California Environmental Protection Agency

~ Air Resources Board

Initial Statement of Reasons for Rulemaking

Proposed Identification of

1,3-Butadiene

as a Toxic Air Contaminant

Staff Report/Executive Summary

Stationary Source Division
May 1992

INITIAL-STATEMENT OF REASONS FOR RULEMAKING

STAFF REPORT/EXECUTIVE SUMMARY

PROPOSED IDENTIFICATION OF 1,3-BUTADIENE AS A TOXIC AIR CONTAMINANT

Prepared by the Staffs of
the California Environmental Protection Agency's Air Resources Board and
Office of Environmental Health Hazard Assessment

May 1992

INITIAL STATEMENT OF REASONS FOR RULEMAKING

STAFF REPORT EXECUTIVE SUMMARY

What is the Initial Statement of Reasons for Rulemaking?

This report is a summary of the information used by the members of the Air Resources Board (ARB or Board) to determine if 1,3-butadiene should be listed as a toxic air contaminant (TAC). The Board will consider the identification of 1,3-butadiene as a TAC at a public hearing in July 1992, in accordance with California Health and Safety Code Section 39662.

Are There Controls Associated with this Rulemaking?

No control measures for 1,3-butadiene are proposed for adoption at this hearing. California law (Health and Safety Code Sections 39650-39666) clearly separates TAC risk assessment (identification) from TAC risk management (control). Therefore, control measures are not considered during the identification phase. If 1,3-butadiene is identified as a TAC by the Board, a report on the need for and appropriate degree of control measures to reduce 1,3-butadiene emissions will be developed in accordance with Health and Safety Code Sections 39665 and 39666. If the Board determines that control of industrial sources are needed, the staff will work closely with all affected parties, actively seeking the participation of industry, the districts, and the public.

What is Contained in the Initial Statement of Reasons for Rulemaking?

The Initial Statement of Reasons for Rulemaking for the Proposed Identification of 1,3-Butadiene as a Toxic Air Contaminant consists of a Staff Report/Executive Summary (which summarizes the scientific basis for the proposed regulation), a discussion of the environmental and economic impacts of the proposal, the Findings of the Scientific Review Panel (SRP), and the proposed Regulation Order.

The Staff Report/Executive Summary summarizes Parts A and B of the Technical Support Document. Part A is prepared by the California Environmental Protection Agency's (Cal/EPA) ARB staff. It is an evaluation of 1,3-butadiene's emissions, ambient and indoor concentrations, statewide population exposure, and atmospheric persistence and fate. Part B is prepared by the Cal/EPA's Office of Environmental Health Hazard Assessment (OEHHA) staff. It assesses the health effects of 1,3-butadiene.

Part C (also part of the Technical Support Document) consists of the ARB/OEHHA staff responses to public comments on draft versions of the report. The Technical Support Document has been developed pursuant to California Health and Safety Code Sections 39660-39662 (Article 3 of Chapter 3.5 of Part 2 of Division 26 of the Health and Safety Code, titled "Identification of Toxic Air Contaminants").

What are the Requirements of Health and Safety Code Sections 39660-39662?

Health and Safety Code Section 39655 requires the use of the following criteria for prioritizing substances for evaluation as possible toxic air contaminants (TACs): 1) risk of harm to public health, 2) amount or potential amount of emissions, 3) manner of usage, 4) persistence in the atmosphere, and 5) ambient concentrations.

Once a substance is selected to enter the TAC identification process, the ARB requests relevant information from the public and a written evaluation of available health effects information (Part B of the Technical Support Document) from the OEHHA. The OEHHA staff's evaluation is required to contain an estimate of the threshold exposure level above which the substance causes or contributes to adverse health effects. In the case where no threshold of significant adverse health affects can be determined, the OEHHA is required to state the range of risk to humans resulting from current or anticipated exposure.

Simultaneous with the preparation of the OEHHA health evaluation, the ARB staff prepares an exposure assessment (Part A of the Technical Support Document) that includes information on the substance's usage, emissions or potential emissions, environmental persistence, and available ambient and indoor exposure levels.

Following a public comment period, the Staff Report/Executive Summary and Parts A, B. and C of the Technical Support Document are formally reviewed by the SRP at a public meeting. Upon reviewing the data, assessments, and conclusions of the report and ascertaining that appropriate scientific methods were used to gather and analyze the data presented, the SRP submits written findings to the Board. At a public hearing, the Board decides whether or not the evidence in the Technical Support Document supports the identification of the substance as a TAC and, if so, whether there is evidence of a threshold exposure below which adverse effects are not expected to occur. Once a substance is identified as a TAC and listed in Section 93000 of Title 17 of the California Code of Regulations, the ARB staff prepares a report on the need and appropriate degree of regulation pursuant to Sections 39665-39668 of the Health and Safety Code.

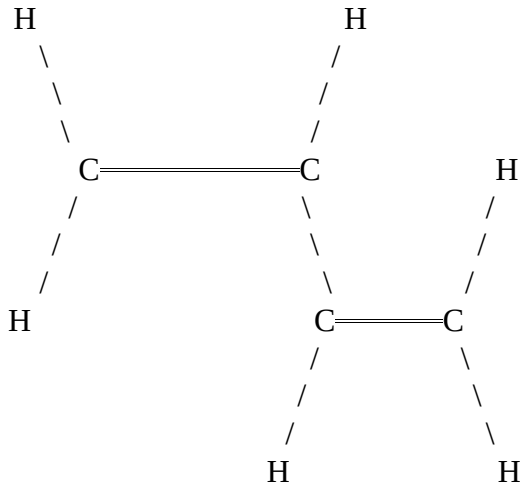
What is a Toxic Air Contaminant?

Health and Safety Code Section 39655 defines a toxic air contaminant 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."

What is 1.3-Butadiene?

1,3-Butadiene is a flammable, colorless gas with a pungent odor. It is known by a variety of synonyms including bivinyl, divinyl, erythrene, vinylethylene, biethylene, and pyrrolylene.

1,3-Butadiene is commercially produced in the United States; however, it is not commercially produced in California. The chemical formula of 1,3-butadiene is C4H6. Its structure is:



1,3-Butadiene has a molecular weight of 54.09 grams per mole. At 25° Celsius, 1 part per billion by volume (ppbv) of 1,3-butadiene equals 2.21 micrograms per cubic meter of 1,3-butadiene; 1 microgram per cubic meter of 1,3-butadiene equals 0.45 ppbv.

Does the ARB Staff Recommend 1,3-butadiene Be Identified as a Toxic Air Contaminant by the Board?

Yes. Furthermore, the OEHHA staff concludes that there is not sufficient scientific evidence at this time to support the identification of an exposure level below which no significant adverse health impacts are anticipated; the OEHHA staff therefore recommends that 1,3-butadiene be treated as having no identified threshold below which cancer would not be expected to occur.

Why Does the ARB Staff Recommend 1,3-butadiene Be Identified as a Toxic Air Contaminant?

The staffs of the ARB and OEHHA have reviewed the available scientific evidence on the presence of 1,3-butadiene in the atmosphere of California and its potential adverse effect on public health. The ARB staff has determined that 1,3-butadiene is emitted from a variety ofsources and can be detected in the ambient air throughout California. It is highly mobile in the environment, and is not naturally removed or detoxified at a rate that would significantly reduce public exposure. Furthermore, the OEHHA staff agrees with the United States Environmental Protection Agency (US EPA) and the International Agency for Research on Cancer (IARC) that 1,3-butadiene is a “probable human carcinogen." The OEHHA staff has concluded that at ambient concentrations, 1,3-butadiene may cause or contribute to an increase in mortality or serious illness and may therefore pose a potential hazard to human health. Based on the OEHHA's findings of carcinogenicity and the results of their risk assessment, the OEHHA staff concludes that 1,3-butadiene meets the definition of a toxic air contaminant.

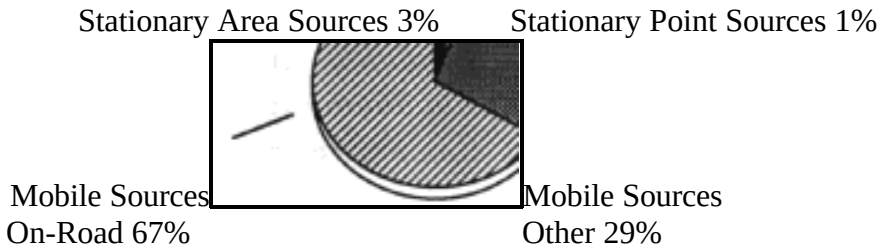
How Much 1,3-butadiene Is Released into California's Outdoor Air?

Total emissions of airborne 1,3-butadiene from quantified sources in California are estimated to be 3,900 tons per year.

What Are the Sources of Outdoor Airborne 1,3-Butadiene?

The incomplete combustion of petroleum-derived fuels by mobile sources accounts for approximately 96 percent of the statewide emissions of 1,3-butadiene. Vehicles that are not equipped with functioning exhaust catalysts emit far greater amounts of 1,3-butadiene than do vehicles with functioning catalysts.

Stationary area sources can emit 1,3-butadiene in the exhaust of boilers, heaters, internal combustion engines, and turbines during activities such as agriculture, manufacturing, residential fuel combustion, and oil and gas production. Stationary point sources can emit 1,3-butadiene during stationary engine fuel combustion, petroleum refining, the production of certain fungicides, and styrene-butadiene copolymer production for the manufacture of tires, hoses, paint latex, carpet backing, and other rubber products.



Are Emissions of 1.3-Butadiene Expected to Increase in the State?

No, they are expected to decrease from mobile sources, California's largest source of outdoor 1,3-butadiene emissions. A large percentage (67 percent) of the 1,3-butadiene emitted to outdoor air comes from on-road motor vehicle exhaust. Vehicles with effective catalysts have dramatically lower emissions of 1,3-butadiene than uncatalyzed vehicles do. Based on data from the ARB's emission factor and emission inventory models (EMFAC7EP and BURDEN7C) and estimates of emissions from other mobile sources, total organic gas emissions from California's mobile sources (the basis of the 1,3-butadiene estimate) are expected to steadily decrease through the year 2010. These decreases are a result of motor vehicle regulations that have already been implemented (e.g., ARB's 1975 light-duty passenger vehicle catalyst regulation**),** and regulations that have been adopted and have future implementation dates.

Vehicles now sold in California are required to meet a standard of 0.39 grams per mile of non-methane organic gas. Starting in 1993, 40 percent of the new light-duty passenger vehicle

fleet must meet a standard of 0.25 grams per mile; in 1994, 80 percent; and by 1995, 100 percent of the light-duty passenger vehicle fleet must meet the 0.25 grams per mile standard for non-methane organic gas. A regulation adopted by the ARB in 1990 ("Low-Emission Vehicles/Clean Fuels") will result in significant reductions in non-methane organic gases from light-duty and medium-duty vehicles by requiring a growing percentage of the new vehicle fleet to meet "low emission vehicle" standards through compliance options that include the use of "clean fuels" (e.g., compressed natural gas). This program will be “phased in” over the period 1994 through 2003.

The ARB's 1991 “Phase-2" reformulated gasoline regulations include a comprehensive set of standards which will affect eight gasoline properties, including: Reid vapor pressure, aromatic hydrocarbon content, oxygen content, benzene content, olefin content, sulfur content, T90 distillation temperature (the temperature at which 90 percent of a given volume of gasoline would evaporate), and T50 distillation temperature. Use of reformulated gasoline (required statewide April 1996) is expected to result in significant reductions of benzene and 1,3-butadiene emissions from engines burning reformulated gasoline.

What Are the Ambient Concentrations of 1.3-Butadiene in the State?

The statewide population-weighted exposure to ambient (outdoor) airborne 1,3-butadiene (based on data from the ARB's toxic monitoring network) is estimated to be an average 0.37 ppbv, or 0.82 microgram per cubic meter. Average air basin exposure estimates range from a low of 0.22 pbbv (0.48 microgram per cubic meter) in the South Central Coast Air Basin to a high of 0.44 ppbv (0.97 microgram per cubic meter) in the South Coast Air Basin.

The 1987 South Coast Air Quality Study (co-sponsored by the ARB) provided a unique opportunity to characterize gaseous and particulate pollutants in the basin under well-documented meteorological conditions. The data represents samples taken over 1-hour periods, while the

tonics monitoring network data represents averaged samples taken over a 24-hour period. Data developed during the study (representing the period of June 1987 through December 1987) indicate that the 1-hour average 1,3-butadiene concentrations from the eight monitoring sites in the South Coast Air Basin were 1.6 ppbv (3.53 micrograms per cubic meter), with minimum hourly concentrations that ranged from below the level of detection of 0.1 ppbv (0.22 microgram per cubic meter) to a high of 17.7 ppbv (39.1 microgram per cubic meter).

What About Indoor Exposure to 1.3-Butadiene?

Indoor air is almost certainly the major route of exposure to 1,3-butadiene for individuals exposed to indoor environmental tobacco smoke (ETS). Based on measurements of 1,3-butadiene in a tavern, bar, and unventilated lab, the estimated dose of 1,3-butadiene inhaled in 3 hours in a high tobacco smoke environment could range from 10 to 60 micrograms. In contrast, the estimated dose of 1,3-butadiene inhaled in 3 hours during exposure to the statewide average outdoor concentration of 0.37 ppbv (0.82 microgram per cubic meter) is about 2.6 micrograms.

