some of the problems with using human data. Although the REL is based on a single study of 36 exposed humans, hydrogen cyanide is a known metabolic poison and needs a chronic REL. However the commentator does not suggest an alternative study for the development of the REL. The existing animal studies would likely result in a much lower chronic REL due to their subacute/subchronic duration and to the HCN levels tested. OEHHA would be pleased to review any superior study and would also encourage USEPA to do the same.

Chemical Manufacturers Association (CMA) - Ethylene Glycol Panel

Comments on the chronic REL for **ethylene glycol** were received from the Ethylene Glycol Panel of the Chemical Manufacturers Association (CMA). Panel members include BASF Corporation, the Dow Chemical Company, Eastman Chemical Company, Huntsman Corporation, Occidental Chemical Corporation, Shell Chemical Company, and Union Carbide Corporation. OEHHA staff developed a chronic REL of 400 $\mu g/m^3$ based on a 37 day inhalation exposure of prison volunteers to ethylene glycol.

Comment 1. The technical support document provides an ethylene glycol chronic inhalation reference exposure level (REL) of 400 $\mu g/m^3$. However, the Panel notes that the calculations used to derive the REL are flawed. In addition, the uncertainty factors used in the ethylene glycol assessment are overly conservative and should be lowered. As outlined below, using the appropriate values in the calculations and more appropriate uncertainty factors, the chronic inhalation REL for ethylene glycol should be set at 730 $\mu g/m^3$, or higher.

In the technical support document, there appears to be an error in the formula used to derive "ppm" exposure values from "mg/m^3"values. For example, the document indicates that the proposed REL of 0.2 ppm ethylene glycol would be equivalent to 400 $\mu g/m^3$. However, based on the appropriate formula, indicated below, 0.2 ppm ethylene glycol would actually be equivalent to 0.508 mg/m^3, or 508 $\mu g/m^3$.

$$mg/m^3 \text{ at } 25`C \text{ and } 760 \text{ mm Hg} = \frac{\text{ppm x molecular weight (EG = 62.07)}}{24.45}$$

$$1 \text{ ppm} = 2.53865 \text{ mg/m}^3$$

$$0.2 \text{ ppm} = 0.508 \text{ mg/m}^3 \text{ or } 508$$

Response. The formula used to convert ppm to mg/m^3 in the document is correct. The human equivalent concentration of 16.7 ppm was divided by a UF of 100 to get an REL of 0.167 ppm or 423 $\mu g/m^3$. Unfortunately both values were subsequently rounded separately to 0.2 ppm and 400 $\mu g/m^3$. When the 0.167 ppm is rounded first to 0.2 ppm, the equivalent value is 508 $\mu g/m^3$, which is rounded to 500 $\mu g/m^3$. Based on this comment OEHHA staff will revise the chronic REL to 500 $\mu g/m^3$.

Comment 2. The exposure levels used as the basis for the REL are inappropriately reported in the technical support document. According to the support document, the basis of the REL is the Wills *et al.* (1974) study, with the REL calculated from the average exposure level from the No Observed Adverse Effect Level (NOAEL). The Wills study, "Inhalation of Aerosolized Ethylene Glycol by Man," is a human study that was conducted by NASA to evaluate the irritant and systemic effects from continuous exposure to ethylene glycol aerosols. During the main study, "twenty volunteers were exposed during 20 to 22 hours per day to aerosolized ethylene glycol in mean daily concentrations between 3 and 67 mg/m^3." However, in its review of the Wills study, the technical support document indicated that the upper end of the exposure concentrations was only 49 mg/m^3 (reported as 20 ppm in the technical document), rather than 67 mg/m^3 (or 26.4 ppm).

Using the correct NOAEL value of 67 mg/m^3 in the derivation equation and the appropriate conversion factor between "mg/m^3 and "ppm", the calculated human equivalent concentration should be 22 ppm. (See footnote 2.)

Response. OEHHA staff used the highest mean value of 49 mg/m^3 (20 ppm) as the No-Observed-Adverse-Effect-Level (NOAEL), not the highest high value of 67 mg/m^3 (rounded from 66.8). (See Table 1 from Wills et al. below.) OEHHA staff believe that 49 mg/m^3 is the appropriate NOAEL.

Table 1. Concentrations of Ethylene Glycol in the Air within the Exposure Chamber

	Concentration of ethylene glycol in air, mg/m^3		
Days	Low	High[a]	Mean
1-7	3.6	75.0	37
8-14	18.8	44.8	29
15-21	0.8	41.6	17
22-28	3.5	49.2	23
29-35	20.6	**66.8**	**49**
36-37	14.4	39.0	31

[a] This column does not include the very high concentrations maintained for comparatively brief periods.

Comment 3. The subchronic uncertainty factor should be reduced from 10 to 3. EPA's IRIS database states that an uncertainty factor "of 3 is used for extrapolation from subchronic to chronic duration due to limited progression between short-term and subchronic exposure and due to rapid metabolism." Ethylene glycol has been shown to have rapid metabolism. Moreover, ethylene glycol respiratory irritation response is due to short-term exposure and does not appear to worsen at subchronic exposure duration, which is supported by the observation in the Wills study with humans exposed up to 30 days.

Response. OEHHA staff consider 30 days to be too short to be considered for a factor less than 10. This length of 30 days is considered by OEHHA staff to be subacute for humans. It is only 0.12% of the 70-year human life span versus 4.2% of a rodent's 2-year life span. Although respiratory irritation might not worsen, the factor of 10 protects against other known systemic effects that may occur over long-term exposure to ethylene glycol in the other 99+% of the human life span.

Comment 4. In addition, it should be noted that the American Conference of Governmental Industrial Hygienists (ACGIH) also considered the Wills study when recommending an occupational exposure level for ethylene glycol. Because of the reported irritation of the upper respiratory tract at 140 mg/m^3 (the LOAEL), ACGIH selected 100 mg/m^3 as the ethylene glycol TLV ceiling value. To the Panel's knowledge, no systemic toxic or irritant effects have been reported in humans from inhalation of ethylene glycol at concentrations less than 100 mg/m^3.

With the corrected human equivalent concentration and the appropriate uncertainty factors applied, the chronic inhalation REL for ethylene glycol should be at least 730 $\mu g/m^3$ (0.287 or 0.3 ppm).

Response. Comment noted. The TLV is for healthy workers and may not protect sensitive individuals in the general population. Furthermore, as stated above, OEHHA staff do not consider 30 days to be a subchronic exposure for humans, although respiratory irritation might not worsen with longer exposure.

Comment 5. The summary section indicates that the critical effects include eye irritation in humans. However, the Wills study did not report any eye irritation, but only respiratory irritation.

Response. The Wills et al. (1974) study did not report any eye irritation. OEHHA staff will delete the reference.

Comment 6. SECTION III - MAJOR USES AND SOURCES: The Panel is concerned with some of the listings in this section which appear to be incorrect, and potentially, dangerously misleading. For example, the technical support document indicates that ethylene glycol is used as a vehicle in some pharmaceutical preparations or as a flavoring, which are inappropriate and certainly not uses recognized by the Panel members. Attached is an information sheet developed by the Panel that provides an overview of recognized uses of ethylene glycol.

Response. This information is from the ethylene glycol file in HSDB and is based on the 1965 edition of Ethel Browning's Toxicology and Metabolism of Industrial Solvents. The file was reviewed by a scientific review panel in 1990. The Ethylene Glycol Panel should make its information available to the National Library of Medicine, maintainers of the HSDB.

Comment 7. SECTION IV - EFFECTS OF HUMAN EXPOSURE. The technical support document includes a review of the Laitinen *et al* study (1995). Attached is the Panel's critique of the Laitinen study. As you will note, given the concerns raised on the clinical chemistry and the analytical methodology; and the findings of ethylene glycol in the control samples, the findings of this study would have to be considered questionable at best. The Panel is also providing a copy of its recent information sheet, "Ethylene Glycol: Research Shows that Normal Skin Contact Is Not Expected to Cause a Health Hazard", which provides an overview of available dermal data on ethylene glycol. In fact, the American Conference of Governmental Industrial Hygienists concluded that a "skin notation", a designation referring to potential exposure by the dermal route, was not necessary for ethylene glycol, given its negligible absorption through the skin

Response. Laitinen et al. made a tentative conclusion from their study of 10 car mechanics: "Therefore, it seems that ethylene glycol is absorbed by skin contact." The study was published in a respected occupational journal and may be questioning the accepted belief about skin

absorption. The point is not critical to the development of the chronic REL. The critique should be sent to the authors of the article or to the journal *Occupational Medicine (Oxford)*.

Comment 8. SECTION V - EFFECTS OF ANIMAL EXPOSURE. The first paragraph of this section includes a review of the DePass study, which indicates that the exposure levels for both rats and mice were 0, 0.04, 0.2, or 1 g/kg/day, and that there were no effects observed in mice. Therefore, the NOAEL for mice would be 1 g/kg/day (1000 mg/kg/day), and not 40 mg/kg/day, as reported in the last sentence.

Response. OEHHA staff regret the error and will change the sentence.

Comment 9. The fourth paragraph of this section includes a review of the Tyl mouse nose-only inhalation study. The technical support document indicates that the NOAEL for maternal effects in this study was 500 mg/m^3, based on increased kidney weights. However, the significance of the kidney weight change has been questioned. The study author herself has concluded "The absence of any treatment-related maternal renal lesions is not unexpected since the mouse appears resistant to ethylene glycol-induced nephrotoxicity after short term exposure, especially since the systemic exposure in the present study is lower than that previously employed (see for example, Price et al., 1985). There is no apparent explanation for the increased maternal kidney weights observed at 1000 and 2500 mg/m^3 by nose-only exposure, in the absence of any microscopic lesions." The Panel believes that the control values were abnormally low in this study. There were no statistically significant differences for relative kidney weights at 1000 mg/m^3 and there were no absolute or relative weight differences in three other inhalation studies at concentrations as high as 2500 mg/m^3. Given that there were no microscopic lesions in the kidneys, that the findings have not been reproduced, and that the author has indicated no apparent explanation for the findings, the kidney weight change should not be used as the basis for the maternal NOAEL in the Tyl study.

Response. Comment noted. OEHHA staff will review the Tyl study in mice, especially the data on controls. However, the true NOAEL for the Tyl study in mice will not influence the chronic REL, which is based on a NOAEL of 50 mg/m^3 (20 ppm) found in a study of respiratory tract irritation in people. The Tyl study also will not influence the respiratory irritation endpoint.

Chemical Manufacturers Association – Isopropanol Panel

Comments on the chronic REL for **isopropanol** (IPA) were made by the Isopropanol Panel of the CMA in a letter from Courtney M. Price dated January 29, 1998. The IPA Panel includes all major U.S. manufacturers of IPA, including: Exxon Chemical Company, BP Chemical Company, ARCO Chemical Company, Shell Chemical Company and Union Carbide Corporation. OEHHA developed a chronic REL of 2,000 $\mu g/m^3$ based on a subchronic (13 week) inhalation study in mice and rats by Burleigh-Flayer et al. (1994).

The Panel believes that IPA should not be listed as an air toxic. Accordingly, the Panel has filed a petition with the California Air Resources Board requesting that IPA be removed from the California list of air toxics. The petition explains that an extensive database exists for IPA and demonstrates that this chemical poses low toxicological concerns. Indeed, IPA is not regulated by the federal government under any environmental statute based on toxicity concerns. A copy of the petition is attached to the comments.

The Panel further asserts:

- OEHHA's chronic toxicity summary for IPA omits discussion of the results of the rat and mouse chronic inhalation studies, which, along with numerous other studies, were conducted by the Panel pursuant to a test rule under Section 4 of the Toxic Substances Control Act (TSCA).

- Any chronic REL for IPA should be derived from the rat chronic inhalation study. This study was of chronic duration by the most relevant route of exposure and demonstrates the lowest "critical toxic effect" of the IPA studies - exacerbation of chronic progressive nephropathy - with a NOAEL of 500 ppm. Applying OEHHA's REL methodology to this NOAEL results in an REL of 7.3 mg/m^3 (3.0 ppm).

- The toxicity summary as currently written bases the critical effect for the chronic REL on changes that are not adverse or relevant to human health. Specifically, OEHHA relies on hyaline droplets in the kidneys of male rats, although the toxicity summary states that these effects are not relevant to humans, and on increased relative liver weights in female mice, although the summary states that this effect may be considered more of a metabolic response than a toxic effect.

- The toxicity summary also credits several questionable findings that the Panel believes should not be included in the toxicity summary. Specifically, the IPA chronic toxicity summary lists three indicators of chronic adverse effects: the development of tolerance of narcosis, blood chemistry changes and reduced fetal body weights. The Panel, however, questions the relevance of the first two effects and the validity of the third effect. The Panel therefore believes that OEHHA should remove references to these questionable findings from its chronic toxicity summary for IPA.

The following Appendices were included:

APPENDIX A. OECD SIDS Dossier for Isopropanol
APPENDIX B. OECD SIDS Initial Assessment Report (SIAR) for Isopropanol
APPENDIX C. Petition of the CMA Isopropanol Panel to Remove Isopropanol from the California List of Air Toxics, Submitted to the California Air Resources Board January 29, 1998
APPENDIX D. Kapp *et al.*, (1996). Isopropanol: Summary of TSCA Test Rule Studies and Relevance to Hazard Identification. *Regulatory Toxicology and Pharmacology* 23: 183-192.
APPENDIX E. Burleigh-Flayer, H., Garman, R., Neptun, D., Bevan, C., Gardiner, T., Kapp, R.,. Tyler, T., and Wright G. (1997). Isopropanol vapor inhalation oncogenicity study in Fischer 344 rats and CD - 1 mice. *Fundam. Appl.* Toxicol., 36:95-111.

Comment 1. Under a Toxic Substances Control Act (TSCA) test rule, Panel members have sponsored extensive studies of IPA, including chronic inhalation studies in rats and mice. All of these studies have been published in the peer-reviewed literature. In addition, the Panel recently worked collaboratively with the United States Environmental Protection Agency (EPA) to prepare a SIDS dossier and SIDS Initial Assessment Report (SIAR) for IPA. Copies of the SIDS dossier and SIAR are attached to these comments as Appendices A and B, respectively.

IPA should not be regulated as an air toxic. The California Air Toxic "Hot Spots" Program lists over 400 chemicals, including IPA, as air toxics for which emissions must be reported and a risk assessment may be required. The Panel believes, however, that IPA is not properly listed as an air toxic. An extensive database exists on the toxicity of IPA and demonstrates that this chemical poses low toxicological concerns. Indeed, IPA is not regulated by the federal government under any environmental statute based on toxicity concerns. IPA is not listed as a federal hazardous air pollutant (HAP), nor is it listed as a "hazardous waste" under the Resource Conservation and Recovery Act (RCRA), a "hazardous substance" under the Comprehensive Environmental Response, Compensation and Liability Act (CERCLA), or a "toxic chemical" under Section 313 of the Emergency Planning and Community Right-to-Know Act (EPCRA). 2 IPA's workplace exposure limits (OSHA permissible exposure limit of 400 ppm) confirm that it is relatively non-toxic.

Moreover, IPA has relatively low photochemical reactivity and has been approved as a substitute for ozone-depleting substances (ODSs). Thus, the removal of IPA from the California air toxics list would facilitate pollution prevention efforts in California, while its retention on that list merely results in unnecessary and wasteful expenditures. Because no toxicological basis exists for listing IPA as an air toxic, and because its listing may have an adverse effect on pollution prevention efforts in California, the Panel has submitted a petition to the California Air Resources Board (CARB) requesting that IPA be removed from the air toxics list. A copy of that petition is included as Appendix C. At a minimum, however, OEHHA should revise its chronic toxicity summary and REL for IPA as described below.

As described in the Panel's petition to remove IPA from the California list of air toxics, EPA has determined that IPA does not meet the criteria for listing under Section 313 of EPCRA; only "isopropyl alcohol (manufacturing - strong acid process") is included on this list. However, the strong acid process is no longer used in the United States.

Response. Isopropanol is currently listed as a Hot Spots chemical and therefore a chronic REL is being proposed. If isopropanol is delisted in California as a Toxic Air Contaminant, then it would likely be removed from the Hot Spots list and the chronic REL would be withdrawn.

Comment 2. The chronic toxicity summary for IPA should discuss the chronic inhalation studies in mice and rats. Because of the extensive studies of IPA conducted by the Panel under a TSCA Section 4 test rule, IPA's human health hazard potential has been extremely well-characterized. The studies that were included in this testing program are identified in Table 1. A review article, which includes citations to several publications of individual studies, is attached as Appendix D.

The EPA's Office of Pollution, Prevention and Toxics (OPPT) has already carefully reviewed all of these studies, and the Agency's assessment of these studies is reflected in a risk management (RM 1) review of IPA, as well as in the SIDS Initial Assessment Report (SIAR) prepared by the Panel and approved by EPA as part of the OECD SIDS program. Moreover, the Panel has striven to ensure that all data produced from its studies are published in the peer-reviewed literature.

TABLE 1. Health Effects Testing for IPA TSCA Section 4 Test Rule Studies

	Test Submission Of Final Report
Mutagenicity Study: Mammalian Cells in Culture	06/90
Developmental Toxicity Studies in Rats and Rabbits	12/90
Subchronic Inhalation Toxicity Studies in Rats and Mice	03/91
Mutagenicity Study: In Vivo Cytogenetics: Micronucleus	03/91
Acute and Subchronic Neurotoxicity Studies in Rats	03/91
Oral and Inhalation Pharmacokinetics Studies in Rats and Mice	03/91
Developmental Neurotoxicity Study in Rats	08/91
Reproductive Toxicity Study in Rats	05/92
Chronic Studies in Rats and Mice	06/94

OEHHA's chronic toxicity summary discusses several of these studies but fails to include the chronic inhalation studies conducted on rats and mice. Indeed, the summary states that a "weakness of the data base for" IPA is that "[n]o long-term studies, spanning a majority of the life span of the test animal, have been performed with isopropyl alcohol." OEHHA's incorrect statement, coupled with its failure to include a discussion of the most recent and relevant studies in the IPA chronic toxicity summary, creates the misleading impression that IPA is a high-production volume chemical that has not been adequately tested. That impression is wrong and should be corrected. In the end, the risk assessments conducted under the Hot Spots program will only be as good as the underlying assumptions and chemical-specific data on which OEHHA relies. It is critical that the most accurate and reliable health hazard information be used. The Panel therefore believes strongly that, if IPA is included in the Hot Spots program, then the technical support document should utilize the chronic studies sponsored by the Panel under TSCA Section 4.

Response. OEHHA prefers to base its RELs on peer-reviewed articles that have been published in the medical and toxicological literature. The chronic study appeared in the peer-reviewed literature after staff had completed its literature review. A summary of the chronic study has been added to the section on Effects of Animal Exposure. The chronic study has been used as the basis of a revised chronic REL.

Comment 3. Any chronic REL for IPA should be derived from the rat chronic inhalation study conducted under TSCA Section 4. Because OEHHA apparently was unaware of the chronic studies of IPA, the proposed IPA REL was calculated using a subchronic inhalation study. Clearly, however, the recent chronic inhalation studies in mice and rats (Burleigh-Flayer et al., 1997), which were conducted in accordance with EPA test guidelines, are more suitable for calculating a chronic REL than the subchronic studies. The results of the chronic studies are summarized below. [OEHHA staff have omitted the commentator's summary of the (negative) results about carcinogenicity since they are not relevant to the chronic REL.]

With regard to systemic chronic toxicity, Burleigh-Flayer *et* al. report that equivocal minimal to mild kidney effects, including renal tubular dilation, were observed in mice. The incidence of renal tubular proteinosis was generally significantly increased for all male and female treatment groups relative to controls; however, the majority of affected animals showed minimal degrees of tubular proteinosis *(i.e.*, only a few tubules affected), there was no concentration-related gradient in either the frequency or severity of this change, and there was no corresponding evidence of alterations to the glomeruli. Mild to moderate degrees of tubular dilation were observed in a small number of females in the 2500 and 5000 ppm groups, but were significantly increased only for the 5000 ppm group. Moreover, this finding was not duplicated in male mice (a significant increase was seen only for the 500 ppm group, but not at the higher dose levels), nor was it accompanied by evidence of tubular cell degeneration or urinary outflow obstruction.

Kidney effects also were observed in the rat following chronic exposure. These effects included some organ weight changes and an exacerbation of chronic progressive nephropathy that occurred in both male and female rats at 2500 and 5000 ppm. Chronic progressive nephropathy is a spontaneous kidney disease of unknown etiology that occurs commonly in aged rats. Although the human health relevance of this condition is unknown, the study researchers considered the effect to be treatment-related and adverse to the rat. (It was considered to be the likely cause of early mortality in some male rats). Exposure to 500 ppm IPA did not produce any effects on the kidney, and is considered the NOAEL for this study.

The Panel believes that rat chronic inhalation study (Burleigh-Flayer *et al, 1997)* should be considered the "critical study" for derivation of an REL for IPA. The study was of chronic duration (two years), by the most relevant route of exposure (inhalation) and demonstrates the lowest "critical toxic effect" of the IPA studies - the exacerbation of chronic progressive nephropathy that occurred in both male and female rats at 2500 and 5000 ppm. This effect should be considered the "critical toxic effect" because it was the likely cause of early mortality in some rats and the study researchers considered the effect to be treatment-related and adverse.

As described above, equivocal effects to the kidney (tubular proteinosis and dilation) were also reported in mice following chronic exposure to IPA vapor. However, it was not clear whether these effects were treatment-related, nor was it clear whether they were adverse effects. Accordingly, the Panel does not consider these effects to be as appropriate for consideration as the "critical toxic effect."

As noted above, OEHHA has generally adopted EPA's RfC methodology for calculating chronic RELs. *See* EPA, *Methods for Derivation of Inhalation Reference Concentrations and Application of Inhalation Dosimetry*, EPA/600/8-90/066F (October, 1994).

As described above, at 2500 and 5000 ppm, the rats experienced chronic progressive nephropathy. Thus, 2500 ppm should be considered the LOAEL for kidney effects, and 500 ppm can be considered the NOAEL. The NOAEL then must be converted into mg/m , and dosimetrically adjusted to provide a human equivalent concentration (HEQ NOAEL, accounting for the non-continuous duration of the dosing in the inhalation study. Id. at 4-2 1.

Critical Study:	IPA Chronic Rat Inhalation Study (Burleigh-Flayer *et al* 1997)
Critical Toxic Effect:	Chronic progressive nephropathy
Study NOAEL (ppm):	500 ppm
Study NOAEL (mg/m3):	500 ppm x 60.11 */24.45 = 1229 mg/m^3
NOAEL[ADJ]:	1229 x 6/24 x 5/7 = 220 mg/m^3
NOAELWCI:	NOAEL[ADJ] x [b:a lambda(a)/b:a lambda(h)]**
	220 mg/m^3 x 1 = 220 mg/m^3

The molecular weight of IPA is 60.11. For water-soluble compounds such as IPA, a factor of one is used.

Consistent with EPA's RfC methodology, OEHHA's methodology involves the application of uncertainty factors to the NOAELWC1 to arrive at an REL that is designed to be protective of the general population receiving repeated daily exposures over the course of a lifetime. Uncertainty factors can range from one (no uncertainty) to ten (the highest value), with a median of three, for five separate categories of uncertainty: (1) protection of sensitive human subpopulations; (2) extrapolating from animal data to humans; (3) extrapolating from subchronic to chronic exposure levels; (4) extrapolating from a LOAEL to a NOAEL; and (5) accounting for an incomplete data set. [According to EPA, however, a composite (maximum) uncertainty factor involving four areas of uncertainty would be 3,000, reflecting the fact that these factors are interdependent.]

An uncertainty factor of 10 typically is used to protect sensitive human subpopulations. For extrapolation from animal data to humans, EPA recommends, and OEHHA has used, an uncertainty factor of three when relying on default dosimetric adjustments in deriving a NOAEL [HEC] from the NOAEL. The Panel has applied these default adjustments to the chronic IPA study. No uncertainty factor is necessary to extrapolate from a LOAEL to a NOAEL, as a clear NOAEL was identified in the IPA study and was used in these calculations.

Similarly, the inhalation study was of chronic duration, so no uncertainty factor is necessary to extrapolate from subchronic to chronic. [Nor is an uncertainty factor needed to account for an incomplete database. As identified in these comments, and generally described in OEHHA's chronic toxicity summary for IPA, a large database exists for IPA, covering a broad range of endpoints and routes of exposure.] Accordingly, an uncertainty factor of 30 would be applied to the NOAEL [HEC, to derive an REL for IPA.

$$\text{REL} \quad 220\ \text{mg/m}^3 / 30 = 7.3\ \text{mg/m}^3\ (3.0\ \text{ppm})$$

Response. OEHHA agrees with the commentator that the 1997 chronic inhalation study in rats and mice by Burleigh-Flayer et al., which became available too late to be included in the October 1997 draft, is superior to the 1994 subchronic study originally used. OEHHA has based a revised chronic REL for isopropanol on the 1997 study and arrived at the same REL calculated by the commentator. The critical effects occurred in the kidney. Therefore we are revising our document accordingly.

Comment 4. OEHHA should remove references to questionable findings from its chronic toxicity summary for IPA. For the reasons discussed above, the Panel believes that, if an REL is to be derived for IPA, OEHHA should use the chronic inhalation study. However, the Panel also believes that modifications to the IPA chronic toxicity summary are necessary to ensure that the document accurately describes the potential human health effects of IPA. As noted above, the chronic toxicity summary should include a discussion of the chronic inhalation studies. In addition, the Panel believes that the toxicity summary as currently written bases the critical effect for the chronic REL on changes reported in the subchronic inhalation study that OEHHA itself has acknowledged are not adverse or relevant to human health. The toxicity summary also credits several questionable findings from the subchronic inhalation and other studies that the Panel believes should not be included in the toxicity summary. OEHHA derived its chronic REL for IPA from a subchronic inhalation study in rats and mice (Burleigh-Flayer *et al.* 1994). OEHHA states that the critical effects are increased relative liver weight (10 percent over controls) in female mice and hyaline droplets in the kidneys of male rats. Elsewhere in the toxicity summary, however, OEHHA states that the "hyaline droplets found in kidneys of male rats has been shown to be a male rat-specific phenomenon and is not considered to be relevant to human risk assessment." The toxicity summary also states: “Many studies also noted increased liver and kidney weights in exposed animals but with no observable relevant pathology. This change may be considered more of a metabolic response, rather than a toxic effect, of the alcohol.”

The Panel agrees that the kidney effect (hyaline droplets) is not relevant to humans, and that the increased liver weights (and kidney weights in other studies) are more properly considered a metabolic response to exposures to high doses of the alcohol. Because of their equivocal nature, the Panel believes that the increased liver weights and hyaline droplets should not be listed as critical effects for derivation of the chronic REL for IPA.

The toxicity summary also discusses results of some studies that the Panel believes are of questionable validity. Specifically, the IPA chronic toxicity summary lists three sensitive

indicators of IPA chronic adverse effects: the development of tolerance to narcosis, blood chemistry changes and reduced fetal body weights. The Panel, however, questions the relevance of the first two effects and the validity of the third effect, and therefore believes that these effects should not be discussed in the summary.

The first effect, tolerance to narcosis, is questionable as an indicator of chronic adverse effect. Narcosis is clearly adverse, but the diminishing of this effect following repeated exposure (likely through enzyme adaptation) would appear to be a positive adaptation for the animal and not an adverse effect. The second effect, hematological changes (anemia) reportedly was observed in the cited studies, Burleigh-Flayer *et al* (1994) and USEPA/OTS (1986) (actually a BIBRA study). However, anemia was not observed in the IPA chronic study, even though the chronic study involved concentrations as high as that at which the effect was observed by Burleigh-Flayer *et al.* (1994) and the higher equivalent concentration that produced the effect in USEPA/OTS (1986). The fact that the chronic study did not corroborate this finding calls into question the reliability of the anemia effect as an indicator of chronic toxicity.

Response. As indicated above, a summary of the chronic inhalation study has been added to the section on Effects of Animal Exposure. The summary notes the lack of anemia in the chronic study, although it occurred in the subchronic study. OEHHA has reviewed the chronic REL summary for other possible revisions.

Comment 5. The third effect, reduced fetal body weight, is an appropriate indicator of chronic adverse effects. Nonetheless, the conclusion in the toxicity summary that this effect occurred at doses lower than those that caused maternal toxicity is questionable when the data are examined as a whole. Specifically, the OEHHA chronic toxicity summary concludes that the LOAEL for fetal body weight effects in the Tyl *et* al. (1994) study was 400 mg/kg. The data presented in the manuscript do not support this conclusion, however, showing statistically significant changes in fetal body weight only at 800 and 1200 mg/kg, doses which also produced maternal lethality. There was no effect on fetal body weight at 400 mg/kg; thus, this dose level should be considered the NOAEL.

Moreover, although Nelson *et* al. (1988) reported reduced fetal weights at all vapor concentrations (3500, 7500, 10000 ppm) and effects on maternal body weight at only the top two vapor concentrations, this study's assessment of maternal toxicity is suspect given that the recent subchronic and chronic inhalation toxicity studies clearly identified narcotic effects in animals (non-pregnant) at 2500 ppm (Burleigh-Flayer *et* al., 1994, 1997). Nelson may have simply missed these narcotic effects in his study. Although Nelson *et* al. (1988) reported a very slight reduction in fetal weight in the 3500 ppm fetuses (approximately 3%), the study researchers discounted this finding in their discussion as not selective developmental toxicity.

However, except for a small but statistically significant decrease in fetal weight in the case of isopropanol, no effect was detected with either solvent at 3500 ppm. These data indicate that neither of these alcohols is a selective developmental hazard to the developing conceptus.

Similarly, in the two BIBRA studies (reported by OEHHA as US EPA/OTS studies), maternal toxicity also apparently was present at the dose levels at which reduced fetal body weights were reported.

The OEHHA toxicity summary further cites Beyer (1992) (or Bevan *et al.,* 1995) as showing a LOAEL for developmental effects at a dosage that did not produce parental toxicity (1000 mg/kg). In fact, however, several parental effects were observed in this study at 1000 mg/kg, including increases in body weights in females, increases in liver weights without structural changes in females, increases in liver weights with hepatocellular hypertrophy in a few males, and increases in kidney weight and structural changes in males. Nonetheless, OEHHA's interpretation may be correct in that these effects may not be adverse to the animal such that fetal effects were seen in this two-generation reproductive toxicity study in the absence of clear parental toxicity. It should be noted, however, that the developmental toxicity study in rats (Tyl *et al.,* 1994) found clear maternal toxicity (lethality, decreased body weight) at only a slightly higher dosage than was used in Beyer (1200 mg/kg).

(The Panel has access only to abstracts of these studies, rather than to the studies themselves. Thus, it is difficult to interpret the study results and assess fully the adequacy of the studies. Nonetheless, the abstracts report that in both studies, animals had reduced food and water intake at the middle and high dose levels (1.0 and 2.0, and 1.25 and 2.5 percent IPA in drinking water in the reproductive and teratogenicity studies, respectively). The reproductive and embryotoxicity study reports decreased body weights in the middle and high dose female animals, while the teratogenicity study reports decreased body weights only in the high dose females. These results are somewhat surprising in that the middle dose animals in the teratogenicity study were exposed to higher concentrations of IPA than the middle dose animals in the reproductive and embryotoxicity study.)

The Panel believes that a weight-of-the-evidence evaluation of these studies leads to a conclusion that IPA may produce only equivocal minimal selective toxicity to the developing fetus at high doses, but that delays in development generally occur only at levels that produce parental toxicity. The Panel requests that OEHHA reevaluate its discussion on developmental toxicity in light of these comments. The Panel further requests that OEHHA revise its final toxicity summary for IPA to remove references crediting questionable findings to ensure that the public is not misled about the potential human health effects of IPA exposure.

Response. As indicated above, a summary of the chronic inhalation study has been added to the section on Effects of Animal Exposure. OEHHA has reviewed the chronic REL summary for possible revisions. The chronic REL has been revised and the critical effect for the revised REL is kidney lesions as described in the 1997 chronic inhalation study.

In regard to developmental studies, especially fetal body weight effects, OEHHA is very concerned about the possible biological importance of even small weight decrements in (small) animal fetuses, even when the decrements may not be statistically significant. In humans, the logarithm of infant mortality (death before the infant's first birthday) increases linearly as birth weight decreases from 3500 to 1000 grams (Hogue *et al*., 1987; Rees and Hattis, 1994). This log-linear relationship exists on both sides of the birthweight of 2500 g conventionally used as a

cutoff defining low birth weight. There is no evidence for a threshold or break in the curve in this region. Thus any reduction in human birth-weight is a cause for concern since it increases the risk of infant mortality. (Hogue CJ, Buehler JW, Strauss LT, Smith JC. Overview of the National Infant Mortality Surveillance (NIMS) project--design, methods, results. Public Health Rep 1987 Mar-Apr;102(2):126-138; Rees DC, Hattis D. Chapter 8. Developing Quantitative Strategies for Animal to Human Extrapolation. In: Principles and Methods of Toxicology. Third Edition. AW Hayes, editor. New York: Raven, 1994). In the absence of certainty, OEHHA staff take the health protective approach that the reduced weight effect in animal fetuses may be biologically significant and may have import for humans. Tyl et al. (1994) used Dunnett's test to show statistically significant diffrerences between the controls and the 800 and 1200 mg/kg dosed animals (Table 1 of Tyl et al., 1994). Analysis of the data by a trend test showed a highly statistically significant trend ($p<0.001$). In each comparison of rat fetal weight (combined, males only, females only) the fetuses in the 1200 mg/kg dosed group weighed less than those in the 800 mg/kg group, which in turn weighed less than the 400 mg/kg fetuses, which weighed less than the controls. Based on the Hogue et al. results, OEHHA considers the 400 mg/kg group to set a biological LOAEL even if not statistically a LOAEL by a test such as Dunnett's.