The amount of 1,3-butadiene inhaled in a ETS environment will vary due to the number of cigarettes smoked, room size, room ventilation rates, and other factors. The following table provides concentrations and accompanying doses for the limited indoor non-residential smoking environments for which data are available:

3 Hour Estimated Inhaled Doses of 1,3-butadiene in Smoking Environments

LOCATION	INDOOR CONCENTRATION (ppbv)	INDOOR CONCENTRATION (microgram/cubic meter)	ESTIMATED INHA DOSE (microgram/exposu
Tavern	5.0-8.6	11-19	35-60
Bar	1.5-2.0	3.3-4.5	10-14
Unventilated Lab	4.3	9.5	30
Well-Ventilated Lab	0.3	0.75	2.4

In contrast to the measured concentrations in heavy smoking environments, the range of levels of 1,3-butadiene in more typical environments is not as well defined. A recent study sponsored by the ARB measured indoor levels of 1,3-butadiene in 62 Northern California homes during the summer of 1990. Only 5 of the 62 homes had 1,3-butadiene concentrations above the study's limit of detection of 0.54 ppbv (1.2 micrograms per cubic meter). Results indicate that the concentrations in these 5 homes ranged from 1.22 to 4.53 ppbv (2.7 to 10.0 micrograms per cubic meter). In 4 of the 5 homes with measurable 1,3-butadiene, 20 to 40 cigarettes reportedly were smoked during the monitoring period. The source of 1,3-butadiene in the other home with measurable 1,3-butadiene is uncertain. Of the homes in this study, 57 (92 percent) had average indoor concentrations that were less than 0.54 ppbv (1.2 micrograms per cubic meter), and smoking occurred in 16 of those homes. Further studies are needed to quantify typical indoor 1,3-butadiene concentrations.

Are There Near-Source Exposures to 1,3-butadiene in the State?

Some Californians may be exposed to near-source, or “hot spot” concentrations of 1,3-butadiene added to the average ambient concentration of 1,3-butadiene. "Hot spot" exposure to airborne 1,3-butadiene may significantly increase the cancer risk to individuals living near sources. Sources of “hot spot" emissions of 1,3-butadiene may include facilities using butadiene to make resins and polymers, synthetic rubber manufacturing facilities, chemical production facilities, petroleum refineries, and stationary fuel combustion sources.

The US EPA has roughly estimated that 1,3-butadiene exposure for individuals living near two facilities in California may be almost double the average exposure experienced by individuals not living near these facilities. However, the ARB staff does not have enough emissions and exposure information available at this time to validate the US EPA's estimate. This information is expected during 1992, when the ARB staff will evaluate 1,3-butadiene emissions data reported by

facilities under the AB 2588 Air Toxics “Hot Spot” emissions reporting program. If the Board identifies 1,3-butadiene as a toxic air contaminant, this “hot spot” information will be used during the risk management phase to help determine priority and need for control of sources emitting 1,3-butadiene.

Are There Other Routes of Exposure to 1.3-Butadiene?

Water ingestion, polybutylene pipes, and some foods have been examined as possible contributors to 1,3-butadiene exposure. To date, only minor amounts of 1,3-butadiene have been detected in one food (olive oil) packaged in bottles made from butadiene polymers.

How Long Does 1,3-butadiene Remain in that Atmosphere?

The atmospheric residence time of 1,3-butadiene is estimated to range from a few hours (because of reactions with hydroxyl radicals present in polluted atmospheres during the day) to approximately 12 hours (because of reactions with nitrate radicals present in polluted atmospheres at night). Thus, 1,3-butadiene is often confined to the local airshed in which it is emitted.

What are the Health Effects of 1,3-butadiene Exposure?

The health effects of 1,3-butadiene exposure have been reviewed and evaluated to determine whether 1,3-butadiene meets the definition of a toxic air contaminant. The following text summarizes the OEHHA staff's findings regarding the health effects of 1,3-butadiene exposure:

Carcinogenic and Potentially Carcinogenic Effects

The OEHHA staff has concluded that at ambient concentrations. 1,3-butadiene may cause or contribute to an increase in mortality and illness, thereby posing a potential hazard to human

health. The OEHHA staff recommend that 1,3-butadiene be considered a carcinogen without an identified threshold below which cancer would not be expected to occur.

Studies of rodents exposed to parts-per-million concentrations of airborne 1,3-butadiene indicate that it is taken up rapidly by the body and distributed with metabolites to all tissues. This distribution can result in cancer in multiple sites, including the heart, lung, mammary gland, ovaries, forestomach, liver, pancreas, thyroid, testes, and hematopoietic system. It is only one of two chemicals (the other being the fungicide Captafol) known to induce cancer in the heart of laboratory animals.

Epidemiological studies of production workers exposed to 1,3-butadiene provide limited evidence of an increased risk of death from hematologic neoplasms, especially leukemia and other lymphomas.

Non-Carcinogenic Health Effects

The OEHHA staff has concluded that it is unlikely that noncarcinogenic and nonmutagenic adverse health effects would be caused by the levels of 1,3-butadiene currently found in the ambient air.

Studies of rodents exposed to parts-per-million concentrations of airborne 1,3-butadiene indicate that it is taken up rapidly by the body and distributed with metabolites to all tissues. Fetotoxic, genotoxic, and reprotoxic effects were observed only at 1,3-butadiene concentrations hundreds to thousands of times higher than those found in the ambient air.

What is the Risk Assessment for Exposure to 1.3-Butadiene?

The staff of the OEHHA recommends that the unit risk of 3.7×10^{-4} per part per billion (1.7×10^{-4} per microgram per cubic meter) be considered the "best value of the upper bound of

risk." This value is based on data from a recent bioassay in mice. This unit risk, coupled with a lifetime exposure to 1 microgram per cubic meter, would yield 170 excess cancers per million people. The use of this "best value" with California's average ambient 1,3-butadiene exposure concentration of 0.37 ppbv (0.82 microgram per cubic meter) yields 140 excess cancers per million people exposed throughout their lives. It can be estimated that up to 4,200 1,3-butadiene-induced cancers would occur statewide among a population of 30 million people exposed to current ambient concentrations throughout their lives. The "best value" unit risk is a plausible upper bound estimate based on health-protective assumptions; the actual risk may be significantly lower.

The upper limit estimate of the number of potential excess cancers due to outdoor airborne 1,3-butadiene exposure ranges from 4 to 300 additional cancers per million people exposed throughout their lives, based on California's present average ambient 1,3-butadiene exposure concentration of 0.37 ppbv (0.82 microgram per cubic meter), and an excess cancer risk range of 9.8×10^{-6} to 8×10^{-4} per part per billion (4.4×10^{-6} to 3.6×10^{-4} per microgram per cubic meter). This range of risk is based on data from studies in rats and mice. The upper limit estimate of the number of potential excess cancers for the South Coast Air Basin (with an average exposure concentration of 0.44 ppbv or 0.97 microgram per cubic meter) is 4 to 350 additional cancers per million people exposed throughout their lives.

“Hot spot” exposure to airborne 1,3-butadiene may increase the cancer risk to individuals living near sources. The U.S. EPA has roughly estimated that lifetime cancer risks for individuals living near two facilities in California may be increased above the ambient risk by 10 to 100 excess cancers per million people exposed to the maximum 1,3-butadiene concentrations near the facilities. Emissions reporting under the Air Toxics “Hot Spots” Information and Assessment Act (AB 2588) will provide facility emissions information that will be used during the risk management phase if 1,3-butadiene is identified as a toxic air contaminant by the Board.

Based on the information available on 1,3-butadiene-induced carcinogenicity and the results of the risk assessment, the OEHHA staff conclude that 1,3-butadiene 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.

What Are the Alternatives to Identifying 1,3-butadiene? as a Toxic Air Contaminant?

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 1,3-butadiene as a toxic air contaminant is to not identify it as such. The ARB staff is not recommending this alternative because 1,3-butadiene meets the statutory definition of a toxic air contaminant.

What Would Be the Environmental Impact of the Identification of 1,3-butadiene as a Toxic Air Contaminant?

The identification of 1,3-butadiene as a toxic air contaminant through the AB 1807 risk assessment program is not expected to result in any adverse impact on the environment. The Board's identification of 1,3-butadiene as a toxic air contaminant and the subsequent analysis of the need to control emissions in the AB 1807 risk management program may result in the adoption of control measures pursuant to Health and Safety Code Sections 39665 and 39666. In considering the adoption of control measures, the ARB will consider all potential impacts of the measures on human health, as well as the potential benefits to public health by reducing 1,3-butadiene emissions. Therefore, the identification of 1,3-butadiene as a toxic air contaminant may ultimately result in control measures that will 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.

What Would be the Economic Impact of the Identification of 1,3-butadiene as a TAC?

The Board's identification of 1,3-butadiene will not directly affect 1,3-butadiene production and use facilities. Should the Board decide to formally identify 1,3-butadiene as a TAC, it will enter the control phase of the program. During the development of control measures, the impact of these measures on businesses will be fully assessed by the ARB and the air pollution control districts in a public forum where the need, degree, and cost of control would be evaluated.

While the majority of 1,3-butadiene emitted to California's atmosphere is a result of the incomplete combustion of mobile source fuel, 1,3-butadiene emissions can also occur from stationary facilities combusting fuel, refining petroleum, or using butadiene for the production of chemicals, resins, polymers, and copolymer products. There may be indirect economic impacts on these types of sources associated with individual district rules. Districts have the authority to require that public exposure to particular toxic substances not exceed levels deemed by the district to be protective of public health. Some districts' permitting requirements mandate that no new or modifying facilities exceed a specific risk level based on the OEHHA recommended cancer risk numbers for specific toxic substances. These requirements may result in operators needing to purchase control equipment or being denied a permit.

Districts are required by state legislation to implement the AB 2588 Air Toxics “Hot Spots” Information and Assessment Act of 1987 (AB 2588; Health and Safety Code Sections 44300-44384). This Act establishes a program to inventory emissions of toxic air pollutants and to assess the risk to public health caused by these emissions. Under this program, districts prioritize, require risk assessments, and determine which facilities must notify the public of potential health risks posed by emissions of toxic substances that are on the “Hot Spots” program list. The program is supported by fees paid by the affected industries. The fees are specified by state and district regulations which are updated each year through a public process,

including public workshops and public hearings. 1,3-butadiene is on the “Hot Spots" toxic substances list, and qualified facilities must report their emissions of 1,3-butadiene and pay program fees. The amount of “Hot Spots” fees due from facilities that emit 1,3-butadiene will not be affected by the identification of 1,3-butadiene as a toxic air contaminant.

Some districts include AB 2588 Air Toxics “Hot Spots” program-listed substances in their list of sources and substances subject to or affected by permitting rules. Other districts may add 1,3-butadiene upon its identification as a toxic air contaminant. If districts add facilities that emit 1,3-butadiene to their permit program, the affected facilities may be required to pay new or additional district permitting fees. Districts must conduct socioeconomic analyses, as well as public workshops and hearings before identified toxic air contaminants are added to their programs and new or additional fees are assessed. Because the OEHHA's recommended potency number is slightly lower than the U.S. EPA's, fewer sources may be affected by district rules.

Finally, Health and Safety Code Section 42311 authorizes local air pollution control districts to assess permit fees to recover the cost of district programs required or authorized by state law. Most districts charge facilities fees to cover the cost of developing and implementing the controls required by Health and Safety Code Section 39666. The amount of these fees are generally determined by the district resources needed to implement the control requirements. The fees are established by district rulemaking action, and districts must conduct socioeconomic impact analyses, workshops and public hearings before adopting or revising any fee rule. The ARB is not proposing controls for 1,3-butadiene at this time. If 1,3-butadiene is identified as a toxic air contaminant by the Board as a result of the risk assessment, it will enter the risk management phase where control technology and costs will be considered in detail by the ARB and local districts. Public workshops and hearings will held before 1,3-butadiene control measures are adopted by the Board or the districts.

What are the Findings of the Scientific Review Panel?

Findings of the Scientific Review Panel on
THE REPORT ON 1,3-BUTADIENE
As Adopted at the Panel's March 19, 1992 Meeting

In accordance with the provisions of Health and Safety Code Section 39661, the Scientific Review Panel (SRP) has reviewed the report ("Proposed Identification of 1,3-butadiene as a Toxic Air Contaminant") of the staffs of the Air Resources Board (ARB) and the Office of Environmental Health Hazard Assessment (OEHHA) on the public exposure to, and health effects of 1,3-butadiene. The Panel also reviewed the public comments received on this report. Based on this review, the SRP finds that the report on 1,3-butadiene is without serious deficiencies and agrees with the staffs of the ARB and OEHHA that:

1. There is evidence that exposure to 1,3-butadiene results in carcinogenicity. The International Agency for Research on Cancer (IARC), the United States Environmental Protection Agency (U.S. EPA), and the U.S. Occupational Safety and Health Administration (OSHA) have found that 1,3-butadiene causes cancer in animals. The IARC and the U.S. EPA have classified 1,3-butadiene as a possible (Group 2B) and probable (Group B2) human carcinogen, respectively, on the basis of sufficient evidence for carcinogenicity in animals and inadequate evidence in humans. However, it is our understanding that, based on three recent epidemiology studies, the IARC will upgrade its human evidence evaluation to "limited" this year, and categorize 1,3-butadiene as a probable (Group 2A) human carcinogen. The OSHA has found that exposure to 1,3-butadiene is associated with an increased risk of death from cancer of the lymphohematopoietic system, and has classified 1,3-butadiene as a potential occupational carcinogen.

2. Because 1,3-butadiene is listed as a hazardous air pollutant under Section 112 of the United States Clean Air Act of 1990, identification of 1,3-butadiene as a toxic air contaminant is required by the California Health and Safety Code Section 39655.

3. Based on available scientific information, a level of 1,3-butadiene exposure below which no carcinogenic effects are anticipated cannot be identified.