Similar to the commentator, OEHHA staff have difficulty when faced with reviewing only the abstracts of studies. It is difficult to base a REL on a one paragraph abstract.

Comment 6. (CONCLUSION). For the reasons set forth in these comments, the Panel believes that IPA should be removed from the list of "air toxics" subject to the "Hot Spots" program. At a minimum, however, OEHHA should derive a chronic REL for IPA using the chronic rat inhalation study, rather than the subchronic study. OEHHA also should remove language from its chronic toxicity summary for IPA that suggests that this chemical has not been adequately studied, and should remove references crediting questionable findings from this document.

Response: OEHHA appreciates the thoroughness of the comments. OEHHA presently has no authority to remove IPA from the Hot Spots list. However, OEHHA has revised its chronic REL for IPA using the rat chronic inhalation study, as suggested by the comment. OEHHA has also reviewed the chronic REL summary and made possible revisions. For example, since a chronic inhalation study is now available, OEHHA included a summary of the study and deleted the statement that no long-term chronic study was available.

Chemical Manufacturers Association - Ketones Panel

The Chemical Manufacturers Association (CMA) Ketones Panel submitted comments on January 28, 1998 in response to the draft chronic reference exposure level (REL) for **methyl ethyl ketone (MEK)**. In the draft TSD OEHHA proposed use of the USEPA Reference Concentration (RfC) of 1,000 $\mu g/m^3$ (0.3 ppm) as the chronic REL. The RfC is based on decreased mean fetal body weight in mice.

Comment 1. OEHHA should re-calculate the REL for MEK using current EPA methodology for deriving inhalation reference concentrations (RfCs). Using current EPA methodology, the REL should be 3.3 mg/m^3 (slightly greater than 1 ppm). The uncertainty factor for MEK for interspecies extrapolation should be 3 and not 10. In the Technical Support Document, OEHHA states that the RELs were developed using the United States Environmental Protection Agency's (EPA) methodology for deriving RfCs. EPA's RfC calculation for MEK is reported in the Integrated Risk Information System (IRIS), an EPA on-line database containing health risk and EPA regulatory information. OEHHA thus based its REL calculations for MEK on the methodology reflected in IRIS. EPA derived the RfC for MEK using a developmental toxicity study (Schwetz et al.; Mast et. al. 1989). RfCs are calculated by applying various "uncertainty factors" (UFs) to account for the uncertainty of applying results from animal testing to humans and for any lack of unequivocal data. The RfC calculation listed in IRIS for MEK was performed in 1992, and uses a default UF of 10 for interspecies extrapolation. In 1994, EPA published new guidance for deriving RfCs. *See* EPA Office of Research and Development, "Methods for Derivation of Inhalation Reference Concentrations and Application of Inhalation Dosimetry," EPA No. 600/8-90/066F (Oct. 1994). The new guidance states that, if standard default dosimetric adjustments have been made, an UF of 3 should be used for interspecies extrapolation rather than an UF of 10. Since the 1994 RfC Guidance was issued, the Agency has used this approach for setting a number of RfCs in the IRIS database. The IRIS database clearly indicates that default dosimetric adjustments were made in the case of MEK. However, because the RfC was established before the 1994 RfC Guidance was adopted, an UF of 10 for interspecies extrapolation, rather than an UF of 3, was used to derive the RfC that is currently listed in IRIS. Using the UF of 3 for interspecies extrapolation reduces the total uncertainty factor from 3,000 to 900, and produces a corrected RfC value of 3.3 mg/in^3 (slightly greater than 1 ppm).

Response. All USEPA Reference Concentrations (RfCs), available when the Technical Support Document (TSD) on chronic Reference Exposure Levels was drafted in October 1997, are being proposed as chronic RELs. RfCs are already used by the USEPA and by California's Department of Toxic Substances Control and were earlier incorporated by reference in Appendix F of the Emissions Inventory Criteria and Guidelines for the Air Toxics "Hot Spots" Program for use in screening risk assessments in the Hot Spots Program. These Guidelines were effective July 1, 1997. The Risk Assessment Advisory Committee (RAAC) recommended that CalEPA harmonize where possible with USEPA on risk assessment. Governor's Executive Order W-137-96 concerned the enhancement of consistency and uniformity in risk assessment between Cal EPA and USEPA. Use of RfCs as chronic RELs was one action that OEHHA took to address the RAAC recommendation and to implement the Executive Order. RfCs released after October 1997, including ones that are revisions of those in the October 1997 draft, will be evaluated for

use in the Hot Spots program. OEHHA staff will review the scientific basis of each RfC when it becomes available and determine whether the scientific literature cited in the RfC is appropriate. Appropriate RfCs will be submitted to the SRP for their review and possible endorsement.

In the case of the MEK RfC, OEHHA agrees that the use of a 10-fold intraspecies factor in this case differs from the method more recently supported by USEPA.

Comment 2. This corrected RfC should be considered a conservative value because it is designed to allow continuous exposure for a lifetime of 70 years without adverse effect. Moreover, in the case of MEK, a modifying factor of 3 for incomplete database probably is excessive. A 2-generation reproductive effects study in rats using 2-butanol, which is rapidly converted metabolically to MEK, has been conducted and in fact was used by EPA to derive an oral reference dose (RfD) for MEK. Further, experience with other compounds shows that an UF of 10 for lack of a chronic study usually is higher than necessary (Dourson, M.L. and Stara, L.F., 1983, "Regulatory history and experimental support of uncertainty (safety) factors", *Regulatory Toxicology and Pharmacology,* 3: 224-238).

Response. OEHHA agrees that there is reason to eliminate a modifying factor of 3 for incomplete database. OEHHA has not used modifying factors in the development of its chronic RELs.

Comment 3. EPA has not revised the MEK IRIS data since 1992, and therefore has not re-calculated the RfC for MEK using the 1994 Guidance methodology. The IRIS database RfC for MEK, as a result, is outdated and inaccurate. Because OEHHA used an UF of 10 for MEK in its calculations for interspecies extrapolation, it appears that OEHHA relied on the outdated RfC methodology as reflected in IRIS. The 1994 Guidance clearly dictates that the correct UF for interspecies extrapolation for MEK should be 3. OEHHA should therefore re-calculate the REL for MEK using an UF of 3 for interspecies extrapolation.

Response. OEHHA reevaluated the RfC and proposed REL, which were derived from the data of Schwetz and associates (1991). Using slight differences in approach preferred by OEHHA, a chronic REL value of 10 ppm (30,000 $\mu g/m^3$) was derived. However, this reanalysis presents a situation where the short-term exposure study of Schwetz is no longer the most appropriate study to evaluate potential chronic health effects, since the 90 day inhalation study of Cavender and associates (1983) reported adverse effects at levels close to that reported by Schwetz. From the Cavender study a revised chronic REL of 4 ppm (10,000 $\mu g/m^3$) was derived (table below).

OEHHA revised chronic REL

Study	Cavender *et al*., 1983
Study population	Rats
Exposure method	Inhalation for 90 days
Critical effects	Increased liver weight and relative kidney weight in males and females
LOAEL	5,041 ppm
NOAEL	2,518 ppm
Exposure continuity	6 hours/day; 5 days/week
Average experimental exposure	449.6 ppm for NOAEL group
Human equivalent concentration	449.6 ppm for NOAEL group (gas with systemic effects, based on RGDR = 1.0 using default assumption that lambda (a) = lambda (h))
Exposure duration	90 days
LOAEL UF	1
Subchronic UF	3
Interspecies UF	3
Intraspecies UF	10
Cumulative UF	100
Chronic inhalation reference exposure level (REL)	4 ppm (4,000 ppb; 10 mg/m^3; 10,000 µg/m^3)

Chemical Manufacturers Association (CMA) - Metal Catalysts Panel

Comments on the Determination of Chronic Toxicity Reference Exposure Levels were received from the Metal Catalysts Panel of the Chemical Manufacturers Association (CMA) in a letter dated January 29, 1998. The CMA Metal Catalysts Panel represents firms manufacturing, using, or reprocessing metal-bearing catalysts, including nickel and nickel compounds. Member firms were Akzo Nobel Chemicals Inc., CRI International, Inc., Criterion Catalyst Co., LP, Crosfield Catalysts, Engelhard Corporation, Gulf Chemical & Metallurgical, Haldor Topsoe, Inc., OM Group, Inc., United Catalysts Inc., and W.R. Grace & Co.

The Panel supported comments separately submitted on the Guidelines and proposed REL for **nickel** by the Nickel Development Institute, the Nickel Producers Environmental Research Association, and Inco United States, Inc (see below). OEHHA proposed a chronic REL of 0.05 $\mu g/m^3$ for nickel based on respiratory system and immune system toxicity.

Chemical Manufacturers Association - Olefins Panel

Comments on the chronic REL for **propylene** were received from Courtney M. Price, on behalf of the Olefins Panel of the Chemical Manufacturers Association (CMA), in a letter dated January 29, 1998.

In addition to the comments below, the commentator provided a list of the references cited. This list is available upon request. The commentator also provided two slides of data in an appendix. These slides were presented by Dr. James Swenberg of CMA in March of 1996 regarding ethylene and ethylene oxide research. The appendix is also available upon request.

OEHHA developed a chronic inhalation REL of 3,000 $\mu g/m^3$ for propylene based on an inhalation study in rats.

Comment 1. The OEHHA summary for propylene should emphasize the minimal severity and species-specificity of the nasal effects and the NOEL of 10,000 ppm in a 14-week study. The observed nasal effects for propylene are minimal, reversible, and have been observed in only one species. OSHA does not regulate propylene inhalation, and ACGIH has classified propylene as a simple asphyxiant [ACGIH 1997, p.34] -- that is, an essentially inert gas that can cause asphyxiation at high levels due to dilution of oxygen in the atmosphere, but which otherwise does not have significant physiologic effects [ACGIH, 1997]. The nasal effects in the rat provide a basis for deriving a chronic REL simply because they are the only observed adverse effects. OEHHA should emphasize to users of the REL document, however, the minimal severity of the effects and the fact that they have been reported only for one species in one study.

Quest *et al*. (1984; NTP, 1985) reported the appearance of nasal cavity changes in rats following chronic exposure to both the low exposure level of 5,000 ppm and the high exposure level of 10,000 ppm, while there were no such effects observed in mice exposed to the same levels. Based on a review of these data, it is clear that chronic exposure to 5,000 ppm propylene can cause some minimal effects in the nasal cavity of rats (but not mice). These effects include increased incidence of inflammation, not otherwise specified (NOS), with no obvious dose-response relationship, and of squamous metaplasia, again with no obvious dose-response relationship; and increased incidence of epithelial hyperplasia in females only. The lack of a clear dose-response effect complicates the interpretation of the significance of these observations, in particular for inflammation, NOS, where the high exposure level incidences were similar to control values. In addition, epithelial hyperplasia, which was observed in one sex only, generally is reversible upon cessation of treatment.

Overall, the minimal severity of these effects, the general reversibility of epithelial hyperplasia, and the lack of dose-response relationship for the other effects indicate that 5,000 ppm represents a borderline LOAEL/NOAEL. In addition, the 14-week subchronic exposure data (NTP, 1985) demonstrated a clear NOEL of 10,000 ppm, with no effects in nasal cavity of rats, at either 5,000 or 10,000 ppm exposure levels, thus emphasizing the minimal response following chronic exposure to 5,000 ppm. The Panel believes that the OEHHA summary for the propylene REL should emphasize these points.

Response. OEHHA staff agree with the commentator that the effects observed at 5000 ppm in rats, as reported by Quest *et al.*, are of low severity. Staff also agree that the lack of a clear dose-response for some of the observed toxic effects (e.g., squamous metaplasia and inflammation) complicates the interpretation of those particular findings. However, it is important to note that with the squamous metaplasia, a statistically significant response was obtained at the low dose in both sexes and at the high dose in females, therefore a propylene-related effect cannot be ruled out. In addition, while the inflammatory lesions were statistically significant only for low-dose male rats, the incidence among high-dose males was of similar magnitude, suggesting that a plateau effect had occurred over the range of concentrations tested.

OEHHA acknowledges that the toxicity effects observed by Quest *et al.* are of low severity, both in the text of OEHHA's document and in the calculation of the REL, where an uncertainty factor of 3 is used instead of the usual 10 to account for the magnitude of the effect observed at a LOAEL compared with a NOAEL. In regards to epithelial hyperplasia, it may be generally reversible upon cessation of exposure. This is consistent with the determination that this LOAEL is of lower severity. However, in the Quest/NTP study, animals were not observed following exposure, so it is unclear what would have happened in this case. It is important to note that chronic RELs are based on continuous life-time exposures, where reversiblity of tissue damage upon cessation of exposure would not come into play.

The study by Quest/NTP, conducted in rats and mice, was the only chronic inhalation toxicity investigation found for propylene. There are no data on toxic responses from chronic inhalation to propylene from any other species, including humans. While the results of this study do suggest that a species difference exists between rats and mice in response to the inhalation of propylene, the authors also suggest that the difference could be due to a better compensatory reflex apnea defense mechanism, as documented in B6C3F1 mice exposed to formaldehyde. If so, the mice may not have taken in as much of the compound into the respiratory tract, thereby resulting in less tissue damage and a lesser degree of respiratory tract toxicity than that observed in rats.

In regards to OSHA and ACGIH, these organizations develop guidance for healthy workers exposed to chemicals during a normal workweek. The chronic REL is intended to protect against continuous lifetime ambient exposure for the entire population, including those individuals who may be more sensitive to the toxic effects of airborne chemicals. Those individuals include infants, children, the elderly, and those with compromised respiratory systems.

Comment 2. OEHHA should calculate the Human Equivalent Concentration based on the total respiratory tract surface area. OEHHA calculated a Human Equivalent Concentration (HEC) by using a calculated regional gas dosage ratio (RGDR) of 0.21, based on extrathoracic respiratory surface area and a minute volume derived from body weight. The Panel believes that using an RGDR is a plausible method to estimate an equivalent human exposure, but that the RGDR should be based on total respiratory tract surface area, not just on extrathoracic respiratory tract surface area.

The nasal cavity effects seen in rodents are expected to be, in large part, due to the fact that rats are obligate nose-breathers. Humans, however, are not obligate nose-breathers, and generally are mouth- and nose-breathers. The equivalent target tissue for irritation effects in humans would be the entire respiratory tract. Therefore, the Panel believes that if one is to estimate an HEC with an RGDR, the RGDR should be calculated using the surface area for the entire respiratory tract to better approximate the conditions of dose to humans. Thus, based on the methodology described in the Technical Support Document, the calculation of an RGDR for a gas with respiratory effects would be as follows:

$$\text{RGDR} = \frac{(\text{Minute volume})_{rat}/(\text{Minute volume})_{human}}{(\text{Surface area})_{rat}/(\text{Surface area})_{human}}$$

The values used by EPA for minute volumes of rats and humans are 2.3996×10^{-4} m^3/min and 0.0138 m^3/min, respectively (EPA, 1994). Using the values cited in the OEHHA Technical Support Document, the total respiratory tract surface areas are 3,440 cm^2 and 543,400 cm^2, for rat and human, respectively. Thus, the calculation for the RGDR would be:

$$\frac{(2.3996 \times 10^{-4})/(0.0138)}{(3{,}440/543{,}400)} = \frac{.017388406}{.006330512} = 2.746761399$$

Use of this factor (2.75) would imply that the exposure level in humans required to result in an equivalent dose per surface area is higher than the exposure level in rats. This is logical as the surface area for the human respiratory tract is much larger than the rat one, about 158 times larger, compared with the 59-fold difference in minute volume. Therefore it would take a much higher exposure concentration in humans than in rats to result in an equivalent dose per surface area for humans. However, to be conservative, the Panel recommends that OEHHA use a factor of 1 to convert the rat LOAEL to a human equivalent concentration.

Response. OEHHA staff determined that the critical effects in the Quest et al. (1984) report were in the extrathoracic portion of the respiratory system, especially the nasal cavity. Thus staff made the HEC adjustment for a gas with extrathoracic respiratory effects and thus calculated a RGDR of 0.21. Staff then used an interspecies UF of 3 since some of the uncertainty/variability in the interspecies extrapolation was subsumed in the HEC correction. That "the equivalent target tissue for irritation effects in humans would be the entire respiratory tract" is possible but since there are no data in humans it is only a guess. In the absence of human data OEHHA staff take a public health protective approach and assume that the target tissue is the same one seen in animals, the nasal epithelium, and use the suggested approach for such an effect.

Comment 3. OEHHA should consider eliminating some uncertainty factors and should acknowledge the conservatism of the propylene REL. Using the foregoing recommendations to derive a chronic inhalation REL for propylene would result in the following values:

Study	Quest *et al.*, 1984; NTP, 1985
Study population	50 rats/group/sex, 300 total

Exposure method	Discontinuous whole body inhalation exposure (0 or 4,985 or 9,891 ppm)
Critical effects	Respiratory tract irritation: species-specific, non-dose-related squamous metaplasia (males and females); epithelial hyperplasia (females only); non-dose-related inflammation (males only) of nasal cavity.
LOAEL/NOAEL	4,985 ppm
NOEL 14-week	10,000 ppm
Exposure continuity	6 hr/d, 5 d/wk
Exposure duration	2 years
Average experimental exposure	890 ppm
Human equivalent concentration	890 ppm (based on entire respiratory tract surface area comparison)
LOAEL uncertainty factor	3 (minimal effects, low severity, reversible or non-dose related)
Subchronic uncertainty factor	1
Interspecies uncertainty factor	3
Intraspecies uncertainty factor	10
Cumulative uncertainty factor	100
Inhalation reference exposure level	8.9 ppm

This value is very conservative. OEHHA applied an uncertainty factor of 3 for use of a low severity LOAEL. The Panel believes that the minimal effects noted at the borderline LOAEL/NOAEL exposure level of 5,000 ppm, the reversibility of the epithelial hyperplasia, and the lack of dose-response for effects such as squamous metaplasia, would support a lower uncertainty factor than the factor of 3 for use of a LOAEL uncertainty factor. Indeed, OEHHA has noted the need to develop a more sophisticated method to address low severity effects. In the case of propylene, the Panel believes that application of the LOAEL uncertainty factor is very conservative.

Furthermore, OEHHA applied an uncertainty factor of 3 for interspecies variation, in addition to the adjustment of the exposure level to a human equivalent concentration. As discussed above, however, the Panel believes an appropriate rat to human conversion would give a HEC that is higher than the rat LOAEL by a factor of 2.75. Thus, use of a factor of 1 for the HEC is already conservative.

The cumulative effect of these uncertainty factors provides an extremely conservative REL. The Panel believes that OEHHA should consider eliminating the uncertainty factors for the LOAEL and for interspecies variation. If OEHHA nevertheless persists in using uncertainty factors of 3 for these parameters, then the REL discussion should point out the very conservative nature of the propylene REL.

Response. OEHHA has used a modified LOAEL to NOAEL uncertainty factor of 3. A factor of 1 would indicate no effect while the data clearly show some effect. OEHHA acknowledges the

need for additional research in order to implement a more sophisticated approach than what we are doing at present. This is stated in our document.

The lower interspecies UF of 3 is used in those cases where an HEC adjustment has been applied since part of the interspecies adjustment involves different configurations of the respiratory system.

The chronic REL must address uncertainties in the available data. Unfortunately, there are very limited data available on the toxicity of propylene. No long-term human toxicologic or epidemiologic studies were located in the literature. No reproductive/developmental data for humans or animals are available. If a better study becomes available, we will use it as the basis for a better health value.

Chemical Manufacturers Association - Phenol Regulatory Task Force

Comments on the chronic REL for **phenol** were made by the CMA's Phenol Regulatory Task Group (Task Group) in a letter dated January 29, 1998. The Task Group is comprised of the major domestic manufacturers of phenol that represent approximately 95 percent of United States production of the chemical. Task Group members include: Allied Signal Inc., Aristech Chemical Corporation, Dakota Gasification Company, Dow Chemical Company, Georgia Gulf Corporation, GE Plastics, GIRSA, Inc., JLM Industries, Inc., Kalama Chemical, Inc., Merichem Company; Shell Chemical Company; and Texaco Refining & Marketing. Associate members are: Borden Inc. and Procter & Gamble. OEHHA proposed a chronic REL of 600 $\mu g/m^3$ for phenol based on reports of systemic effects in mice, rats and monkeys inhaling phenol.

The Task Group urges OEHHA to withdraw its draft chronic toxicity summary and proposed reference exposure level (REL) for phenol. The studies on which OEHHA has relied are inadequate to derive a REL, and the draft chronic toxicity summary does not reflect accurately phenol's potential health effects. All data bearing on phenol's chronic health effects, including data recently generated by members of the Task Group, should be reviewed before OEHHA publishes its chronic toxicity summary or issues a final REL. In restricting its comments to the toxicity summary and related REL, however, the Task Group does not endorse the risk assessment practices, policies, and methods set forth in those Guidelines in whole or in part. Moreover, the Task Group reserves the right to challenge OEHHA's use of the Guidelines to assess or regulate any chemical, including phenol. OEHHA staff provide detailed responses to the appended comments below.

Comment 1. *The studies on which OEHHA relies are not adequate to derive a REL*. OEHHA has based its proposed REL of 0.2 parts per million (ppm) (600 micrograms per cubic meter ($\mu g/m^3$)) for phenol on the findings of two subchronic inhalation studies performed in animal species: Sandage (1961) and Dalin and Kristofferson (1974). These studies are an inadequate basis for deriving a REL. The Sandage (1961) study in rhesus monkeys, rats, and mice detected no adverse effects following continuous exposure to phenol at 5 ppm for 90 days.

The Dalin and Kristofferson (1974) study in rats exposed continuously to phenol concentrations of 26 ppm reported some signs of neurological impairment, but the signs did not last during the whole exposure period and therefore were not considered to be severe. Moreover, the results are inconsistent with those of Deichmann et al. (1944). In the Deichmann et al. study involving inhalation exposure to 26 to 52 ppm phenol, no overt neurological signs were found in mice or rats after 88 and 74 days of exposure. For these reasons, the Task Group believes, that studies on which OEHHA relies are not adequate to derive a REL for phenol, and the proposed REL should be withdrawn.

Even if the studies on which OEHHA relies were an adequate basis for deriving a REL, the approach OEHHA uses to estimate risk does not accurately reflect phenol's potential chronic inhalation hazard. Using the no observed adverse effect level (NOAEL) of 5 ppm

referenced in its draft summary, a comparable human equivalent concentration of approximately 28 ppm would be derived, which, in turn, gives rise to a REL of approximately 1.0 ppm (using the identical uncertainty factors employed by OEHHA).

Moreover, based upon the available toxicity database for phenol, the occupational Safety and Health Administration (OSHA) has established an exposure limit of 5 ppm 8-hour time-weighted average. Identical standards have been established by the National Institute for Occupational Safety and Health (NI0SH) and the American Conference for Governmental Industrial Hygienists (ACGIH). Adjustment of the occupational exposure standard of 5 ppm for continuous rather than workday exposure would derive a REL of 1.0 ppm - a value which is, the Task Group believes, far more defensible than the standard of 0.2 ppm proposed by OEHHA.

REL calculations using human equivalent concentrations are appended as Attachment 1. The Panel notes that OEHHA has computed an HEC of 5 ppm, which is equivalent to the NOAEL. Because OEHHA has not set forth its HEC calculations, the Panel is unable to comment upon these calculations or their effect on that HEC.

Accordingly, RELs derived using existing occupational standards, or animal studies together with appropriate human equivalent concentrations, would yield roughly the same value - one that is considerably higher than that proposed by OEHHA. The Task Group believes that OEHHA's proposed REL is not supported by the data on phenol's toxicity, and will mislead the public about the health significance of exposure to low levels of phenol in the ambient air.

Response. OEHHA staff do not understand the basis for the commentator converting the animal NOAEL of 5 ppm into an HEC of 28 ppm. OEHHA determined that phenol is a gas with systemic effects. We used an RGDR of 1 using default assumptions. Thus the Human Equivalent Concentration (HEC) is the same as the average animal exposure concentration, in this case 5 ppm. The methodology is explained in the introductory chapter of the Technical Support Document and in the 1994 document U.S. Environmental Protection Agency Methods for Derivation of Inhalation Reference Concentrations and Application of Inhalation Dosimetry (EPA/600/8-90/066F. Office of Research and Development, Washington, DC). With this methodogy it is rare for the HEC to be greater than the average animal exposure.

In regard to TLVs and PELs, neither USEPA nor OEHHA use work-place standards to calculate RfCs and RELs. In regard to the TLV of 5 ppm, time adjustment to continuous exposure from 40 to 168 hours would result in a value of 1.2 ppm. However, TLVs are usually not NOAELs. They are usually LOAELs and in some cases FELs (Frank Effect Levels) for healthy workers. In the case of phenol the TLV is based on a study of 8 human volunteers who were exposed to from 1.6 to 5.2 ppm phenol by face mask for 8 hours on one day (Piotrowski, 1971). The study did not report any irritation of the eyes, nose and throat. Thus based on this experiment 5.2 ppm (20,002 $\mu g/m^3$) is a NOAEL for an acute exposure. OEHHA used this study as the basis of its acute REL for phenol. For a chronic REL, allowances for extrapolating from a 1 day acute exposure to chronic exposure (for which

precedents are lacking) and for exposing sensitive individuals such as infants, children, asthmatics and the elderly would need to be made.

Other human studies have reported workplace levels of 3.3 ppm phenol (Ohtsuji and Ikeda, 1972), 4 ppm phenol (Connecticut Bureau of Industrial Hygiene, undated), and 1.22-4.95 ppm phenol (Ogata *et al*., 1986). The studies do not mention adverse health effects or years of exposure. For comparison with the REL proposed, assume, for example, that 4 ppm is a human chronic NOAEL. A worker inhales 10 cubic meters of air per workday, so the level of phenol averaged over 7 days would be 1.43 ppm (4 x 10/20 x 5/7). Applying an uncertainty factor of 10 to account for variability in sensitivity in the human population results in a REL of 143 ppb or 550 $\mu g/m^3$, in good agreement with the 600 $\mu g/m^3$ REL derived from animal studies. The comment time-adjusted the TLV of 5 ppm to 1 ppm (3,850 $\mu g/m^3$) but did not make any allowance for variability in sensitivity in the human population. Thus OEHHA is still proposing 600 $\mu g/m^3$ as the chronic REL for phenol.

Comment 2. *Before publishing its chronic toxicity summary and REL, OEHHA must consider all information on phenol's health effects including data being generated by task group members*. The California Toxic Air Contaminant Program provides that OEHHA "shall evaluate the health effects of and prepare recommendations regarding ... toxic air contaminants." In conducting its evaluation, OEHHA must "consider all available scientific data," including, but not limited to, data provided by state and federal agencies, private industry, and public health and environmental organizations. The evaluation must include an assessment of the availability and quality of data on health effects, including potency, mode of action, and other biological factors. OEHHA's Guidelines are intended to help implement this statutory requirement. OEHHA's draft toxicity summary does not, however, review and evaluate all available information on phenol's health effects. Studies or information not reviewed by OEHHA include:

Argus Research Laboratories (1997): In this study conducted in rats exposed by gavage to high levels of phenol, which has been provided to OEHHA by the Non-Prescription Drug Manufacturers Association (NDMA) the only evidence of developmental toxicity observed was a decrease in fetal body weight and an increased incidence of one minor skeletal variation at the high dose level (360 mg/kg/day) only, a dose level associated with serious maternal toxicity. No developmental toxicity was observed at 60 or 120 mg/kg/day, despite the occurrence of significant maternal toxicity at the 120 mg/kg/day level.

ATSDR Toxicological Profile for Phenol (Sept. 1997): OEHHA's chronic toxicity summary for phenol relies heavily on an eight-year old ATSDR toxicological profile. OEHHA apparently has not reviewed, and does not reference, the updated profile for phenol, which was released by ATSDR for public comment in September 1997. The1997 draft profile summarizes a large number of studies addressing phenol's potential health effects that are not included in the 1989 profile.

CMA Pharmacokinetic Study: In this study, provided to EPA by Task Group members in *1994*, no phenol was detected in the blood of rats exposed to phenol in the ambient air at concentrations of 25 ppm, or in drinking water at concentrations of 5,000 ppm. This study demonstrated that, under both exposure conditions, phenol is readily conjugated and detoxified.

The Task Group also urges OEHHA not to publish its chronic toxicity summary, or issue a final REL for phenol, until after it has reviewed additional data generated recently by Task Group members, as well as data that are being generated and which will be available soon. The most recent schedule for completing these data is appended as Attachment 3. These studies are being conducted pursuant to an enforceable consent agreement entered into between the United States Environmental Protection Agency (EPA) and some members of the Task Group to satisfy certain testing proposed by EPA. The testing is intended to characterize phenol's potential for subchronic neurotoxicity, developmental toxicity, reproductive toxicity, and respiratory toxicity and will provide additional data upon which to base a REL. Data completed to date, pursuant to the ECA, include the following:

Subacute Rat Inhalation Study: No toxic effects were detected in the respiratory system of rats exposed to phenol concentrations up to 25 ppm for two weeks. A copy of this study is appended as Attachment 3.

Subchronic Drinking water Study in the Rat: No neurotoxic effects were reported in rats exposed to phenol in drinking water at doses up to 5,000 ppm.

Task Group members also are conducting a two generation reproductive toxicity study in the rat. In its chronic toxicity summary, OEHHA commented that "[n]o multi-generational studies evaluating reproductive or developmental effects under chronic exposure conditions could be identified. " The ongoing CMA study will provide definitive data on this endpoint. CMA is also conducting a neurotoxicity study. These studies will be available no later than, and October 17, 1998, respectively. The Task Group urges OEHHA to defer publishing the chronic toxicity summary, or issuing a final REL for phenol, until it has received and reviewed the data. Publication of the summary and REL at this time would be contrary to the intent embodied in the Health and Safety Code that OEH11A use all available information to characterize toxic air contaminants.

Response. OEHHA appreciates the additional information on phenols. But their relevance to developing a chronic inhalation REL is limited.

Argus Research Laboratories (1997). This is not an inhalation study but a recent gavage study (not yet published in the peer-reviewed literature), that addresses developmental endpoints. It would appear to have limited relevance to developing a chronic inhalation REL.

ATSDR Toxicological Profile for Phenol (Sept. 1997). The chronic REL Technical Support Document was released in October 1997 and thus staff did not have the opportunity to

thoroughly review the ATSDR profile. However, OEHHA did its own literature searches for phenol and referred to ATSDR as a convenient summary document.

CMA Pharmacokinetic Study. In this unpublished study, no phenol was detected in the blood of rats ex posed to phenol in air at 25 ppm, or in drinking water at 5,000 ppm. This study indicates that phenol is readily conjugated and detoxified, but it is not applicable to developing a chronic inhalation exposure level.

Subacute Rat Inhalation Study. No toxic effects were detected in the respiratory system of rats exposed to phenol concentrations up to 25 ppm for two weeks. This result is consistent with the results of the subchronic studies OEHHA staff used to develop the chronic REL.

Subchronic Drinking Water Study in the Rat. It is not clear how a subchronic drinking water study in the rat is relevant to the chronic REL especially since the length of the study is not specified.

Task Group's two generation reproductive toxicity study in the rat due June 17, 1999. OEHHA staff is not willing to delay release of the chronic REL until this report is released. However, we will be happy to review it when it is released to determine if the chronic REL should be revised.

Task Group's a neurotoxicity study due October 17, 1998. As of February 1999 OEHHA staff have not received a copy of this study. However, we will be happy to review it when it is released to determine if the chronic REL should be revised.

Comment 3. *OEHHA should revise its draft toxicity summary to describe more accurately phenol's potential chronic health effects*. The Task Group urges OEHHA to revise its draft chronic toxicity summary for phenol to characterize accurately phenol's potential chronic health effects. In particular, OEHHA has not accurately described phenol's potential developmental and reproductive effects. In its summary, OEHHA reviewed two studies conducted by Jones-Price et al. (1983) ("Teratologic evaluation of phenol in CD-1 rats" and "Teratologic evaluation of phenol in CD-1 mice. Research Triangle Institute) and notes that, in the first study, pregnant rats dosed with 0, 30, 60, and 120 mg/kg/day phenol on gestation days 6-15 exhibited reduced fetal body weight in a dose-related manner. The second study notes that in the fetus, reduced growth, decreased viability, and an increased incidence of cleft palate was also observed at the highest dose.

The Jones-Price et al. (1983a) study does not support the conclusion that phenol causes developmental /reproductive toxicity, for the following reasons.

- EPA has expressed "low confidence" in the Jones-Price et al. studies in its Integrated Risk Information System (IRIS) database because of the gavage nature of dosing.

- A seven percent decrease in fetal body weight (the only endpoint apparently affected in the study, which evaluated dozens of endpoints) is close to the limits of statistics to discern a significant decrease in body weight in a study of this type (five-six percent is the limit). Thus, the effect is at the border of being detectable.

- A seven percent decrease in fetal body weight may not be biologically significant. Decreases in fetal body weight of this small magnitude are usually readily reversible and of no functional consequence.

The average fetal body weight of the control group in this study was high compared to historical controls. This observation calls into question whether the apparent decrease in fetal body weight at the high dose was real and repeatable. The authors did not consider the historical control data in their interpretation of the fetal body weight data.

The authors did however consider historical control data in the interpretation of malformation data. The incidence of malformations was also unusually high in the controls in this study. Based on the comparison with historical controls, the authors concluded phenol had no significant effect on malformation data. A similar comparison with historical controls would show that phenol had no significant effect on fetal weight.

The preliminary range-finding study for the NCTR (1983) rat study showed no effect on fetal body weight at much higher doses. Although the statistical power of the preliminary study was less than that of the full study, one would expect to see some indication of a reduction in fetal weight at the higher doses.

A statistically significant difference was observed at the high dose only. Thus, a clear dose-response relationship was not demonstrated.

The litter size was 12 percent greater in the high dose group than in the control group. An inverse relationship between fetal weight and litter size is well recognized. While a 12 percent increase in litter size is not normally sufficient to explain a significant decrease in fetal weight, given that the decrease was at the limit of statistical significance, one cannot rule out the possibility that increased litter size may have played some role in the difference in fetal weight. Additionally, the combination of the heavier weight control group, plus the increased litter size, may have been sufficient to result in a statistically significant (although minimal) decrease in fetal weight.

Response. In regard to fetal weight differences, the weight decrement of 7% was statistically significant. A weight difference of 7% may be biologically meaningful in a very small, developing animal. The weight decrement of 7% might not be biologically significant if the loss is generally distributed. If it were specific to some organ or system, it could be. In the absence of certainty, OEHHA takes the health protective approach that the effect may be biologically significant. However, the difference in fetal body weights between the experiment cited and historical controls could mean that there was really no difference. Also, as pointed out in the comment, the increase in litter size may also have affected the fetal body

weight. OEHHA prefers to use articles from the peer-reviewed scientific literature to develop RELs since such discrepancies are often noticed by peer reviewers. USEPA's low confidence in the study is a conclusion of their peer review.

A further concern with fetal weight reduction is that in humans the logarithm of infant mortality (death) increases linearly as birth weight decreases from 3500 to 1000 grams (Hogue *et al*., 1987; Rees and Hattis, 1994). This log-linear relationship exists on both sides of the weight (2500 g) conventionally used as a cutoff defining low birth weight. There is no evidence for a threshold. Thus any reduction in fetal weight is a cause for concern. (Hogue CJ, Buehler JW, Strauss LT, Smith JC. Overview of the National Infant Mortality Surveillance (NIMS) project--design, methods, results. Public Health Rep 1987 Mar-Apr;102(2):126-138; Rees DC, Hattis D. Chapter 8. Developing Quantitative Strategies for Animal to Human Extrapolation. In: Principles and Methods of Toxicology. Third Edition. AW Hayes, editor. New York: Raven, 1994)

Comment 4. The chronic toxicity summary should also be revised to correct or clarify the following: *Include Phenol Exposure Levels for End-Points*: In the section of the toxicity summary entitled Effects of Animal Exposures, OEHHA describes a number of subchronic and chronic studies conducted with phenol and concludes that their findings indicate pulmonary damage, liver damage, renal damage, neurological effects, as well as various other chronic effects. The introductory paragraph does not, however, reference the exposure at which these findings were induced. Absent these data, the public cannot accurately assess whether phenol presents any health risk. OEHHA should revise its discussion of the animal studies to indicate levels of exposure at issue and thereby provide a more meaningful and less misleading summary of phenol's potential health effects.

Response. Many toxicological effects occur at fairly high concentrations. OEHHA staff look for the most sensitive toxic endpoint in humans or in an animal that is considered to react like humans to the chemical. Thus, the concentrations used or estimated in the key study are regularly included as well as our estimate of what the LOAEL and NOAEL are. In addition phenol concentrations are given for many of the other studies cited. In OEHHA's chronic toxicity summary for phenol the concentrations used in many of the studies are specifically mentioned.

Comment 5. *OEHHA's Conclusion About the Greater Toxicity of Inhalation Exposure is Not Borne Out by CMA's Study:* OEHHA states that "[c]omparison of the three routes of exposure found that oral exposure was less effective at producing systemic toxic effects possibly due to the rapid metabolism of phenol to sulfate and glucuronide conjugates by the gastrointestinal tract," and that "inhalation is a sensitive route of exposure for laboratory animals." Data from CMA's pharmacokinetic study, however, indicate that under either oral or inhalation exposure, phenol is readily conjugated and detoxified. No phenol was detected in the blood of rats at inhalation concentrations of 25 ppm or after drinking water exposure to concentrations of 5,000 ppm. The Task Group therefore urges OEHHA to revise its

conclusion regarding phenol's purported greater sensitivity through the inhalation route to reflect the findings of CMA's study.

Response. OEHHA based its conclusion on the publications of Deichmann et al. (1944) and NTP (1980) concerning toxic effects of phenol. The comment does not state whether in the CMA study toxic effects were seen in rats ex posed to phenol in air at 25 ppm or in drinking water at 5,000 ppm. The CMA study itself does not state adverse effects by these routes. In fact 25 ppm was probably chosen for inhalation since Deichmann et al. reported no effects at 26 ppm. The CMA study did find transient muscle twitching in rats administered phenol by a third route, gavage, at 150 mg/kg phenol. In any case two free standing NOAELs for different routes of exposure are not an adequate basis to conclude that the routes do not differ in effects. Although other organ systems are more sensitive, phenol would be irritating to the respiratory system at high levels, an effect not likely to be ameliorated by rapid sulfation or glucuronidation. The phenomenon of irritancy would not be tested by measuring phenol concentrations in the blood. There is no indication given that objective measures of irritancy were taken in the CMA rat study. It is difficult to know when a laboratory animal is experiencing irritation until it is rather pronounced.

Comment 6. *OEHHA Should Provide a Statistical Analysis of Study Endpoints:* OEHHA does not apply any statistical analyses of study endpoints, or otherwise describe the strength of the association between exposure to phenol and the relevant effect. Absent such information, the public cannot properly assess the predictive power of the study and its relevance to human exposure.

Response. It has not been customary to provide a statistical analysis of all study endpoints and to mention all concentrations used. Usually an endpoint is mentioned only if it has been adversely affected consistently in exposures to the chemical under study. The toxicity summaries are brief summaries of the literature. It would not be useful to entertain statistical analyses of every study mentioned.

Comment 7. *Correct the Reference to the Jones-Price et al. (1983b) Study:* OEHHA states that increased mortality was detected in rats in this chronic developmental effect study. The study in which increased maternal mortality was detected was conducted with mice, not rats, and the draft should be corrected accordingly.

Response. OEHHA has changed the animal from rats to mice in the summary of the Jones-Price et al. (1983b) study.

Chemical Manufacturers Association – Phthalate Ester Panel

Comments on the chronic REL for **diethylhexylphthalate** (DEHP) were made by the Phthalate Ester Panel of the Chemical Manufacturers Association in a letter dated January 29, 1998. OEHHA proposed a chronic REL for DEHP of 10 $\mu g/m^3$ based on a rat subchronic inhalation study by Klimisch et al. (1992) which found increased liver weight plus the appearance of lung alveolar thickening and foam-cell proliferation.

Comment 1. D*iethylhexylphthalate***.** OEHHA should adjust the DEHP REL to reflect the known greater sensitivity of the rat and should revise its discussion of the effects of DEHP.

Response. OEHHA staff are not aware of available data useful in assessing the relative susceptibility of humans and rats to inhaled DEHP. The commentator does not specify any. It has been the practice of OEHHA as well as USEPA and other authoritative bodies to consider the most sensitive species when estimating potential human health risks based on animal data.

Comment 2. DEHP has very low vapor pressure, so that the study (Klimisch *et al*., 1992) used to derive the REL was performed using a specially-generated aerosol. OEHHA should make that distinction clear to readers.

Response. OEHHA agrees that the vapor pressure of DEHP is low. However air contaminants, such as DEHP, that exist mostly or entirely as particulates rather than vapors may still be present at levels hazardous to human health. The low vapor pressure of DEHP was indicated or reflected at several locations in the document. (1) Mass concentration units ($\mu g/m^3$) were not converted to volume concentration units (ppb) in the Chronic Toxicity Summary (p. A-186). (2) The vapor pressure is reported in the Physical and Chemical Properties Summary (p. A-186). (3) The particulate nature of DEHP administered by Klimisch and associates (1992), as well as by Schmezer and colleagues (1988), was described in the experimental summary (p. A-187). The review of the study of Merkle and associates (1988) did not specifically mention the particulate nature of the administered DEHP. Text is being added at several locations in the document to emphasize the particulate nature of DEHP at concentrations experimentally studied.

Comment 3. OEHHA also should emphasize that the effects observed in Klimisch, et al., were reversible upon cessation of treatment.

Response. The apparent resolution of adverse findings after an eight-week post-exposure period was noted in the document (A-187). Additional text is being added to "Section VI. Derivation of Chronic Reference Exposure Level" to clarify this point. However, it should be noted the OEHHA chronic reference exposure levels specifically address the potential health effects from continuous lifetime exposures. Issues specific to

intermittent or less-than-lifetime exposures such as resolution of adverse effects over time are not addressed in this document.

Comment 4. OEHHA has incorrectly identified liver weight increase as a critical effect in this study, and should list only the lung effects as a critical effect. In addition, OEHHA has incorrectly characterized other studies as demonstrating a potential for DEHP inhalation to cause pulmonary effects. OEHHA should revise its discussion to correct these mischaracterizations.

Response. Relative liver weights were significantly increased in males and females at the LOAEL dose in the Klimisch et al. (1992) study, and absolute liver weights were significantly increased at that dose. No histopathological evidence of liver toxicity was noted, and the authors considered the effects secondary to toxicity at other sites. The text in the document is being changed to emphasize that the primary adverse effects observed at the LOAEL dose were increased lung weights, accompanied by foam-cell proliferation and alveolar septi thickening.

Comment 5. OEHHA should not apply an interspecies uncertainty factor to derive the REL. Extensive data demonstrate that primates are far less susceptible to DEHP effects than the rat. Indeed, OEHHA should adjust the REL by a factor of 0.2 to account for the known difference between primates and rats.

Response. The existing database of relative toxicity of substances toward different species does not support assuming species more closely linked evolutionarily will respond to chemical exposures more similarly than distantly related species. Among the two most commonly studied rodent species, rats and mice, large differences in susceptibility have frequently been reported. Also relative species susceptibility observed for oral exposures may differ from that observed for inhalation exposures, in part because of large species difference in lung anatomy.

Chemical Manufacturers Association (CMA) - Propylene Glycol Methyl Ether Panel

Comments on the chronic REL proposed for **propylene glycol methyl ether** (PGME) were received from the Propylene Glycol Methyl Ether Panel of the Chemical Manufacturers Association (CMA). The proposed chronic REL is the USEPA RfC of 2,000 $\mu g/m^3$ based on neurotoxicity.

Comment 1. The TSD proposes an REL for PGME of 2,000 $\mu g/m^3$ (0.6 ppm) by explicitly accepting U.S. EPA's Reference Concentration (RfC) for the compound. EPA derived this RfC by applying an uncertainty factor of 300 (factors of 10 each for the absence of a chronic study and intraspecies variability, and a factor of 3 for interspecies differences) to the 1,000 ppm No Effect Levels (NOEL's) in the Landry (1983) rat and rabbit 13-week inhalation studies. The observed effects of note at 3,000 ppm were

transient sedation in both species that resolved after the first one-two weeks of exposure and an adaptive hepatocellular swelling in rats with no evidence of degenerative changes.

Response. USEPA Reference Concentrations (RfCs), available when the chronic REL TSD was drafted in October 1997, are being used as chronic Reference Exposure Levels (RELs). RfCs are already in use by California's Department of Toxic Substances Control and by the USEPA and were earlier incorporated by reference in Appendix F of the Emissions Inventory Criteria and Guidelines for the Air Toxics "Hot Spots" Program for use in screening risk assessments in the Hot Spots Program. These Guidelines were effective July 1, 1997. The RfCs were recommended for use by OEHHA by the Risk Assessment Advisory Committee (RAAC) and used in response to Governor Wilson's Executive Order W-137-96 which concerned the enhancement of consistency and uniformity in risk assessment. RfCs released after October 1997 will be evaluated for use by reviewing each new RfC as it becomes available. Acceptable RfCs will be submitted yearly to the SRP for review and possible endorsement.

Comment 2. The Panel has recently completed lifetime studies of PGME in rats and mice exposed to 0, 300, 1,000 or 3,000 ppm PGME vapor for two years. As in the Landry study, sedation was observed at 3,000 ppm for both species, but, again, the effects had resolved by the second week of the study. PGME did not cause a dose-related increase in tumors in males or females of either species. Lifetime NOELs of 300 ppm were determined for both rats and mice.

We recommend employing this chronic study in the REL determination to eliminate the need for a subchronic vs. chronic uncertainty factor of 10 in the RfC/REL calculation. Because this study has just recently been completed, the final laboratory report has not yet been issued. However we will send the full report to you as soon as possible.

Response. Staff appreciates receiving the documentation of a chronic study of exposure to PGME and looks forward to the full report. A lifetime NOEL of 300 ppm from an exposure continuity of presumably 6 hours per day, five days per week results in an average experimental exposure of 54 ppm and a human equivalent concentration (HEC) of 54 ppm. Applying a total uncertainty factor of 30 (3 for interspecies UF and 10 for intraspecies UF) to the HEC results in a REL of 1.8 ppm, 3-fold higher than the RfC of 0.6 ppm. Staff will reconsider the proposed REL when the study is finalized. OEHHA staff encourage the commentator to submit the study to a peer-reviewed journal in order to increase the acceptability of the results.

Comment 3. The Panel has also recently completed a two-generation reproduction study of PGME in rats. We enclose that study report. As you will see, the NOEL for fertility and reproductive effects was 1,000 ppm; no effects were seen in the parents at 300 ppm. Decreased female fertility and reproductive effects were found at the highest concentration tested, 3,000 ppm PGME. These effects were associated with general toxicity and apparent resultant nutritional stress by the mothers and offspring at this high

concentration of PGME and not thought to be due to direct toxicity to reproductive organs.

Response. Staff appreciates the information on these endpoints. Both the proposed REL/RfC and a REL based on the chronic study described above should be protective against adverse reproductive effects.

Chemical Manufacturers Association – Hydrocarbon Solvents Panel (Xylenes)

Comments on the chronic REL for **xylenes** were made by the Hydrocarbon Solvents Panel of the Chemical Manufacturers Association. The Panel's members include: CITGO Petroleum Company, Exxon Chemical Company, Koch Chemical Company, Mobil Chemical Company, Phillips 66 Chemical, Shell Chemical Company, and Sun Company. OEHHA developed a chronic REL of 700 $\mu g/m^3$ from a study of 175 xylene-exposed factory workers by Uchida et al. (1993). Critical effects were nervous system effects as well as irritation of the eyes, nose and throat.

Comment 1. The Chemical Manufacturers Association (CMA) Hydrocarbon Solvents Panel has reviewed the Reference Level (REL) proposed for xylenes. We find the human study upon which that value is based seriously flawed and urge California not to establish an REL based on that study. We also include comments on the Technical Support Document's (TSD's) discussion of animal developmental studies of xylenes.

Response. Responses to the substantive issues are provided below.

Comment 2. THE PROPOSED REL. California proposes an REL of 0.05 ppm (200 $\mu g/m^3$) for xylenes. That value is derived from the Uchida et al. (1993) study of factory workers, which OEHHA interprets as finding a Lowest Observed Adverse Effect Level (LOAEL) of 14.2 ppm and no NOAEL. The critical effects specified are increases in the prevalence of eye irritation, sore throat, floating sensation and poor appetite. OEHHA converts the reported average 14.2 ppm workplace exposures to a continuous lifetime exposure of 5.1 ppm and then applies an uncertainty factor of 100 (10 for the absence of a NOAEL and 10 for intraspecies difference) to obtain the 0.05 ppm REL.

SIGNIFICANT FLAWS IN THE UCHIDA STUDY. The Uchida study suffers from a number of serious deficiencies and limitations in the design and reporting that render it unreliable for risk assessment.

Absence of Well-Documented Health Effects Data. First, Uchida, *et al.* used a subjective symptom questionnaire to assess health effects and thus did not obtain well-documented or reliable data on health effects. Symptom questionnaires may be substantially influenced by response bias and are therefore not reliable indicators of adverse health effects. The authors reported no control for such a potential bias (e.g., blinded interview), nor any validation of the subjective survey (e.g., a neurobehavioral or irritancy assessment).

Response. As noted by the comment, the subjective reports are not objectively verified by other measures. We agree that such verification would provide additional confidence in the subjective reports. The Uchida et al. (1993) article does not indicate whether the survey was blinded. We also agree that blinded interviews reduce the likelihood of inadvertent bias; the comment therefore raises a substantial potential limitation of this study. Here, the simplicity of the task and of the questions mitigates the potential for

such bias. Furthermore, and more importantly, the overall prevalence of subjective symptoms during work was greatly increased in the workers exposed to xylene as compared to the controls (19.2% versus 4.0%). With respect to many individual symptoms (e.g., eye irritation, nasal irritation, sore throat, floating sensation, and headache) the differences between exposed and unexposed workers were as great or greater than the overall prevalence. The magnitude of the observed differences makes such bias an unlikely explanation. The magnitude of the response differences also reduces concerns regarding the lack of an objective validation of the subjective complaints.

Comment 3. Other apparent problems with the symptom survey are an inherent bias to irritation and CNS symptoms (especially Part 1) and a duplication of like symptoms (e.g., dizziness, floating sensation, drunken feeling). This problem may have resulted in inflated prevalence results in the exposed workers.

Response. The comment asserts that there was an inherent bias toward irritation and CNS symptoms in the questionnaire. The sensory and subjective symptoms of Part 1 of the questionnaire (unusual smell, unusual taste, and face flushing aside) solely relate to irritative and CNS depressant effects. The Part I survey results are therefore experimentally limited to finding only effects related to irritancy and CNS depression. This limitation is not a bias that would affect the validity of the results as to the health effects covered by the survey. This limitation was appropriate given the known ability of many solvents to cause irritation and CNS depression. However, the absence (except for facial flushing) from the list of other symptoms not associated with the known health effects of xylene exposure is of some relevance. The presence of other unrelated symptoms on the list could have served as an internal control for false positive results. Here, the prevalence of the likely unrelated facial flushing symptom was not increased. In addition, the prevalence of the drunkenness symptom (the most severe symptom of CNS depression) was not increased.

As the comment points out, the duplication of like symptoms in the questionnaire has the potential to inflate the <u>overall</u> prevalence rates. However, this duplication is substantially mitigated by the means of calculating prevalence rates which takes the number of questions into account. The prevalence rate for a group is calculated by dividing the number of affirmative answers by the group by the number of people in the group and dividing that result by the number of questions asked.

Here, the key question is whether or not the inflation in overall prevalence rates could have biased the results so as to produce a false positive rate for the study as a whole. This clearly is not the case. If one considers only those workers who report no symptoms, duplicative symptoms would not be an issue. For symptoms during work, the great majority of controls (189 out of 241 unexposed workers or 78%) report no symptoms; by contrast only a very small percentage of the exposed workers (37 out of 175 or 21%) report no symptoms. Although the overall prevalence rates at issue here include unusual smell and unusual taste as symptoms, OEHHA would not consider these

sensations as symptoms of toxic injury. However, the individual results for these two 'symptoms' suggests that they do not importantly contribute to the overall prevalence rates. Nevertheless, while the overall prevalence rates are positive, our draft document did not base its analysis on the overall prevalence rates alone. Uchida et al. (1993) also presented the prevalence results for each individual symptom that was significantly elevated. Our conclusions were also based on those data. Duplication of like symptoms does not affect those prevalence rates.

Comment 4. Odor may also have contributed to the subjective response results of the exposed workers. Dalton et al. (1997) recently demonstrated that both perceived odor and cognitive expectations about a chemical can significantly affect the reporting of health symptoms.

Response. In Dalton et al. (1997) subjects with a *positive* bias (having been told that the test substance was beneficial) reported less irritation from short-term exposures to acetone (800 ppm) or phenylethyl alcohol. However, subjects with a *negative* bias (having been told the substances were harmful over the long term) evinced no consistent differences from subjects with a neutral bias (having been given no health hazard information). The Uchida et al. (1993) study participant biases are likely either neutral or negative. The comment is not clear as to the source of the cognitive expectations regarding xylene or as to which symptoms they would be relevant. To the extent those expectations are based upon the actual experience of workers, they are of much less concern with respect to confounding.

The Dalton et al. (1997) analysis also posited that, if irritancy is primarily a function of both odor intensity and cognitive expectations, then odor intensity should be a predictor of irritancy. With respect to acetone, the test compound, this correlation held up. However, Dalton et al (!997) could not well control for the possibility that the irritancy of acetone (at a test concentration of 800 ppm) affected the reports of its odor intensity.

Interestingly, Doty et al. (1977) (cited in Dalton et al. (1997)) tested p-xylene for its ability to intranasally stimulate the trigeminal nerve in anosmic subjects. Trigeminal nerve stimulation relates to the irritancy or pungency of a compound as opposed to its odor. These anosmic subjects rated p-xylene on average as a 3.69 in overall intensity (the intensity scale ranged from very weak which had a value of one to very strong with a value of nine) as measured along several attributes (strength, pleasantness, warmth, safety). Thus, when p-xylene was actually tested, odor was not necessary to detect the trigeminal response to p-xylene.

The relationship between odor and pungency is apparently a complex one. Cometto-Muniz et al. (1990) (cited in Dalton et al. (1997)) reported that the odor threshold and pungency threshold for eight aliphatic alcohols (methanol to octanol) varied from 23-fold to 10,000-fold.

Furthermore, in Uchida et al. (1993), the subjective complaints of the workers over the prior three months were also elevated. While odor may arguably have contributed to the subjective responses of the exposed workers, odor is much less likely to account for the symptoms experienced while workers are not on the job. In addition, as to on-the-job complaints, the sensation of unusual smell was not reported to be significantly increased.

Comment 5. Limited Exposure Data. Second, the Uchida study's assessment of worker exposures is similarly problematic. The study relied on a single point estimate, one 8-hour air sample (time-weighted average, TWA), to characterize "chronic" solvent exposure. The TWA concentrations of xylene did not indicate maximum concentrations, which the authors admitted might have influenced the subjective symptom prevalence.

Also, no evaluations for other non-solvent exposures were included, although workers may have been exposed to such materials in rubber boot production (e.g., adhesives), plastic-coated wire production (e.g., metals), or printing work (e.g., pigments).

Response. For each exposed worker in the study, Uchida et al. (1993) assessed exposure over the period of an entire shift. The LOAEL of 14.2 ppm is based upon the geometric mean of 175 such exposure measures taken on the day before the questionnaire was administered. The measurements and survey instruments are therefore very close in time. We therefore have high confidence in the representativeness of those measurements.

With respect to the prevalence of symptoms on the job, the comment presents a substantial uncertainty as to the interpretation of the findings. Acute exposures to xylene can cause irritation of the eyes and respiratory tract as well as CNS depression. Therefore, with respect to the prevalence of symptoms reported on the job, there is uncertainty whether some of the symptoms with a potentially quick onset (eye irritation) relate to the peak exposures as much as to the 8-hour average exposures. Other symptoms (e.g., sore throat, headache) may not be as responsive to peak concentrations. With respect to symptoms outside of work, short-term variations in xylene exposure are much less a concern and the 8-hour time weighted average is more clearly a reasonable measure of exposure.

The comment suggests that perhaps other exposures could have accounted for the observed results. The study specifically addressed the potential for confounding by a variety of other solvents, which could produce a similar spectrum of effects. The spectrum of effects found here parallel closely with those previously reported for xylene; it seems improbable therefore that this same spectrum of relatively typical effects would be due to a confounding agent. Furthermore, with respect to subjective symptoms at work, very few of the exposed workers were without symptoms. Thus, each of the three workplaces studied would have to have been independently subject to such confounding. This appears particularly unlikely.

Comment 6. Failure to Assess Exposure Variations and Worker Hygiene Practices. Third, the relationship of duration of exposure to health effects was not specifically assessed, as the authors did not attempt to differentiate acute from chronic effects through evaluation of changes in symptom magnitude over time. Variations in an individual's length of time on the job or changes in work assignments over time could have resulted in differences in exposure that would have affected interpretation of chronic health effects.

Response. As noted in the comment, the Uchida et al. (1993) study does not well differentiate whether the observed adverse effects are the result of a long-term chronic exposure or are simply short term effects repeatedly occurring as the result of daily repeated exposures. The characterization of the effects as chronic or something much less than chronic is therefore uncertain.

It is not likely that symptoms away from the job, as compared to symptoms while at work, relate to peak exposures on any given day. Symptoms away from the job more likely relate to the prior cumulative exposure/duration and not peak concentrations.

The comment is correct that the available information does not distinguish whether any given symptom is more closely related to the prior shift's average exposure, the prior week's average exposure, or the prior year's average exposure. If we assume that <u>only</u> the prior day's exposure contributed to the observed symptoms, then the dose response analysis below would suggest the <u>daily</u> REL which would be protective of health. If we assume, at the opposite extreme, that the prior annual exposure accounts for the observed symptoms, the dose response analysis below provides the annual (chronic) REL value that would be protective of public health. The difference in the magnitude of these two RELs is small. Their principal practical difference relates to the time frame for which they would be applied. A daily REL of 0.07 ppm would result in a hazard index greater than one if exposures exceeded that level for any given day in a year. A chronic REL of 0.05 ppm would allow prolonged excursions above the 0.07 ppm level providing that they were balanced by exposure periods equally below 0.05 ppm such that the ground level concentration divided by the REL would be less than one.

	Daily Exposure	Annual Exposure
LOAEL:	14.2 ppm	14.2 ppm
Average exposure concentration*	7.1 ppm	5.1 ppm
LOAEL uncertainty factor	10	10
Subchronic uncertainty factor	1	1
Intraspecies uncertainty factor	10	10
Cumulative uncertainty factor	100	100
Inhalation reference exposure level	0.07 ppm	0.05 ppm
Applicable period	Daily	Annually

*For daily exposure, the standard continuity adjustment factor of 10/20 was applied. For annual exposure, the standard factors of 10/20 x 5/7 were applied.

Thus, with respect to dose response assessment, knowing whether or not the observed effects are subacute or chronic (or something in between) is not essential, in this context, to derive a reasonable and health protective chronic REL.

Comment 7. Finally, the authors did not comment on the workers' hygiene practices at the factories, specifically whether the workers wore gloves or instead had dermal contact with multiple solvents and other chemicals. Substantial dermal exposure would have complicated an accurate estimation of the worker exposure.

Response. One of the symptoms reported to be increased in both men and women was rough skin. This symptom would be consistent with solvent dermatitis and would therefore indicate the likelihood of skin contact. Thus, as noted in the comment, dermal absorption is a potential confounding effect. However, in a companion paper, Uchida et al. (1993) have extensively analyzed the relationship between urinary metabolite measurements and the actual xylene air levels for these same workers. Their analyses showed a very strong correlation between the measured air levels and the urinary metabolite levels. The parameters of the curve relating urinary metabolite levels to these air concentrations indicated that at the reported median air concentration of 14.2 ppm, the great bulk of the urinary metabolite levels would be predicted by the air concentration data. Thus, use of the air concentration data as the estimate of exposure is appropriate. Furthermore, dermal exposure relates much more to systemic effects than to such site-of-contact effects as eye and respiratory tract irritation.

Comment 8. There are a number of other less serious problems in the study. These problems, however, cannot be critically assessed because of a lack of sufficient detail given in the report. Taken together, the above problems in the health and exposure assessments of the Uchida study do not permit definitive conclusions of causality of adverse health effects associated with xylene exposure. This study should not be used for risk assessment of xylenes.

Response. The study's subjective symptom survey does have limitations. The principal limitation, for our purposes, is that the key study data comes only from the results of a subjective symptom questionnaire. However, the magnitude of the differences observed for prevalence rates for many of the on-the-job symptoms reported to be increased in exposed workers as compared to unexposed workers (e.g., eye irritation: 25.1% v. 6.6%; nasal irritation: 40.6% v. 9.1%; sore throat: 31.4% v. 4.6%; floating sensation: 49.7% v. 8.3%; and headache: 22.9% v. 6.6%) strongly supports their use. For several of the symptoms while not at work over the past three months, there were also substantial differences in symptom prevalence in both men and women and in combination (forgetfulness: 33 v. 18%; nightmare: 40% v. 19%; anxiety: 12% v. 3%; inability to concentrate: 12% v. 3%). (These numerical values are estimated from graphical data in

Uchida et al. (1993), figure 2.) Given the potential for xylene to affect the CNS system in other studies, these symptoms are also of concern.

The temporal relationship between effects and exposure is not known. For some of the effects (eye irritation, nasal irritation), very short-term exposures may be more relevant than the longer term exposure history. For effects while not on the job, this concern is not as great. However, for the purposes of protecting the public health, this uncertainty as to the relevant exposure time frame is not a practical barrier to use of the study.

The exposure information itself is strong. Each of the 175 exposed subjects exposures were monitored on the day before their survey was conducted. The companion paper indicates that dermal exposure was not a major contributor to dose. The lack of complete industrial hygiene therefore is a minor limitation.

For these reasons, the study is suitable for use in deriving a chronic REL.

Comment 9. MISCHARACTERIZATION OF DEVELOPMENTAL EFFECTS. The TSD's discussion of the xylene animal developmental toxicity database does not include a critical and complete review of the xylene developmental toxicity studies. It thus suggests the erroneous conclusion that xylenes cause adverse developmental effects. There are substantial experimental design and data interpretation flaws in the cited studies that must be considered, and the well-conducted Biodynamics (1983) study, which did not find xylene to be a developmental toxicant, is not even cited. If you would like a copy of the Biodynamics study, as referenced, please contact Barbara Francis at (703)741-5609.

Response. The documentation for xylene devoted one paragraph in the TSD to its potential developmental toxicity. OEHHA did not purport to develop and provide an overall weight-of-the-evidence determination as to the potential developmental toxicity of xylene in one paragraph. The document itself offers no conclusion of its own regarding the overall weight of the evidence as to the potential developmental toxicity of xylene. The document does however put the reader on notice as to the existence of a substantial body of evidence that bears on the developmental toxicity of xylene.

The document quotes the 1995 ATSDR review, Toxicological Profile of Xylenes, thusly: "ATSDR concluded that the body of information available for the developmental effects are consistent with the hypothesis that xylene is fetotoxic and many fetotoxic responses are secondary to maternal toxicity." The paragraph then went on to briefly summarize some of the findings of the major studies bearing on the question of developmental toxicity. A principal purpose of the document is to provide background information relevant to the selection of the key study for dose response purposes. As with other health effects, the developmental toxicity data for xylene are presented with dose response information to make clear the margin of exposure between the proposed REL and other reported adverse effects.

Any inference that OEHHA inappropriately failed to cite the Biodynamics (1983) study would be incorrect. The ATSDR addressed the Biodynamics (1983) study in its review and did not find that it indicated the absence of an adverse effect on development. The ATSDR rather included the Biodynamics (1983) study in a group of studies which "had limitations that made them difficult to assess." (ATSDR p. 56). As a general rule, OEHHA prefers information published in the peer reviewed literature.

While OEHHA does not purport to offer a complete and comprehensive review of the developmental toxicity literature in this one paragraph, OEHHA has critically examined the available scientific literature. The summary is not in error as to the findings it presents. OEHHA did not offer an overall interpretation of those findings. Those findings, on their face, however, do provide evidence tending to support a conclusion that exposure to xylene may have adverse effects on development, perhaps at levels associated with maternal toxicity.

Yet, as the comment points out, there are some animal studies that did not find substantial adverse effects on fetal development. OEHHA agrees with the comment that this important and complicated subject matter merits more detail. We will therefore expand the treatment at issue. However, it is still not OEHHA's objective to develop an overall conclusion as to the weight of the evidence bearing on the developmental toxicity of xylene.

Comment 10. Inadequate Study Design. The first serious problem with the cited studies is inadequacies in study design. Unusual exposure durations were used (e.g., oral dosing of 3 times per day; continuous 24-hour exposures), suggesting excessive handling of the animals and possible stress-induced changes that could affect body weight gain/loss and food consumption (Marks et al., 1982). Continuous treatment in 24-hour exposures generally results in a higher incidence of growth and retardation characterized by decreased mean fetal body weights (Hudak and Ungvary, 1978).

Response. In general, all experimental manipulations of laboratory animals have an assumed potential to affect the study results. Therefore, it is necessary to have controls that match the experimental group, save for the alteration in one condition, so as to allow a contrast to be made on the one altered condition. In the Marks et al. (1982) study, the potential effect of the experimental manipulation (thrice daily doses by gavage) of the animals can not be denied. However, since Marks et al. (1982) included a vehicle control group in their study, this potential source of confounding was eliminated.

Hudak and Ungvary (1978) did not report that continuous treatment in 24 hour exposures generally results in a higher incidence of growth and retardation characterized by decreased mean fetal body weights. There, the untreated controls had fetal weight outcomes essentially the same as the air controls subjected to the exposure chamber manipulations. Furthermore, Hudak and Ungvary (1978) found 230 ppm xylene to have

no effect on mean fetal weight. In that study, xylene increased the incidence of skeletal anomalies and provided some evidence for retarding skeletal development.

Thus, the study design concerns raised by the comment are not a serious problem in the two example studies offered by the comment.