4. Based on a health protective interpretation of the available scientific evidence, the upper bound of the lifetime excess cancer risk resulting from 1,3-butadiene exposure ranges from 9.8×10^{-4} to 8×10^{-3} per ppb [4.4×10^{-6} to 3.6×10^{-4} per microgram per cubic meter ($\mu g/m^3$)]. This range of risk is based on data from studies in rats and mice. The best value of the upper bound of risk is 3.7 x 10-4 per ppb (1.7×10^{-4} per $\mu g/m^3$). This value is based on data from a recent bioassay in mice. Appendix I compares the best value of the upper bound

1,3-butadiene cancer unit risk with those of other compounds reviewed by the SRP. These 95 percent upper bound lifetime risk estimates are health-protective estimates; the actual risk may be much lower.

5. Mobile sources (both on- and off-road) are responsible for the majority of the identified emissions of 1,3-butadiene. Mobile sources that do not have a functioning exhaust catalyst emit far greater amounts of 1,3-butadiene than do mobile sources with functioning catalysts. Stationary sources contribute to ambient concentrations of 1,3-butadiene during petroleum refining, fuel combustion, production of certain chemicals, and the manufacturing of styrene-butadiene copolymer products.

6. Based on data collected by the ARB's ambient toxic air contaminant monitoring network from 1988 through 1989, the estimated mean annual population weighted outdoor ambient exposure for California is 0.37 ppbv (0.82 $\mu g/m^3$).

7. Based on the ARB emission inventory, areas that may be expected to have 1,3-butadiene levels higher than the mean statewide concentration are near facilities using 1,3-butadiene for the production of resins and polymers, synthetic rubber manufacturing facilities, chemical production facilities, petroleum refineries, stationary fuel combustion sources, and congested freeways. New data from the AB2588 Air Toxics “Hot Spots" emissions reporting program should be used to evaluate “hot spot" exposures if 1,3-butadiene is identified as a toxic air contaminant.

8. Based on its gas-phase reactivity with the hydroxyl radical, ozone, and the nitrate radical, 1,3-butadiene's estimated tropospheric lifetime ranges from a few hours to about 12 hours.

9. Limited indoor monitoring for 1,3-butadiene indicates that individuals exposed to indoor environmental tobacco smoke (ETS) are almost certainly exposed to higher concentrations of 1,3-butadiene indoors than outdoors. The estimated dose for an individual spending three hours in an ETS environment is 10 to 60 μg. The same individual, spending three hours in outdoor air at the statewide average 1,3-butadiene concentration of 0.37 ppbv (0.82 $\mu g/m^3$), will experience an estimated dose of 2.6 μg.

10. Studies of mice exposed to ppm concentrations of 1,3-butadiene indicate that 1,3-butadiene is taken up rapidly by the body and distributed with metabolites to all tissues. This distribution can result in cancer in multiple sites, including the heart, lung, mammary gland, ovaries, forestomach, liver, pancreas, thyroid, testes, and hematopoietic system. Exposure to 1,3-butadiene at higher concentrations ($\geq$ 1,000 ppm) is associated with tumors in the rat. Although it is not included in the calculations for the risk assessment, it is important to note that 1,3-butadiene is one of only two chemicals (the other being the fungicide Captafol) known to induce cancer in the heart of laboratory animals.

11. Epidemiological studies of production workers exposed to 1,3-butadiene provide limited evidence of an increased risk of death from hematologic neoplasms, especially leukemia and other lymphomas. Adverse health effects other than cancer are not expected to occur at mean statewide outdoor ambient concentrations.

12. Based on the OEHHA staffs best value cancer unit risk of 3.7×10^{-4} per ppb (1.7×10^{-4} per $\mu g/m^3$), and the ARB staff's population-weighted outdoor ambient exposure of 0.37 ppbv (0.82 $\mu g/m^3$), up to 140 potential excess cancers per million are predicted if exposed to this level over a 70-year lifetime. This corresponds to an excess cancer burden of up to 4,200 cancers statewide (based on a population of 30 million people).

13. Based on the available scientific evidence, we conclude that 1,3-butadiene should be identified as a toxic air contaminant.

For these reasons, we agree with the ARB staff recommendation to its Board that 1,3-butadiene be listed by the ARB as a toxic air contaminant.

I certify that the above is a true and correct copy of the findings adopted by the Scientific Review Panel on March 19, 1992

//s//

_____

Dr. James N. Pitts, Jr.
Chairman, SRP

APPENDIX I

SUBSTANCES APPROVED BY THE SCIENTIFIC REVIEW PANEL FROM 1984 TO 1992
(in order of cancer potency)

Dioxins	3.8×10^{1}	Particulate Matter
Chromium VI	1.4×10^{-1}	Particulate Matter
Cadmium	4.2×10^{3}	Particulate Matter
Inorganic Arsenic	3.3×10^{-3}	Particulate Matter
Nickel	2.6×10^{-4}	Particulate Matter
1.3-Butadiene	**1.7×10^{-4}**	**3.7×10^{-4}**
Ethylene Oxide	8.8×10^{-5}	1.6×10^{-4}
Vinyl Chloride	7.8×10^{-5}	2.0×10^{-4}
Ethylene Dibromide	7.1×10^{-5}	5.5×10^{-4}
Carbon Tetrachloride	4.2×10^{-5}	2.6×10^{-4}
Benzene	2.9×10^{-5}	9.3×10^{-5}
Ethylene Dichloride	2.2×10^{-5}	8.9×10^{-5}
Perchloroethylene	8.0×10^{-6}	5.4×10^{-5}
Formaldehyde	6.0×10^{-6}	7.0×10^{-6}
Chloroform	5.3×10^{-6}	2.6×10^{-5}
Trichloroethylene	2.0×10^{-6}	1.1×10^{-5}
Methylene Chloride	1.0×10^{-6}	3.5×10^{-6}
[Asbestos	1.9×10^{-4} per 100 fiber/m^3]	

* Perchloroethylene's unit risk is under review by the OEHHA.

PROPOSED REGULATION ORDER

Amend Titles 17 and 26, California Code of Regulations, Section 93000 to read as follows:

93000. Substances Identified as Toxic Air Contaminants.

Each substance identified in this section has been determined by the State Board to be a toxic air contaminant as defined in Wealth and Safety Code Section 39655. If the State Board has found there to be a threshold exposure level below which no significant adverse health effects are anticipated from exposure to the identified substance, that level is specified as the threshold determination. If the Board has found there to be no threshold exposure level below which no significant adverse health effects are anticipated from exposure to the identified substance, a determination of "no threshold" is specified. If the Board has found that there is not sufficient available scientific evidence to support the identification of a threshold exposure level, the "Threshold" column specifies "None identified."

Substance	Threshold Determination
Benzene (C_6H_6)	None identified
Ethylene Dibromide ($BrCH_2CH_2Br$; 1,2-dibromoethane)	None identified
Ethylene Dichloride ($ClCH_2CH_2Cl$; 1,2-dichloroethane)	None identified
Hexavalent Chromium (Cr(VI))	
Asbestos [asbestiform varieties of serpentine (chrysotile) riebeckiute (crocidolite) cummingtonite-grunerite (amosite), tremolite, actinolite, and anthophyllite]	None identified
Dibenzo-p-dioxins and Dibenzofurans chlorinated in the 2,3,7 and 8 positions and containing 4,5,6, or 7 chlorine atoms	None identified
Cadmium (metallic cadmium and cadmium compounds)	None identified
Carbon Tetrachloride (CCl_4; tetrachloromethane)	None identified
Ethylene Oxide (1,2-epoxyethane)	None identified

Substance	Threshold Determination
Methylene Chloride (CH_2Cl_2;Dichloromethane)	None identified
Trichloroethylene	None identified
Chloroform ($CHCl_3$)	None identified
Vinyl Chloride (C_2H_3Cl;Chloroethylene)	None identified
Inorganic Arsenic	None identified
Nickel	None identified1
Perchloroethylene (C_2Cl_4;Tetrachloroethylene)	None identified[1]
Formaldehyde (HCHO)	None identified[1]
1,3-Butadiene (C_4H_6)	None identified

NOTE: Authority cited: Sections 39600, 39601 and 39662, Health and Safety Code. Reference: Sections 39650, 39660, 39661 and 39662, Health and Safety Code.

1. Nickel, Perchloroethylene, and formaldehyde are not part of this regulatory action. The Board has adopted three separate amendments to Section 93000, Title 17, California Code of Regulations, identifying nickel, Perchloroethylene, and formaldehyde as toxic air contaminants; however, those three amendments have not been submitted to the Office of Administrative Law for review and filing with the Secretary of State.

California Environmental Protection Agency

Air Resources Board

Proposed Identification of Diesel Exhaust as a Toxic Air Contaminant

Part A
Exposure Assessment

State of California
Air Resources Board
Stationary Source Division

May 1992

BOARD VERSION

REPORT TO THE AIR RESOURCES BOARD ON THE PROPOSED IDENTIFICATION OF 1,3-BUTADIENE AS A TOXIC AIR CONTAMINANT

"PART A" TECHNICAL SUPPORT DOCUMENT
PUBLIC EXPOSURE TO, SOURCES AND EMISSIONS OF
1,3-BUTADIENE IN CALIFORNIA

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May 1992

BOARD VERSION
REPORT TO THE AIR RESOURCES BOARD ON 1,3-BUTADIENE

Part A - Public Exposure To, Sources and Emissions of Atmospheric 1,3-Butadiene in California

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I.
INTRODUCTION

According to Section 39655 of the California Health and Safety Code, a toxic air contaminant (TAC) 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." This report, Part A of the Technical Support Document Proposed Identification of 1,3-Butadiene as a Toxic Air Contaminant, prepared by the staff of the California Environmental Protection Agency's (Cal/EPA) Air Resources Board (ARB), is an evaluation of the compound's emissions, ambient (outdoor) and indoor concentrations, statewide population exposure, and atmospheric persistence and fate. The Part B report, prepared by the staff of the Cal/EPA's Office of Environmental Health Hazard Assessment (OEHHA), assesses the health effects of 1,3-butadiene. The ARB will consider the Technical Support Document, the findings of the Scientific Review Panel (SRP), written comments, and public testimony to determine if 1,3-butadiene should be identified as a TAC.

A. SUMMARY OF CALIFORNIA HEALTH AND SAFETY CODE SECTIONS 39660-39662 (IDENTIFICATION OF TOXIC AIR CONTAMINANTS)

Section 39650 (f) of the California Health and Safety Code requires the use of the following criteria for prioritizing substances for evaluation as possible toxic air contaminants: 1) risk of harm to public health, 2) amount or potential amount of emissions, 3) manner of usage, 4) persistence in the atmosphere and, 5) ambient concentrations.

Once a substance is selected to enter the TAC identification process, the ARB requests relevant information from the public and a written evaluation of available health effects information (Part B) from the OEHHA. The OEHHA staff's evaluation is required to contain an estimate of the threshold exposure level above which the compound causes or contributes to adverse health effects. In the case where no threshold of significant adverse health effects can be determined, the OEHHA is required to state the range of risk to humans resulting from current or anticipated exposure.

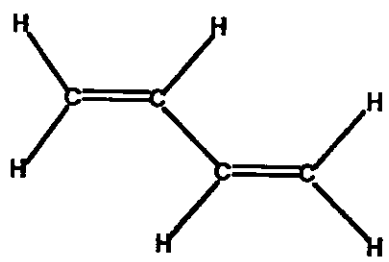
Simultaneous with the preparation of the OEHHA health evaluation, the ARB staff prepares an exposure assessment (Part A) including information on the substance's usage, emissions or potential emissions, environmental persistence, and available outdoor and indoor exposure levels.

Following a public comment period, the Staff Report/Executive Summary and Parts A, B, and C (the public comments and staff responses document) are formally reviewed by the SRP at a public meeting. Upon reviewing the data, assessments, and conclusions of the report and ascertaining that appropriate scientific methods were used to gather and analyze the data presented, the SRP submits written findings to the ARB (the Board). At a public hearing, the Board decides whether or not the evidence in the Technical Support Document supports the identification of a substance as a toxic air contaminant, and if so, whether there is evidence of a threshold exposure below which adverse effects are not expected to occur. Once a substance is identified as a TAC and listed in Section 93000 of Title 17 of the California Code of Regulations, the ARB staff prepares a report on the need and appropriate degree of regulation pursuant to Sections 39665-39668 of the Health and Safety Code.

B. 1,3-BUTADIENE AS A CHEMICAL COMPOUND

1,3-Butadiene is an unsaturated aliphatic hydrocarbon containing two double bonds. It has the chemical formula C_4H_6 (Figure I-1), and is also known as bivinyl, divinyl, α,γ-butadiene, erythrene, vinylethylene, biethylene and pyrrolylene.

Figure I-1
Structure of 1,3-Butadiene



The majority of California's ambient (outdoor air) emissions of 1,3-butadiene are the result of the incomplete combustion of gasoline and diesel fuels. The ARB staff estimates that approximately 3,900 tons of 1,3-butadiene are emitted annually into California's air.

II.

PHYSICAL AND CHEMICAL PROPERTIES OF 1,3-BUTADIENE

At room temperature, 1,3-butadiene is a flammable, colorless gas with a pungent, aromatic odor. It is insoluble in water, slightly soluble in methanol and ethanol, and soluble in organic solvents such as benzene and ether (US EPA, 1989). 1,3-Butadiene is not known to contribute to either global warming or to the depletion of stratospheric ozone. Some of the physical and chemical properties of 1,3-butadiene are shown in Table II-1.

Table II-1
Physical and Chemical Properties of 1,3-Butadiene

Property	Value	Reference
Boiling Point	- 4.5° C	Merck Index, 1983
Density (20° C)	0.6211 g/cm^3	US EPA, 1989
Melting Point	- 108.91° C	US EPA, 1989
Molecular Weight	54.09 g/mole	Merck Index, 1983
Vapor Pressure (15.3° C)	2.0 atm	US EPA, 1989
Conversions (25° C)		
1 ppb (by volume)= 2.21 μg/m^3		
1 μg/m^3 = 0.45 ppb (by volume)		

Most of the 1,3-butadiene emitted to California's atmosphere is a result of the incomplete combustion of gasoline and diesel fuel in motor vehicles. During combustion of the fuel, 1,3-butadiene is expected to be formed primarily by hydrogen atom abstraction from a saturated carbon atom on a straight-chain alkene. Dryer and Brezinsky (1986) have shown that 1,3-butadiene is a significant intermediate oxidation product of n-octane, while Venkat et al. (1982) have shown that butadiene is present as an intermediate in the oxidation of the fuel components benzene, toluene, and ethylbenzene.