Comment 11. Unreliable Exposure Information. Second, in many of the studies (i.e., Hudak and Ungvary, 1978; Haas and Jacobsen, 1993), test atmospheres were not monitored continuously, and thus the exposure data provided are not reliable. Exposure test atmospheres should be monitored continuously and the method of generation should be well-documented.

Response. These comments relate potential limitations affecting some of the studies. However, the limitations bear mostly on the question of dose response and not hazard identification. OEHHA did not use the studies in its quantitative dose response assessment.

Comment 12. Species-Specific Problems. Third, the test species utilized in some of the studies may have also influenced the test results. Rabbits are known to show inherent erratic body weight gain and loss during gestation; therefore, the effects observed in the studies with this animal (e.g., Ungvary and Tatrai, 1985) must be interpreted cautiously. Some of the studies were also conducted with mice, a species known to show more variable types and incidences of spontaneous malformations compared to rats or rabbits. It is doubtful that the laboratories conducting these studies possessed the considerable experience that is necessary to work with the evaluations on the small fetus of a mouse.

Response. With respect to species differences in the variation associated with an experimental measure, increased variability within one species reduces the statistical power of experiments with that species and therefore increases the likelihood of a null result for such experiments. Thus, in the face of such increased variability, it is particularly the null result that needs to be appraised with caution.

It is difficult to respond to the comment's declaration: "It is doubtful that the laboratories conducting these studies possessed the considerable experience that is necessary to work with the evaluations on the small fetus of a mouse." However, it seems improbable that <u>all</u> the laboratories that utilized the mouse as the test species fell below a reasonable competency standard.

Regardless, poor technique is more likely to produce false null results than false positive ones. With respect to whether or not a given laboratory possessed the requisite skill to conduct studies in the mouse, poor execution of an experimental procedure may affect the accuracy or precision of an observation. Where poor precision occurs, the variance of the measures is increased and the power to discern an affect is reduced. Thus, poor precision would reduce experimental power and tend toward the null result. Where

poor accuracy is alternatively alleged, all of the measures tend to be skewed in the same way. Thus, as the exposed and control groups would be inaccurate in the same way, the differences between them would still be informative.

The blinding of experimental procedures provides further insurance as to the validity of the experimental results. Typically, the experimenters in these studies were blinded and did not know the treatments received by the test animals at the time of observation. Thus, to the extent (if any) the experimental procedures were poor, it is unlikely that the differences would reflect poor technique compounded by a subjective bias.

Comment 13. Improper Statistical Analyses. Fourth, the appropriateness of the statistical analyses employed in the cited studies is a major concern. The statistical analyses for the majority of the studies considered only the number of fetuses affected. Reproductive toxicologists now consider the litter the appropriate independent unit for statistical evaluation (USEPA, 1991).

Response. The comment is correct. The litter is the preferred unit of analysis for statistical comparisons between groups. The unit of analysis is a factor to be considered in evaluating each study's findings. It is possible that, if most or all of the adversely affected fetuses were in one or a few litters, the statistical comparison of control and experimental groups on the basis of individual fetuses would be misleading. Where the differences between experimental and control groups are sufficiently large, or where the findings have been replicated between studies, or within parts of studies, or where there is evidence of dose response, the opportunity to be misled (as to either a false positive or a false null) is much reduced.

Comment 14. Incorrect Interpretation of Certain Variants and Malformations. Finally, OEHHA's interpretation of the data from these studies is flawed. In several of the studies (e.g., Haas and Jacobsen, 1993; Hudak and Ungvary, 1978; Ungvary et al., 1980), skeletal variants (e.g., rudimentary ribs, fused sternebrae) were observed. Skeletal variations are not adverse developmental effects and, in rats, they have been found to be reversible (Chernoff, et al., 1991; Harris and De Sesso, 1994; Kimmel and Wilson, 1973). Skeletal variants such as rudimentary ribs and fused sternebrae should not be considered biologically significant in the absence of other conventional signs of embryotoxicity (e.g., increased malformations, increased embryolethality or decreased fetal weight). These variants are usually not regarded as harmful developmental toxic effects, but instead may be indicative of non-test-material-related stress due to the exposure regimen. They would only be considered toxic effects if a significant dose-related increase above controls (historical and concurrent) were observed.

Response. OEHHA did not interpret, but only presented, the available scientific information.

Comment 15. In addition, certain malformations such as cleft palate often occur spontaneously in mice as a result of environmental changes during critical stages of development. Thus, the biological significance of increased incidence of cleft palate in teratology studies must be evaluated carefully.

Response. Comment noted. The biological significance of any malformations must be carefully addressed in their interpretation. Where unexposed controls and exposed experimental groups are compared, the spontaneously occurring adverse effects should be of similar magnitude and incidence in each group. However, we agree with the comment that the mechanism of any increase in malformations in the experimental group should be evaluated carefully, especially with respect to extrapolating any findings to humans. Whether or not a malformation or other adverse effect represents a direct action upon reproduction or is secondary to a general maternal toxicity bears more upon the characterization of the toxic insult than its practical meaning. Yet, if the mechanism of the developmental insult in the test species is well understood and not thought likely to be relevant to humans, it would be inappropriate to regard the exposure on that basis alone as a potential human developmental toxicant.

The ATSDR quotation in the document does indicate that most of the adverse fetal effects occur at doses near to or causing maternal toxicity.

Comment 16. And, finally, historical control data, which were not considered, should always be considered when interpreting the significance of skeletal variants and malformations. This will ensure the findings truly exceed the range of control values for a larger population.

Response. Historical control data bear on the replicability of the concurrent control data. In general, as to control or experimental group data, where results have been replicated they warrant a greater degree of confidence.

The absence of historical control data is not a serious limitation in general. Where test animals are randomly assigned to control and experimental groups at the start of an experiment, it is the concurrent controls that are more likely to closely match the experimental groups for all appreciated and unappreciated variables at the start of the experiment. It is the comparison between the concurrent controls and experimental groups which is most probative.

Furthermore, individual studies often incorporate more than one control group. For instance, in Hudak and Ungvary (1978), the study design incorporated three different control groups (untreated controls, 24-hour air exposures in test chambers on days 9-14 of pregnancy, and 8-hour air exposures in test chambers on days 1-21 of pregnancy). These three control groups gave closely similar results that increased the confidence in each.

In other studies, the dose response information itself supports the results achieved in the null group. For instance, in Marks et al. (1982), the dose response relationship for average fetal weight itself afforded strong support to the accuracy of the control value (0 ml/kg-d - 0.982 g; 0.06 ml/kg-d – 0.982 g; 1.2 ml/kg-d – 0.975 g; 2.4 ml/kg-d – 0.861 g; 3.0 ml/kg-d – 0.785 g; 3.6 ml/kg-d – 0.708 g).

Comment 17. The above points demonstrate there are substantial weaknesses in the cited studies that diminish reliable interpretations of the data and conclusions on developmental toxicity. There is available a more definitive and well-conducted developmental toxicity study for xylene (Biodynamics, 1983) (copy attached). This study does not indicate that xylene is a developmental toxicant. We urge California to include this study in their assessment and to take account of the issues discussed above in their discussion of the other developmental studies.

Response. The most consistent adverse effect seen in the different developmental toxicity studies is decreased fetal weights. Different studies, including the Biodynamics (1983) study, have reported different NOAELS/LOAELs for this adverse effect on fetal development:

Study	Strain/Species	Exposure Duration	NOAEL	LOAEL
Mirakova et al. (1983)	"white" rat	6 h/day	2.3 ppm	12 ppm
Hass (1993)	Wistar rat	6 h/day	200 ppm	
Bio/dynamics (1983)	CrL-CD (SD) BR rat	6 h/day	250 pm	500 ppm
Shigeta et al. (1983)	ICR mice	6 h/day	230 ppm	460 ppm
Hudak et al. (1978)	CFY rat	24 h/day	230 ppm	
Ungvary et. (1985)	CFY rat	24 h/day		60 ppm
Ungvary et. (1985a)	CFY rat	24 h/day		140 ppm
Ungvary et al. (1985)	CFLP mice	24 h/day	120 ppm	230 ppm

With respect to NOAEL and LOAEL values, the Mirakova et al. (1983) is clearly an outlier. The ATSDR has suggested that this study may have been influenced by poor animal husbandry. Partially, for these reasons, OEHHA chose not to base its chronic REL upon the Mirakova et al. (1983) study.

Overall, the available data suggest that the 24 hour exposure regimens result in lower NOAELs than the 6 h/day exposure regimens. Regardless, these other observed NOAELs are sufficiently high so as to indicate the proposed chronic REL should provide an adequate margin of exposure. Thus OEHHA is proposing 700 $\mu g/m^3$ as the chronic REL for xylenes.

Geysers Geothermal

Comments on the chronic REL for **hydrogen sulfide** were made by the Geysers Geothermal Association (GGA), a non-profit, mutual benefit corporation with a membership of almost 300 companies and individuals participating in the production and utilization of geothermal energy at The Geysers geothermal field. In the draft document OEHHA proposed use of the U.S. EPA RfC of 0.7 ppb (0.9 $\mu g/m^3$) as the chronic REL.

Comment 1. The Office of Environmental Health Hazard Assessment (OEHHA) has made a great effort to involve input from risk managers and stakeholders throughout the Reference Exposure Level (REL) promulgation process. We support your approach and believe that it is important to seek input from various sources.

Response. OEHHA staff appreciate the comment. The legislation enabling the Hot Spots program included the requirement to obtain and consider input from risk managers and stakeholders.

Comment 2. However, it appears that OEHHA has taken an extremely conservative approach in determining the proposed H_2S REL of 0.7 ppb.

Response. The chronic REL proposed in the draft is a USEPA RfC, which has been available since 1995. The approach to developing RfCs is very similar to the development of chronic RELs. All USEPA Reference Concentrations (RfCs), available when the Technical Support Document (TSD) on chronic Reference Exposure Levels was drafted in October 1997, are being proposed as chronic RELs. RfCs are already used by the USEPA and by California's Department of Toxic Substances Control and were earlier incorporated by reference in Appendix F of the Emissions Inventory Criteria and Guidelines for the Air Toxics "Hot Spots" Program for use in screening risk assessments in the Hot Spots Program. These Guidelines were effective July 1, 1997. The Risk Assessment Advisory Committee (RAAC) recommended that CalEPA harmonize where possible with USEPA on risk assessment. Executive Order W-137-96 concerned the enhancement of consistency and uniformity in risk assessment between Cal EPA and USEPA. Use of RfCs as chronic RELs was one action that OEHHA took to address the RAAC recommendation and to implement the Executive Order. RfCs released after October 1997, including ones that are revisions of those in the October 1997 draft, will be evaluated for use in the Hot Spots program by reviewing the scientific basis of each RfC when it becomes available and by determining whether the scientific literature cited in the RfC is current. Appropriate RfCs will be submitted to the SRP for review and possible endorsement.

However, based on other comments and on OEHHA's assessment of the developmental toxicity data available, OEHHA staff have reviewed the value in the draft document and have calculated a revised chronic REL for hydrogen sulfide of 9 $\mu g/m^3$ (7 ppb).

Comment 3. We are aware that final reports should be available from the Chemical Industry Institute of Toxicology (CIIT) on the reproductive effects and developmental neurotoxicity of H_2S by mid-1998. We request that a final decision on the REL not be made until these studies are completed. A decision of this magnitude should be made in a collaborative setting with input from academia, industry, and regulators. This approach is consistent with OEHHA's development of the proposed REL.

Response. OEHHA staff will be happy to review the studies when they become available and hope that USEPA will do the same. As of January 1999, OEHHA had been provided with only an abstract of a CIIT study on the neurobehavioral and neurochemical effects of hydrogen sulfide. The study involved 5 consecutive days of exposure, which would not be a study length suitable for developing a chronic REL.

Comment 4. We strongly believe that OEHHA's decision to lower the REL to 0.7 ppb should be revisited. Based on the available data, the REL should be set no lower than 7 ppb. We recommend this revisitation based on the following: (1) The California ambient air quality standard for H_2S is 0.03 ppm. (2) The proposed REL of 0.7 ppb is one thousandth of the upper concentration for H_2S naturally occurring in human breath. (3) Low levels of H_2S are rapidly metabolized and detoxified by the human body and, therefore, are unlikely to be a chronic hazard at concentrations at or below the odor threshold. (4) The excessive conservative safety factors used in deriving the USEPA Reference Concentration (RfC), on which the new H_2S REL is based, should be decreased by at least one order of magnitude. (5) New studies on the toxicity of H_2S have been published or initiated since the 1994 USEPA RfC was finalized. We urge OEHHA to delay your decision on the REL until all of the information is updated.

Response. As stated above, OEHHA is now proposing 9 $\mu g/m^3$ (7 ppb) as a chronic REL for hydrogen sulfide. The commentator should also request USEPA to revisit the RfC. To decrease the UFs by an order of magnitude, changing the existing UFs would need to be addressed. If the available reproductive and developmental toxicity data are adequate for U.S. EPA, the modifying factor of 3 can be eliminated. Also if a chronic study were produced, the subchronic uncertainty factor could be eliminated. Thus the REL might increase 3, 10 or 30 fold depending on what studies are available.

Comment 5. The California H_2S ambient air quality standard of 0.03 ppm is based upon the low threshold for odor detection by humans. The Lake County Air Basin, located downwind of The Geysers, has been in attainment with all State and Federal ambient air quality standards, including H_2S, since 1990. This means that the H_2S ambient air quality level is significantly lower than the enforced level of 0.025 ppm. This attainment was accomplished by a cooperative effort of the public, regulatory agencies, and the geothermal industry. A tremendous amount of time, energy, and money was spent achieving this significant accomplishment. The proposed REL will not provide a greater level of public safety.

Response. The California H_2S ambient air quality standard of 0.03 ppm is for a 1 hour averaging time and is being retained as the acute REL in the Hot Spots program. Thus local residents should not experience acute adverse health effects from H_2S. The chronic REL is for much longer exposures and should be less than the acute REL. Although odor is one consideration with H_2S, it is not the only effect and odor is not necessarily a sentinel for other toxicity, since some chemicals have no odor and while for other with an odor adverse effects may occur above or below the odor threshold depending upon the chemical.

Comment 6. The economic impacts of the proposed REL will be enormous throughout California. It certainly could have a significant impact for the Geysers due to the deregulation of the electrical generating industry. Using the proposed REL for Air Toxics scoring purposes could subject some Geysers facilities to perform risk assessments with no corresponding benefit to public health and safety. We are attempting to control costs and remain competitive in a deregulated market. The proposed additional regulatory requirements imperil this goal.

Response. The determination of RELs is a risk assessment process and uses the best science available at the time. The economic impacts are part of risk management considerations. The risk manager can take into account the uncertainty in the REL and the delisting of H_2S as a federal Hazardous Air Pollutant as well as the known toxicity of H_2S, a hazardous gas reported to be the most common cause of sudden death in the workplace.

Lake County Air Pollution Control District

Comments on the **hydrogen sulfide** chronic REL were received from Robert Reynolds, Air Pollution Control Officer for Lake County. In the draft document OEHHA proposed use of the US EPA RfC of 0.7 ppb (0.9 $\mu g/m^3$) as the chronic REL.

Comment 1. Hydrogen sulfide is one of the air pollutants for which an Ambient Air Quality Standard (AAQS) exists, and for which the Recommended Exposure Level (REL) proposed would raise the issue as to whether the AAQS is as comparatively protective of health. The proposed action seems to be inconsistent with Cal-EPA's goal of coordinating programs, avoiding redundancies and lessening paper work. We suggest that either the AAQS should be reviewed and updated or OEHHA and the ARB should at least reconcile and comment on the Hydrogen Sulfide REL and the requirement to be protective of health as part of our state AAQS.

Response. The Ambient Air Quality Standard is for a 1 hour averaging time and is being retained in the Hot Spots program as the acute REL. The chronic REL is a USEPA RfC for long-term exposure. In order to coordinate with USEPA as recommended by the RAAC Committee and to comply with Governor's Executive Order W-137-96, all USEPA Reference Concentrations (RfCs), available when the Technical Support Document (TSD) on chronic Reference Exposure Levels was drafted in October 1997, are being proposed as chronic RELs. RfCs are already used by the USEPA and by California's Department of Toxic Substances Control and were earlier incorporated by reference in Appendix F of the Emissions Inventory Criteria and Guidelines for the Air Toxics "Hot Spots" Program for use in screening risk assessments in the Hot Spots Program.

Comment 2. The unintended consequences of some of the poorly substantiated RELs, especially those based on little or no directly applicable data, may be an erosion of public faith in the protectiveness of historic and ongoing air quality management programs. Potentially, this could result not from "poor science," but the absence of available science and effort in reaching the recommendations proposed by OEHHA.

Response. OEHHA is limited to the available data to develop a REL. Uncertainty factors which account both for known variability between humans and animals and within the human species as well as uncertainty due to extrapolating from LOAELS to NOAEL and from subchronic to chronic are used because there are seldom chronic exposures to sensitive humans available for use. The RfC for H_2S includes uncertainty due to lack of data on reproductive harm and a factor for subchronic to chronic extrapolation. Hopefully interested parties will be motivated to obtain better data.

Comment 3. The uncertainty factors, resulting from the lack of directly applicable information, may be appropriate to accept in some instances, but in those cases where the decision has much consequence in concern for public health protection, or extensive resource and cost demand, an additional effort should be made to increase certainty and the confidence in the recommendations of OEHHA. It would be better to fund necessary

work based in good science and carry it out prior to OEHHA advocacy or required recommendations being published.

Response. Risk assessment always involves uncertainty. The risk manager is expected to include that aspect in his decisions. OEHHA does not have a research budget and thus can only point out the need for more research.

Comment 4. Present state air toxic programs, while providing benefit to the public and industry, fail drastically in that they do not consider the cumulative effect of many sources within an airshed which is typical of the real world. Additionally, they are sometimes based upon a minimal effort that has tremendous downstream costs to others. I believe the subject effort potentially falls into this category and should receive more careful consideration, especially for the RELs proposed that are poorly supported but which are likely to have substantial importance and consequence.

Response. The consideration of cumulative effects within an airshed is of interest and of concern. Such considerations are theoretically possible anywhere and have been recently approached by the USEPA on a national basis (Woodruff TJ, Axelrad DA, Caldwell J, Morello-Frosch R, Rosenbaum A. Public health implications of 1990 air toxics concentrations across the United States. Environmental Health Perspectives 1998 May;106(5):245-51.) OEHHA staff does not have the resources to do such work and doubts that many air districts do either. OEHHA also recognizes that the actual costs of compliance are often less, and even considerably less, than the predictions made by affected stakeholders when a new regulation is proposed. The South Coast Air Basin has continued to thrive in spite of some of the most stringent air quality rules.

Comment 5. OEHHA needs to better identify sources and parties likely to be interested in and affected by the proposed recommendations, and then hold meaningful meetings and considerations which affords easy input. While it is obvious OEHHA has tried to include "stakeholders", "incorporate peer review," etc., I believe the effort should be redoubled and your final action delayed as necessary. Consider having workshops on specific component RELs of concern in those geographical areas of the state where the components are an air management and public exposure concern. In the long run it may save all of us time and effort, and better serve the public.

Response. The TAC program has been in place in California since 1983. The Hot Spots program has been in place since 1987. We have 1500 individuals on our mailing list for Hot Spots and contacted them regarding our Hot Spots documents. The information has been posted on the Internet and we have held public meetings in both the northern and southern parts of the state to present and discuss the issues with stakeholders. In addition we have received public comments. Based on the comments we have revised our document and we are responding to those comments. We believe that we have made every effort to involve stakeholders that have an interest in this process.

Comment 6. The recommendations seem to establish a need to protect against an exposure level that historically has been allowed as part of public policy (AAQS) and at a level that is extremely costly or virtually impossible to measure. This aspect of your recommendations should be identified for each of the RELs. The chosen approach and lack of factual information is likely to result in a great deal of confusion when applied differently than intended, and unnecessary resources going towards paper studies that provide no real benefit to air quality or public health. The worst consequences may be unfounded: fear on the part of the exposed public, wasteful efforts and costs to the regulated, and misused resources by those whom must implement programs based on the OEHHA recommendations.

Response. The CAAQS is for a 1 hour exposure which we had proposed adopting. The chronic REL is for continuous chronic exposures. The two values are not comparable and their uses are not comparable. In a risk assessment the chronic REL is compared to an annualized average, while the CAAQS (acute REL) is compared to the maximum one-hour concentration.

Based on other comments and on OEHHA's assessment of the developmental toxicity data available, OEHHA staff have reviewed the value in the draft document and have calculated a revised chronic REL for hydrogen sulfide of 9 $\mu g/m^3$ (7 ppb).

Metal Finishing Association of Southern California

Comments on the Draft Technical Support Document for the Determination of Noncancer Chronic Reference Exposure Levels were made by the Metal Finishing Association of Southern California (MFASC). The Association commented on 13 chemicals of relevance to their work. The comments included as an attachment a table titled Comparison of Limits for Selected Substances. Most of that information is in the Table included in the response to Comments 1-3.

Comments 1 – 3. For some of the substances, the proposed RELs are considerably different from those used before. … New data (i.e. more recent than 1992) were used to determine RELs for only 3 of the 13 chemicals [of interest to the MFASC]. Thus, the main difference between the new proposed RELs and the previous ones must be the methodology with which the existing data are handled.

For most of the new substances, the new RELs represent a substantial tightening of the regulatory burden. The 1992 REL for cadmium was 350 times less stringent than the proposed 1997 REL. Thus, sources that emit cadmium for which chronic non-cancer health effects were not previously predicted could now be subject to severe new regulatory requirements as a result of the change in the REL.

Response. The previous values were provided and presented by CAPCOA in response to the original Hot Spots Act. Subsequent legislation (Health and Safety Code Sec. 44360) required OEHHA to develop risk assessment guidelines for the Hot Spots program. OEHHA has used methods similar to those of the USEPA. Many of the CAPCOA values were, by contrast, derived from preexisting health risk guidance values (e.g., route-to-route extrapolation of oral Reference Doses, occupational exposure limits). These preexisting values were not originally intended for such purposes. CAPCOA used several indirect and ad hoc methodologies to derive its guidelines from these preexisting values. The CAPCOA effort was not as rigorous or time- and effort-intensive as the OEHHA effort. The comment is therefore correct: Most of the new proposed values represent intentional differences in methodology between OEHHA and CAPCOA. Cases where there would be substantial differences between the CAPCOA values and the proposed OEHHA were to be expected from the outset.

In particular, all the new values are based upon a much more thorough search of the existing scientific literature. The CAPCOA values were derived from preexisting health risk guidance values of quite varying vintage. Therefore, in many cases, the newly derived OEHHA proposed values incorporate additional data which, even if available prior to 1992, were not incorporated in the health risk guidance values upon which the CAPCOA values were based.

For instance, with respect to the case of the cited example of cadmium, in the CAPCOA document the then USEPA IRIS oral reference dose (RfD) was adjusted to an equivalent air concentration on the assumption that the oral and inhalation routes were of similar potency. However, in order to develop the OEHHA REL, OEHHA conducted an

extensive search of the inhalation toxicology literature. Through that effort, OEHHA identified the Lauwerys et al. (1974) key study of renal toxicity in workers exposed to cadmium. Unlike the original USEPA IRIS RfD, the proposed chronic REL value is based upon that 1974 epidemiological study. In 1996, the USEPA revised and greatly lowered its oral RfD for cadmium in light of health hazard information obtained from additional epidemiological studies. This recent change in the RfD supports the original presumption that oral and inhalation toxicities for cadmium would be similar. The new value for the cadmium RfD is therefore more in line with the proposed OEHHA chronic REL value.

The proposed OEHHA REL for cadmium of 0.01 $\mu g/m^3$ is 350-fold lower than the earlier CAPCOA value of 3.5 $\mu g/m^3$. The CAPCOA values were interim guidance values and were superseded when the appropriate governmental health risk assessments were completed. As a TAC, cadmium is regulated as a carcinogen with an OEHHA cancer unit risk value for cadmium is 4.2 E-3 $(\mu g/m3)^{-1}$. (At the proposed chronic REL the estimated lifetime cancer risk would be forty in a million.)

Chemical of Interest to MFASC	OEHHA Proposed REL $\mu g/m^3$	1992 CAPCOA Guidance Value $\mu g/m^3$	Basis of 1992 CAPCOA Value	Ratio of CAPCOA to OEHHA
Beryllium	0.001	0.0048	ACGIH TLV	4.8
Cadmium	0.01	3.5	USEPA IRIS	350
Chromium VI	0.0008	0.002	USEPA HEAST	2.5
Copper	0.02	2.4	USEPA IRIS	120
Hydrogen Chloride	7	7	USEPA IRIS	1.0
Hydrogen Cyanide	3	70	USEPA IRIS	23
Hydrogen Fluoride	30	5.9	ACGIH TLV	0.2
Methylene Chloride	300	3000	USEPA HEAST	0.1
Nickel	0.05	0.24	ACGIH TLV	4.8
Nitric Acid	40	none	(Not listed)	N/A
Perchloroethylene	40	35	USEPA IRIS	0.9
Sodium Hydroxide	2	4.8	ACGIH TLV	2.4
Zinc	0.9	35	Superfund PHEM	39

HEAST: Health Effects Evaluation Summary Table
IRIS: Integrated Risk Information System
TLV: Threshold Limit Value established by the American Conference of Governmental Industrial Hygienists
PHEM: Public Health Evaluation Manual

Finally, OEHHA staff attempt to use the best study of a chemical that it can find in the peer-reviewed literature to develop a chronic REL. When Hazard Indices exceed 1, air district staff consult with OEHHA staff on a case-by-case, chemical-by-chemical basis about the likelihood of adverse health effects. Risk management is an important part of the Air Toxics Hot Spots program.

Comment 4. Many of the proposed RELs may be below detection levels. Thus, it would be impossible to prove that these RELs are not being exceeded through the use of ambient monitoring. Moreover, no future epidemiological studies with such low concentrations would be possible. These values thus represent purely theoretical concentrations with no verifiable basis in reality.

Response. The inability of epidemiological studies to "verify" a chronic REL is a general one. With respect to epidemiological studies, the chronic REL value assumes that there is a threshold concentration below which adverse effects do not occur. The OEHHA values estimate this threshold with a margin for uncertainty. Thus, if the OEHHA REL is satisfactory, epidemiological studies conducted upon populations exposed at, or somewhat above, the chronic REL (or RfC for that matter) should find no adverse effect.

The comment is correct that ambient monitoring could not be used at locations where the ambient levels fell below the analytical detection limits. However, in most circumstances, emission rate information and air dispersion modeling are used to estimate ambient exposures. In the abstract, where exposures substantially exceed the REL and so are more likely to yield evidence of an adverse effect in an epidemiological study, they are also more likely to exceed the limit of detection.

While not optimal, often epidemiological investigations are necessarily conducted in the absence of actual air concentration data. These studies use distance from a source, duration of exposure, or air dispersion modeling as surrogate exposure information that permits persons to be classified by their relative degree of exposure.

The ability of an epidemiological study to detect any difference in a particular effect is much reduced at low levels of exposure. As the magnitude of exposure declines, the magnitude and frequency of any particular effect also declines to a point where any effect becomes hard to discern. Null results from such studies are reassuring but can not meaningfully "verify" a chronic REL, absent an extraordinarily large number of exposed persons in the study. For these practical reasons, epidemiological studies usually target high exposure (e.g., occupational) populations for study. Where epidemiological studies have detected adverse effects of air pollution on sensitive subjects (PM_{10}, ozone), these studies encompassed an extraordinarily large number of exposed persons.

Comment 5. The OSHA PELs cannot be used to assess risk to the public health. Yet, they are another measure of health risk published by an agency other than OEHHA. The new RELs do not reflect the general relationship of health risks for the different compounds. For example, the PELs would indicate that copper is no more hazardous than nickel although neither of these substances are as toxic as other metals. Yet, the new REL for copper is less than half that for nickel.

Response. When OSHA was created in 1973, OSHA adopted the existing ACGIH TLVs for copper and nickel as the OSHA PELs. Those OSHA values have not been revised. Since that time, a great deal of toxicity information on nickel compounds has been generated. The OEHHA nickel REL is based upon new animal data provided by the National Toxicology Program in 1994. This information was unavailable in 1973.

The proposed REL for copper is based upon comparatively limited information. The OEHHA value is based upon the Gleason study of just 3 workers exposed to copper. The study measured copper levels on only one day for three exposure conditions. This study provided a LOAEL of 30 $\mu g/m^3$ and a NOAEL of 8 $\mu g/m^3$ for copper fumes. The only other available inhalation toxicity study was a subchronic mouse study that established a LOAEL of 130 $\mu g/m^3$. Both these studies have substantial limitations. The human data were preferred as they required a much smaller overall uncertainty factor and exposure duration adjustment. If the animal data had been used, an even smaller proposed REL would have been developed. As a result of this limited animal and human information, uncertainty factors and exposure duration adjustments particularly contributed to the very low proposed chronic REL for copper. However, in light of the extent, quality, and coherence of the available toxicity information, OEHHA has reconsidered its derivation of the chronic REL for copper.

Comment 6. Because new health data are not being used, it is important to consider the assumptions especially in light of the uncertainty factors being used by OEHHA in proposing these new RELs. In lieu of corresponding RfCs from the USEPA, OEHHA appears to have applied the most conservative assumptions with regard to uncertainty where that were possible.

Response. For most of the substances under consideration, OEHHA pioneered the development of chronic RELs for environmental exposures. Whether or not the health data for a given substance were 'new', OEHHA's use of the data for this purpose was new for most of the covered substances.

OEHHA followed a methodology closely similar to that of the USEPA. A comparative analysis of the uncertainty factors applied by OEHHA in the development of its RELs and by the USEPA in the development of the similar RfCs does not support the contention that OEHHA excessively applied uncertainty factors in lieu of an available RfC. OEHHA's average cumulative uncertain factor of 134 is in fact approximately one-half the USEPA's average cumulative uncertainty factor of 238.

	Geometric Mean of Uncertainty Factors	
Uncertainty Factor	**OEHHA REL**	**USEPA RfC**
LOAEL	2.6	1.9
Subchronic	2.2	2.1
Interspecies	2.4	2.7
Intraspecies	9.3	8.9
Modifying factor	1	2.4

Average Cumulative UF	**134**	**238**

Comment 7. It is recommended that an independent peer review committee that includes toxicological experts independent of OEHHA review all assumptions used in proposing these RELs before they are adopted for use in regulatory programs.

Response. Pursuant to Health and Safety Code Section 39670, California has established an independent scientific peer review committee to perform just this function. Under this regime, OEHHA's work is submitted to the state's independent Scientific Review Panel on Toxic Air Contaminants.

Comment 8. It is recommended that OEHHA continue to use the 1992 RELs until the USEPA adopts new RfCs or until the proposed RELs are reviewed by an independent peer review committee as suggested above.

Response. We will be submitting this work to the Scientific Review Panel along with the Public Comments and staff responses to the Public Comments. The RELs will not be used until and only if the Scientific Review Panel endorses them.

Pacific Gas and Electric (PG&E)

Comments on the chronic REL document were made by PG&E in a letter dated January 29, 1998. PG&E made comments on the general methodology, the role of California Ambient Air Quality Standards (CAAQS), nitrogen dioxide, formaldehyde, and H_2S.

Comment 1. PG&E requests that the draft chronic Reference Exposure Levels (RELs) be revised to separately identify "known effect levels" and "uncertainty elimination levels", with the idea that risk assessments could be required to calculate hazard indices with respect to both the "known effect levels" and the "uncertainty elimination levels".

Response. OEHHA has based its proposed chronic reference exposure levels (RELs) on methods developed by USEPA in its reference concentration (RfC) program. RELs and RfCs are intended as estimates of levels unlikely to result in observable adverse effects among the general public. They are definitely not "uncertainty elimination levels," and methods to determine absolutely risk-free exposure levels are unknown. The REL document presents observed effects data for most chemicals reviewed that represent examples of "known effect levels." These data give a partial picture of potential adverse effects associated with chemical exposure and are presented to inform risk managers and other readers about these observed effects. Direct comparison of various observed effect levels is difficult because of the great variability in the bases for these data. Some are observations among occupationally exposed workers while others are from experimental animal studies. Exposures may be brief, intermittent, or over an entire lifetime. Effects noted may be mild or severe. They may affect a few susceptible subjects or nearly all exposed individuals. The change in severity and incidence in effects observed may be rapid or gradual with increasing exposures. Studies vary in quality and comprehensiveness and some significant adverse effects may go undetected.

Comment 2. In the toxic air contaminant identification process, H&S 39660(c) requires the Office of Environmental Health Hazard Assessment (OEHHA) to estimate both "the exposure level below which no adverse health effects are anticipated" and "an ample margin of safety." But H&S 39660(a) requires OEHHA merely to 'Prepare recommendations." H&S 39661(a)(1) states that "the state board in consultation with, and with the participation of the office, shall prepare a report", and H&S 39661(c) stipulates that the final regulation adopted by the state board will account "for the factors described in subdivision (c) of Section 39660". State law relies upon OEHHA's technical experts to recommend levels, but also upon the Air Resources Board's (ARB's) elected and appointed "risk managers" to review those levels. But these chronic RELs are not identification documents subject to ARB risk manager control. Rather OEHHA is responding to a separate guidelines mandate in H&S 44360(b)(2) which does not even, cross reference H&S 39660(c). Instead, H&S 44362(b) clearly states that it will be up to the judgment of the districts what level of risk or hazard will be deemed significant.

Response. The Toxic Air Contaminant (TAC) mandate (H&SC Sec. 39660 et seq.) is a separate mandate from the Air Toxics Hot Spots Information and Assessment program

(H&SC Sec. 44300 et seq.). However, there is overlap. All TACs, including the 189 Hazardous Air Pollutants (HAPs) in the Clean Air Act Amendments of 1990 which became TACs in April 1993 as a result of AB2728, are subject to the Hot Spots program (H&SC Sec. 39655(d)). In addition chemicals appearing on other lists are also subject to Hot Spots (H&SC Sec. 44321). OEHHA is the state's expert on health risk assessment (e.g., see Governor's Executive Order W-137-96) and develops health guidance values. The air districts decide how to manage the risks estimated using the values.

Comment 3. The draft chronic Reference Exposure Levels (RELs) include factors of uncertainty that push the draft chronic RELs below current ambient air quality standards (for hydrogen sulfide), and below levels which the Scientific Review Panel concluded caused no adverse effects other than a cancer risk (for formaldehyde). The formaldehyde and hydrogen sulfide RELs are just two examples where high uncertainty factors have been proposed for relatively mild effects even though substantial human exposure data is available.

Response. The hydrogen sulfide ambient air quality standard addresses short-term exposures. OEHHA based its chronic REL for hydrogen sulfide on the USEPA reference concentration (RfC). The USEPA also did not adopt an ambient air quality standard for hydrogen sulfide since it has none, but rather used long-term exposure data.

All USEPA Reference Concentrations (RfCs), available when the Technical Support Document (TSD) on chronic Reference Exposure Levels was drafted in October 1997, are being proposed as chronic RELs. RfCs are already used by the USEPA and by California's Department of Toxic Substances Control and were earlier incorporated by reference in Appendix F of the Emissions Inventory Criteria and Guidelines for the Air Toxics "Hot Spots" Program for use in screening risk assessments in the Hot Spots Program. These Guidelines were effective July 1, 1997. The Risk Assessment Advisory Committee (RAAC) recommended that CalEPA harmonize where possible with USEPA on risk assessment. Governor Wilson's Executive Order W-137-96 concerned the enhancement of consistency and uniformity in risk assessment between Cal EPA and USEPA. Use of RfCs as chronic RELs was one action that OEHHA took to address the RAAC recommendation and to implement the Executive Order. RfCs released after October 1997, including ones that are revisions of those in the October 1997 draft, will be evaluated for use in the Hot Spots program. OEHHA staff will review the scientific basis of each RfC when it becomes available and determine whether the scientific literature cited in the RfC is appropriate. Appropriate RfCs will be submitted to the SRP for their review and possible endorsement.

The RfC for hydrogen sulfide was adopted by USEPA in 1995 and incoporates a 1,000-fold cumulative uncertainty factor. The RfC is derived from a 90-day inhalation study with mice conducted by CIIT (Chemical Industry Institute of Toxicology). The study was well-conducted with many appropriate endpoints examined, but the number of animals tested was small. The critical endpoint for the RfC is nasal histological changes. The RfC was reviewed by OEHHA for general adequacy and accepted, although there is

some concern that the cumulative UF may be too large. OEHHA is reevaluating the hydrogen sulfide RfC and will be discussing this issue with the Scientific Review Panel.

The cumulative uncertainty factor for the formaldehyde REL was only 10-fold. This factor was necessary because sensitive human groups such as children or the elderly may be considerably more susceptible to effects from long-term formaldehyde exposure than were the relatively healthy group of workers described in the Wilhelmsson and Holmstrom reports. Cancer is a separate issue.

Comment 4. The public and their risk managers may have differing perspectives about how feasible or necessary it is to provide so ample a margin of safety. Perspectives may also differ between districts, or over time. OEHHA does not need to obtain risk manager consensus on these uncertainty factors. But OEHHA should design its factors and its hazard identification guidelines to enhance, not reduce, risk manager flexibility.

Response. OEHHA fully acknowledged in the draft chronic REL document the many and varied uncertainties involved in the task of estimating exposure values protective against noncancer health effects. The nomenclature used by OEHHA and USEPA for its values, REL and RfC, respectively, incorporate the term "reference" because of the recognition that no single exposure value can be derived that demarcates "safe" from "hazardous." It is rather intended to be a useful risk management tool in assessing relative and cumulative risks associated with chemical exposures. Thus an important goal of the OEHHA chronic REL effort was to maintain a consistent basis for deriving the various RELs.

Comment 5. We recommend that OEHHA establish chronic "Known Effect Levels" (KELs) at the projected No Observed Adverse Effects Level (NOAEL), and separate "Uncertainty Elimination Levels" (UELs) that consider what added safety or uncertainty factors are finally adopted. We further recommend that OEHHA's chronic hazard evaluation guidelines require that the hazard index be calculated both with respect to the KELs and with respect to the UELs. This would provide the state and public with a uniform database on relative hazards posed by different sources in different districts, as well as a good indication of how much uncertainty there is about those hazards. It would also enable district risk managers to chose whether to relate their significant hazard level to either the KELs or the UELs. While most Districts may be content to relate acceptable levels to OEHHA's UELs for most compounds, some may prefer to relate significance levels for some compounds to the KELs - at least until it becomes feasible to provide the additional margins of safety that uniform adherence to the UELs would likely require.

Response. OEHHA developed its risk assessment methods for noncancer health effects from chronic exposures to be consistent with methods used by USEPA in the development of its reference concentrations. Levels associated with observed adverse effects are reviewed in the document. RELs and RfCs are intended as estimates of levels unlikely to result in observable adverse effects among the general public, but are not

"uncertainty elimination levels." RELs are presented with observed effect data (that could be termed "known effect levels") to inform risk managers and other readers. Direct comparison of various observed effect levels is difficult because of the great variability in the bases for these data. Some are observations among occupationally exposed workers while others are from experimental animal studies. Exposures may be brief, intermittent, or over an entire lifetime. Effects noted may be mild or severe. They may affect a few susceptible subjects or nearly all exposed individuals. The change in severity and incidence in effects observed may be rapid or gradual with increasing exposures. Studies vary in quality and comprehensiveness and some significant adverse effects may go undetected.

Comment 6. Exclude substances regulated by state or federal ambient air quality standards, like NO_2, from the toxic air contaminant hazard evaluations, or if hazard evaluation is deemed necessary, evaluate them only with respect to a chronic REL set at the most applicable adopted ambient standard.

Response. Chronic RELs are for use in the Hot Spots program. Many chronic RELs are for toxic air contaminants (TACs) because TACs are subject to the Hot Spots program. Other Hot Spots chemicals are not currently classified as toxic air contaminants. The ambient air quality standards are generally designed to protect against adverse effects resulting from exposures to concentrations for varying time periods which varies with the standard. The USEPA has an annual standard for nitrogen dioxide of 0.05 ppm (100 $\mu g/m^3$) to "prevent health risk and improve visibility." California has a 1-hour standard for nitrogen dioxide of 0.25 ppm (470 $\mu g/m^3$). OEHHA separately evaluated health effects of short-term exposures (1-hour time-weighted average exposure) and long-term exposures ("annual time"-weighted average exposure) for acute and chronic RELs, respectively. The focus of evaluation and the averaging time for an ambient air quality standards and a REL can differ. OEHHA used the 1-hour California nitrogen dioxide standard as its acute REL. The proposed chronic REL of 20 $\mu g/m^3$ (10 ppb) is based purely on health effects, in this case a 1993 report by Infante-Rivard in which effects in asthmatic children were observed at 15 ppb. This is tabulated in Section VI of the chronic REL summary. It would not be a responsible action for OEHHA to let the chronic REL be set at the annual Federal standard of 0.05 ppm (50 ppb) when adverse effects in children at 15 ppb nitrogen dioxide have been reported in the peer-reviewed literature.

Comment 7. In 1995, there was no place in California where either the federal annual NO_2 standard of 100 $\mu g/m^3$ or the state daily NO_2 standard of 470 $\mu g/m^3$ was exceeded. But ambient concentrations exceeded the proposed NO_2 REL of 20 $\mu g/m^3$ (0.01 ppm) at 90 of 123 monitoring sites during that year. Currently, 80% of 1995 California NO_x emissions were attributed to mobile sources not regulated under the "hot spots" program (see pp. 100-110 of the ARB's annual "Summary of 1995 Air Quality Data", and pp. 34 of the 10/10/97 statewide inventory tables available at http://arbis.arb.ca.gov/emisinv/95inven195inv.htm). Those fuel combustion sources

regulated under the "hot spots" program typically emit much more NO than NO_2, and it would be difficult for NO_x dominated "hot spot" sources to accurately estimate what percentage of their NO emissions might convert to NO_2 before their points of maximum ground level impact.

Response. Issues such as (1) whether ambient concentrations in any particular areas may exceed the health-based risk assessment values, (2) the relative sources of ambient concentrations, or (3) the technical difficulties in estimating emissions or reducing emissions to avoid exceeding such values are not relevant to the development of these values, though these certainly are additional risk management issues. Unlike other exposure values that incorporate such risk management concerns, OEHHA RELs and USEPA RfCs and RfDs are purely health data-based guidance values that ultimately will be one of a number of issues considered by risk managers.

Comment 8. Although NO destroys ozone while converting to NO_2, conversion in the center of an NO dominated plume can be incomplete 60 km downwind (page 8, Air & Waste Management Association paper 95-RAI13A.01, "The Significance of NO_x Emissions from Coal-Fired Power Plants in the Middle Tennessee Area on Tropospheric Ozone, by Prof. Wayne T. Davis, Univ. of Tenn., et al, June 18-23, 1995). Also precise calculation of NO to NO_2 requires data and models that won't be available at most locations, and very costly to acquire and use at those few locations where they might be available.

Furthermore, since the effects of ozone exposures appear more serious than those from NO_2, overestimating conversions might harm rather than protect - if the result was less NO in an area where NO helps to destroy ozone. Meanwhile, much of California is non-attainment of state ambient air quality standards for ozone or fine particulate, and as a result will already require most "hot spots" sized NO_x sources to impose either Best Available Retrofit Technology or All Feasible Control Technology. All of these factors suggest that NO_2 concerns remain more appropriately addressed under the ambient air quality programs, rather than within the chronic hazards portion of the "hot spots" program.

Response. The concerns raised by this comment should be more appropriately addressed in other settings, such as at the risk management level. It was beyond the scope of the OEHHA chronic REL document to address issues such as difficulties in estimating emissions and exposures, relative risks of chemicals, and the fate of chemicals in the ambient air. This document is focused on the development of strictly health-based exposure guidance.

Comment 9. Ambient air quality standard reviews focus immense attention upon one substance. For example, the joint ARB/OEHHA Technical Support Document "Review of the One-Hour Ambient Air Quality Standard for Nitrogen Dioxide" published in December 1992 required 232 pages to address NO_2 data. While OEHHA has prepared an excellent 8 page summary on its proposed NO_2 REL, a summary of that size is unable to

provide the detail on the key study needed to attract critical review, especially when it competes for agency/commentator attention with 750+ other pages on 119 other substances. Where a comprehensive review has already been undertaken, a briefer review should not be allowed to displace it. If desired, OEHHA could ensure NO_2 inclusion in calculated total hazard indices by simply referencing the existing federal annual standard. If a subsequent review were to result in revision of the standard, the REL could also change.

Response. The 1-hour ambient air quality standard for nitrogen dioxide is designed to protect against adverse effects resulting from short-term exposures to peak concentrations and has been endorsed by the Scientific Review Panel for use as an acute REL in the Hot Spots program. The USEPA's annual standard for nitrogen dioxide of 0.05 ppm (100 $\mu g/m^3$) is to "prevent health risk and improve visibility." The proposed chronic REL of 20 $\mu g/m^3$ (10 ppb) is based purely on health effects, in this case a 1993 report by Infante-Rivard in which effects in asthmatic children were observed at 15 ppb. This is tabulated in Section VI of the chronic REL summary. It would not be appropriate for OEHHA to let the chronic REL be set at the annual Federal standard of 0.05 ppm (50 ppb) when adverse effects have been reported in the peer-reviewed literature in children at 15 ppb.

Comment 10. If a chronic REL is proposed for hydrogen sulfide even though an ambient standard exists for that compound, then the existence of the hourly standard should at least reduce the need for a subchronic exposure uncertainty factor.

Response. OEHHA separately evaluated health effects of short-term exposures (1-hour time-weighted average exposure) and long-term exposures (1-year time-weighted average exposure). The proposed chronic REL for hydrogen sulfide is based on the USEPA RfC which was derived from subchronic exposure data. USEPA followed its RfC methodology in determining an appropriate subchronic uncertainty factor. The subchronic uncertainty factor is intended to account for potential differences in the magnitude of adverse effects between those observed in subjects exposed over less than a full lifetime and those that might be experienced by the general public over their entire lifetime. Thus short-term exposure data, even if extensive, do not eliminate uncertainties resulting from a lack of long-term exposure data.

Comment 11. For Hydrogen Sulfide, data showing no adverse effects at 5400 ppb is claimed to justify limiting exposures to 0.7 ppb. In other words, the proposed REL is 7,714 times as stringent as the No Observed Adverse Effects Level (NOAEL). This reflects the use of a factor of eight in extrapolating from mice to humans, and the multiplication of additional "uncertainty" factors amounting to 900, but tabulated as 1,000. Given that the effect that is not observed to occur at the 5400 ppb level was "inflammatory changes in the nasal mucosa", is such a high uncertainty factor necessary?

Response. The magnitude of the difference between concentrations known to cause adverse effects and those without appreciable risk can never be determined with absolute

certainty. Thus USEPA has developed and OEHHA has adopted, with some changes to ensure consistency, default and data-based methods to derive the RfC and REL "reference" levels. In some cases where overall uncertainty is low, a small or even no uncertainty factor has been used. In other cases, several areas of significant uncertainty exist. This results in a large cumulative uncertainty factor that is unsatisfying for all concerned but will require the development of better data to improve the situation. When better data become available, the RELs will be reevaluated and hopefully the use of uncertainty factors can be lessened or in some cases eliminated. Also some humans experience adverse effects of nausea and headache at the 30 ppb one-hour standard and some people may experience adverse effects at a somewhat lower concentration when exposed chronically. The point of using uncertainty factors is to get below these adverse effect levels. Comparing 5400 ppb and 30 ppb, it is no surprise that the extrapolation to the chronic REL must be at least 180-fold and probably more since 30 ppb is a LOAEL for people.

OEHHA is attempting to determine if USEPA inadvertently applied an incorrect uncertainty factor of 10 in the RfC calculation when the text indicated that 3 was appropriate. It is also not clear that a database deficiency factor is warranted. We will be discussing this issue with the Scientific Review Panel.

Comment 12. California previously adopted an ambient air quality standard for hydrogen sulfide at 30 ppb or 42 $\mu g/m^3$, averaged over one hour to protect against annoying odors. It is generally recognized that annual average concentrations are typically ten or more time lower than peak hourly concentrations. The "Toxic Air Pollutant Source Assessment Model for California Air Pollution Control Districts and Applicants for APCD permits" adopted October 1, 1987 uses a multiplying factor of 0.1 for conversion of hourly model results to annual in flat terrain downwash, and presents a similar hourly to annual ratio (4.0/0.4 = 0. 1) when 24 hour complex terrain model results are extrapolated. On page 111-5 of the 1993 California Air Pollution Control Officer's Association Risk Assessment Guidelines, the 0. 1 factor continues to be used for the conversion of hourly screening model results to annual average, and the only examples cited on page 111-13 all had peak hourly concentrations 10 to 15 times the corresponding annual average concentrations. On that basis, the state standard of 30 ppb should be sufficient to protect against chronic exposures of 3 ppb. 3 ppb would be a factor of 1800 below the reported NOAEL, and a factor of 220 below the OEHHA calculated equivalent human NOAEL.

Response. The 10-fold convention used in exposure estimation is based on only commonly observed differences in maximum 1 hour and maximum 1 year average exposure concentrations. This factor does not address differences in health effects that might be observed between a short-term exposure and those over a lifetime. OEHHA separately evaluated health effects of short-term exposures (1-hour time-weighted average exposure) and long-term exposures (1-year time-weighted average exposure). The proposed chronic REL for hydrogen sulfide is based on the USEPA RfC, which was derived from long-term exposure data. The ambient air quality standard for hydrogen sulfide is designed to protect against adverse effects resulting from short-term exposures

to peak concentrations. Therefore the focus of evaluation for the AAQS is different from the chronic REL.

Comment 13. It would be better for everyone if all available compliance resources were devoted towards ensuring continuous compliance with the existing state hourly standard, rather than dividing resources to separately evaluate compliance with separate standards. Therefore PG&E recommends that the Office of Environmental Health Hazard Assessment (OEHHA) not adopt any chronic REL for hydrogen sulfide, but instead rely upon the hourly standard to protect the public from chronic exposures as well.

Response. This concern should be more appropriately addressed in other settings, such as at the risk management level. This document is focused on the development of strictly health-based exposure guidance, and includes chemicals selected from substances of concern identified by the California Air Resources Board. Acute exposure standards are not necessarily protective of the general public exposed over a lifetime.

Comment 14. If OEHHA believes that it must adopt a chronic REL for a substance for which an hourly state standard exists, then we urge OEHHA to reconsider the proposed factors of uncertainty. For example, eliminating the subchronic uncertainty factor (because there is a separate subchronic standard), would be sufficient to raise the REL to 660 ppb / 90 - 7 ppb. If the chronic REL were set at 7 ppb, the hourly 30 ppb standard would remain the governing factor in almost all situations.

Response. OEHHA reviewed the USEPA RfC and concluded that it was adequate for use as an OEHHA chronic REL. There is no basis for eliminating a subchronic uncertainty factor because of the availability of short-term data and exposure guidelines. This factor is eliminated where adequate data on toxicity following long-term exposure are available, which was not the case for hydrogen sulfide.

Comment 15. Our chief concern with such a 7 ppb REL is whether geothermal power plants would be expected to evaluate compliance with such an REL within the secured geothermal steam supplier leasehold. If OEHHA adopts a 7 ppb REL, then we would hope that OEHHA would make provisions within its risk assessment guidelines for adjacent industrial sources to agree among themselves that risks/hazards need only be evaluated outside their common perimeters.

Response. This concern should be more appropriately addressed in other settings, such as at the risk management level. This document is focused on the development of strictly health-based exposure guidance.

Comment 16. OEHHA should use lesser uncertainty factors for natural compounds like formaldehyde for which abundant exposure data exist.

Response. From a risk assessment perspective, the natural occurrence of a chemical is not a factor in estimating risks from exposure. The large health effects database for formaldehyde was considered and is reflected in the relatively small 10-fold cumulative uncertainty factor for formaldehyde.

Comment 17. [OEHHA] should not adopt RELs in conflict with prior Scientific Review Panel identification report conclusions.

The proposed formaldehyde REL is equated to 1 ppb. This is 4.4 times below the mean annual outdoor population weighted exposure that 20 million Californians were deemed subject to in finding #6 on page 17 of the Executive Summary of the "Final Report on the Identification of Formaldehyde as a Toxic Air Contaminant" in July 1992 . On page 18 of that report, Scientific Review Panel finding #10 concludes that "Adverse health effects other than cancer are not expected to occur at mean statewide outdoor ambient concentrations". OEHHA should not adopt a REL that suggests hazards could exist at levels that more exhaustive prior identification report review concluded did not pose a hazard. There are often issues which cannot be adequately evaluated in these more generic REL reviews. OEHHA also appears to be changing an identification report finding outside the established process (see PG&E's comment #1).

The proposed formaldehyde REL is also equated to 2 $\mu g/m^3$, which is 130 times below the identified Lowest Observed Adverse Effects Level (LOAEL), and 45 times below the No Observed Adverse Effects Level (NOAEL). Given the lack of data suggesting that widespread ambient air exposures are causing problems, a lesser combined factor of uncertainty would appear appropriate. Replacing the current factor of uncertainty of 130 relative to the LOAEL with a factor of uncertainty of ten with respect to the NOAEL would raise the REL to 9 $\mu g/m^3$ (6 ppb). This level would be above recent average exposures, and protecting against above average exposures would be more consistent with the identification report findings.

Response. The health effects assessment document that is part of the "Final Report on the Identification of Formaldehyde as a Toxic Air Contaminant" focused on cancer risks associated with formaldehyde exposure, and did not extensively evaluate noncancer health effects. The proposed OEHHA REL for formaldehyde is based on a review of noncancer health effects data. The Scientific Review Panel will review the proposed formaldehyde REL. The magnitude of the REL relative to ambient exposure levels is not an appropriate factor to incorporate into the derivation of the REL, but is an issue of concern to the risk management of formaldehyde exposures. The Panel approved an acute REL for formaldehyde of 94 $\mu g/m^3$.

Comment 18. One key finding in the identification report was that the risk of cancer was the greatest concern. But adoption of a formaldehyde REL of 2 $\mu g/m^3$ would flip flop that conclusion at least in non-residential areas. Both cancer risks and chronic hazards

can be adjusted for hours worked per year of exposure. But chronic hazards are based upon the single worst year, whereas as cancer risks are based upon average exposure over 46 year working lifetime (versus a 70 year nominal lifetime). A single significant figure REL would have to be at least 3 $\mu g/m^3$ before worker risk would be more significant than chronic hazards for workers subject to non-variable exposures.

10 in a million [significant risk]		70 years [nominal]			chronic REL at
----------	x	----------	=	2.54 $\mu g/m^3$	which cancer and
6 in a million/($\mu g/m^3$) [unit risk]		46 years [working]			hazards equate

But most exposures vary from year to year. In our opinion 9 $\mu g/m^3$ offers a reasonable balance - a factor of 10 below the NOAEL, a factor of 2 above the historic exposures found not to cause adverse effects, and a factor of 3 above the level at which steady state sources would calculate more significant chronic hazard indices than cancer risks. Chronic hazards could still govern at sources with more variable emissions.

Response. The availability of relevant data was an important consideration in the development of the chronic RELs. While USEPA frequently uses its limited database factor, OEHHA did not use such a factor.

Comment 19. RELs (or KELs & UELs) should be presented with both all significant figures and with an appropriately rounded number of significant figures. It would be inconsistent to propose a cumulative factor of uncertainty of 1000 or more, while insisting upon the use of multiple significant figures. But even when numbers are highly uncertain, rounding does not render the rounded numbers more accurate, so neither would it be appropriate to insist upon rounding. If one calculates a hazard index of 0.999 relative to a significance level of 1.0 no one should ever round such "insignificant" results up to the significance level.

An OEHHA REL (or as we suggest, an OEHHA UEL) should be specified both as "originally calculated" with all available significant figures, and as rounded where the degree of rounding should reflect the assumed level of uncertainty. For example, compounds employing a factor of uncertainty of 1,000 or more could be rounded to one significant figure, while compounds employing a factor of uncertainty between 1,000 and 10 could be rounded to two significant figures, and compounds employing a factor of uncertainty of 10 or less could be rounded to three significant figures. But the risk assessment guidelines should allow hazard assessors to use either rounded or originally calculated RELs, as long as only "rounded" or only "originally calculated" numbers are used for all of the RELs employed in the same hazard index evaluation.

For NO_2, the calculated median of a 10- 15 range was 12.5, which was rounded to 10. If OEHHA were to adopt that proposed REL (KEL?) for NO_2 with no added

uncertainty factor, then all three significant figures (that is the 12.5 number) should be retained.

Response. The use of one significant figure is consistent with the practice of USEPA in its RfC program. Since OEHHA adopted many USEPA RfCs as RELs, OEHHA adopted USEPA practice of using one significant figure for chronic RELs. Furthermore, additional figures would not be meaningful given the degree of uncertainty associated with the proposed chronic REL values. The values used in the derivation of the RELs are fully presented, and risk managers using the chronic REL guidance may consider this issue as part of their evaluation of health impacts associated with chemical exposures.

Comment 20. The public and risk managers would benefit if risk assessments differentiated between levels actually associated with adverse effects (Known Effect Levels) and levels deemed necessary to provide added protection (Uncertainty Elimination Levels), and if ambient standard pollutants were evaluated as clearly separated chronic background adders rather than as part of a single initially calculated chronic hazard index.

Response. OEHHA has based its proposed chronic reference exposure levels (RELs) on methods developed by USEPA in its reference concentration (RfC) program. RELs and RfCs are intended as estimates of levels unlikely to result in observable adverse effects among the general public. The REL document presents observed effects data for most chemicals reviewed that represent examples of "known effect levels." These data give a partial picture of potential adverse effects associated with chemical exposure and are presented to inform risk managers and other readers about these observed effects. Direct comparison of various observed effect levels is difficult because of the great variability in the bases for these data. Some are observations among occupationally exposed workers while others are from experimental animal studies. Exposures may be brief, intermittent, or over an entire lifetime. Effects noted may be mild or severe. They may affect a few susceptible subjects or nearly all exposed individuals. The change in severity and incidence in effects observed may be rapid or gradual with increasing exposures. Studies vary in quality and comprehensiveness and some significant adverse effects may go undetected.

Comment 21. OEHHA should reconsider its use of the same factors of ten for each level of uncertainty for compounds with both abundant and sparse exposure data. We believe that lower factors of uncertainty should be used, particularly for naturally occurring substances like formaldehyde for which abundant exposure data exist.

Response. Uncertainty factors of between 1 and 10 were used in the chronic REL document, depending on data quality. From a risk assessment perspective, the natural occurrence of a chemical is not a factor in estimating risks from exposure. An "abundance of exposure data" does not provide information for developing a health-based Reference Exposure Level, unless accompanied by a corresponding study of the

health effects of exposure. The large health effects database for formaldehyde was considered and is reflected in the relatively small 10-fold cumulative uncertainty factor for formaldehyde.

Styrene Information and Research Center

Comments on the chronic REL for **styrene** were made by Jeffrey C. Terry, Manager for State Government Relations of the Styrene Information and Research Center (SIRC). OEHHA recommended use of the US EPA Reference Concentration of 1,000 $\mu g/m^3$ based on neurotoxicity in humans as the chronic REL for styrene.

Comment 1. Effective Exposure Level. SIRC supports the evaluation of the effective exposure level the USEPA included in determining the RfC for styrene in its IRIS review. Mutti et al. concluded that the workers with metabolites of up to 150 mmoles/mole appeared to have no significant effects. SIRC recommends, as OEHHA is adopting the USEPA RfC, that OEHHA not include any discrepancy between its analysis of the effective exposure level and that of the USEPA.

Response. While OEHHA has recommended that the USEPA RfC be adopted as the California chronic inhalation REL, OEHHA is also charged under Health and Safety Code Section 39660(c)1 with providing information on the completeness and quality of the available data. This "discrepancy" relates to determination of a LOAEL or NOAEL for use in the dose response assessment. The "discrepancy" has been disclosed as it reflects an important issue associated with California's adoption of the RfC as a chronic REL.

In the Mutti et al (1984b) study, tests for some individuals in the lowest exposure group did provide abnormal results; and, conversely, tests on some of the individuals in the highest exposed groups did not provide abnormal results. The statement that "workers with metabolites of up to 150 mmoles/mole appeared to have no *significant* effects" has meaning in so far as it pertains to statistical comparisons that bear on the experience of groups, not individuals. The mean exposure for the lowest exposure group was 75 mmoles/mole. The value of 150 mmoles/mole represents the designated upper limit of the exposures for this group. It is the mean value of 75 mmoles/mole, and not the designated upper exposure level of the lowest exposure group, which most accurately represents the exposure history of that group. Therefore, 75 mmoles/mole is the appropriate starting point for dose response assessment.

Comment 2. Uncertainty Factors. SIRC understands the difficulty in assessing the Mutti study. However, USEPA's interpretation of the study by imposing a cumulative uncertainty factor of 30 is appropriate. SIRC disagrees with an UF of 10 for intraspecies variability that OEHHA mentions. SIRC quoted the USEPA's IRIS:

" A partial UF of 3 was used for database inadequacy, including the lack of concentration-response information on respiratory tract effects. A partial UF of 3 instead of 10 was used for intraspecies variability since the lower confidence limit of the exposure extrapolation was used and because Perbellini et al. (1988) demonstrated that this biological exposure index (i.e. urinary metabolites) accounts for differences in pharmacokinetic/physiologic parameters such as alveolar ventilation rate. A partial

UF of 3 instead of 10 was also evoked for lack of information on chronic studies as the average exposure duration of the principal study of Mutti et al. (1984) was not long enough (8.6 years) to be considered chronic. The total uncertainty is therefore 30."

SIRC also disagreed with the OEHHA speculation that potential nutritional differences also supported use of the full intraspecies uncertainty factor of 10.

SIRC requested that the inconsistency with the USEPA RfC analysis be taken out of the OEHHA document.

Response. The USEPA based its rationale for a partial intraspecies uncertainty of 3 on its use of the lower 95 percent confidence limit of its estimate of the central tendency value for the air styrene concentrations predicted from the 150 mmole/mole urinary styrene metabolite concentrations observed in the Mutti et al. (1984) study. The USEPA opined that use of this partial uncertainty factor was justified since the urinary metabolites' biological index took into account differences in pharmacokinetic/physiologic parameters and also because use of the lower 95 per cent confidence limit takes into account some of the intraindividual variation in the toxicokinetics of styrene.

OEHHA does not dispute that the urinary metabolites' biological index takes into account differences in pharmacokinetic/physiologic parameters. If a chronic REL were to be expressed in terms of urinary metabolite levels, a partial uncertainty factor of 3 would be appropriate to the extent that the toxicokinetic contribution to intraindividual variance was substantially eliminated by use of a standard based upon urine metabolite levels. However, here, the chronic REL is expressed in terms of a styrene air concentration, not the concentration of styrene metabolites in the urine. The contribution of toxicokinetics to the overall variance is no longer taken into account when the standard is expressed in units of air styrene concentrations, and not urinary styrene metabolite levels.

The USEPA did, however, use the lower 95 per cent level confidence limit of the airborne styrene concentration associated with 150 mmoles/mole (mmoles styrene metabolite per mole of urinary creatinine) in its dose response assessment. The USEPA stated that the choice of this value took into account some of the population variance due to toxicokinetic differences. This value, which was 88% of the central value, is based upon the *standard error* of a mean value. Therefore, this 95% lower confidence limit is an inadequate measure of the range of individual response characteristics, which relate more reliably to the *standard deviation* of the study population. When sample sizes are large, standard errors especially convey very little information about the standard deviation of the population. The Guillemin et al. (1982) study employed a large study population (N = 90). The USEPA methodology could not capture the variability that it sought to take into account when it selected the 95% lower confidence limit of the air styrene concentration associated with the 150 mmoles/mole styrene metabolite level. Therefore, this approach may not have warranted use of a partial uncertainty factor.

While nutritional factors are known to alter the human response to other chemical species, OEHHA's opinion that an intraspecies uncertainty factor of 10 was preferable did not turn on the issue of malnutrition as a potential contributor to the variability of the human response to styrene. The study on which the RfC was based addressed the effects of styrene on an occupational cohort. Clearly, since the USEPA study is based upon a worker population, the issue of malnutrition was a secondary consideration.

Worker studies, as discussed in the OEHHA draft document, do not capture the variability of the general population, which is to be protected by the chronic REL. Working populations are typically healthier than the general population and also do not share its age distribution. Furthermore, the eligibility criteria of the Mutti et al. (1984) study excluded workers with a variety of diseases. Thus, even if the USEPA methodology had captured the magnitude of the intraindividual toxicokinetic variability of the worker population in Mutti et al. (1984), it could not have adequately captured the toxicokinetic variability of a general population comprised of the elderly and children as well as those with medical conditions.

OEHHA disagrees that mention of the Khanna et al. (1994) study indirectly imposes an interspecies uncertainty factor. No such factor was applied. The Khanna et al. (1994) study raises the potential for a greater than usual intraspecies variability due to the effects of malnutrition. Similar human data are not available. The Khanna et al. (1994) study was discussed in the context of what was the most appropriate *intraspecies* uncertainty factor to use in the risk assessment.

UNOCAL Geothermal

Comments on the **hydrogen sulfide** chronic REL were submitted by UNOCAL Geothermal, which has an operation at the Geysers. The comments are those of their consultant, Dr. Charles Lambert. OEHHA proposed use of the US EPA RfC of 0.7 ppb (0.9 $\mu g/m^3$) as the chronic REL.

Comment 1. After thorough review of the H_2S literature and the OEHHA supporting documentation for the proposed dramatic change in REL, I believe strongly that OEHHA's decision to lower the REL to 0.7 ppb should be revisited. The REL for H_2S should be set no lower than 7.0 ppb. This recommendation is based on the following conclusions: (1) The California ambient air quality standard for H_2S is 30 ppb. (2) The proposed REL of 0.7 ppb is one thousand times lower than the upper concentration for naturally occurring H2S in human breath. (3) Low levels of H_2S are rapidly metabolized and detoxified by the human body and therefore unlikely to be a chronic hazard at concentrations at or below the odor threshold. (4) The extremely conservative safety factors used in deriving the USEPA RfC, on which the new H_2S REL is based, should be decreased by at least an order of magnitude. (5) New studies on the toxicity of H_2S have been published or initiated since the 1994 USEPA reference concentration (RfC) was finalized. OEHHA should wait until all of this updated information is in before finalizing the REL.