References for Chapter II

Dryer, F. L., and K. Brezinsky, 1986. A flow reactor study of the oxidation of n-octane and iso-octane. Combust. Sci. Technol., 45, 199.

Merck Index, 1983, 10th Edition, Merck and Co., Inc., Rahway, New Jersey.

US EPA, 1989. Locating and Estimating Air Emissions from Sources of 1,3-Butadiene. EPA-450/2-89-021, December 1989

Venkat, C., Brezinsky, K., and I. Glassman, 1982. High temperature oxidation of aromatic hydrocarbons. Nineteenth Symposium (International) on Combustion, p. 143. The Combustion Institute.

III.

PRODUCTION, USES, AND EMISSIONS OF 1,3-BUTADIENE

1,3-Butadiene is not commercially produced in California, although it is commercially produced elsewhere in the United States. The largest national uses of 1,3-butadiene include the production of synthetic elastomers and adiponitrile.

In California, the majority of 1,3-butadiene emissions are the result of the incomplete combustion of gasoline and diesel fuels by mobile sources. Mobile sources account for approximately 96 percent (3,700 tons, see Table III-1) of the total annual emissions statewide (approximately 3,900 tons) for quantified sources. Stationary point and area sources also emit 1,3-butadiene during fuel combustion. Stationary point sources emit 1,3-butadiene from boilers, heaters, internal combustion engines, and turbines, while stationary area sources emit 1,3-butadiene during agricultural processes, oil and gas production, manufacturing and industrial processes, service and commerce, and residential fuel combustion.

Non-combustion emissions of 1,3-butadiene occur during vehicle tire wear, petroleum refining, and styrene-butadiene copolymer production.

A. PRODUCTION OF 1,3-BUTADIENE

1,3-Butadiene is commercially produced in the United States through one of two methods. The first is the recovery of 1,3-butadiene during ethylene production (from a C_4 hydrocarbon stream). The second process involves the oxidative dehydrogenation of n-butenes. Almost all of the 1,3-butadiene produced in the United States involves the first method. There are no commercial producers of 1,3-butadiene in California (US EPA, 1989). For U.S. production, import, export, and usage of 1,3-butadiene, see Figure III-1.

Table III-1
1,3-Butadiene Emission Inventory[a]

Source Type	Emissions (Tons/Year)	Inventory Year
Mobile Sources		
On-Road Vehicle Fuel Combustion	2,600	1990
Other Mobile Source Fuel Combustion	1,100	1987
Tire Wear	Not Available	
Stationary Area Sources		
Fuel Combustion	110	1987
Stationary Point Sources		
Fuel Combustion	35	1988
Petroleum Refining	9	1989
Styrene-Butadiene Copolymer Production	1.5[b]	1991
Acrylonitrile-Butadiene-Styrene Resin Production	None[c] Expected	1988
Chemical Production	None[c] Expected	
Approximate Total of[d] Identified Emissions:	3,900 TPY	

Notes:

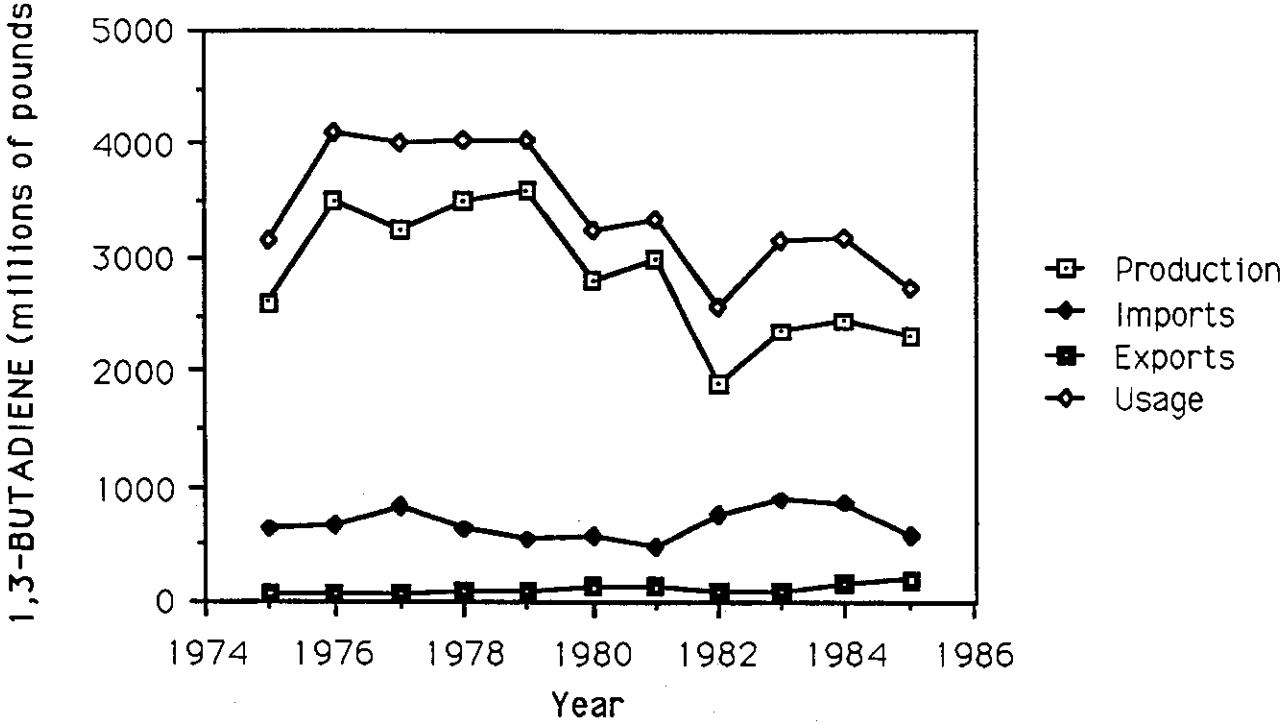
a. Additional emissions will result from unidentified sources and from sources for which emissions have not been estimated (e.g., tire wear).

b. The estimate does not include emissions from the Southern California facility due to a lack of reported data.

c. See text for explanation.

d. The total is not the same as the sum of the emissions due to rounding.

Figure III-1
U.S. Production, Imports, Exports, and Use of 1,3-Butadiene



B. USES OF 1,3-BUTADIENE

The largest use of 1,3-butadiene in the United States is in the production of synthetic elastomers, which include: styrene-butadiene copolymer (SB), acrylonitrile-butadiene-styrene resin (ABS), polybutadiene, neoprene, and nitrile rubber. There are no polybutadiene, neoprene, or nitrile rubber production facilities in California (US EPA, 1989). The US EPA (1989) reports that there are two SB copolymer production facilities and one ABS resin production facility in California.

The second major national use of 1,3-butadiene is in the production of adiponitrile, the raw material used in nylon 6,6 production. Nylon 6,6 is highly resistant to common solvents, alkalis, heat, and impact, and is used to make light weight gears and a number of mechanical parts. The US EPA (1989) reports that there are no adiponitrile production facilities in California.

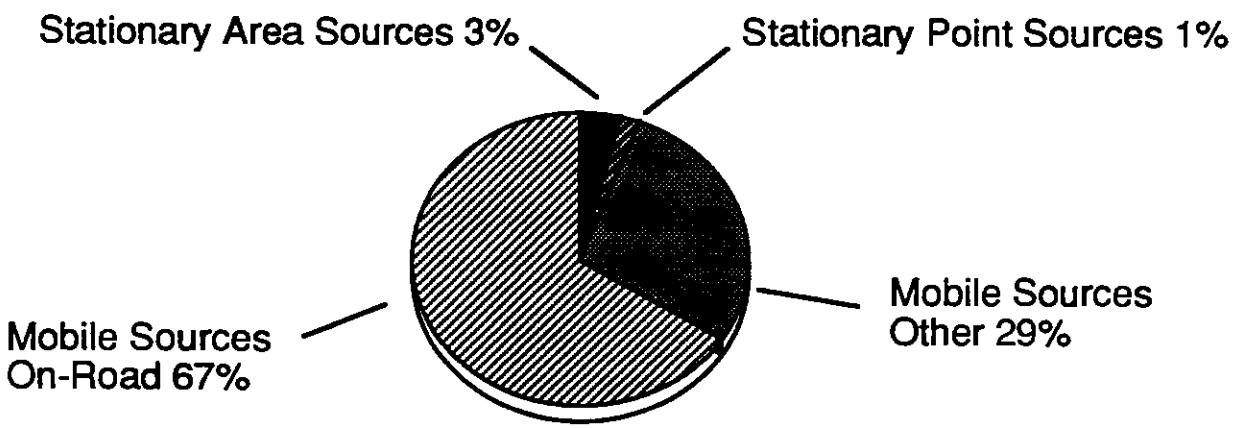
C. EMISSIONS OF 1,3-BUTADIENE

There are approximately 3,900 tons of 1,3-butadiene from quantified sources emitted into California's air each year (see Table III-1). The majority of these emissions are the result of incomplete combustion of

petroleum-derived fuels, rather than the escape of 1,3-butadiene during a chemical manufacturing process.

An estimated 96 percent of California's 1,3-butadiene emissions are the result of the combustion of gasoline and diesel fuels by mobile sources. "On-road" and "other" mobile sources account for 67 percent (2,600 tons) and 29 percent (1,100 tons), respectively, of the total annual statewide estimate of 1,3-butadiene emissions from quantified sources (see Figure III-2).

Figure III-2
California Emissions of 1,3-Butadiene



"Stationary area" sources of fuel combustion account for approximately 3 percent (110 tons) of the total annual statewide estimate of 1,3-butadiene emissions from quantified sources (see Figure III-2).

"Stationary point" sources account for approximately 1 percent (45.5 tons) of the total annual estimate of 1,3-butadiene emissions in California from quantified sources (see Figure III-2). Stationary point sources emit 1,3-butadiene during fuel combustion, petroleum refining, and styrene-butadiene copolymer production.

1. <u>Mobile Sources</u>

Mobile sources account for approximately 96 percent (3,700 tons) of annual 1,3-butadiene emissions. This estimate includes fuel combustion emissions from both on-road vehicles and other mobile sources. Tire wear is also a source of 1,3-butadiene emissions, but is not included in this estimate due to the lack of data.

Mobile sources emit 1,3-butadiene as a result of the incomplete combustion of gasoline or diesel fuel. 1,3-Butadiene emissions increase roughly proportionately to hydrocarbon emissions in engine exhaust.

According to the United States Environmental Protection Agency (US EPA), refineries attempt to minimize the 1,3-butadiene content of gasoline and diesel fuels because the combustion of 1,3-butadiene can cause varnish to form in the engine. Because of the insignificant amount of 1,3-butadiene present in fuel, the US EPA assumes that there will be no 1,3-butadiene emissions from refueling operations or fuel evaporation (US EPA, 1989).

a. On-Road Motor Vehicles

California's on-road motor vehicles emitted approximately 2,600 tons of 1,3-butadiene during 1990 (see Appendix A). On-road motor vehicles include motorcycles, light-duty passenger vehicles, and light-, medium-, and heavy-duty trucks. Emission factors developed for this category were based on data which included 1988 and 1989 model-year vehicles; therefore, the staff estimated 1,3-butadiene emissions using the most recent available activity data (i.e., inventory year 1990). Emission estimates are based on the emission factors, expressed in weight percent of 1,3-butadiene to total organic gases (TOG), and the TOG emissions from individual vehicular classes (ARB, 1990; ARB, 1991a).

Many of California's on-road motor vehicles are equipped with catalysts to reduce exhaust emissions of hydrocarbons (e.g., 1,3-butadiene). Vehicles that are not equipped with functioning catalysts emit far greater amounts of 1,3-butadiene than do vehicles with functioning catalysts. In California, catalyzed vehicles account for approximately 86 percent of the on-road vehicle miles traveled, but are only responsible for an estimated 46 percent of the on-road vehicle fleet's total emissions of 1,3-butadiene (see Appendix A). Uncatalyzed on-road vehicles, accounting for only 14 percent of the on-road vehicle miles traveled, are responsible for approximately 54 percent of the on-road vehicle emissions of 1,3-butadiene. Also, much of the 1,3-butadiene emitted from catalyzed vehicles is emitted when the catalyst is still cool; once the catalyst has reached operating temperature, it dramatically reduces the amount of emitted 1,3-butadiene.

b. **Other Mobile Sources**

California's emissions of 1,3-butadiene from "other" mobile sources are approximately 1,100 tons per year (see Appendix A). This category includes off-road vehicles, boats, trains, ships, aircraft, mobile equipment and utility equipment. Engines in this group are rarely catalyzed for reduction of hydrocarbon exhaust emissions (e.g., 1,3-butadiene); uncatalyzed engines emit far greater amounts of 1,3-butadiene than engines with functioning catalysts. Emission factors for on-road diesel- and gasoline-powered vehicles were assumed to be applicable to similar vehicles and equipment in the "other mobile source" category, and were used to estimate emissions for this category. The 1,3-butadiene emission estimates for this category are based on the 1987 inventory year because 1987 represents the most recent available activity data (ARB 1989a; ARB 1991b).

c. **Tire Wear**

Most of the styrene-butadiene rubber and polybutadiene produced in the United States is used to manufacture tires and tire products. 1,3-Butadiene is released to the atmosphere as the tire wears on the pavement. There is not enough information to develop an emission estimate at this time and thus, emissions from tire wear have not been quantified.