Response. The dramatic change referred to in the comment is the change from the 1 hr CAAQS for H_2S of 30 ppb used in the CAPCOA risk assessment guidance (last updated in October 1993) to the proposed use of the USEPA RfC of 0.7 ppb in the Technical Support Document. All USEPA Reference Concentrations (RfCs), available when the Technical Support Document (TSD) on chronic Reference Exposure Levels was drafted in October 1997, are being proposed as chronic RELs. RfCs are already used by the USEPA and by California's Department of Toxic Substances Control and were earlier incorporated by reference in Appendix F of the Emissions Inventory Criteria and Guidelines for the Air Toxics "Hot Spots" Program for use in screening risk assessments in the Hot Spots Program. These Guidelines were effective July 1, 1997. The Risk Assessment Advisory Committee (RAAC) recommended that CalEPA should harmonize where possible with USEPA on risk assessment. Governor's Executive Order W-137-96 concerned the enhancement of consistency and uniformity in risk assessment between Cal EPA and USEPA. Use of RfCs as chronic RELs was one action, which OEHHA took to address the RAAC recommendation and to implement the Executive Order. RfCs released after October 1997, including ones that are revisions of those in the October 1997 draft, will be evaluated for use in the Hot Spots program by reviewing the scientific basis of each RfC when it becomes available and by determining whether the scientific literature cited in the RfC is current. Appropriate RfCs will be submitted yearly to the SRP for review and possible endorsement.

Comment 2. The odor threshold for H_2S is around 10-20 ppb, while the characteristic "rotten egg" odor associated with H_2S can be clearly noted at ambient concentrations of

30-100 ppb (odor recognition threshold). However, it is not until levels in excess of 50 ppm where irritation of the mucous membranes of the eyes and lungs may start to occur. This is an approximate 1,000 fold margin of safety between the odor threshold and signs of toxicity. The concentrations at which health effects begin to occur are well documented and are the basis for current, national, safe exposure limit concentrations. It is not until levels in excess of 50 ppm where irritation of the mucous membranes of the eyes and lungs may start to occur

Response. The national limits are for workplace exposure, not chronic ambient exposure of the general population including sensitive individuals, the target of chronic RELs. OEHHA staff notes that the ACGIH intends to lower the H_2S TLV from 10 to 5 ppm. The assertion that "it is not until levels in excess of 50 ppm where irritation of the mucous membranes of the eyes and lungs may start to occur" is not held by all observers. Concentrations of hydrogen sulfide that substantially exceed the odor threshold result in the annoying and discomforting physiological symptoms of headache or nausea (Amoore, 1985; Reynolds and Kauper 1985). The perceived intensity of the odor of hydrogen sulfide depends on the longevity of the concentration, and the intensity increases 20% for each doubling of the concentration (Amoore, 1985). Several studies have been conducted to establish the ratio of discomforting annoyance threshold to detection threshold for unpleasant odors (Winneke, 1975; Winneke and Kastka, 1977; Hellman and Small, 1974; Adams *et al.,* 1968; and NCASI, 1971). The geometric mean for these studies is 5, indicating that when an unpleasant odor reaches an average concentration of 5 times its detection threshold, the odor will result in annoying discomfort. Applying the 5-fold multiplier to the mean detectable level, 0.008 ppm, results in a mean annoyance threshold of 0.04 ppm. At the current California Ambient Air Quality Standard (CAAQS) of 0.03 ppm, the level would be detectable by 83% of the population and would be discomforting to 40% of the population. These estimates have been substantiated by odor complaints and reports of nausea and headache (Reynolds and Kauper, 1985) at 0.03 ppm H_2S exposures from geyser emissions. The World Health Organization (WHO) reports that in order to avoid substantial complaints about odor annoyance among the exposed population, hydrogen sulfide concentrations should not be allowed to exceed 0.005 ppm (5 ppb or 7 $\mu g/m^3$), with a 30-minute averaging time (WHO, 1987; National Research Council, 1979; Lindvall, 1970). The RfC of 0.9 $\mu g/m^3$ (0.7 ppb) is for a year's averaging time and is within a factor of 8 of WHO's recommendation for 30 minutes. The RfC seems rather low but was the result of following USEPA's documented procedure for developing RfCs which has evolved during the last 10 years and is compatible with WHO's recommendation for a 30 minute acute exposure. The RfC is based on a study in mice in which animals in the LOAEL group had histopathological inflammatory changes in the nasal mucosa, an endpoint compatible with respiratory irritation.

Comment 3. The major human health concern from hydrogen sulfide is from acute exposures in excess of 50 ppm. There are OEHHA acute RELs in place to deal with such exposure scenarios. There is a significant amount of literature documenting chronic human exposure to hydrogen sulfide. There is no convincing evidence that chronic low-level exposure to H_2S at levels around the odor threshold causes adverse health effects. One study of a community in Rotonia, New Zealand (an area of significant geothermal

activity) showed that no chronic health effects could be identified after long-term exposure to 5 to 1,900 ppb H_2S.

Response. If chronic low-level exposure to H_2S at levels around the odor threshold cause no adverse health effects, we should be able to develop a chronic REL based on that data. The referenced study (Siegel, S.M., Penny, P., Siegel, B.Z. et al. (1986) Atmospheric hydrogen sulfide at the Sulfur Bay wildlife area, Lake Rotonia, New Zealand. *Water Air Soil Pollut.* 28:385-391) should be submitted to the USEPA for review. However, as stated above, WHO reports that in order to avoid substantial complaints about odor annoyance among the exposed population, hydrogen sulfide concentrations should not be allowed to exceed 0.005 ppm (5 ppb) for a 30-minute averaging time.

Comment 4. H_2S has been measured in the human breath at levels of 65-698 ppb, and is the result of normal bacterial activity in the digestive tract. H_2S is also produced in various tissues, including the brain where it is thought to function as a neuromodulator, and also acts as a smooth muscle relaxant. Given the rapid metabolism of H_2S and the low levels naturally produced by the body, the lack of observations of toxicity after chronic exposure to low levels is not surprising. Even the work of Bhambhani and Singh cited in the OEHHA supporting documentation found that healthy subjects could safely exercise at their maximum at hydrogen sulfide concentrations of 5 ppm (5000 ppb) H_2S. In later studies, Bhambhani found small but statistically significant changes in oxygen uptake and increase in blood lactate after exposure to 10 ppm (10,000 ppb) H_2S, a physiologic effect, but not an adverse health effect. No subjective symptoms were reported in this study as subjects breathed either 5 or 10 ppm H_2S through a mouthpiece.

Response. Many toxic chemicals are produced by metabolism: CO, acetaldehyde, formate, NO, and H_2S. The chronic RELs are to protect against low-level, involuntary exposures. OEHHA staff are aware of the Bhambhani studies which are generally acute/subacute studies with normal individuals and thus not useful for developing a chronic REL.

Comment 5. The proposed OEHHA REL of 0.7 ppb for H_2S is based on the USEPA reference concentration (RfC) promulgated in 1994. A number of studies on the toxicity of H_2S have since been published or are near completion. This new information should be incorporated into the final REL.

Response. Comment acknowledged. OEHHA's use of RfCs was explained above. OEHHA and hopefully also USEPA will examine the new information for possible incorporation when it becomes available. We have revised our proposed REL as explained in responses to comments from Geyser's Geothermal using the same study as U.S. EPA, but different uncertainty factors. The new proposed REL is 9 $\mu g/m^3$ (7 ppb).

Comment 6. In both the derivations of the RfC and REL a large uncertainty factor of 1000 is used. A large uncertainty factor is only appropriate when there is a paucity of data. This is not the case for H_2S. Based on the significant amount of human and animal data available, this factor should be decreased by at least an order of magnitude.

The derivation of the REL uses a subchronic-to-chronic uncertainty factor of 10. This should be reduced to 3, based on available data suggesting that the types of lesions found in rodents at high sub-chronic exposures are unlikely to progress with longer duration of exposure. Moreover, given the rapid metabolism and detoxification of H_2S, these subchronic rodent studies performed at high H_2S concentrations (much higher than the OEHHA acute level II REL for H_2S) are not relevant to chronic low level human exposures.

Response. OEHHA agree that the uncertainty factor (UF) is large and close to the maximum uncertainty factor used by USEPA of 3,000. We revised our proposed REL as noted above.

Comment 7. The other significant part of the large uncertainty factor is the modifying factor of 3 used for the "lack of reproductive and developmental toxicity data". I believe this modifying factor is unwarranted. The developmental study cited in the OEHHA documentation demonstrates no developmental effects in rats, even at concentrations (150 ppm) high enough to cause slight maternal toxicity. This is clear evidence that H_2S is a compound the body is capable of metabolizing and detoxifying quite rapidly. Additionally, by mid-1998, final reports should be available from the Chemical Industry Institute of Toxicology (CIIT) on the reproductive effects and developmental neurotoxicity of H_2S. No final decision on the REL should be made until these studies are completed.

Response. This suggestion has merit and should be made to USEPA. There are other developmental studies with H_2S by S. Roth and coworkers in which adverse effects on the developing nervous system were seen at 20-25 ppm, the lowest concentration tested. As noted above, we revised our proposed REL.

Comment 8. OEHHA cites the "lack of adequate long-term human exposure data" as a major area of uncertainty and the reason for the conservativeness of the REL. Humans are constantly exposed to low levels of hydrogen sulfide, and life could not continue without the production of hydrogen sulfide. To set the REL one thousand times lower than the upper concentration for naturally occurring H_2S in human breath seems very conservative. OEHHA did not take this type of human data into consideration in its derivation of the REL. OEHHA uses a high uncertainty factor for a chemical for which there is a large amount of good animal and some human data.

If the subchronic-to-chronic uncertainty factor of 10 is reduced to 3, and a very conservative modifying factor removed, the REL uncertainty factor could be lowered by

an order of magnitude to 100. This would put the REL in the 7.0 ppb range, a concentration at the very limits of odor recognition and found naturally in the human body.

Response. This suggestion also has merit and should also be made to USEPA. However, OEHHA is not willing to unilaterally change USEPA uncertainty factors. OEHHA is harmonizing with USEPA where possible.

Comment 9. I hope the foregoing discussion and data, along with the soon-to-be-completed CIIT studies, will be taken into consideration by OEHHA before setting the final REL. The economic impacts of the proposed REL could be enormous throughout California. The final decision on the REL should therefore be made in a collaborative setting with industry. OEHHA has made a great effort to involve input from risk managers and stakeholders throughout the process

Response. Many of the comments above need to be made to the USEPA. OEHHA will certainly review the CIIT studies when they become available. However, based on this comment and other comments about the hydrogen sulfide chronic REL and on OEHHA's own assessment of the developmental toxicity data available including a study on spontaneous abortion published in 1998, OEHHA staff have reviewed the value in the draft document and have calculated a revised chronic REL for hydrogen sulfide of 9 $\mu g/m^3$.

Western States Petroleum Association (WSPA)

Comments on the Determination of Chronic Toxicity Reference Exposure Levels were received from WSPA in a letter dated January 29, 1998. WSPA made comments on the general methodology and on the chronic RELs for **benzene** and **hydrogen sulfide**. WSPA is a trade association representing members engaged in all aspects of the exploration for, and production, refining, transportation and marketing of, petroleum and petroleum products in the western United States.

Comment 1. WSPA is pleased to note the extent to which OEHHA has attempted to harmonize their approach for calculating RELs with that of USEPA. WSPA agrees that uncertainties exist in the characterization and quantification of potential health effects in humans, especially when extrapolating from animal studies. However, the potential to significantly overestimate the likelihood of these effects by compounding uncertainty factors must also be recognized. OEHHA has recognized the utility of pharmacokinetics in tempering the use of uncertainty factors in REL calculations. Indeed, information about the biochemical mechanism of the chosen toxic endpoint in key studies can be extremely important in reducing uncertainties regarding intraspecies and interspecies variability, and the likelihood of enhanced susceptibility based upon age, gender, etc. OEHHA should give greater consideration to the role of mechanistic data in the parent document and in calculating selected RELs.

Although WSPA generally agrees with OEHHA on the basic methodology for calculating inhalation RELs, we find that the choice of key study and the application of the REL methodology give rise to a number of concerns with certain chemical-specific RELs. The attached comments address WSPA's concerns with the proposed REL calculations for benzene and hydrogen sulfide. We understand that the Olefins panel of the Chemical Manufacturers Association (CMA) will submit comments on certain other RELs, some of which are also of interest to WSPA. Specifically, WSPA shares CMA's interest in the proposed RELs for ethylene, 1,3-butadiene and propylene. Rather than duplicate CMA's comments on these chemicals in this submittal, we will instead incorporate their comments herein by reference.

Response. Comment noted. OEHHA used the best study it could find in the medical and toxicological literature prior to the release of the document in October 1997. In the case of isopropanol a superior study appeared in 1997 and has been used to revise the REL. In other cases OEHHA used scientific judgment with which others might not agree. In the case of hydrogen sulfide OEHHA used the USEPA’s chronic REL as part of its effort to harmonize with USEPA. OEHHA has contacted the USEPA to determine if USEPA made an error in calculating the RfC. OEHHA addressed the comments of the Chemical Manufacturers Association - Olefins Panel in a response above.

Comments on General Methodology

Comment 2. OEHHA should not include abstracts of presentations or posters as references in this document (e.g., Alexeeff et al., 1997; Foureman, et al ., 1995; Gillis, et al., 1997; Kadry, et al., 1995; Khodair et al., 1995; Mitchell, et al., 1993; Schmidt et al., 1997 and Swartout, 1997). A number of other citations are to secondary references such as book chapters. The value of such citations is extremely limited since the methodology and conclusions cannot be evaluated. In addition, these studies and their conclusions have not been subject to peer review. OEHHA should not include such citations as supporting documentation for these guidelines.

Similarly, journals from the former Soviet Union are of little use as citations (e.g., Chizikov, 1973). These documents are not available in English and cannot be translated in time to provide the opportunity for comment. In addition the frequent use of nonstandard terminology and the generally poor quality of English used in the abstract translations when present have not been subject to peer review outside of the former Soviet Union as evidenced the studies cited in these journals.

Response. OEHHA has attempted to use the best studies it could find. We would prefer peer-reviewed journal articles for all cases. However this is not always possible. We have used well-conducted, unpublished industry studies for the development of RELs if these had the best data available. USEPA used an unpublished study (at the time of promulgation of the RfC) to develop the RfC for MTBE. OEHHA also used as the basis for different RELs a case report (one individual) published in the peer-reviewed literature, a NIOSH Health Hazard Evaluation, and research institute and government (e.g., NTP) reports. In one case we based a REL on studies published in Russian that had been summarized by NIOSH. But we did not base any REL on just an abstract.

Comment 3. P. 11, Section 1.4, Para. 3: RELs may appropriately be based upon the most sensitive endpoint unless there is data demonstrating that the endpoint in question is not relevant. This will usually not be an issue for RELs based upon human studies. In the case of RELs based upon animal studies, there may be scientifically sound reasons why the most sensitive endpoint in an animal study is not relevant to human populations. These reasons may be based upon biochemical mechanism, metabolic pathways or pharmacokinetics. This possibility should be acknowledged by OEHHA here as it is in Section 2.1.2.

Response. The introduction is only an overview. The elaboration of this specific point has been done in the Hazard Identification section as noted by the comment.

Comment 4. P. 11, Section 1.4, Para 4. Under AB-2588, the Hazard Index is based on estimated exposure levels derived from air dispersion models, not from measurements as stated in the document.

Response. Comment noted. The exposure levels could be derived from measurements, but they rarely are.

Comment 5. P. 12, Section 1.5, Para 1. In the discussion of susceptible sub-populations, the reference to increased exposures should be deleted. Differences in susceptibility are the result of variations in physiological or biochemical processes characteristic of a specific sub-population. Variations in exposure are relevant to risk calculations but should be accounted for as such. Accounting for variations in exposure within a subject population is the subject of a companion technical support document, *Exposure Assessment and Stochastic Analysis.*

Response. OEHHA staff can not totally agree with this suggestion and will not delete reference to exposure differences. At the same external exposure there may be different susceptibilities due to physiological processes such as the increased absorption of ingested lead by children compared to adults and of inhaled pollutants due to the increased breathing rates of children relative to adults.

Comment 6. P. 13, Section 1.6. The last sentence would benefit from some additional clarification.

Response. The last sentence states: "Thus, human exposures of greater than 8 years are not adjusted either in their calculation or application." OEHHA has added text clarifying the concept.

Comment 7. P. 17, Section 2.1.2, Para 2. In the discussion of relevance of animal data to human response, OEHHA should include mechanism of action with pharmacokinetics and metabolism as information useful in selecting the relevant animal model.

Response. OEHHA will include mechanism of action as information useful in selecting the relevant animal model.

Comment 8. P. 17, Section 2.1.2, Para 3. Although useful information may be obtained from studies that may not conform to every detail of sound design or comply with a rigorous application of Good Laboratory Practices, clearly regulatory standards should not be based on poorly designed or executed studies. WSPA hopes that OEHHA would agree that data from studies of questionable scientific validity should only be considered in the calculation of RELs if supported by data from separate valid studies.

Response. OEHHA would prefer to only use studies that conform to every detail of sound design or comply with a rigorous application of Good Laboratory Practices. If such a study is not available for a chemical, we must act to protect public health by using the best data we can find. Hopefully affected parties will be motivated to get better data.

Comment 9. P. 19, Section 3.1.1, Para 2. The first sentence needs to have a reference and more discussion to support the allegation of such a high potential for undetected adverse effects. Clearly the statistical power of a study to detect an incidence of an adverse effect will vary with the size of the study populations. The concern that a relevant endpoint may not have been detected is reasonable if a chronic REL is being calculated from a short term study. WSPA believes that concern is addressed adequately by the inclusion of an uncertainty factor for a less-than-chronic study, especially in the light of numerous other uncertainty factors which are applied because of concerns that *may be true,* but are not known to be true.

Response. The commentator apparently does not agree that the NOAEL may be associated with an incidence of adverse effects of 1 to 20%. One relevant reference is the paper by Leisenring and Ryan (1992) which is given at the end of the paragraph 2. They report that "average risk levels associated with the NOAEL may be substantial." Another reference is Crump (1984) which is cited elsewhere in the introduction. It is true that the sample size in part determines statistical power. That is the basis for the statements in the 2 papers cited.

OEHHA prefers to address the subchronic to chronic differences separately from the LOAEL/NOAEL consideration.

Comment 10. Studies should be evaluated for thoroughness in considering appropriate endpoints before they are used for REL calculation. This is another area in which consideration of pharmacokinetic and mechanistic information can be useful. Increasing the degree of uncertainty adjustment because one can never answer all possible "what if" scenarios is not a sound basis for calculation of useful health based standards.

Response. OEHHA has evaluated all relevant studies for use in the REL calculations. The database for most chemicals is limited. Uncertainty factors are used when insufficient data are available to support the use of chemical-specific and species-specific extrapolation factors. The human intraspecies factor is in many ways a variability factor since humans are known to be variable in response to chemicals. While the default factor of 10 may be too large for some chemicals it is probably not adequate for others (Hattis D. 1996. Variability in susceptibility – how big, how often, for what responses to what agents? Environmental Toxicology and Pharmacology. 2:133-145). As one example, in a study of DNA adducts due to PAHs the interindividual variability was about 24-fold (Dickey C et al. Variability in PAH-DNA adduct measurements in peripheral mononuclear cells: implications for quantitative cancer risk assessment. Risk Anal 1997;17(5):649-656).

Comment 11. P. 20, Para 2. Although WSPA agrees with the general approach in addressing the uncertainty in NOAEL to LOAEL relationships, the comparison of

NOAELs and LOAELs is not as straightforward as implied in the discussion. The ratio of LOAEL to NOAEL will usually be an overestimate. This is unavoidable unless a study has many dose levels. Discussions of population statistics notwithstanding, the study NOAEL will usually underestimate the true value. This is especially true when there is large variation among dose levels. Again, when there are large differences among dose levels it is also likely that the LOAEL determined will be higher than the true value. The portion of the Alexeeff et al., 1997 study that compared LOAELs for serious effects to NOAELs for all effects should not be used to justify large uncertainty corrections, since comparably based parameters were not being considered. The comparison based on mild effects has a firmer theoretical foundation and showed ratios within the 10-fold range. Unfortunately the methodology of this study could not be evaluated for these comments since the reference is to a meeting abstract.

Response. OEHHA agrees that in practice the comparison of NOAELs and LOAELs is not straightforward. Based on a review of the literature by ATES staff the SRP recommended in December 1998 that staff use a LOAEL to NOAEL default adjustment factor of 6 instead of 10 for acute RELs. The Alexeeff et al. (1997) report is available as Appendix F of the final Acute Reference Exposure Level Technical Support Document. ATES has considered whether a LOAEL to NOAEL factor other than 10 is justified for chronic RELs also and has described its approach in Section 3.1.2 of the Introduction. As examples in which a factor other than 10 was used, in the propylene chronic REL developed by OEHHA, after doing the RGDR adjustment, a LOAEL to NOAEL factor of 3 was used due to low severity of the adverse effect. For the sulfuric acid REL OEHHA used 3 due to slight, low incidence adverse effects. Finally, for silver the LOAEL to NOAEL factor was 1 because there were the effects were cosmetic without associated adverse health effects.

OEHHA disagrees with the comment that the LOAEL and NOAEL are generally underestimates. There is no basis for this statement. A NOAEL is sometimes incorrectly viewed as an estimate of a threshold level for adverse effects. However, a NOAEL could be associated with a substantial (1-20%) but undetected incidence of adverse effects among the exposed population, or alternatively it could be lower than a true population threshold (Gaylor DW. Incidence of developmental defects at the no observed adverse effect level (NOAEL). Regul Toxicol Pharmacol 1992 Apr;15(2 Pt 1):151-160; Leisenring W, Ryan L. Statistical properties of the NOAEL. Regul Toxicol Pharmacol 1992 Apr;15(2 Pt 1):161-171).

Comment 12. Page 20, Para 3. WSPA agrees with the OEHHA scheme for use of an intermediate uncertainty factor, but suggests that EPA grade 6 be the cutoff for a low severity effect since by definition the changes have no functional effect on the organism.

Response. OEHHA staff have weighed the cutoff level thoroughly and decided that level 5 was appropriate. Degenerative or necrotic tissue changes are considered serious, even though they are not accompanied by an apparent decrement in organ function.

Comment 13. Page 23, Para 1. It is not clear why 3 + 3 equals 10. OEHHA should provide some justification for adding intermediate uncertainty factors in this manner.

Response. Uncertainty factors are multiplied, not added. The factor of 3 is really 3.16, the square root of 10, which has been rounded to 3. When 2 of these are multiplied together, each is actually the square root of 10 and the product is therefore 10.

Comment 14. P. 27, Section 3.4.1.2, Para 2. OEHHA should justify the use of a 10-fold factor rather than the HEC in the absence of chemical- and species- specific information. There is no thermodynamic reason to expect that the blood:air partition coefficients between species will vary to any great extent. Therefore, unless OEHHA has data to indicate that this is not the case, the calculation of an HEC seems to be warranted.

Response. OEHHA is unable to calculate an HEC in the absence of a blood:air partition coefficient for the specific chemical. Rather than using the HEC adjustment and the lower interspecies UF of 3, the interspecies default value of 10 is used.

Comment 15. P. 28, Section 3.4.2. The use of the Schmidt et al., 1997 reference to justify the default use of a 10-fold uncertainty factor for animal to human extrapolation is inappropriate. This reference is to an abstract and neither the methodology nor the interpretation of the results can be adequately evaluated.

Response. The report of Schmidt et al. is consistent with known data on interspecies uncertainty factors. The report was presented at the 1997 Annual Meeting of the Society of Toxicology. It is unfortunate that there are limited analyses of this type in the peer-reviewed literature.

Comment 16. P. 29, Section 3.5, Para 1. The phenomenon is not indicative of hyper-susceptibility and should not be part of this discussion. Idiosyncratic response refers to a response, which is qualitatively different than that seen in study populations. The true idiosyncratic response does not necessarily occur at doses or exposures below those at which the "normal" response is seen.

Response. OEHHA staff believe that the inclusion of idiosyncratic response is appropriate. According to Casarett and Doull's Toxicology (4th edition, p. 16), the chemical idiosyncratic response "is usually qualitatively similar to that observed in all individuals but may take the form of extreme sensitivity to low doses or extreme insensitivity to high doses of the chemical." In addition, allergic hypersensitivity is also considered by OEHHA to be an idiosyncratic response.

Comment 17. P. 30, Para 1. While the default use of a 10-fold uncertainty factor is consistent with USEPA default methodology, OEHHA should recognize that this is an area in which the consideration of basic mechanisms of toxicity can reduce uncertainty. OEHHA should consider an intermediate uncertainty factor for those chemicals for which the chronic REL is based upon direct irritation as the endpoint. Since direct irritation responses do not involve sources of population variation such as metabolism, pharmacokinetic considerations or enzyme mediated responses, the opportunity for inter-individual variation within a population is much smaller than for systemic effects. An intermediate UF of 3 would be consistent with OEHHA's approach for developing intermediate UFs for LOAEL to NOAEL conversions.

Response. There are a number of studies indicating wide variability in the population in response to irritant chemicals (e.g., formaldehyde). There are no data indicating less variability for irritants than for other toxicants that we are aware of. The commentator presents an attractive hypothesis but without supporting data.

Comments on Specific Chemical RELs: Benzene

For benzene OEHHA developed a chronic REL of 60 $\mu g/m^3$ based on hematologic effect seen in the Tsai et al. (1983) study of 303 male refinery workers.

Comment 18. WSPA takes issue with a number of points in the calculation of the REL for benzene and believes that the REL should be at least 2-fold higher than the OEHHA estimate. While we agree on the choice of toxicological endpoint we disagree with the exposure estimate chosen as the NOAEL in the Tsai et al. study and with some of the supporting documentation for the resultant REL. In addition, we believe that there is biochemical information supporting the use of an UF for intraspecies variation less than the default value of 10.

The Tsai et al. (1983) retrospective epidemiology study of refinery workers exposed to benzene was chosen by OEHHA as the key study and the endpoint of depressed red and white blood cell counts as the critical effect for this REL. An examination of the WBC and RBC counts of a subset of 303 workers, approximately 75% of the total cohort, indicate that all counts, including multiple counts on many workers over the course of their employment in a benzene-exposed job, were within normal limits. The study authors stated that the overall median benzene exposure of this group as determined by personal monitors was 0.53 ppm. OEHHA chose this value as the NOAEL for the group. If the value of the median exposure is 0.53 ppm, then that means that 50% of all exposures were greater than that value. Since Tsai et al. reported that all blood counts were within normal limits, 0.50 ppm is clearly an underestimate of the NOAEL for this group. Tsai et al also determined, as reported by OEHHA, that 85% of all exposures to benzene in their study were less than or equal to 1.0 ppm. It is this latter figure that WSPA believes should be used as the NOAEL for the hematologic effects of benzene in this cohort. Other considerations notwithstanding, making this adjustment would increase the REL by an effective value of 2.

Response The comment is correct in pointing out that Tsai et al. (1983) determined that 84% of all exposures to benzene in their study were less than or equal to 1.0 ppm. This statistic applies to the entire study population. Tsai et al. (1983) reported the central tendency for each of the three reported subgroups as median exposure values: "benzene-related" subgroup - 0.53 ppm; "other benzene" subgroup - 0.24 ppm; and "all others" - 0.07 ppm. The central tendency of a value, and not its upper exposure range, most accurately represents the population exposure history. The benzene chronic REL is based upon the median (half higher/half lower) exposure value (0.53 ppm) reported for the highest exposed subgroup ("benzene-related") without a reported adverse health effect in the Tsai et al. (1983) study.

The chronic REL value is based upon the medical surveillance program results obtained for the subset of workers who worked in the benzene areas. These workers mostly included those assigned to the "benzene related" category (benzene, aromatic distillate hydrogenation (ADH), ethylene, and cumene) with a median exposure of 0.53 ppm. However, workers assigned to the "other benzene" category (pumps, docks) with a median exposure of 0.24 ppm were also included. Therefore, it appears that the choice of 0.53 ppm to represent the median exposure history of the medical surveillance population is likely to be somewhat above the actual median for this group. However, since the "benzene-related" subgroup included in that surveillance population could reasonably be considered to have shown no ill effects since the whole group showed no ill effects, the use of the 0.53 ppm value is justified.

Comment 19. Citing the existence of a healthy worker effect, OEHHA applies the default 10-fold UF for intraspecies variation to the NOAEL adjusted for continuous exposure to calculate the final REL. While acknowledging that a healthy worker effect for mortality or all causes and cardiovascular diseases existed, with regard to those factors known to be important for the hematotoxicity of benzene there is no reason to believe that the variation within the population of the Tsai et al., study was less than in the general population, including the very young and the elderly. To produce a toxic effect, benzene must first be metabolized to active metabolites by cytochrome P-450 system, specifically Cyp2EI (Medinsky et al., 1997). In addition it has also been reported by Martyn Smith (but not yet peer-reviewed) that the enzyme DT-diaphorase, or reductase-NQO 1, is important in the detoxification of quinones such as those generated as metabolites of benzene. In neither case can WSPA imagine a medical condition caused by a variant form (or degree of activity) of either of those enzymes that would disqualify an individual from employment in a refinery and through its omission contribute to a "healthy worker" effect. In other words, there is no reason to believe that the worker population studied by Tsai et al., differed from the general population in these two critical aspects. In fact, if the very young or very old differ from the general population in the activity of Cyp-IIE I, it is most likely in the direction of having lower than average levels of this enzyme, since P-450 mediated metabolism is generally agreed to be decreased in infants and the elderly. In the absence of other plausible bases for the

full 10 fold default intraspecies UF, WSPA believes that an intermediate factor of 3 or 5 could be used and remain health protective of sensitive individuals.

Response. The application of the 10-fold intraspecies uncertainty factor was not predicated upon the existence of a healthy worker effect in the study populations. The parenthetical statement following the listing of the intraspecies uncertainty factor in the derivation section was misleading and will be deleted. The intraspecies uncertainty factor reflects the fact that the study populations typically do not and can not (given study size limitations and the non-random selection of workers pursuant to occupational qualifications and requirements) capture the variability of the general population's susceptibility to toxic injury. The healthy worker effect observed in the Tsai et al. study suggests the presence of prior selection. However, the absence of a healthy worker effect would not demonstrate that the workers were, in fact, representative of the broader population. Here, for instance, the Tsai et al. (1983) study reported the noncancer chronic endpoints only for males.

The Tsai et al. (1983) study also lacked a comparison of the exposed group to matched controls. Thus, although none of the observed hematology parameters were considered clinically abnormal, there was limited ability to detect changes in mean values attributable to exposure. By way of illustration, in the Rothman study[1] which compared a control group to exposed groups, even where the ranges of the reported hematology parameters were similar for control and exposed groups, the mean values for several health effects (e.g., reduced white blood cell counts, reduced absolute lymphocyte counts) were statistically different. Rothman et al. (1996) reported a LOAEL of 7.6 ppm which is just slightly more than 10-times the NOAEL from Tsai et al. (1983). However, even considering the lack of a comparison group, the Tsai et al. (1983) study remains suitable for use in risk assessment. It followed a large number of workers over a long period of time and included up to four hematological tests per year for each worker. However, since the Tsai et al. (1983) study did not compare an exposed to an unexposed population, and especially since it did not provide dose response information, the Tsai et al. (1983) study provides limited information as to population variability. These limitations militate against use of a smaller intraspecies uncertainty factor.

As indicated by the comment, recent research by Rothman et al.[2] (published after the comments were submitted) indicates that known genetic variants in the enzyme pathways which activate benzene (CYP2E1) and then detoxify (NQ01) its metabolites affect the potential to develop benzene poisoning. In this Rothman et al. (1997) case-control study, individuals with benzene poisoning (abnormal blood counts) were 7.8-fold more likely than other workers to have enzyme genotypes which would result in fast activation and slow detoxification. Since the majority of the workers in the case control study were not fast activators and slow detoxifiers, other factors are likely important to the development of benzene poisoning. It is improbable that these enzymes are the only substantial source of variation in the human poisoning response to benzene exposure.

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[1] Rothman et al. American Journal of Industrial Medicine 29:236-246 (1996).
[2] Rothman et al. Cancer Research 37, 2839-2842, 1977

Given uncertainties as to the range of human susceptibility, an uncertainty factor of ten for intraspecies variation is appropriate.

Comment 20. A number of studies have been cited by OEHHA as support for the accuracy of the proposed REL of 0.02 ppm. The Cody et al. (1993) study was mentioned as correlating decreased WC and RBC counts in a subset of the Pliofilm cohort with median, job-specific exposures to benzene in the range of 30 to 54 ppm. For the comparison, the lower value of 30 ppm was selected by OEHHA and a hypothetical REL of 0.01 ppm calculated as if this study had been selected as the critical study. The Cody et al, (1993) study used the Crump and Allen exposure estimates for the Pliofilm cohort as a basis for the 30-54 ppm median exposure estimates. Again, by definition, 50% of the exposure estimates for the subject workers were above 30 ppm, using the Crump and Allen estimates. In addition, the exposure estimates for the Pliofilm cohort calculated by Paustenbach et al. (1992) are significantly higher than those of Crump and Allen for most job categories and years and have been acknowledged as superior by the senior author of the Crump and Allen reference, Crump (1994). Typically, Paustenbach et al. (1992) exposure estimates were 50% to 100% higher than those of Crump & Allen on a job-specific basis. This strongly suggests that the 30 ppm exposure estimate used by OEHHA for Cody et al. (1993) should be considered a significant underestimate of the majority of exposures in that study, and the calculated REL should be revised upward accordingly.