2. <u>Stationary Point Sources</u>

Stationary point sources also have significant emissions, a specific location, and are separately reported from the mobile source inventory. Stationary point sources emit 1,3-butadiene during fuel combustion, petroleum refining, styrene-butadiene copolymer production, acrylonitrile-butadiene-styrene resin production, and chemical production.

The staff estimates statewide emissions of 1,3-butadiene from stationary point sources to be approximately 46 tons per year.

a. **Fuel Combustion**

Combustion sources emit 1,3-butadiene as a result of the incomplete combustion of the fuel similar to the processes associated with motor vehicle exhaust. Sources of fuel combustion include (but are not limited to) boilers, heaters, internal combustion engines, and turbines. The staff estimates statewide emissions of 1,3-butadiene from fuel combustion sources to be

approximately 35 tons per year (see Appendix A). This estimate is based on the TOG emissions from different types of combustion fuels (ARB, 1989c), speciation profiles, and emission rates of 1,3-butadiene.

b. Petroleum Refining

The Oil & Gas Journal lists 31 petroleum refining facilities in California (Oil & Gas, 1989). Of these facilities, there are 5 facilities in the Bay Area Air Quality Management District for which 1,3-butadiene emissions data are available (BAAQMD, 1989a). Using these data and the design capacities of all 31 refineries to process crude oil, the staff estimates air emissions of 1,3-butadiene from petroleum refining processes statewide to be approximately 9 tons per year (see Appendix A).

c. Styrene-Butadiene Copolymer Production

Styrene-butadiene (SB) copolymers are made up of giant molecules formed by the union of simple molecules of styrene and 1,3-butadiene. SB copolymers can be either solid or an elastomer emulsion, depending on the composition and the extent of drying in the process.

The two types of solid copolymers are styrene-butadiene rubber and styrene-butadiene elastomer. Styrene-butadiene rubber typically contains more than 45 percent 1,3-butadiene, while styrene-butadiene elastomer typically contains more styrene. Emissions for both these types of solid SB copolymers are not differentiated. Solid SB copolymers are commonly used in tires, tire products, and mechanical rubber goods.

Elastomer emulsion SB copolymer is commonly referred to as styrene-butadiene latex. The emulsion process is the same process used for solid SB copolymers except that the emulsion breaking (coagulation) and drying steps are omitted. Latex is commonly used in foam products, carpet and textile backing, paper coatings, and adhesives.

There are two latex production facilities in California (one in Northern California and one in Southern California), and no solid SB copolymer production facilities. In 1984, the Northern California facility and its four affiliate facilities (located in other states) had a combined capacity to produce 175,000 megagrams (192,900 tons) of latex per year. In 1984 the

Southern California facility had the capacity to produce 25,000 megagrams (27,560 tons) of latex per year (US EPA, 1989).

Emission information reported to the Bay Area Air Quality Management District show that the Northern California facility is estimated to emit approximately 1.5 tons of 1,3-butadiene per year. Approximately 2,555 pounds of the 1,3-butadiene emissions came from the latex process and tank leaks, while an estimated 533 pounds of the 1,3-butadiene emissions came from equipment leaks. There are no available data to estimate emissions from the Southern California facility at this time, thus, the statewide emission estimate only includes emissions from the Northern California facility.

d. Acrylonitrile-Butadiene-Styrene Resin Production

Acrylonitrile-butadiene-styrene (ABS) resins are used to make a variety of plastic components including: automotive parts, pipes and fittings, appliances, electronics, business machines, telecommunications, construction, toys, recreational products, and plastic modifiers (US EPA, 1989). There are nine facilities in the United States producing ABS resins. At least five of these facilities use polybutadiene instead of 1,3-butadiene.

The only facility producing ABS resins in California is in Southern California. This facility had the capacity (in 1988) to produce 32,000 megagrams (35,000 tons) of resin per year, using polybutadiene as the raw material for ABS production. According to the US EPA, no 1,3-butadiene emissions are expected from ABS resins production using polybutadiene as a feedstock (US EPA, 1989). Thus, there are no 1,3-butadiene emissions expected from ABS resins production facilities in California.

e. Miscellaneous Uses of 1,3-Butadiene in Chemical Production

There are at least 20 facilities nationwide producing chemical products from 1,3-butadiene. According to the US EPA, a northern California chemical production facility is the only facility in California holding a permit to manufacture a chemical product from 1,3-butadiene (US EPA, 1989). The product is a fungicide; it is produced in a pressurized system which is vented to an incinerator on a continuous basis. The facility has not produced the fungicide since 1987 (BAAQMD, 1989b). Presently there are believed to be no 1,3-butadiene emissions from the production of fungicide in California.

3. Stationary Area Sources

Stationary area sources individually emit small amounts of 1,3-butadiene, but collectively result in significant emissions and are reported separately from either the point source or the mobile source inventory.

1,3-Butadiene emissions occur when stationary area sources combust petroleum-derived fuel (1,3-butadiene is a product of incomplete combustion). Area sources include agricultural activities, oil and gas production facilities, manufacturing and industrial sources, services and commerce, and residential fuel combustion. Based on TOG emissions from different fuel combustion sources (ARB, 1989a) and 1,3-butadiene emission rates, the staff estimates statewide 1,3-butadiene emissions to be approximately 110 tons per year from area sources of fuel combustion (see Appendix A).

D. EMISSION PROJECTION

Approximately 96 percent of California's outdoor 1,3-butadiene comes from mobile sources (both on-road motor vehicles and other mobile sources). Based on data from the ARB's emission factor and emission inventory models (EMFAC7EP and BURDEN7C) and estimates of emissions from other mobile sources, total organic gas exhaust emissions from California's mobile sources (the basis of the 1,3-butadiene estimate) are expected to steadily decrease through the year 2010. These decreases are a result of motor vehicle regulations that have already been implemented, and regulations that have been adopted and have future implementation dates.

Vehicles now sold in California are required to meet a standard of 0.39 grams per mile of non-methane organic gas. Starting in 1993, 40 percent of the new light-duty passenger vehicle fleet must meet a standard of 0.25 grams per mile; in 1994, 80 percent; and by 1995, 100 percent of the light-duty passenger vehicle fleet must meet the 0.25 grams per mile standard for non-methane organic gas. A 1990 ARB regulation ("Low-Emission vehicles/ Clean Fuels") will result in reductions in non-methane organic gases from light-duty and medium-duty vehicles by requiring a growing percentage of the new vehicle fleet to meet "low emission vehicle" standards through compliance options that include the use of "clean fuels" (e.g., compressed natural gas). This program will be "phased in" over the period 1994 through 2003.

The ARB's 1991 "Phase 2" reformulated gasoline regulations include a comprehensive set of standards which will affect eight gasoline properties, including: Reid vapor pressure, aromatic hydrocarbon content, oxygen content, benzene content, olefin content, sulfur content, T90 distillation temperature (the temperature at which 90 percent of a given volume of gasoline would evaporate), and T50 distillation temperature. Use of reformulated gasoline (required statewide April 1996) is expected to result in significant reductions of benzene and 1,3-butadiene emissions from engines burning reformulated gasoline.

E. INDOOR SOURCES OF 1,3-BUTADIENE

Environmental tobacco smoke (ETS) is the primary source of 1,3-butadiene in the indoor environment. Brunnemann et al. (1989) measured 1,3-butadiene concentrations of 205 to 361 μg/cigarette in sidestream smoke from seven different types of cigarettes. Concentrations of 1,3-butadiene in mainstream smoke ranged from 16 to 75 μg/cigarette. Lofroth et al. (1989) found 1,3-butadiene concentrations to average 400 μg/cigarette in a chamber where sidestream smoke had been directed from machine-smoked cigarettes. Another source of 1,3-butadiene indoors is vehicle exhaust which can infiltrate into homes near busy freeways or busy roadways. Information on exposure to 1,3-butadiene from indoor sources can be found in Chapter IV C and E.

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IV.
EXPOSURE TO 1,3-BUTADIENE

A. AMBIENT MONITORING FOR 1,3-BUTADIENE

The ARB's toxic monitoring network is comprised of 20 toxic monitoring stations (see Figure IV-1), nine of which are located in Southern California (south of Bakersfield), while the other 11 are located in the northern portion of the state. The monitoring sites and equipment locations were selected in accordance with the US EPA's site criteria document Probe Siting Criteria for Ambient Air Quality Monitoring, found in Appendix E of 40 CFR Chapter 1, Part 58. Data used in the exposure analysis were collected during the period of July 1988 through June 1989. The data for this period (hereafter referred to as "the study period") represent the most recent period for which the available data are known to be of consistent and verifiable quality. Individual 24-hour samples were collected and subsequently analyzed for 1,3-butadiene using gas chromatography with photoionization and a flame ionization detector. (For a complete description of the standard operating procedure for the determination of 1,3-butadiene in ambient air, please see Appendix B).

B. AMBIENT CONCENTRATIONS OF 1,3-BUTADIENE

The 1,3-butadiene data for the study period represents 20 sites and a percentage of all possible days during the sampling period. The months during which data are available for each site are summarized in Table IV-1.

1. Study Period Data Collected

The number of samples available per site during the study period range from 12 to 24 in Northern California, and 12 to 23 in Southern California. Sampling days were randomly selected; the Northern and Southern California sites averaged 1.6 observations per month.

Only 12 percent of the observations were below the limit of detection (LOD). In August 1988, the MLD staff changed the analysis technique for 1,3-butadiene resulting in a lowering of the LOD from 0.10 ppbv

Figure IV-1
The ARB's Toxic Monitoring Network for 1,3-Butadiene
(July 1988--June 1989)

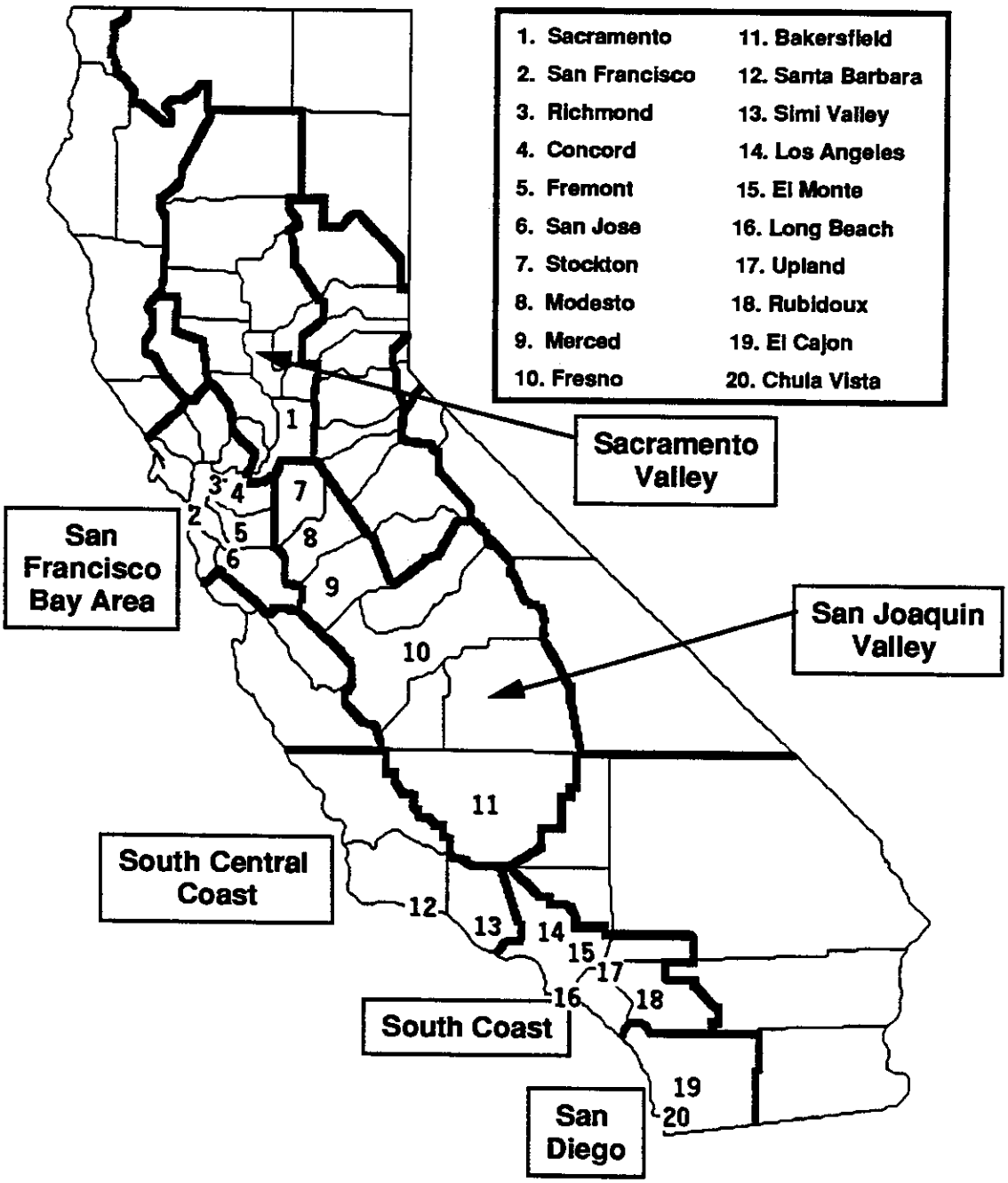


Table IV-1
Summary of 1,3-Butadiene Data*
July 1988 - June 1989

Air Basin / Site Location	J	A	S	O	N	D	J	F	M	A	M	J	Samples
South Coast Air Basin													
El Monte	o	o	o	o	o	o	o						12
Los Angeles	o				o	o	o	o	o	o	o	o	15
Long Beach	o	o	o	o	o	o	o	o	o		o	o	20
Rubidoux	o	o	o	o	o	o	o	o	o	o	o	o	20
Upland	o	o		o	o	o	o	o	o	o	o	o	21
South Central Coast Air Basin													
Santa Barbara	o			o	o	o	o	o	o	o	o	o	16
Simi Valley	o			o	o	o	o	o	o	o	o	o	23
San Diego Air Basin													
Chula Vista	o	o	o	o	o	o	o	o	o	o	o	o	22
El Cajon	o	o	o	o	o	o	o	o	o	o	o	o	20
San Francisco Bay Area Air Basin													
Concord	o			o	o	o	o	o	o	o	o	o	20
Fremont	o	o	o	o	o	o	o	o	o	o	o	o	22
Richmond	o	o	o	o	o	o	o		o	o	o	o	20
San Francisco	o				o	o	o	o	o	o	o	o	17
San Jose	o			o	o	o	o	o	o	o	o	o	20
San Joaquin Valley Air Basin													
Bakersfield	o	o	o	o	o	o	o	o	o	o	o	o	21
Fresno	o	o		o	o	o	o	o	o	o	o	o	20
Merced	o			o	o	o		o	o				12
Modesto	o	o	o	o	o	o	o	o	o	o	o	o	24
Stockton	o			o	o		o	o	o	o	o	o	16
Sacramento Valley Air Basin													
Citrus Heights	o			o	o	o	o	o	o	o	o	o	17

* An "o" indicates that at least one sample was collected during the month.