Response. The comment is correct that OEHHA used the 30 ppm value which is the low end of the range of median values (30 – 54 ppm) used in the Cody et al. (1993) study. OEHHA notes this fact in our document. The purpose of the discussion and related dose response calculation from Cody et al. (1993) was to assess our use of the Tsai et al. (1983) free standing NOAEL as the basis for the proposed chronic REL. The dose response analysis of the Cody et al. (1993) data resulted in a derived comparative REL of 0.01 ppm. This value was close enough to the proposed chronic REL of 0.02 ppm to indicate that reliance upon the free standing NOAEL reported for Tsai et al. (1983) study, a study which lacked a control group for comparison, would not yield results which were inconsistent with those which might be derived from the Cody et al. (1993) study. For these purposes, 30 ppm was selected as the most health conservative median value. However, whether the dose response analysis of the Cody et al. (1993) data was based upon the lowest or highest end of the range, or something in between, would not affect our conclusion that the results achieved with either the Cody et al. (1993) or the Tsai et al. (1983) data would be consistent. This conclusion satisfied prudential concerns regarding the selection of a study with a free-standing NOAEL as the key study.

Utterback and Rinsky[3] (1995) have critically evaluated in detail the Paustenbach et al. (1992) reanalyses of the Pliofilm cohort exposures. They reported that the Paustenbach et al. (1992) exposure estimates are greatly skewed by the use of unrealistically large dermal absorption factors and unrealistically large estimates of the exposed body surface area. They also provided substantial evidence also that the

[3] Utterback, David F. and Rinsky, Robert A. American Journal of Industrial Medicine 27:661-676 (1995).

Paustenbach et al. (1992) reanalyses inadequately incorporated the available historical airborne exposure information. Furthermore, Utterback and Rinsky (1995) noted that the Paustenbach et al. (1992) reconstructions entailed exposures which were on the order of 100 to 200 ppm and lasted as long as a decade. Yet, there was no epidemic of hematopoietic disorders which would be expected from chronic exposures to these high doses. Furthermore, given the repeated visits by industrial hygiene inspectors from the State of Ohio to evaluate the benzene exposures, it seems unreasonable to conclude that there were persistent exposures to benzene grossly over the recommended level.

Nevertheless, ultimately the exposure history uncertainties for the Pliofilm cohort favor our use of the Tsai et al. (1983) study for purposes of dose-response assessment. To adjust the proposed chronic REL upward, as suggested by the comment, on the basis of analyses of the Pliofilm cohort would in fact negate the selection of the Tsai et al. (1983) cohort as the basis of the dose response assessment and import the uncertainties associated with analyses of the Pliofilm cohort into the proposed chronic REL.

Comment 21. OEHHA briefly mentions the study of Kipen et al. (1988), continues on to a discussion of the Cody et al. (1993) study and never returns to Kipen. The reason for this is unclear. The Cody study clearly identifies itself as a sequel to the earlier Kipen et al. study which was designed to respond to criticisms of the earlier study (See Hornung et al., 1989). The results of the Kipen et al study were never invalidated and should be more thoroughly considered by OEHHA. That study identified a median exposure for benzene exposed workers subject to WBC & RBC counts as 75 ppm during the early 1940s. The Cody et al. study was limited to workers for whom pre-employment physicals were available as a baseline. The results of the Kipen et al. study indicate that the effect on WBC and RBC counts disappeared after the 1940's when regulatory levels and additional engineering controls decreased benzene exposures below 50 ppm. The longer term of follow-up for workers in this study versus those in the Cody study support the use of a smaller UF for subchronic to chronic extrapolation and support a higher REL than the current proposal.

Response. The Kipen et al. (1988) study reported that as benzene exposure levels fell during the 1940's worker blood counts went up. However, Hornung et al. (1988) challenged the Kipen analysis on the basis that similar temporal trends showed up in preemployment blood samples. Thus, the analyses in Kipen et al. (1988) with respect to the time period in question were subject to confounding. Kipen et al. (1988) did not closely analyze the reported effect of benzene exposure for later periods when exposures were lower and the preemployment blood count trends were stable. However, the Ward et al. (1996) study did apply a more sensitive nested case control methodology to the same study population and reported a relationship between benzene exposure and reduced white blood cell counts whether or not the pre-1947 data was included. Thus, the Ward et al. (1996) study reported an association for exposures occurring after 1947, a time when the pre-employment blood values had stabilized and when the benzene exposures were much reduced and less uncertain. Given, the Ward et al. (1996) study

findings and the primary focus of the Kipen et al. (1993) study on the earlier exposure period, there was no reason to use the Kipen et al. (1993) study as the basis for the REL.

The proposed chronic REL for benzene of 0.02 ppm was derived using a subchronic to chronic uncertainty factor of one. The derivation of the comparative chronic REL from the Cody et al. (1993) study used a subchronic to chronic uncertainty factor of ten. This factor of ten was based upon the fact that the Cody et al. (1993) study focused upon the blood dyscrasias developing over the first year of exposure.

Comment 22. The Ward et al. (1996) study is cited in support of the 0.53 ppm NOAEL chosen by OEHHA. This study analyzed the same data set as that of Kipen et al. (1988) and Cody et al. (1993) with the important exception that the exposure estimates for the individuals from whom the blood count data were obtained were based upon the work of Rinsky et al. (1987). This was the earliest set of exposure estimates for the Pliofilm rubber-worker cohort and used the most simplistic set of exposure assumptions, i.e., that the exposures of the workers in the early 1940s were essentially the same as those in the 1970s. A later exposure estimate was made by Crump and Allen in 1984 for the OSHA benzene risk assessment and is the one used by Cody et al., 1993 cited by OEHHA. The most recent assessment is that of Paustenbach et al. (1992) which has been accepted by Crump as the most thorough and best assessment to date (Crump, 1994). Both the Crump and Allen and the Paustenbach exposure assessments conclude that the workers in this cohort had much higher exposures during the 1940s than estimated by Rinsky et al. (Paustenbach, et al., 1992). Accordingly, the Ward et al. (1996) study cannot be used for accurate quantitative assessment of exposures or any calculations based on those exposures.

Response. The proposed chronic REL is not based upon the Ward et al. (1996) study. Our document briefly describes the Ward et al. (1996) study and concludes that the results are not inconsistent with each other. While the comparisons at issue are directed toward the proposed chronic REL value, the principal value of these comparisons was in assessing the reasonableness of OEHHA's choice of the Tsai et al. (1983) study as the source of the NOAEL value to be used in deriving the chronic REL. This confirmation was of particular importance as the Tsai et al. (1983) study provided a free-standing NOAEL with respect to frank toxicity. Given that the key study lacked a control group for internal comparison, the power to detect an adverse effect based upon a comparison of means was reduced. These factors reduced confidence in null results reported for the Tsai et al. (1983) study. Therefore, OEHHA sought to compare the proposed chronic REL obtained using the Tsai et al. (1983) study to those obtained from other studies which reported LOAELs but not NOAELs.

As stated above, Utterback and Rinsky[4] (1995) have critically evaluated in detail the Paustenbach et al. (1992) reanalyses of the Piofilm cohort exposures. They reported that the Paustenbach et al. (1992) exposure estimates are greatly skewed by the use of unrealistically large dermal absorption factors and unrealistically large estimates of the

[4] Utterback, David F. and Rinsky, Robert A. American Journal of Industrial Medicine 27:661-676 (1995).

exposed body surface area. They also provided substantial evidence that the Paustenbach et al. (1992) reanalyses inadequately incorporated the available historical airborne exposure information. Furthermore, Utterback and Rinsky (1995) noted that the Paustenbach et al. (1992) reconstructions entailed exposures which were on the order of 100 to 200 ppm and lasted as long as a decade. Yet, there was no epidemic of hematopoietic disorders which would be expected from chronic exposures at these high levels. Furthermore, given the repeated visits by industrial hygiene inspectors from the State of Ohio to evaluate the benzene exposures, it seems unreasonable to conclude there were persistent exposures to benzene grossly over the recommended level.

Comment 23. In the general discussion of animal data and in the last paragraph of Page A-63, the work of Farris et al., (1997) is not discussed. Specifically, with respect to the report of Baarson et al., (1984) the Farris study demonstrated that 10 ppm was a NOAEL for any hematologic effects after 8 weeks of inhalation exposure in mice. The response of the study animals was assessed at 1, 2, 4, and 8 weeks at 4 exposure concentrations with recovery groups. Although not 13 weeks in length, the pattern of the data in this study strongly supports 10 ppm as a true NOAEL for benzene in this species.

Response. OEHHA thanks the commentator for pointing out the more recent Farris et al. (1997) reference. The document will be updated to include a summary of the information provided by Farris et al. (1997). The Baarson et al. (1984) study utilized C57Bl mice. The Farris et al. (1997) study utilized $B6C3F_1$ mice. Any differences in the results of these two studies may reflect the difference in the strains used. Baarson et al. reported marked depression in erythropoietic colony formation after 178 days of exposure to benzene. The Baarson et al. (1984) study also reported decreases in circulating red blood cells and lymphocytes at 60 and 178 days of exposure. The NOAEL for a species is determined by the most sensitive strain. Here, C57B1 is the most sensitive strain. Although the pattern of the data in the Farris et al. (1997) study may support 10 ppm as a true NOAEL for benzene in mice (or at least this strain), 8 weeks is not a chronic exposure.

Comment 24. In summary, WSPA believes that evaluation of the full data set, including the most recent animal data, available mechanism-based information and a full assessment of the Pliofilm worker hematologic data supports the assertion that the chronic REL for benzene should be 2- to 6-fold higher than the current proposal.

Response. OEHHA staff appreciate the thoroughness of the comments and hope that we have adequately addressed them. In accordance with the above responses and information, OEHHA continues to prefer the proposed chronic REL.

References cited by commentator for benzene

Farris, G. M., Robinson, S. M., Gaido, K. W., Wong, B. A., Wong, V. A., Hahn, W. P., and Shah, R. S. (1997) Fund. and Appl. Toxicol., 36,119-129.

Paustenbach, D. J., Price, P. S., Ollison, W., Blank. C., Jernigan, J. D., Bass, R. D., and Peterson, H. D. (1992) J. Toxicol. Environ. Health, 36, 177-23 1.

Rinsky, R. A., Smith, A. B., Hornung, R., Filoon, T. J., Young R. J., Okun., A. H., and Landrigan, P. J. (1987) N.E.J. M., 316 1044-1050.

Comments on Specific Chemical RELs: Hydrogen Sulfide

[In the draft TSD OEHHA proposed use of the US EPA RfC of 0.7 ppb (0.9 $\mu g/m^3$) as the chronic REL for hydrogen sulfide.]

Comment 25. OEHHA used the 1994 USEPA IRIS database as the basis for their calculation and documentation. On July 1, 1995, USEPA updated the IRIS database record for H_2S. Additional studies on H_2S have been published or initiated since that date. OEHHA should review this new information and revise their recommendation accordingly.

OEHHA recommended that the inhalation reference exposure level for H_2S should be 0.7 parts-per-billion (0.9 $\mu g/m^3$). The calculation and documentation of this value have numerous flaws. WSPA recommends that OEHHA consider a value between 9 and 30 ppb H_2S as the reference exposure level in California. The California ambient air standard for H_2S is 30 ppb to prevent nuisance odor situations. There is no evidence or expectation of toxicity at this concentration. The odor detection level for H_2S is approximately 9 ppb (AIHA, 1989), although this is highly variable. A reference level between 9 and 30 ppb is adequate to prevent adverse health effects as well as community annoyance due to the odor of H_2S (Amoore, 1985). OEHHA should acknowledge that the basis for a reference exposure level in this range is based on aesthetic (odor) rather than toxicity concerns.

Response. All USEPA Reference Concentrations (RfCs), available when the Technical Support Document (TSD) on chronic Reference Exposure Levels was drafted in October 1997, are being proposed as chronic RELs. RfCs are already used by the USEPA and by California's Department of Toxic Substances Control and were earlier incorporated by reference in Appendix F of the Emissions Inventory Criteria and Guidelines for the Air Toxics "Hot Spots" Program for use in screening risk assessments in the Hot Spots Program. These Guidelines were effective July 1, 1997. The Risk Assessment Advisory Committee (RAAC) recommended that CalEPA harmonize where possible with USEPA on risk assessment. Governor's Executive Order W-137-96 concerned the enhancement of consistency and uniformity in risk assessment between Cal EPA and USEPA. Use of RfCs as chronic RELs was one action that OEHHA took to address the RAAC recommendation and to implement the Executive Order. RfCs released after October 1997, including ones that are revisions of those in the October 1997 draft, will be

evaluated for use in the Hot Spots program by reviewing the scientific basis of each RfC when it becomes available and by determining whether the scientific literature cited in the RfC is current. Appropriate RfCs will be submitted to the SRP for review and possible endorsement. OEHHA also noted that IRIS currently lists the RfC as 1 $\mu g/m^3$.

Comment 26. The 1995 USEPA documentation of their inhalation reference concentration (RfC) contains a numerical error. The summary (Section I.B. 1) reports a total uncertainty factor of 1000 and a modifying factor of 1. In Section I.B.3, USEPA explains, "The uncertainty factor of 1000 reflects a factor of 10 to protect sensitive individuals, a factor of 10 to adjust from subchronic studies to a chronic study, and a factor of 10 for both interspecies conversion and database deficiencies." However, this is contradicted in Section I.B.4 when, after a long discussion about the progression of respiratory irritation from repeated exposure, USEPA reports, "On this basis, the standard uncertainty factor of 10 for subchronic-to-chronic extrapolation is reduced by half to a threefold factor." USEPA obviously forgot to use the 3X uncertainty factor in their final calculation. OEHHA also used this subchronic-to-chronic uncertainty factor of 10. This should be reduced to 3.

Response. OEHHA confirms the finding that the text of the IRIS document contains the reference to reducing the subchronic to chronic UF to 3. This comment should also be directed to the USEPA.

Comment 27. A single uncertainty factor of 10 for both interspecies conversion and database deficiencies is unusual. USEPA typically uses separate values for each item. OEHHA followed suit using 3 for interspecies and 3 for a modifying factor because of the lack of reproductive and developmental toxicity data.

We believe that a modification factor of 3 is unwarranted for several reasons. H_2S is a product of bacterial action in the human body. H_2S concentrations exceeding the proposed RfC have been measured in human mouth air, saliva, and periodontal pockets (Blanchette and Cooper, 1976, Rosenberg et al. 1991, Person, S., 1992, Coil and Tonzetich, 1992). Intestinal gas can contain H_2S far in excess of the proposed RfC (Kirk, E. 1949, EPA 1990, Beauchamp et al. 1984). H_2S is emitted from saltwater marshes, animal waste, landfills, rice fields, and by geothermal activity (EPA 1993). Ambient air concentrations of H_2S from natural sources have been estimated to be 0.11 to 0.33 ppb (EPA 1993). Because of our endogenous production of H_2S and its ubiquitous presence in the environment, there is no reason to use a modifying factor to prevent unforeseen effects at low part-per-billion concentrations.

Response. OEHHA staff agree that a single uncertainty factor of 10 for both interspecies conversion and database deficiencies is unusual. In regard to the modifying factor OEHHA staff generally have not used them in the development of RELs. However, there are many substances produced normally in the body that can be hazardous to other parts of the human organism. The hydrochloric acid in the stomach (present in the stomach as

a normal constituent made by the body rather than bacteria) can etch the enamel from teeth when regurgitated and cause serious respiratory problems when aspirated. The body produces carbon monoxide as a result of heme metabolism. Formate is also produced endogenously. Route of exposure must also be considered; for example, hexavalent chromium and beryllium are much less toxic by the oral route compared to the inhalation route. Finally, the modifying factor of 3 was applied by USEPA because of a deficiency of information on the effects of hydrogen sulfide on development, a database deficiency not necessarily related to our endogenous production of H_2S and its ubiquitous presence in the environment. The estimated ambient air concentrations of H_2S from natural sources between 0.11 and 0.33 ppb are below the RfC of 0.7 ppb. (For comparison ozone is another ubiquitous chemical whose background levels of 0.01 to 0.04 ppm are fairly close to the air quality standards of 0.08 to 0.12 ppm.)

Comment 28. Also, by mid-1998, final reports should be available from ongoing studies on the reproductive effects and developmental neurotoxicity of H_2S. Those studies are sponsored by the American Petroleum Institute and can be forwarded to OEHHA if desired. These studies should directly address the reasons that EPA and OEHHA used a modification factor of 3 to calculate their chronic exposure level.

Response. As of January 1999, the only study that OEHHA has been furnished with is an abstract of a 5 consecutive day exposure study on neurobehavioral and neurochemical effects carried out at the CIIT. This study is unlikely to be the basis for a chronic REL.

Comment 29. We agree with the OEHHA interspecies uncertainty factor of 3. Irritant gases like H_2S have a steep dose-response curve for respiratory effects across species. However, data are available that should significantly change the documentation presented by OEHHA. The citation of Bhambhani and Singh (1991) should be deleted as the results reported were not due to H_2S. A larger and more carefully controlled study by Bhambhani et al. (1994) found no effects in human subjects exposed to 5 ppm H_2S**.** Other studies by Bhambhani et al. (1996 and 1997) in human subjects did observe small but statistically significant changes after exposure to 10 ppm H_2S. OEHHA also cites Bhambhani and Singh, 1985, to suggest, "... either that humans are more sensitive to H_2S, or that the measurements in laboratory animals are too crude to detect subtle measures of irritation." OEHHA should note that Bhambhani and Singh (1985) reported only subjective symptoms. No objective measures of respiratory irritation were done. In the subsequent work in Bhambhani's laboratory, no subjective symptoms are reported in their publications as subjects breathed either 5 or 10 ppm H_2S through a mouthpiece. No objective measurements of respiratory irritation were part of the experimental design.

Response. Bhambhani and Singh (1991) conclude that “healthy young male subjects could safely exercise at their maximum metabolic rates while breathing 5.0 ppm H_2S without experiencing a significant reduction in their maximum physical work capacity during short-term incremental exercise.” Bhambhani et al. (1996) found that exposure to H_2S at 5 ppm “might inhibit aerobic metabolism during exercise in healthy men, thereby

increasing their dependency on anaerobic metabolism." Unfortunately the commentator does not indicate what the results are due to, if not due to H_2S.

OEHHA considers that 3 is appropriate as a subchronic to chronic uncertainty factor for the hydrogen sulfide study used (CIIT, 1983). Also OEHHA does not agree with the U.S. EPA position that there is a lack of data on developmental effects of hydrogen sulfide. In addition to the Saillenfait et al. (1989) study in rats in the chronic REL summary, there are several other developmental studies available by S.H. Roth and colleagues on the effects of hydrogen sulfide on the developing nervous system. Also there is now available an epidemiological study by Xu et al. (1998) which is described in our revised summary. Using these changes OEHHA staff calculate a chronic REL of 9 $\mu g/m^3$ for hydrogen sulfide.

References cited by commentator for hydrogen sulfide

AIHA. 1989. American Industrial Hygiene Association. Odor Thresholds for Chemicals with Established Occupational Health Standards. 1989

Amoore JE. 1985. The Perception of Hydrogen Sulfide Odor in Relation to Setting an Ambient Standard. CARB Contract A4-046-33, April 1985.

Bhambhani, et al. 1994. Comparative Physiological Response of Exercising Men and Women to 5 PPM Hydrogen Sulfide Exposure. Am. Ind. Hyg. Assoc. J. (55) /November 1994.

Bhambhani, et al. 1996. The Effects of 10-ppm Hydrogen Sulfide Inhalation on Pulmonary Function in Exercising Men and Women. J Occup Environ Med 38:1012-1017,1996.

Bhambhani, et al. 1997. Effects of 10-ppm Hydrogen Sulfide Inhalation in Exercising Men and Women - Cardiovascular, Metabolic, and Biochemical Res ses. J of Environ Med 39:122-129 1997.

Blanchette AR and Cooper AD, 1976. Determination of hydrogen sulfide and methyl mercaptan in mouth air at parts-per-billion level by gas chromatography. Anal Chem 48(4):729-731 1976.

Beauchamp et al, 1984, A critical review of the literature on hydrogen sulfide toxicity. Crit Rev Toxicol 13(l):25-97 1984.

Coil JM and Tonzetich J, 1992. Characterization of volatile sulphur compounds production at individual gingival crevicular sites in humans. J Clin Dent 3(4):87-103, 1992.

Kirk E, 1949. The quantity and composition of human colonic flatus. Gastroenterology 12(5):782-794, 1949.

Person S, 1992. Hydrogen sulfide and methyl mercaptan in periodontal pockets. Oral Microbiol Immunol 7:378-379,1992.

Rosenberg et al, 1991. Halitosis measurement by an industrial sulphide monitor. J Periodontol 62(8):487-489, 1991.

USEPA, 1990. Health Assessment Document for Hydrogen Sulfide. Office of Health and Environmental Assessment, Environmental Criteria and Assessment Office, RTP, NC EPA/600/8-86/026A

Nickel Development Institute, NiPERA, and INCO United States, Inc.

Comments on the chronic REL proposed for nickel were made by the Nickel Development Institute, the Nickel Producers Environmental Research Association (NiPERA), and INCO United States, Inc. in a letter dated January 27, 1998. OEHHA proposed a chronic inhalation REL of 0.05 $\mu g/m^3$ for nickel for respiratory system and immune system toxicity.

Introductory comment. The Comments focus on OEHHA's derivation of a noncancer chronic Reference Exposure Level (REL) for nickel and nickel compounds based on the results of a two-year bioassay of nickel sulfate hexahydrate. As explained in the Comments, it is scientifically inappropriate to establish a single REL to be applied to metallic nickel and all nickel compounds. Such an approach ignores the well-documented differences in toxicity among the various forms (or species) of nickel. Accordingly, using OEHHA's methodology, we have calculated separate RELs for nickel sulfate hexahydrate (and other soluble nickel species), nickel oxide (and other insoluble nickel species), and nickel subsulfide. Because they are far and away the predominant nickel species in the ambient air of California, the RELs for nickel sulfate and nickel oxide are the most relevant of the RELs for purposes of the Air Toxics "Hot Spots" Program.

Nickel subsulfide constitutes a negligible fraction of total nickel in the ambient air. Consequently, the REL for nickel subsulfide has no practical relevance under the Air Toxics "Hot Spots" Program. Metallic/elemental nickel also constitutes a negligible percentage of the total nickel to which California residents may be exposed via inhalation, so there is no need to calculate a REL for metallic nickel. Furthermore, there are no inhalation data from which such a REL could be calculated directly. It is clear, however, that metallic nickel is less toxic than the nickel compounds for which RELs can be calculated. Hence, if a REL is set for metallic nickel, it would have to be at least as high or higher than the highest REL for any of these nickel compounds.

Response. OEHHA responds to the substantive points on each issue below.

Comment 1. Establishing a single REL for elemental nickel and all inorganic nickel compounds ignores the importance of speciation in evaluating the toxicity of the different forms of nickel. Each compound or species of a metal has its own physico-chemical properties that dictate how it behaves under a given set of conditions, including interactions with biological organisms. This point holds even if the free metal ion is assumed to be the toxic species, because the different physico-chemical properties of various forms of the metal will largely determine the extent to which the free metal ion can be made bioavailable and delivered to a relevant biological site within an organism.[a]

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[a] See Conard, B., "Is Nickel Safe? A Toxicology Primer," in Pyrometallurgical Operations Environment Vessel Integrity in High-intensity Smelting and Converting

As U.S. EPA has emphasized: "Speciation and associated solubility of metal species ... are key factors that influence the bioavailability of metals" and their "fate, transport, and uptake in various media (e.g., plant tissue, animal tissue) and receptors."[b]

It is not surprising, then, that different forms of nickel exhibit different toxicities. For example, the oral LD50 value for nickel carbonyl is 50 mg/kg body weight; for nickel sulfate hexahydrate, it is 300 mg/kg - while for green nickel oxide and nickel subsulfide, it is >5000 mg/kg.[c] While the LD50 values relate to acute toxicity, speciation also is important in evaluating chronic toxicity and potential carcinogenicity of the various forms of nickel. U.S. EPA, for example, has distinguished among different nickel species for purposes of cancer classifications.[d] So has the American Conference of Governmental Industrial Hygienists ("ACGIH"), which recently adopted three different carcinogen classifications for different nickel species as part of its Threshold Limit Value (TLV) program.[e] Similarly, in the two-year bioassay studies referenced by OEHHA in the TSD, the U.S. National Toxicology Program (NTP) found markedly different results with regard to the potential carcinogenicity of nickel subsulfide, nickel oxide, and nickel sulfate hexahydrate in rats and mice.[f]

Processes. C. Diaz, et al., Editors. Proceedings of the Nickel-Cobalt 97 International Symposium-Vol. 111, August 1997, Sudbury, Ontario.

[b] Hazardous Waste Identification Rule, 60 Fed. Reg. 66344, 66363/1-2 (December 21, 1995).

[c] See Reagan, E.L. Acute oral toxicity study in rats with green high temperature nickel oxide. Journal of the American College of Toxicology 11(6):689, 1992; Reagan, E.L. Acute oral LD(50) study in rats with nickel sulfate hexahydrate. Journal of the American College of Toxicology11(6):685, 1992; Reagan, E.L. Acute oral toxicity study in rats with nickel subsulfide. Journal of the American College of Toxicology 11 (6):691, 1992.

[d] See 51 Fed. Reg. 34135 (September 25, 1986); see also 59 Fed. Reg. 15504 (April 1, 1994) (EPA divides nickel species into four subsets for purposes of setting proposed de minimis emissions levels and establishing toxicity rankings) under a Clean Air Act hazardous air pollutant program.

[e] Insoluble nickel compound and nickel subsulfide were designated Category Al - Confirmed Human Carcinogens. Soluble nickel compounds were placed in Category A4 – Not Classifiable as a Human Carcinogen. Elemental/metallic nickel was placed in Category A5 – Not Suspected as a human Carcinogen. See ACGIH, 1997 TLVs ® and BEIs® at 42-43 (Notice of Intended Changes). These proposed TLV recommendations and carcinogen classifications were ratified as "adopted" values by the ACGIH Board of Directors on November 1, 1997.

[f] Nickel subsulfide showed clear evidence of carcinogenic activity in male and female rats, and no evidence of carcinogenicity in mice of either sex. Nickel oxide showed some evidence of carcinogenic activity in male and female rats but no dose-response between the mid- and high-dose groups, no evidence of carcinogenic activity in male mice, and only equivocal evidence in female mice. Nickel sulfate hexahydrate showed no evidence

Speciation has long been recognized as a critical factor in evaluating the noncancer chronic toxicity of nickel as well. Thus, in setting time-weighted average TLVs for long-term occupational exposure, ACGIH historically differentiated between metallic nickel and insoluble nickel compounds on the one hand (TLV = 1.0 mg Ni/m^3) and soluble nickel compounds on the other (TLV = 0. 1 mg Ni/m^3).[g] The most recent update of the TLVs draws additional distinctions among the different forms of nickel.[h]

A large body of research has been conducted to elucidate the relative respiratory toxicities of the primary nickel compounds in animals. Much of the most relevant recent work in this area has been performed by the same researchers who conducted the NTP bioassays of nickel compounds referenced by OEHHA in the TSD. (Benson et al. 1985; 1986a; 1986b; 1987; 1988; 1989; 1990; and Dunnick, 1989; 1995). These studies have related the toxicity of nickel compounds to their water solubility and subsequent clearance from the respiratory tract, which is most rapid for water soluble nickel (and nickel chloride) followed by nickel subsulfide and nickel oxide.[i]

The observed toxicity for soluble nickel compounds is due to a direct cytotoxic action of the nickel ion, released in the fluid of the alveolus, on the stromal cells in the lung.[j] Insoluble nickel compounds, by contrast, do not readily release nickel ions in the alveolar fluid. Instead, they elicit a large macrophage "hyperplasia," and their toxicity is related to the phagocytosis of the insoluble particles and the subsequent release of nickel

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of carcinogenic activity in male or female rats and no evidence in male or female mice. See 61 Fed. Reg. 66054-66057 (December 16, 1996).

[g] See ACGIH, 1997 TLVs® and BEIs® at 30. The U.S. Department of Labor's Occupational Safety and Health Administration (OSHA) adopted this same distinction for its permissible exposure limits (PELs) in 1989. See 54 Fed. Reg. 2332, 2946 (January 19, 1989). However, the PELs for nickel and nickel compounds, along with hundreds of other updated PELs, were set aside by the U.S. Court of Appeals for the Eleventh Circuit in 1992 for reasons unrelated to the specific values that OSHA had adopted for the different forms of nickel. See AFL-CIO v. OSHA 965 F.2d 962 (11th Cir. 1992).

[h] See ACGIH, 1997 TLVs® and BEIs® at 42 (setting different numerical TLV values for elemental/metallic nickel, insoluble nickel compounds, and soluble nickel compounds/nickel subsulfide). As noted above, these proposed TLVs were ratified as adopted values on November 1, 1997. See note 6, supra.

[i] See Dunnick, J. K., *et al.* Lung toxicity after 13-week inhalation exposure to nickel oxide, nickel subsulfide, or nickel sulfate hexahydrate in F344/N rats and B6C3F1 mice. Fundamental and Applied Toxicol 12: 584-594, (1989)

[j] See Benson, J.M., *et al.* Biochemical responses of rat and mouse lung to inhaled nickel compounds. Toxicology 57(3):255-266 (1989).

ions inside the cell.[k] Nickel subsulfide has been shown to be appreciably more cytotoxic in vivo than nickel oxide.[l] In fact, the toxicity of nickel oxide approximates the particle overload effects seen with compounds such as carbon black rather than the direct stromal or phagocytic cell cytotoxicities seen with soluble nickel compounds and nickel subsulfide, respectively.[m]

Against this background, it is difficult to understand how OEHHA can justify establishing a single REL for elemental nickel and all nickel compounds based on toxicological results for nickel sulfate hexahydrate. OEHHA's apparent rationale for doing so is that in the NTP studies, nickel sulfate hexahydrate, nickel subsulfide, and nickel oxide "all showed similar non-carcinogenic effects in rats and mice.[n] That may well be, but that does not mean they cause the effects by the same mechanism or at the same dose level. In fact, as indicated above, soluble and insoluble nickel compounds exert their toxicity by different mechanisms, and the dose levels for the various compounds in the NTP studies were not the same.[o] Moreover, elemental/metallic nickel was not administered in the NTP studies at all, so there is no basis for concluding that it would produce the same toxic effects and at the same dose as nickel sulfate hexahydrate.

In sum, it is not appropriate to apply a REL derived from rodent studies of nickel sulfate hexahydrate to all other forms of nickel - including insoluble nickel species and metallic nickel. To do so would be like calculating a REL for methanol and applying it to ethanol, ethylene glycol, and all other "alcohols." Just as the various forms of nickel have different acute toxicities and carcinogenic potentials, they have different noncancer chronic toxicity profiles. By assigning a single nickel sulfate-derived REL to elemental nickel and all inorganic nickel compounds, OEHHA has ignored the fundamental importance of speciation in evaluating the potential toxicity of the different forms of nickel. We urge OEHHA to reconsider this overly simplistic approach and to establish species-specific RELs instead.

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[k] See Dunnick, J. K., *et al.* Comparative carcinogenic effects of nickel subsulfide, nickel oxide, or nickel sulfate hexahydrate chronic exposures in the lung. Cancer Res. 55:5251-5256 (1995).

[l] See Benson J.M., *et al.* Comparative acute toxicity of four nickel compounds to F-344 rat lung. Fundamental and Applied Toxicology 7(2):340-347 (1986).

[m] See Dunnick, J. K. *et al.* Comparative carcinogenic effects of nickel subsulfide, nickel oxide, or nickel sulfate hexahydrate chronic exposures in the lung. Cancer Res.55:5251-5256 (1995); Oller A.R., M. Costa, and G. Oberdorster. Carcinogenicity assessment of selected nickel compounds. Toxicol. Appl. Pharmacol. 143(l):152-66 (1997).

[n] See TSD Appendix A at A-538.

[o] In the two-year NTP studies, the LOAEL for nickel sulfate hexahydrate (0.06 mg Ni/m^3) was roughly one-half of the LOAEL for nickel subsulfide (0. 11 mg Ni/m^3) and an order of magnitude lower than the LOAEL for nickel oxide (0. 5 mg Ni/m^3).

Response. The risk assessment for nickel compounds proceeded under a presumption that compounds comprised of the same inorganic element will have somewhat similar health effects and potencies. The presumption does not require that the toxicities be identical and, in fact, acknowledges the existence of differences by selecting the most sensitive effect of the most potent chemical species in order to assure protection over a broad chemical class. All results, including null results, should be evaluated to determine whether they do establish a substantial difference amongst the species in the class. The evidence sufficient to sustain speciation of a substance would correspondingly also sustain the development of any needed, alternative, health-risk guidance values.

In addition, the question of speciation needs to be addressed, not in general, but with respect to the particular health endpoints of concern and the agents to be speciated. Chemical species differences which are material with respect to one mechanism of action or health effect may not be similarly material for another. For example, with respect to inhalation exposures, speciation of a particular form of nickel may be warranted for one effect of concern (e.g., lung irritation) or route of exposure (oral, inhalation) but not another (e.g., lung cancer).

The comment offers several lines of evidence which support the speciation of nickel compounds with respect to the derivation of a noncancer, chronic REL based upon respiratory toxicity. The comment cites several kinds of indirect and direct toxicological evidence (differences amongst nickel compounds with respect to physico-chemical properties, acute oral toxicities, inhalation carcinogenic potential, and respiratory system effects and mechanisms) to support the speciation of nickel compounds in general and with respect to the proposed REL.