(0.22 $\mu g/m^3$) to 0.04 ppbv (0.09 $\mu g/m^3$). Observations below the LOD are referred to as partially observed data (POD). The number of POD samples for each site are included in Table IV-2.

Table IV-2

Estimated Mean[a] Annual 1,3-Butadiene Concentrations (in ppbv*)
Using Four Methods for Estimating Values Below the LOD
July 1988 - June 1989

Air Basin Site Location	POD=0 Mean	POD=0.02 Mean	POD=0.04 Mean	Gleit Mean	POD Samples
South Coast Air Basin					
El Monte	0.58	0.58	0.58	0.58	0
Los Angeles	0.47	0.47	0.47	0.47	0
Long Beach	0.38	0.38	0.38	0.38	1
Rubidoux	0.23	0.24	0.25	0.35	3
Upland	0.29	0.29	0.29	0.29	0
Basin Summary	0.41	0.42	0.42	0.42	4
South Central Coast Air Basin					
Santa Barbara	0.30	0.30	0.30	0.30	1
Simi Valley	0.13	0.13	0.14	0.14	5
Basin Summary	0.22	0.22	0.22	0.22	6
San Diego Air Basin					
Chula Vista	0.15	0.16	0.17	0.17	5
El Cajon	0.36	0.37	0.38	0.37	3
Basin Summary	0.26	0.27	0.28	0.27	8
San Francisco Bay Area Air Basin					
Concord	0.23	0.24	0.24	0.24	2
Fremont	0.19	0.20	0.20	0.20	3
Richmond	0.15	0.16	0.17	0.16	3
San Francisco	0.21	0.21	0.22	0.21	2
San Jose	0.42	0.42	0.42	0.42	1
Basin Summary	0.24	0.25	0.25	0.25	11
San Joaquin Valley Air Basin					
Bakersfield	0.28	0.28	0.28	0.28	2
Fresno	0.34	0.35	0.36	0.35	2
Merced	0.26	0.26	0.27	0.27	3
Modesto	0.27	0.28	0.29	0.28	4
Stockton	0.28	0.29	0.29	0.29	1
Basin Summary	0.29	0.29	0.30	0.29	12
Sacramento Valley Air Basin					
Citrus Heights	0.30	0.31	0.31	0.31	4

a. Means are computed as the sum of all observations divided by the total number of observations. This method is used in order to compare the effects of the different LOD values.

* 1 ppbv = 2.21 $\mu g/m^3$

POD were reported for each site (except for El Monte, Los Angeles, and Upland). Even though no value is reported when a concentration is below the LOD, important information is still available. The fewer POD there are, the less significant their impact on the analysis. Because removing these values from the dataset would bias the sample statistics and exposure estimates, four methods for dealing with POD were compared: (1) replacement of the values below the LOD with zero; (2) replacement of the values below the LOD with half the LOD; (3) replacement of the values below the LOD with the LOD; and (4) replacement of the values below the LOD with estimated order statistics (Gleit, 1985). The results of this comparison are presented in Table IV-2.

Since the 1,3-butadiene dataset has some data from the period when the LOD was 0.10 ppbv (0.22 $\mu g/m^3$) and some data when the LOD was 0.04 ppbv (0.09 $\mu g/m^3$), replacement values were calculated for each site and subset of data with a different LOD. Gleit's estimate was calculated for a subset with the higher LOD first, then the POD from the period of higher LOD were replaced by the estimated replacement value. Next, the same procedure was used to estimate a replacement value for any remaining POD. Both subsets of data for each site were then combined to form the overall dataset representing each site.

2. <u>Site-Specific Concentrations</u>

The range of concentrations sampled at each site during the study period are summarized in Table IV-3. Minimum, maximum, median and mean concentrations, as well as the standard deviation are reported for each site. Mean 1,3-butadiene concentrations were calculated as the mean of available monthly means to provide equal weighting for each month when the number of samples per month varies. Standard deviations were calculated from monthly means so they could be compared with the annual mean concentrations.

The site-specific ranges of the ambient 1,3-butadiene concentrations listed in Table IV-3 are plotted in Figure IV-2. Minimum concentrations range from POD at 17 of the 20 sites to 0.10 ppbv (0.22 $\mu g/m^3$) at El Monte, Los Angeles, and Upland. Maximum concentrations range in value from 0.34 ppbv (0.75 $\mu g/m^3$) at Richmond to 1.70 ppbv (3.75 $\mu g/m^3$) at San Jose.

Table IV-3
Summary of Ambient 1,3-Butadiene Data (in ppbv)
July 1988 - June 1989

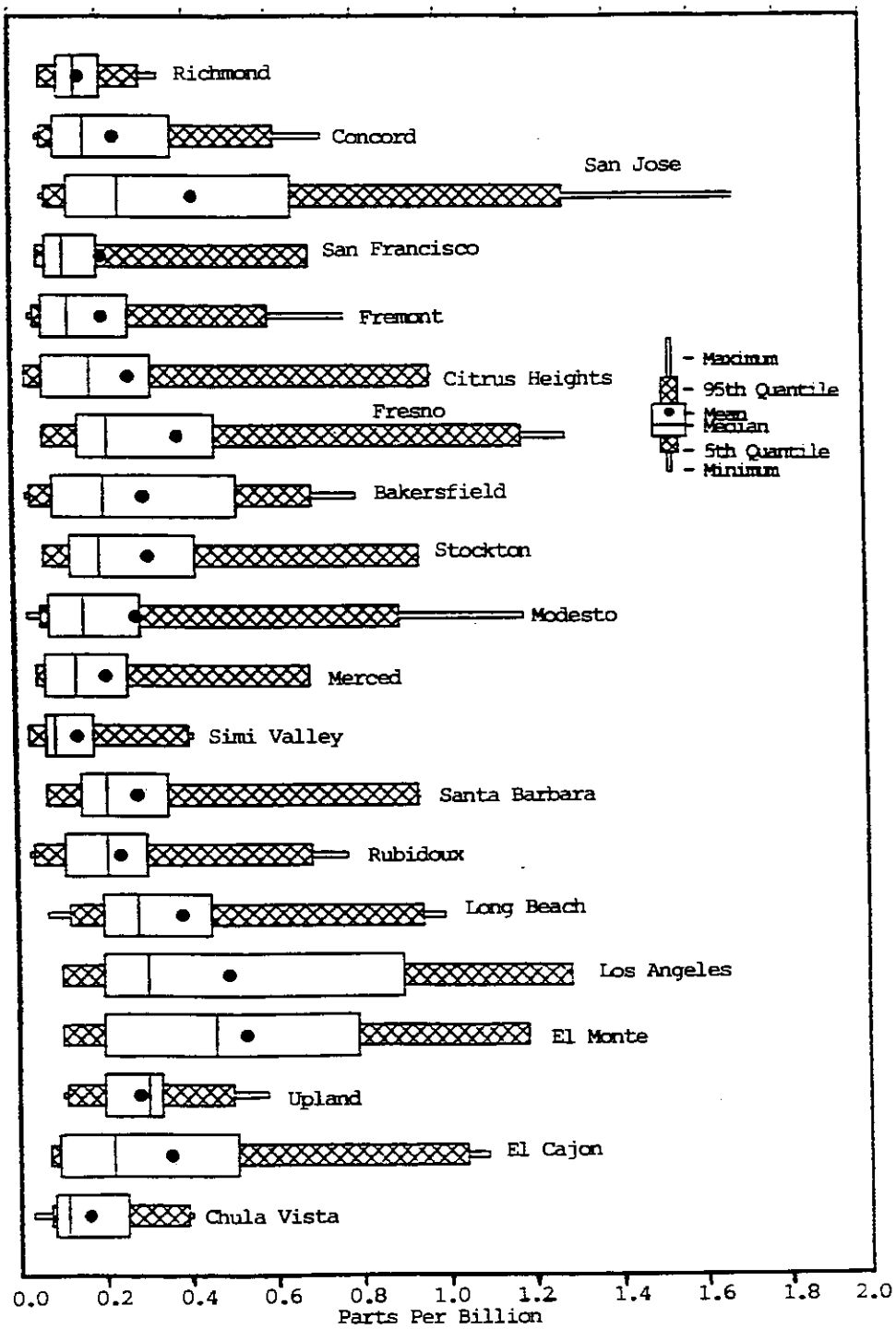
Air Basin Site Location	Minimum Conc.	Maximum Conc.	Median Conc.	Mean[a] Conc.	Standard[b] Deviation
South Coast Air Basin					
El Monte	0.10	1.20	0.46	0.58	0.37
Los Angeles	0.10	1.30	0.30	0.47	0.30
Long Beach	POD	1.00	0.28	0.38	0.26
Rubidoux	POD	0.77	0.21	0.25	0.19
Upland	0.10	0.58	0.30	0.29	0.12
Basin Summary	POD	1.30	0.31	0.39	0.26
South Central Coast Air Basin					
Santa Barbara	POD	0.94	0.21	0.30	0.16
Simi Valley	POD	0.41	0.09	0.14	0.11
Basin Summary	POD	0.94	0.20	0.22	0.14
San Diego Air Basin					
Chula Vista	POD	0.40	0.11	0.17	0.11
El Cajon	POD	1.10	0.22	0.37	0.35
Basin Summary	POD	1.10	0.21	0.27	0.26
San Francisco Bay Area Air Basin					
Concord	POD	0.73	0.17	0.24	0.13
Fremont	POD	0.78	0.13	0.20	0.18
Richmond	POD	0.34	0.15	0.16	0.08
San Francisco	POD	0.70	0.12	0.21	0.17
San Jose	POD	1.70	0.25	0.42	0.33
Basin Summary	POD	1.70	0.15	0.25	0.20
San Joaquin Valley Air Basin					
Bakersfield	POD	0.80	0.21	0.28	0.23
Fresno	POD	1.30	0.22	0.35	0.27
Merced	POD	0.69	0.14	0.27	0.22
Modesto	POD	1.20	0.16	0.28	0.27
Stockton	POD	0.95	0.20	0.29	0.20
Basin Summary	POD	1.30	0.20	0.29	0.26
Sacramento Valley Air Basin					
Citrus Heights	POD	0.98	0.18	0.31	0.32

a. Site means were calculated as the mean of available monthly means. Basin means are the mean of the site means.

b. Site standard deviations were calculated from the monthly means. Basin standard deviations are pooled values of the standard deviations across sites within a basin.

* 1 ppbv = 2.21 $\mu g/m^3$

Figure IV-2
Mean Annual 1,3-Butadiene Concentrations
Plotted Using Extended Box Plots
(July 1988--June 1989)



Mean annual concentrations ranged from a minimum of 0.14 ppbv (0.31 $\mu g/m^3$) at Simi Valley to a maximum of 0.58 ppbv (1.28 $\mu g/m^3$) at El Monte. Basin averages range from a minimum of 0.22 ppbv (0.48 $\mu g/m^3$) in the South Central Coast Air Basin to a maximum of 0.39 ppbv (0.86 $\mu g/m^3$) in the South Coast Air Basin.

3. Basin-Wide Mean Concentrations

The highest basin-wide mean concentration occurred in the South Coast Air Basin (0.42 ppbv = 0.93 $\mu g/m^3$), followed by the San Joaquin Valley Air Basin (0.29 ppbv = 0.64 $\mu g/m^3$), San Diego Air Basin (0.27 ppbv = 0.60 $\mu g/m^3$), San Francisco Bay Area Air Basin (0.25 ppbv = 0.55 $\mu g/m^3$), and South Central Coast Air Basin (0.22 ppbv = 0.48 $\mu g/m^3$).

4. Population-Weighted Exposure Estimates

Mean population-weighted exposure estimates were calculated using the study period 1,3-butadiene data. Exposures for the South Coast Air Basin and San Francisco Bay Area Air Basin were estimated by interpolating station values to census tract centroids. For the other air basins, a basin-wide mean concentration was estimated from the means for all sites in the basin. It was then assumed that all people in those counties with a sampling site were exposed to this estimated basin-wide mean concentration. Population data used in the exposure analysis represent 1980 census data updated to 1985 levels. The results of the exposure analysis are summarized in Table IV-4.

Included in Table IV-4 are Bootstrap confidence bounds (Efron, 1982), provided as a measure of uncertainty in the exposure estimates. The larger the difference between the mean annual exposure estimate and the upper and lower bounds, the greater the uncertainty in the estimate; the bounds are not provided as an upper or lower bound to actual exposure. The upper and lower bounds for each site were calculated by resampling the monthly means.

The mean population-weighted 1,3-butadiene exposure estimate for California is 0.37 ppbv (0.82 $\mu g/m^3$). A total of 20.3 million people are estimated to reside in the study areas. Basin-specific, population-weighted mean concentrations range from a minimum of 0.22 ppbv (0.48 $\mu g/m^3$) in the South Central Coast Air Basin to a maximum of 0.44 ppbv (0.97 $\mu g/m^3$) in the South Coast Air Basin.

Table IV-4
Summary of 1,3-Butadiene Population-Weighted Exposure Estimates
July 1988 - June 1989

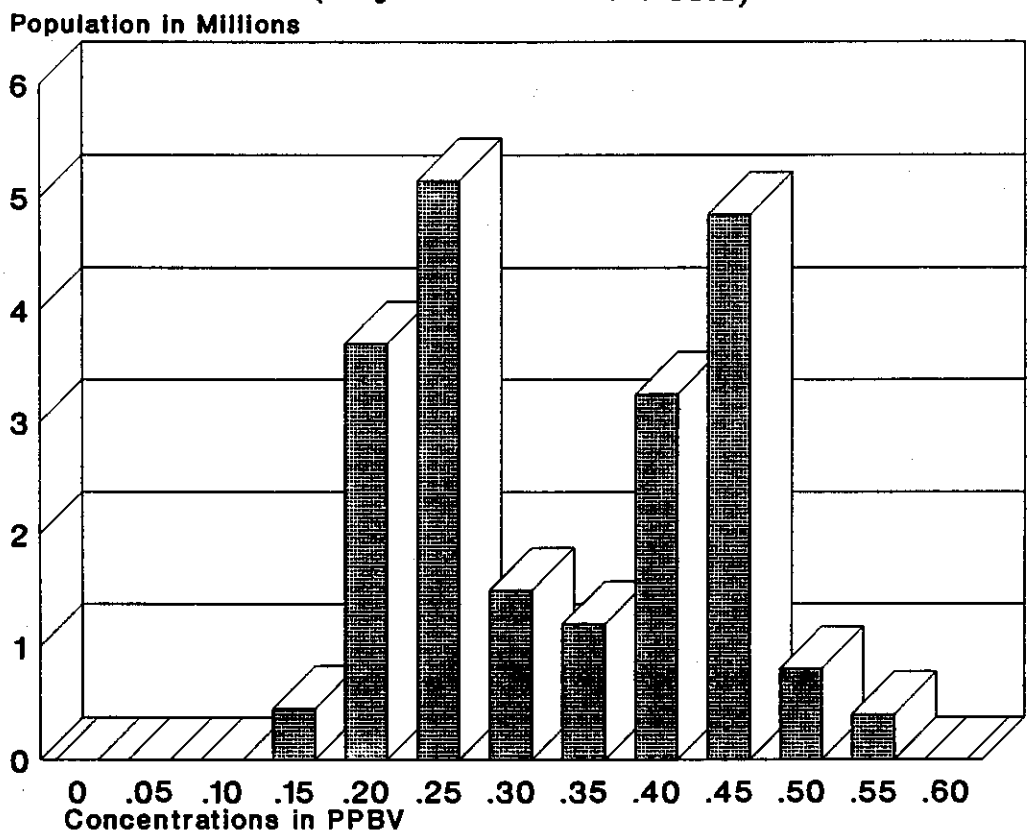
Air Basin Site Location	Upper Bound (ppbv)	Mean Exposure (ppbv)	Lower Bound (ppbv)	Population
South Coast Air Basin				
El Monte	0.80	0.58	0.34	
Los Angeles	0.62	0.47	0.30	
Long Beach	0.48	0.38	0.23	
Rubidoux	0.31	0.25	0.14	
Upland	0.37	0.29	0.22	
POPULATION-WEIGHTED EXPOSURE FOR SoCAB		**0.44**		10,092,133
South Central Coast Air Basin				
Santa Barbara	0.37	0.30	0.20	
Simi Valley	0.20	0.14	0.08	
POPULATION-WEIGHTED EXPOSURE FOR SCCAB		**0.22**		925,822
San Diego Air Basin				
Chula Vista	0.19	0.17	0.10	
El Cajon	0.55	0.37	0.20	
POPULATION-WEIGHTED EXPOSURE FOR SDAB		**0.27**		2,135,872
San Francisco Bay Area Air Basin				
Concord	0.27	0.24	0.15	
Fremont	0.27	0.20	0.12	
Richmond	0.18	0.16	0.11	
San Francisco	0.33	0.21	0.12	
San Jose	0.60	0.42	0.25	
POPULATION-WEIGHTED EXPOSURE FOR SFBAAB		**0.25**		4,394,374
San Joaquin Valley Air Basin				
Bakersfield	0.37	0.28	0.15	
Fresno	0.51	0.35	0.22	
Merced	0.44	0.27	0.14	
Modesto	0.39	0.28	0.14	
Stockton	0.35	0.29	0.16	
POPULATION-WEIGHTED EXPOSURE FOR SJVAB		**0.29**		1,901,243
Sacramento Valley Air Basin				
Citrus Heights	0.50	0.31	0.15	
POPULATION-WEIGHTED EXPOSURE FOR SVAB (Sac.County only)		**0.31**		889,806
OVERALL POPULATION-WEIGHTED EXPOSURE		**0.37**		20,339,250

* 1 ppbv = 2.21 $\mu g/m^3$

Figure IV-3 shows the total number of people exposed to various mean annual 1,3-butadiene concentrations (rounded off to the nearest 0.05 ppbv). The peak at 0.45 ppbv (1 $\mu g/m^3$) represents the population in the South Coast Air Basin, while the peak at 0.25 ppbv (0.55 $\mu g/m^3$) represents the remaining five air basins.

The overall geographic mean 1,3-butadiene concentration was 0.29 ppbv (0.64 $\mu g/m^3$). This value is approximately 22 percent lower than the population-weighted exposure estimate of 0.37 ppbv (0.82 $\mu g/m^3$), indicating that the highest concentrations of 1,3-butadiene tended to be in the areas of higher population density.

Figure IV-3
Estimated Mean Annual 1,3-Butadiene Exposure
(July 1988--June 1989 Data)



C. SOUTH COAST AIR QUALITY STUDY (SCAQS) 1,3-BUTADIENE CONCENTRATIONS DATA

The 1987 SCAQ Study (the first comprehensive study of air pollution in the South Coast Air Basin) provided a unique opportunity to characterize gaseous and

particulate pollutants in the basin under well-documented meteorological conditions. The sampling and analysis methods used in the study resulted in a data base that represents the 1,3-butadiene concentrations for a specific hour of a sampling day (as opposed to the data from the ARB's toxic monitoring network which represents 24-hour averages).

SCAQS air samples for the gaseous pollutants were drawn, over the period of 1 hour, into SUMMA polished stainless steel canisters. Analysis was performed using a gas chromatography/flame ionization detector. The data base represents the period of June 1987 through December 1987 (SCAQS, 1991). Average 1,3-butadiene concentrations from the eight monitoring sites in the SoCAB were 1.6 ppbv (3.53 $\mu g/m^3$), with minimum concentrations that ranged from below the limit of detection (LOD) of 0.1 ppbv (0.22 $\mu g/m^3$) to a high of 17.7 ppbv (39.1 $\mu g/m^3$). In comparison, data derived from the ARB's toxics monitoring network (July 1988 through July 1989 from samples drawn over 24 hours on random days) for the SoCAB show minimum, average, and maximum concentrations of below LOD, 0.39, and 1.3 ppbv, respectively (see Table IV-3).

D. INDOOR AIR CONCENTRATIONS OF 1,3-BUTADIENE

A limited number of studies have measured 1,3-butadiene concentrations indoors. Lofroth et al. (1989) investigated the chemical composition of environmental tobacco smoke (ETS) in a tavern where commercial cigarettes were smoked by occupants. The tavern was approximately 180 cubic meters in volume and was variously occupied by 5 to 25 individuals, many of whom were smoking. The 1,3-butadiene concentrations were from 2-hour sampling periods (both indoors and outdoors) on two separate study days. For the first study day, the 1,3-butadiene concentration was 8.60 ppbv (19 $\mu g/m^3$) indoors and less than the LOD (0.45 ppbv or 1 $\mu g/m^3$) outdoors. For the second study day, investigators measured 4.98 ppbv (11 $\mu g/m^3$) 1,3-butadiene indoors and 0.45 ppbv (1 $\mu g/m^3$) outdoors. Similar findings were obtained by the same investigators in a related chamber study using machine-smoked cigarettes.

Brunnemann et al. (1989) sampled air in a bar where an average of 5 to 15 cigarette smokers were present at any time. Continuous sampling over a 3-hour period yielded 1,3-butadiene concentrations ranging from 1.49 to 2.04 ppbv (3.3 to 4.5 $\mu g/m^3$).

A recent study sponsored by the ARB measured indoor levels of 1,3-butadiene in 62 Woodland California homes during the summer of 1990 (Sheldon, 1991). Air samples were collected in 6-liter SUMMA polished steel canisters. The sample was slowly drawn into the evacuated canister over 24 hours via a restrictive orifice attached to the canister valve. Interestingly, only 5 homes (8.1 percent of those sampled) had 1,3-butadiene concentrations above the LOD (0.54 ppbv or 1.2 $\mu g/m^3$). Results indicate that the concentrations in these homes ranged from 1.22 to 4.53 ppbv (2.7 to 10.0 $\mu g/m^3$). In 4 of the 5 homes with measurable 1,3-butadiene, 20 to 40 cigarettes reportedly were smoked during the monitoring period. The source of 1,3-butadiene in the other home with measurable 1,3-butadiene is uncertain.

An initial expectation is that homes in which many cigarettes were reportedly smoked would have measurable levels of 1,3-butadiene. However, smoking occurred in 16 of the 57 homes where indoor concentrations of 1,3-butadiene were less than the LOD. There are several possible reasons why 1,3-butadiene was not detected in more study homes with ETS. Since the study was designed to measure multiple air pollutants in the same sample, the LOD for 1,3-butadiene was relatively high (0.54 ppbv or 1.2 $\mu g/m^3$). Some of the homes may have had very high air exchange rates during the monitoring period; this can have a major effect on indoor pollution levels. Brunnemann et al. (1989) measured 1,3-butadiene in a 16 cubic meter laboratory (both with and without ventilation) while 5 to 10 cigarettes were being machine smoked. When the lab was unventilated, the 1,3-butadiene concentration was 4.30 ppbv (9.5 $\mu g/m^3$). When the lab was ventilated at 12 air exchanges per hour, the 1,3-butadiene level was 0.34 ppbv (0.75 $\mu g/m^3$). Another possible reason for the many measurements below the LOD in the Woodland study is that 1,3-butadiene is known to react with acrolein, another component of ETS (Harke et al., 1970, as reported by Brunnemann et al., 1989).

In the Woodland pilot study (November 1989), 3 of the 4 homes sampled had measurable levels of 1,3-butadiene (Sheldon et al., 1990). The measured indoor concentrations ranged from below the LOD of 0.05 ppbv (0.12 $\mu g/m^3$) up to 0.47 ppbv (1.04 $\mu g/m^3$). It is not known if ETS was present in these homes. The indoor concentrations were lower than the measured outdoor concentrations of 0.67 and 0.76 ppbv (1.48 and 1.67 $\mu g/m^3$).

E. ESTIMATES OF EXPOSURE FROM INDOOR AIR

Information on 1,3-butadiene concentrations in homes or public buildings is limited at this time. Indoor concentrations of 1,3-butadiene are primarily dependent upon the presence of ETS. On any given day, 43 percent of adult non-smokers report that they are near ETS. The proportion of non-smoking youths (age 12 through 17) exposed to an ETS environment on any given day is even higher at 64 percent (Jenkins et al., 1992). Therefore, indoor air is almost certainly the major route of exposure to 1,3-butadiene for individuals who are exposed to an ETS environment.

Exposure estimates for short-term exposure in a bar or tavern are presented in Table IV-5. Concentrations of 1,3-butadiene measured by Lofroth et al. (1989) and Brunnemann et al. (1989) are the basis for the short duration calculation. The exposure duration used is 3 hours, the average time spent in a bar or nightclub (Jenkins et al., 1992). The assumed breathing volume is 1.06 cubic meters per hour (based on an average breathing volume of 20 cubic meters of air per day). An average of 3 cubic meters of air are breathed during 8 hours of sleep, while an average 1.06 cubic meters per hour are breathed during the remaining 16 hours of the day (International Commission on Radiological Protection, 1975). Based on measurements of 1,3-butadiene in a tavern, bar, and an unventilated lab, the estimated dose of 1,3-butadiene inhaled in 3 hours in an ETS environment could range from 10 to 60 μg. The exposure estimate for a well-ventilated indoor environment (ventilated lab) with ETS is much lower at 2.4 μg of 1,3-butadiene during 3 hours. The exposure estimate for an individual inhaling the statewide outdoor average concentration of 1,3-butadiene for 3 hours is 2.6 μg.

Restaurants also provide opportunities for exposure to an ETS environment. Restaurants are visited by 35 percent of the California population for an average duration of 81 minutes on any given day (Jenkins et al., 1992). Elevated indoor 1,3-butadiene concentrations in such places can lead to a large increase in total exposure for the individual who spends time in these microenvironments. The actual amount of 1,3-butadiene inhaled from ETS exposure will vary due to room size, room ventilation rates, the number of cigarettes smoked, and other factors.