With respect to differences in physico-chemical properties, OEHHA agrees that the different nickel species vary greatly (from soluble to insoluble) with respect to their physico-chemical properties. OEHHA also agrees, as discussed in our 1991 risk assessment document[5], that "the different physico-chemical properties of various forms of the metal will largely determine the extent to which the free metal can be made bioavailable and delivered to a relevant biological site within an organism." OEHHA also agrees with the comment that the different nickel species present different acute *oral* toxicities which may relate to their solubility. For instance, insoluble compounds are more likely to simply pass through the digestive tract without absorption.

With respect to inhalation exposures, it is less clear how solubility will affect the lung response. After inhalation, both insoluble and soluble compounds are directly deposited into the lung. It might be presumed that more soluble species will be more quickly removed from the lung and that such compounds would in general be less harmful than the insoluble forms of nickel thought to be retained by the lung. However,

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[5] Office of Environmental Health Hazard Identification, Identification of Nickel and Nickel Compounds as Toxic Air Contaminants. 1991

the NTP non-cancer, chronic inhalation toxicity findings for (insoluble) nickel subsulfide, (insoluble) nickel oxide, and (soluble) nickel sulfate suggest otherwise and do not support such easy interpretations. In fact, much of the discussion of solubility/bioavailability related differences in mechanisms cited by the comment were offered by the original investigators as possible reasons to explain the apparently greater toxicity (carcinogenicity) of the insoluble nickel species in the rat. Here, the same data would be offered to argue for a lesser non-cancer, chronic respiratory toxicity. Clearly, such indirect evidence needs to be particularly interpreted with caution.

The comment cites, as indirect evidence of the merits of its position, that the American Conference of Governmental Industrial Hygienists (ACGIH), the Occupational Safety and Health Administration (OSHA), and the United States Environmental Protection Agency (USEPA) have speciated nickel compounds. With respect to the ACGIH, the ACGIH is a non-governmental body that develops exposure guidelines, which are not regulatory standards, to limit occupational exposures, not environmental exposures. The ACGIH holds that its limits are not to be used for any other purpose including the "evaluation and control of community air pollution nuisances." Not withstanding this distinction, OEHHA in responding below to the major substantive issues has indicated where and why OEHHA differed with the ACGIH approaches.

With respect to OSHA, the current OSHA position reflects the statutorily mandated blanket adoption, upon its creation over 25 years ago, of the ACGIH guidelines existing at that time. The OSHA position on nickel speciation is therefore not independent of the ACGIH.

OSHA in the cited (and overturned) 1989 rulemaking proposed to adopt as its own standards the updated ACGIH guideline values for the various nickel compounds. That effort was a small part of a much larger effort to adopt, with correspondingly minimal critical review, as governmental standards over 400 other occupational exposure guidelines developed by the ACGIH. While that ruling was overturned on general principles which were not "specifically" related to nickel, the findings of the court bar any reasonable inference that the OSHA rulemaking adequately reached the scientific merits as to whether or how nickel compounds might be speciated. The federal court stated in its conclusion "It is clear that the analytical approach used by OSHA in promulgating its revised Air Contaminants Standard is so flawed that it cannot stand. OSHA not only mislabeled this a "generic" rulemaking, but it inappropriately treated it as such. The result of this approach is a set of 428 inadequately supported standards. OSHA has lumped together substances and affected industries and provided such inadequate explanation that it is virtually impossible for a reviewing court to determine if sufficient evidence supports the agency's conclusions. The individual substances discussed in this opinion are merely examples of what is endemic in the Air Contaminants Standard as a whole." [AFL-CIO v. OSHA, 965 F.2d 962]. OSHA simply did not reach the merits of nickel speciation in the overturned effort. Nor, in that effort, did OSHA propose to adopt the recommendations of the National Institute for Occupational Safety and Health (NIOSH) for an occupational Reference Exposure Level of 15 $\mu g/m^3$ for nickel compounds.

The USEPA quotation [60 Fed. Reg. 66344, December 21, 1995] cited by the comment, ”Speciation and associated solubility of metal species … are key factors that influence the bioavailability of metals” and their “fate and uptake in various media (e.g., plant tissue, animal tissue) and receptors,” is uncontroversial as a general proposition (see above). Here, we are concerned with inhalation exposures to nickel compounds and their effects at the site of exposure, the lung. Issues related to environmental fate and transport that often are greatly affected by solubility considerations and that are important to the context of the proffered quotation (hazardous waste regulations) are clearly not involved.

By contrast, the Agency for Toxic Substances Disease Research (ATSDR), in its 1997 document Toxicological Profile for Nickel (updated), recommended a Minimal Risk Level of 0.2 $\mu g/m^3$ for nickel compounds. The ATSDR, in stating that the MRL value (derived from the same NTP nickel sulfate study used by OEHHA in this proposed rulemaking) was most appropriate for use in evaluating the health risks associated with soluble nickel compounds, did not limit its application to only soluble compounds.

Footnote d of the comment states that the USEPA speciated nickel compounds in a Clean Air Act rulemaking [51 Federal Registrar 34135]. However, in that rulemaking, the USEPA relied upon exposure information to exclude nickel carbonyl and nickel subsulfide from its regulatory efforts. It did not address nickel speciation according to relative solubilities.

The question of speciation needs to be addressed, not in general, but with respect to the particular health endpoints of concern, the agents to be speciated, and the route of exposure. With respect to the differential carcinogenicity of nickel compounds by inhalation, OEHHA believes that this evidence is not closely on point as to the non-cancer health effects of nickel compounds. OEHHA also believes that the comment overstates what is known about the relative carcinogenic potential of various nickel species either in animals:

NTP Cancer Bioassay Results in $B6C3F_1$ Mice.

Nickel subsulfide and nickel sulfate each gave null results in the mouse inhalation carcinogenicity bioassays. Nickel oxide provided equivocal results. These results make the mouse results moot with respect to the speciation of these compounds as to their carcinogenic potential.

NTP Lung Cancer Observations in F344/N Rats.

As noted in the comment, nickel sulfate hexahydrate did not cause lung cancers in the F344/N rat by inhalation. Nickel subsulfide did cause lung cancers in rats of both sexes and nickel oxide provided some evidence of lung cancers in rats of both sexes. In accord with the comments, OEHHA accepts these experimental results. However, in stating that the "(NTP) found markedly different results with regard to the potential carcinogenicity of nickel subsulfide, nickel oxide, and nickel sulfate hexahydrate in rats

and mice," the comment gives undue weight to the null results obtained for nickel sulfate given the assay conditions and results. The question as to whether or not the NTP bioassays do reliably distinguish the carcinogenic potency of soluble nickel compounds from the insoluble nickel compounds deserves further examination.

In order to either qualitatively or quantitatively distinguish the carcinogenic potential of nickel sulfate from that of nickel subsulfide and nickel oxide, not only the results, but the conditions, of the NTP bioassays for these compounds need to be compared. The issue turns on the relative meaning of the null result for nickel sulfate. This difference in outcomes for the three nickel species may reflect any of three general possibilities: 1. qualitative differences in effect among the three compounds, 2. a lower potency for nickel sulfate, or 3. the relative power of the different bioassays to detect a carcinogenic effect under their test conditions.

The conditions of the rat bioassay make it difficult to distinguish whether the difference for nickel sulfate represents a qualitative difference in carcinogenic potential, a small quantitative difference in potency, or no difference in potency. The highest exposure concentration for nickel sulfate was equivalent to the lowest exposure concentration for nickel subsulfide (0.11 mg nickel/m^3). The differences in incidence for lung adenomas and carcinomas combined among the various groups at these exposure levels were small: For nickel sulfate, the incidences were 1/54 female rats and 3/55 male rats. For nickel subsulfide the incidences were 6/53 and 6/53. The incidences of lung cancer in the rats exposed to nickel oxide at a level equivalent to 0.5 mg nickel/m^3 were even lower: 0/53 and 1/53 for females and males respectively. Such small differences do not well distinguish the carcinogenic potential of nickel sulfate from nickel subsulfide and, especially, nickel oxide. It is not possible to reliably distinguish between the possibility that the null result for nickel sulfate reflects no carcinogenic potential, a slightly lesser potency than that of nickel subsulfide, or the possibility that the nickel sulfate bioassays lacked the experimental power to detect an effect of nickel sulfate which is of equal magnitude to that of nickel subsulfide.

OEHHA does not fault the NTP bioassay procedures. Nickel sulfate possessed greater pulmonary toxicity than the other two nickel species in the 13-week range finding studies. This toxicity limited the range of exposure levels at which nickel sulfate could be tested and reduced the sensitivity of the bioassay to detect a carcinogenic effect at concentrations of interest. In addition, in the two year bioassay, the toxic responses in the lung of rats and mice exposed by inhalation to nickel sulfate were less severe than those in the lungs of rats and mice exposed to either nickel oxide or nickel subsulfide. Nickel sulfate may not have been tested as close to the maximally tolerated exposures as were the other two compounds.

Nor does analysis of the available lung burden information help with interpreting the differences in results among the three nickel species. Exposure to nickel sulfate (0.11 mg nickel/m^3) was associated with a lung burden of 1-2 μg Ni/g lung at 15 months. Exposure to the insoluble nickel subsulfide at (0.11 mg nickel/m^3) was associated with a lung burden of 4 μg nickel/g lung at 15 months. However, nickel oxide did substantially

accumulate in the rat lung over the course of the two-year bioassay. Therefore, nickel oxide exposure (0.5 mg nickel/m^3) gave a much higher lung burden of about 300 μg nickel/g lung at 15 months. This result, coupled with the equivocal effects found for nickel oxide, suggests that the toxicokinetics of the nickel species can not reliably guide interpretations of the observed differences in response amongst these three species of nickel compounds.

Based upon these uncertainties in the available information, the null result in the nickel sulfate bioassays, when contrasted to the results obtained for nickel subsulfide and nickel oxide, is not sufficient to reliably distinguish, even with respect to the rat lung, the carcinogenic potential of nickel sulfate from either nickel subsulfide or nickel oxide.

OEHHA also believes that the comment overstates what is known about the relative carcinogenic potential of the various nickel species in humans[6,7,8]. OEHHA[9] has similarly determined that the available epidemiological studies do not demonstrate material and substantial differences amongst the nickel species studied as to their potential carcinogenicity for the respiratory tract.

The proposed chronic REL is based upon the 1994 NTP rat inhalation study using nickel sulfate hexahydrate. It is with respect to the respiratory effects observed in that study that the speciation of nickel compounds needs to be addressed. To that end, the

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[6] Report of the International Committee on Nickel Carcinogenesis in Man. Scand. J. Work Environ. Health 16(1):1-82 (1990).

The ICNCM summary position with respect to soluble nickel compounds is found on p.70 of their report: Their conclusion begins "There was strong evidence, primarily based upon the large excesses observed for electrolysis workers of the Kristiansand, Norway refinery that exposure to soluble nickel was associated with increased respiratory cancer risk." In their overall conclusion to the document, the ICNCM report states (p.74, Concluding Remarks, first paragraph) "There was also evidence that soluble nickel exposure increased the risk of these cancers (lung and nasal cancers) and that it may enhance risks associated with exposure to less soluble forms of nickel.

[7] International Agency for Research on Cancer, Nickel and Nickel Compounds, IARC Monographs 49, p. 410.

IARC also evaluated the carcinogenicity of nickel and nickel compounds, and largely based upon the epidemiological evidence assembled by the ICNCM, concluded that there was "sufficient evidence in humans for the carcinogenicity of nickel sulfate, and of the combinations of nickel sulfides and oxides encountered in the nickel refining industry."

[8] This point also speaks to the conclusions drawn by the American Conference of Governmental Industrial Hygienists, and cited by the comment, with respect to speciation of nickel compounds and carcinogenicity. Based upon the NTP bioassay results, the ACGIH concluded, "It is clear that carcinogenicity varies with the form of nickel used."(TLV Recommendation, p.8). Therefore, as the ACGIH sought to address cancer and non-cancer hazards, this conclusion of the ACGIH implicitly influenced their decision to speciate.

[9]Office of Environmental Health Hazard Identification, Identification of Nickel and Nickel Compounds as Toxic Air Contaminants. 1991

comment marshaled the above indirect evidence. This indirect evidence is not determinative to the extent that it is not known how any differences in physico-chemical properties, acute toxicity, or carcinogenicity relate to the chronic respiratory system health effects upon which the REL is based and their particular mechanisms.

B. Direct Evidence Regarding Speciation

OEHHA concurs that the NTP chronic inhalation bioassays provide the most reliable information with respect to the speciation of nickel compounds as to their chronic inhalation health effects. The results to be compared were obtained under very similar laboratory conditions and protocols and also under the auspices of the NTP, which provides assurances that the results for the different species are readily comparable and highly reliable. This information bears directly on dose-response differences amongst nickel species with respect to chronic inhalation injury to the rat lung. Indeed, the experiments were designed with speciation in mind. However, the dose response analyses of the comment (discussed below) gave alternative chronic RELs for (insoluble) nickel oxide (0.33 $\mu g/m^3$) and (soluble) nickel sulfate (0.29 $\mu g/m^3$) that are within 10% of each other. Within the range of scientific uncertainty, the values are not distinguishable and, at the relevant level of one significant digit, in fact, they are the same (0.3 $\mu g/m^3$). Thus, by the comment's analyses, there would be no practical consequences to speciation. Any material differences would also be insubstantial.

However, OEHHA disagrees (see response to Comment 2 below) with the comment's approach to dose response assessment for soluble nickel and continues to believe that OEHHA's proposed value of 0.05 $\mu g/m^3$ is more appropriate. The difference between the value for nickel oxide of 0.33 $\mu g/m^3$ and the value for nickel sulfate of 0.05 $\mu g/m^3$ is over 6-fold and would be clearly substantial regardless of the extent to which the similar respiratory effects of nickel oxide and nickel sulfate are mediated by the same or different mechanisms.[10] Therefore, if the comment's dose response assessment satisfies the OEHHA chronic REL guidelines, it would be appropriate to provide a separate REL for nickel oxide which reflects this difference. OEHHA responds to the comment's derivation of proposed alternative RELs for nickel oxide, nickel subsulfide, and metallic nickel below (see response to comment 3).

Comment 2. In calculating the REL for nickel sulfate hexahydrate, OEHHA made an unwarranted dose adjustment. In calculating the REL for nickel sulfate hexahydrate, OEHHA made an adjustment to translate the 6 hours/day/week intermittent exposure regimen of the rats in the NTP study to a continuous exposure scenario. Such an adjustment is justified for substances whose toxic effects are mainly duration-dependent, rather than concentration-dependent, and that are slowly excreted from the body. A dose

[10] Regardless of expected differences in the efficacy of soluble and insoluble forms of nickel as vehicles for the Ni ion, the *Benson* and *Dunnick* references relied upon by the comment each found that both soluble and insoluble nickel species caused macrophage hyperplasia and chronic active inflammation of the lung in rats and mice.

adjustment of this sort is not appropriate, however, for a substance like nickel sulfate hexahydrate whose effects are mainly concentration-dependent and that is rapidly metabolized and excreted. Thus, as stated by the Agency for Toxic Substances and Disease Registry (ATSDR) in its Guidance for Derivation of Minimal Risk Levels (which correspond in terms of purpose and basic derivation technique to OEHHA's RELs): "When the critical effects are mainly dependent on the exposure concentrations and the substance being tested is rapidly metabolized and/or excreted, dose adjustment is inappropriate."

Response. Many studies have shown that the effects of nickel on the lung are concentration-dependent. Effects such as fibrosis, which are observed only upon long duration exposure, suggest the accumulation of harm over time. These studies do not examine how the duration of any assumed concentration-dependent mediating effects, such as active inflammation, relate to the chronic outcomes.

With respect to inflammation, the data also indicate duration-dependence:

Comparison of the 13-week inhalation study results for nickel sulfate hexahydrate to the 2 year study results with respect to chronic inflammation of the rat lung[11]:

Study duration:		13 weeks		2 years	
Nickel sulfate air concentration (mg/m^3):		0.25	0.5	0.25	0.5
Lung Chronic inflammation					
	Males	0/10	0/10	42/53	46/53
	Females	0/10	0/10	49/52	52/53

These results show an effect of duration of exposure.

Furthermore, given a lung half-life of from one to three days in the rat, the potential for nickel sulfate to concentrate in the rat lung over the course of several days of administration seems clear. Most of the time-adjustment factor relates to the 6-hour/day exposure regimen which entailed a four-fold adjustment factor to reflect that for only one-fourth of the time were the animals exposed each day. The remainder of the adjustment factor represents a $7/5^{ths}$ term to account for the lack of study exposures during weekends. Even if the effects of nickel sulfate on the lung were related solely to the concentration of nickel sulfate in the lung, a time adjustment factor of at least four-fold for partial exposures each day would be appropriate. In addition, in the instance of a lung half-life of one to three days, the smaller seven-fifths adjustment for two day gaps in exposure during the weekends would be appropriate.

This analysis is consistent, in fact, with the ATSDR's own treatment of the nickel sulfate data in the document, Toxicological Profile for Nickel,[12] cited by the comment. There,

[11] National Toxicology Program, Toxicology and Carcinogenesis Studies of Nickel Sulfate Hexahydrate in F333/N Rats and B6C3f1 Mice.

[12] ATSDR, Toxicological Profile for Nickel (September 1997 update), p. 32.

in developing a Minimal Risk Level for nickel compounds, the ATSDR adjusted these nickel exposures by factors of four and seven-fifths to account for the exposure gaps.

Comment 3. Setting RELs for Nickel Oxide, Nickel Subsulfide, and Metallic Nickel. The NTP two-year bioassay results for green nickel oxide and nickel subsulfide can be used to derive RELs for those substances as well. In contrast to nickel sulfate, excretion of these compounds is moderate to slow; hence, a dose adjustment to translate intermittent exposure concentrations into continuous exposure concentrations may be appropriate. In addition, because NOAELs were not identified in those studies, LOAELs must be used to derive the RELs. Consequently, a LOAEL uncertainty factor must be applied as part of the calculation. Under OEHHA's methodology, a LOAEL uncertainty factor of 10 is applied where the adverse effects are severe, and a LOAEL uncertainty factor of 3 is applied when the adverse effects are mild. In the NTP studies, lesions were scored on the following severity scale: 1 = minimal; 2 = mild; 3 = moderate; 4 = marked. Bearing these factors in mind, RELs for nickel oxide and nickel subsulfide may be derived as follows.

A. Nickel Oxide.

In the two-year NTP study, the LOAEL for green nickel oxide based on chronic inflammation in the lung was 0.5 mg Ni/m^3, and the lesions were graded as 1.6 in male rats and 1.7 in female rats, indicating that the inflammation was in the minimal to mild range.[p] Accordingly, under OEHHA's methodology, a LOAEL uncertainty factor of 3 should be applied in calculating the REL for nickel oxide. The resulting value is 0.33 mg Ni/m^3 calculated as follows:

LOAEL:	500 μg Ni/m^3
Exposure continuity:	6 hours/day, 5 days/week
Exposure duration:	104 weeks
Average experimental exposure:	89.5 μg Ni/m^3 (using dose adjustment factor of 0.179)
Human equivalent concentration:	30 μg Ni/m^3 (using RDDR = 0.29)
LOAEL uncertainty factor:	3
Interspecies uncertainty factor:	3
Intraspecies uncertainty factor:	10
Cumulative uncertainty factor:	90
Inhalation REL:	0.29 μg Ni/m^3

[p] See NTP, Toxicology and Carcinogenesis Studies of Nickel Oxide in F344/N Rats and B6C3F1 Mice, Technical Report Series No. 451 (NIH Publication No. 96-3367, July 1996) at 63-64, Table 14. The nickel oxide concentration of 0.62 mg/m^3 was equivalent to 0.5 mg Ni/m^3. See id. at 40.

$$REL = \frac{500\ \mu g\ Ni/m^3 \times 0.179 \times 0.29}{3 \times 3 \times 10} = \frac{30\ \mu g\ Ni/m^3}{90} = 0.33\ \mu g\ Ni/m^3$$

In the absence of additional compound-specific data, it would be reasonable to apply the REL for nickel oxide as a surrogate for other insoluble nickel compounds (e.g., nickel carbonate) and sparingly soluble nickel compounds (e.g., nickel hydroxide) as well.

B. Nickel Subsulfide.

In the two-year NTP study, the LOAEL for nickel subsulfide based on chronic inflammation in the lung was 0. 11 mg Ni/m', and the lesions were graded as 1.8 in male rats and 1. 7 in female rats, indicating that the inflammation was in the minimal to mild range.[q] Accordingly, under OEHHA`s methodology, a LOAEL uncertainty factor of 3 should be applied in calculating the REL for nickel subsulfide. The resulting value is 0.06 μg Ni/m3, calculated as follows:

LOAEL	110 μg Ni/m^3
Exposure continuity:	6 hours/day, 5 days/week
Exposure duration:	104 weeks
Average experimental exposure:	19.7 μg Ni/m^3 (using dose adjustment factor of 0.179)
Human equivalent concentration:	5.7 μg Ni/m^3 (using RDDR = 0.29)
LOAEL uncertainty factor:	3
Interspecies uncertainty factor:	3
Intraspecies uncertainty factor:	10
Cumulative uncertainty factor:	90
Inhalation REL:	0.06 μg Ni/m^3

$$REL = \frac{110\ \mu g\ Ni/m^3 \times 0.179 \times 0.29}{3 \times 3 \times 10} = \frac{5.7\ \mu g\ Ni/m^3}{90} = 0.06\ \mu g\ Ni/m^3$$

Response: OEHHA disagrees with the comment's use of a LOAEL uncertainty factor of three in deriving the nickel oxide REL. In order to use a LOAEL uncertainty factor of three rather than ten, OEHHA requires first that the effect of concern be of slight severity and second that less than half the animals exposed at the LOAEL be affected.[13] These criteria help to insure that the LOAEL is likely to be closer to the NOAEL than otherwise.

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[q] See NTP, Toxicology and Carcinogenesis Studies of Nickel Subsulfide in F344/N Rats and B6C3F, Mice, Technical Report Series No. 453 (NIH Publication No. 96-3369, July 1996) at 63-64, Table 14. The nickel subsulfide concentration of 0. 15 mg/m^3 was equivalent to 0.11 $mgNi/m^3$. See id. at 40.

[13] OEHHA, Cal/EPA. Determination of Chronic Noncancer Reference Exposure Levels, p. 20, October 1997 Draft for Public Comment.

OEHHA agrees with the comment that the pulmonary effects at issue were classified by the NTP as being in the minimal to mild range (1.6 - 1.7) at the LOAEL dose. However, the OEHHA guidance also requires that the effect be observed in less than half the subjects at the LOAEL dose. In the NTP study, for male and female rats combined, the frequencies of chronic lung inflammation were greatly increased for the LOAEL group (105/106) and as compared to the controls (46/105). It could be argued that about one-half the LOAEL incidence reflects a contribution from the background rate and that the background rate should be excluded. If this were done, it would still be found that nearly all the remaining animals (constituting more than half of the original groups) were affected. In addition, if the expected control/background were excluded, it would also be appropriate to correct for their influence on the overall severity score. If this correction were made, an adjusted effect severity score of slightly greater than 2.0 (a mild effect) would result.

Applying the LOAEL uncertainty factor of 10 would result in a proposed REL for nickel oxide of 0.1 μg Ni/m^3. This value is twice the value (0.05 μg Ni/m^3) developed by OEHHA.

The comment stated that its nickel oxide alternative REL of 0.33 μg Ni/m^3 should also be used to regulate other insoluble and partially soluble forms of nickel: "In the absence of additional compound specific data, it would be reasonable to apply the REL for nickel oxide as a surrogate for other insoluble nickel compounds (e.g., nickel carbonate) and sparingly soluble nickel compounds (e.g., nickel hydroxide) as well". OEHHA disagrees. In setting inhalation exposure RELs for groups of compounds, OEHHA uses the most sensitive strain, species, sex, chronic endpoint, and agent for each group of substances. The comment's proposed alternative REL of 0.33 μg/m^3 for nickel oxide is also substantially different from the comment's REL of 0.06 μg/m^3 for (insoluble) nickel subsulfide. Therefore, it would be inappropriate to apply the nickel oxide value to all other "insoluble and sparingly soluble" nickel compounds. The comment's alternative value for nickel subsulfide of 0.06 μg/m^3 would be preferred for all other forms of insoluble nickel compounds[14].

Furthermore, as the value proposed for (insoluble) nickel subsulfide is not substantially different from that proposed by OEHHA for (soluble) nickel sulfate, regardless of any extent to which there are any differences in the mechanisms of action or

[14] This result would not be changed even if the ARB were to exclude nickel subsulfide from regulation on the strength of the comment's assertion that no one was exposed to nickel subsulfide in California. Nickel subsulfide's membership in the set of "insoluble and poorly soluble" nickel compounds is simply a matter of physico-chemical fact, not regulatory fiat. The relevance of the health findings for nickel subsulfide to other members of the "insoluble and poorly soluble" set of nickel compounds stands regardless of where ever else nickel subsulfide might be consigned in a regulatory scheme. The comment also requests that if a REL is to be provided for metallic nickel that the REL be at least as high as that of nickel oxide (0.33 ug/m^3). However, for these reasons, OEHHA also believes that the proposed REL value of 0.05 ug/m^3 should be applied to metallic nickel as well.

effect, there would also be no reason to even further speciate nickel compounds with respect to the chronic REL.[15]

However, for similar reasons to the nickel oxide case, OEHHA also disagrees with the comment's use of a partial LOAEL factor of three in the dose response assessment for nickel subsulfide. OEHHA considers a value of ten to have been more appropriate. The combined incidence of adverse effects for male and female rats in the nickel subsulfide LOAEL group was much greater than that seen in controls with respect to lung fibrosis (98/106 vs. 2/106) and chronic inflammation (104/106 v. 16/106); the average severity of the chronic inflammation in the LOAEL group (2.5) indicated mild to moderate effects. If a LOAEL factor of ten were applied to the nickel subsulfide dose-response assessment, the resulting REL value would be 0.02 ug Ni/m^3.

In developing its response to these comments, OEHHA has identified the following possible REL values:

Form of Nickel	REL Value	Based Upon	Cumulative Safety Factor
Nickel oxide:	0.1 μg Ni/m^3	LOAEL	300
Nickel sulfate:	0.05 μg Ni/m^3	NOAEL	30
Nickel subsulfide	0.02 μg Ni/m^3	LOAEL	300

The above results, which span a four-fold range, are generally consistent with the presumption that compounds comprised of the same inorganic elements will have somewhat similar health effects and potencies. In providing a REL for "Nickel and Nickel Compounds," OEHHA prefers to use the REL value derived from the soluble nickel data, and not the value derived from the nickel subsulfide data. Nickel sulfate and nickel subsulfide produced similar chronic, noncancer pulmonary effects of similar severity. The NOAEL in the nickel sulfate study was below the LOAEL in the nickel subsulfide study. This preference for the REL value derived from the nickel sulfate study data eliminates the additional uncertainty inherent in the use of the nickel subsulfide study data with its higher LOAEL.

However, the results of the NTP studies and these dose response analyses do support the speciation of nickel oxide. The health effects data for nickel oxide indicate that its adverse pulmonary effects were less severe (absence of fibrosis, lower chronic lung inflammation severity scores) at higher doses than the pulmonary effects observed for nickel sulfate and nickel subsulfide. The higher chronic REL value for nickel oxide of 0.1 μg/m^3 reflects these dose response differences. Furthermore, while it is based upon a LOAEL, the lower severity of the adverse health effects at the LOAEL mitigates some of the uncertainty associated with use of a LOAEL rather than a NOAEL. OEHHA therefore concludes that 0.1 μg/m^3 is an appropriate REL for nickel oxide.

[15] This result speaks to the ACGIH's assertion that speciation of nickel is warranted as soluble nickel compounds pose a greater risk of pulmonary inflammation than insoluble compounds (TLV documentation, page 9).

Comment 4. Having calculated a REL for nickel subsulfide, we would like to add an important caveat. The vast preponderance of nickel emitted to the ambient air in California is in the form of nickel sulfate and oxidic nickel.[r] Nickel subsulfide constitutes a negligible fraction of total nickel in the ambient air; thus, the general population in California will have virtually no exposure to nickel subsulfide. Consequently, the REL for nickel subsulfide has no practical relevance under the Air Toxics "Hot Spots" program.

Response. To the extent that the predominant exposures to nickel in California are to oxidic nickel and nickel sulfate, the inclusion and provision of RELs for other nickel compounds in the regulatory scheme is without practical consequences. However, to the extent that the inclusion of other nickel compounds in the regulatory scheme dissuades the substitution of one regulated nickel species by another unregulated species (e.g. nickel sulfate by nickel chloride), the inclusion of these other nickel compounds protects the public health. In addition, to the extent to which there are or may develop exposures to these other forms of nickel, the inclusion of other nickel compounds serves to directly protect the public health.

Comment 5. Metallic nickel has not been the subject of an inhalation toxicology study, so there are no data from which a REL for metallic nickel can be calculated directly. And there really is no reason to establish a REL for metallic nickel because members of the general population (for whom RELs are established) are not exposed to metallic nickel via inhalation. Furthermore, because of its limited bioavailability[s] and the largely negative findings of epidemiological investigations, it is generally accepted that metallic nickel is less toxic than nickel compounds. Thus, ACGIH's recently adopted TLV for elemental/metallic nickel is 15 times higher than the TLVs for soluble nickel compounds and nickel subsulfide and 7 ½ times higher than the TLV for insoluble nickel compounds.[t] Similarly, while the International Committee on Nickel Carcinogenesis in Man (ICNCM) concluded that workplace exposures to certain nickel compounds have been associated with increased risks of lung and nasal cancers, it found no evidence that metallic nickel was associated with increased lung and nasal cancer risks."[u]

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[r] See CARB, Proposed Identification of Nickel As A Toxic Air Contaminant: Technical Support Document Part A (June 199 1) at A-6 through A- 12 and Table 11- 1.

[s] Since elemental/metallic nickel is not soluble in water, it cannot become bioavailable without first undergoing an oxidizing chemical reaction - referred to as corrosion - that produces a different nickel species.

[t] See ACGIH, 1997 TLVs© and BEIs© at 42. As noted above, these proposed TLVs were ratified as adopted values on November 1, 1997. See note 6, supra.

[u] See Report of the ICNCM, 16 Scandinavian J. Work, Environ. & Health, February 1990 at 74. The

In these circumstances, there is no need to set a REL for metallic nickel, and we suggest that none be set. If OEHHA is determined to identify a REL for metallic nickel, however, it seems clear that, even using the most conservative assumptions, the REL should be at least as high - indeed, higher - than the REL for nickel oxide, i.e., equal to or greater than 0.33 μg Ni/m^3. This REL clearly would be far lower than is necessary to protect against any chronic health hazards that might be associated with inhalation exposure to metallic nickel. But, in the absence of relevant studies from which a REL for metallic nickel can be calculated directly, applying the REL for insoluble nickel oxide to insoluble metallic nickel may be viewed as the most logical, if overly conservative, approach - particularly since metallic nickel, as noted above, must undergo an oxidizing reaction before it can become bioavailable.

Response. With respect to speciation of elemental nickel, the comment also states: "Moreover, elemental/metallic nickel was not administered in the NTP studies at all, so there is no basis for concluding that it would produce the same toxic effects and at the same dose as nickel sulfate hexahydrate." The comment is correct, but applies the wrong test. The risk assessment for nickel compounds proceeded under a presumption that compounds comprised of the same inorganic elements will have somewhat similar health effects and potencies. As elemental/metallic nickel was not administered in the NTP studies at all, those studies provide no basis for concluding that elemental/metallic nickel would not produce similar harmful effects.

The comment also correctly points out that the ICNCM study found "no evidence that metallic nickel was associated with increased lung and nasal cancer risks". However, OEHHA disagrees with the comment that this is a reason to not set a chronic REL for metallic nickel. The California chronic RELs are not set to protect against cancer. They are meant to protect against non-cancer, chronic health effects. The lack of carcinogenicity findings therefore is not determinative. Given the absence of comparable inhalation toxicity studies for metallic nickel, this presumption is not overcome as to respiratory effects from inhalation exposures.

With respect to whether to apply a nickel oxide REL value to metallic nickel, while there are physico-chemical differences between metallic nickel and soluble nickel, there are also physico-chemical differences between metallic nickel and insoluble forms of nickel. OEHHA selects the most sensitive effect of the most potent chemical species in order to assure protection over a broad chemical class. Nickel sulfate and nickel subsulfide, and not nickel oxide, are the most potent forms of nickel with respect to inhalation exposures. The nickel sulfate REL value therefore applies to metallic nickel.

International Agency for Research on Cancer (IARC) also has distinguished between metallic nickel and nickel compounds in assigning cancer classifications, with metallic nickel being classified as having a much lower carcinogenic potential than nickel compounds. See IARC Monographs on the Evaluation of Carcinogenic Risks to Humans, Vol. 49 (1990) at 411.

As indicated above, to the extent that the predominant exposures to nickel in California are to oxidic nickel and nickel sulfate, the inclusion and provision of RELs for other nickel compounds in the regulatory scheme is without practical consequences. However, to the extent that the inclusion of other nickel compounds in the regulatory scheme dissuades the substitution of one regulated nickel species by another unregulated species (e.g., nickel sulfate by nickel chloride), the inclusion of these other nickel compounds protects the public health. In addition, to the extent to which there are, or may develop, exposures to these other forms of nickel, the inclusion of other nickel compounds serves to directly protect the public health.

Finally, facilities subject to the Hot Spots program do not speciate their emissions of metals. Without such speciation, different RELs for different forms of nickel would be of toxicological interest but not of practical use in the Hot Spots Program.