Residential concentrations data for 1,3-butadiene are too limited at this time to generate exposure estimates. However, it is apparent that the potential for indoor exposure can exceed outdoor exposure. Further measurements are needed in a wide variety of homes and offices and over different seasons in order to quantify typical indoor butadiene exposures. Such measurements may also help to further identify the relationships between smoking and indoor levels, and between indoor and outdoor levels of 1,3-butadiene.

Table IV-5
3 Hour Estimated Inhaled Doses of 1,3-Butadiene in Environments with ETS

LOCATION	REFERENCE	INDOOR CONCENTRATION (ppbv)	INDOOR CONCENTRATION (μg/cubic meter)	ESTIMATED INHALED[d] DOSE (μg/exposure)
Tavern	1	5.0-8.6[a]	11-19	35-60
Bar	2	1.5-2.0[a]	3.3-4.5	10-14
Unventilated Lab	2	4.3[b]	9.5	30
Ventilated Lab	2	0.3[c]	0.75	2.4

References: 1) Lofroth et al., 1989. 2) Brunnemann et al., 1989.

a: Range of 2 samples. b: Mean of 4 experimental runs. c: Mean of 2 samples.
d: Estimated dose =
indoor concentration x air breathed/hour x # of hours at that location
(μg/cubic meter) (1.06 cubic meters/hr) (3 hours)

F. NEAR-SOURCE ("HOT SPOT") EXPOSURE

Some Californians may be exposed to near-source, or "hot spot" concentrations of 1,3-butadiene added to the average ambient concentration of 1,3-butadiene. "Hot spot" exposure to airborne 1,3-butadiene may significantly increase the cancer risk to individuals living near sources. Sources of "hot spot" emissions of 1,3-butadiene may include facilities using butadiene for production of resins and polymers, synthetic rubber manufacturing facilities, chemical production facilities, petroleum refineries, and stationary fuel combustion sources.

The US EPA has roughly estimated that 1,3-butadiene exposure for individuals living near two facilities in California may be almost double the average exposure experienced by individuals not living near these facilities (see Part B's Section 5.2 on Hot Spots). However, the ARB staff does not have enough emissions and exposure information available at this time to validate the US EPA's estimate. This information is expected during 1992, when the ARB staff will evaluate 1,3-butadiene emissions data reported by facilities under the AB 2588 Air Toxics "Hot Spots" emissions reporting program. If the Board identifies 1,3-butadiene as a toxic air contaminant, this "hot spot" information will be used during the risk management phase to help determine priority and need for control of sources emitting 1,3-butadiene.

G. OTHER ROUTES OF 1,3-BUTADIENE EXPOSURE

1. Water Ingestion

Detectable concentrations of 1,3-butadiene have been reported in drinking water by the International Agency for Research on Cancer (IARC, 1986). The investigators referenced by IARC did not say how much 1,3-butadiene had been detected, only that it had been.

1,3-Butadiene has been listed as a carcinogen under the California Safe Drinking Water and Toxic Enforcement Act of 1986 (Proposition 65; Health and Safety Code Section 25249.5). Substances listed under Proposition 65 have not necessarily been measured in water, but are listed due to their toxicity and their potential release into water. According to the California Department of Health Services (DHS), 1,3-butadiene has not been found in California's ground water. Polybutylene pipe (used in water systems) was investigated by the DHS as a possible source of 1,3-butadiene contaminating water supplies (Spath, 1989). It was not found in tests of water from these types of pipes (Cooper, 1989).

2. Food

There is limited information regarding the level of 1,3-butadiene in food. Butadiene is used to make food packaging, vegetable oil bottles, chewing gum bases, and lids for certain containers such as those for yogurt and cottage cheese. McNeal and Breder (1987) investigated the migration of 1,3-butadiene from food containers to the food. They reported that for the five different types of containers tested, 1,3-butadiene was detected in the contents of only one of the containers (olive oil, which contained about 8 to 9 nanograms of 1,3-butadiene per gram of oil). No butadiene was detected in chewing gum bases.

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V.

ATMOSPHERIC FATE AND PERSISTENCE OF 1,3-BUTADIENE

A. PERSISTENCE OF 1,3-BUTADIENE

The atmospheric persistence and fates of chemical compounds emitted into the troposphere are governed by a number of chemical and physical removal processes (Finlayson-Pitts and Pitts, 1986). For the great majority of organic compounds (including 1,3-butadiene) the most important removal processes are photolysis, reaction with hydroxyl (OH) and nitrate (NO_3) radicals, and reaction with ozone (O_3).

1. Chemical Removal Mechanisms

The atmospheric lifetime (τ) of a chemical is defined as the time to decay to a concentration of 1/e (=0.368) of that initially present. Also,

$$\frac{1}{\tau} = \frac{1}{\tau_{chemical}} + \frac{1}{\tau_{physical}}$$

where $\tau_{chemical}$ and $\tau_{physical}$ are the lifetimes of the chemical with respect to only chemical reaction and only physical loss processes, respectively. These chemical and physical lifetimes are composites of those for the individual loss processes, for example:

$$\frac{1}{\tau_{chemical}} = \frac{1}{\tau_{photolysis}} + \frac{1}{\tau_{OH}} + \frac{1}{\tau_{NO_3}} + \frac{1}{\tau_{O_3}} \ldots$$

where $\tau_{photolysis}$, τ_{OH}, τ_{NO_3} and τ_{O_3} are the lifetimes with respect to photolysis, reaction with the OH radical, reaction with the NO_3 radical, and reaction with O_3, respectively.

The reactive loss processes are determined by the rate constants, k_x, for reaction and the ambient atmospheric concentrations, [X], of the reactive intermediates. For example:

$$\frac{1}{\tau_{OH}} = k_{OH}[OH]$$

For photolysis, the photolysis rate depends on the absorption cross section (σ), the photolysis quantum yield (ϕ), and the radiation intensity (J), all of which are wavelength dependent:

$$\frac{1}{\tau_{photolysis}} = k_{photolysis} = \int_{\sim 290\ nm}^{\sim 800\ nm} \sigma_\lambda\ \phi_\lambda J_\lambda d_\lambda$$

a. Reaction with OH Radicals

The available kinetic data of Lloyd et al. (1976), Atkinson et al. (1977), Barnes et al. (1982), Ohta (1983), Atkinson and Aschmann (1984) and Liu et al. (1988) are given in Table V-1 and are plotted in Arrhenius form in Figure V-1 (both adapted from Atkinson, 1989). The room temperature rate constants from Lloyd et al. (1976), Atkinson et al. (1977), Barnes et al. (1982), Ohta (1983), Atkinson and Aschmann (1984) and Liu et al. (1988) are in very good agreement. Furthermore, the temperature dependencies obtained by Atkinson et al. (1977) and Liu et al. (1988) are in good agreement.

A unit-weighted least-squares analysis of the absolute rate constant data of Atkinson et al. (1977) and Liu et al. (1988) at temperatures less than 500 K yields the Arrhenius expression of:

$$k(1,3\text{-butadiene};\ T < 500\ K) = (1.44^{+0.54}_{-0.40}) \times 10^{-11}\ e^{(448 \pm 117)/T}\ cm^3\ molecule^{-1}\ s^{-1}$$

over the temperature 299-483 K, where the indicated errors are two least-squares standard deviations.

The relative rate data of Atkinson and Aschmann (1984) for a series of alkenes and dialkenes, when combined with the literature absolute rate constants (Atkinson, 1989), leads to the recommendation of:

$$k(1,3\text{-butadiene}) = 6.78 \times 10^{-11}\ cm^3\ molecule^{-1}\ s^{-1} \quad \text{at 295 K.}$$

Combined with the above temperature dependence, this yields the recommended Arrhenius expression of:

$$k(1,3\text{-butadiene};\ T < 500\ K) = (1.48^{+0.56}_{-0.42}) \times 10^{-11}\ e^{(448 \pm 117)/T}\ cm^3\ molecule^{-1}\ s^{-1}$$

TABLE V-1

Rate Constants k and Arrhenius Parameters ($k = Ae^{-B/T}$) for the Gas-Phase Reactions of the OH Radical with 1,3-Butadiene

$10^{12} \times A$ (cm^3 molecule^{-1} s^{-1})	B (K)	$10^{12} \times k$ (cm^3 molecule^{-1} s^{-1})	at T (K)	Teohnique	Reference	Temperature range covered (K)
		67.6 ± 13.6	305 ± 2	RR [relative to k(n-butane) = 2.62×10^{-12}][a]	Lloyd et al. (1976)	
14.5	-468 ± 151	68.5 ± 6.9 57.2 ± 5.7 43.3 ± 4.4	299.5 347.2 424.0	FP-RF	Atkinson et al. (1977)	299-424
		65.0	300	RR [relative to k(ethene) = 8.44×10^{-12}][a]	Barnes et al. (1982)	
		61.6 ± 1.5	297 ± 2	RR [relative to k(propene) = 2.65×10^{-11}][a]	Ohta (1983)	
		68.8 ± 2.2	297 ± 2	RR [relative to k(2-methyl-2-butene) = 8.72×10^{-11}][a]	Ohta (1983)	
		67.8 ± 2.2	295 ± 1	RR [relative to k(propene) = 2.68×10^{-11}][a]	Atkinson and Aschmann (1984)	

(continued)

TABLE V-1 (cont.)

$10^{12} \times A$ (cm^3 molecule^{-1} s^{-1})	B (K)	$10^{12} \times k$ (cm^3 molecule^{-1} s^{-1})	at T (K)	Technique	Reference	Temperature range covered (K)
		61 ± 6	313	PR-RA	Lin et al. (1988)	313-1203
		50 ± 5	333			
		51 ± 5	338			
		46 ± 5	373			
		41 ± 4	393			
		47 ± 5	408			
		42 ± 4	438			
		35 ± 4	483			
		29 ± 3	563			
14 ± 1	-440 ± 40 (313-623 K)	30 ± 3	623			
		30 ± 3	673			
		24 ± 3	723			
		20 ± 2	773			
		17 ± 2	873			
		15 ± 2	923			
		11 ± 1	1023			
		10 ± 1	1053			
		6.5 ± 0.6	1153			
		6.9 ± 0.7	1173			
		7.7 ± 0.8	1203			

[a]Rate constant recommendations taken from Atkinson (1988).

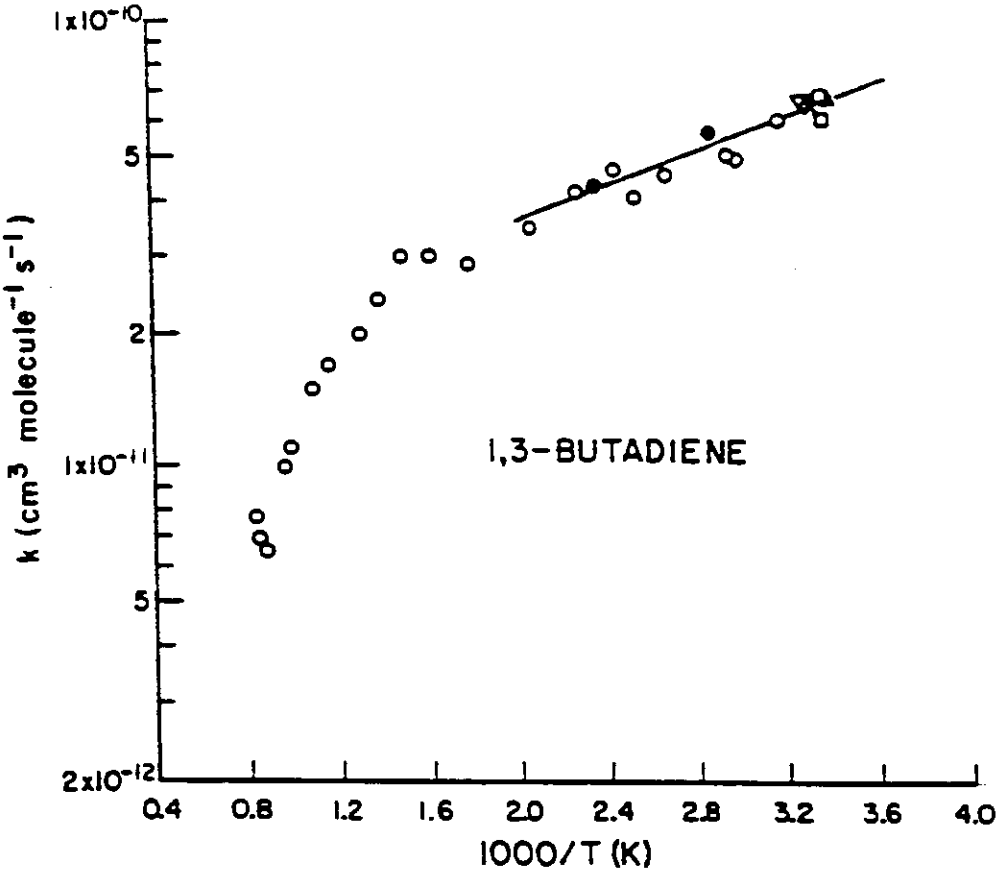


Figure V-1 Arrhenius plot of rate constants for the reaction of the OH radical with 1,3-butadiene. (▽) Lloyd et al. (1976); (●) Atkinson et al. (1977); (x) Barnes et al. (1982); (□) Ohta (1983); (△) Atkinson and Aschmann (1984); (○) Liu et al. (1988); (———) recommendation (see text).

over the temperature range 295-483 K, where the indicated errors are two least-squares standard deviations, and

$$k(1,3\text{-butadiene}) = 6.66 \times 10^{-11}\ \text{cm}^3\ \text{molecule}^{-1}\ \text{s}^{-1} \text{ at } 298\ \text{K},$$

with an estimated overall uncertainty at 298 K of ± 20 percent. Using the Arrhenius expression given above, the OH rate constant at 265 K is

$$8.03 \times 10^{-11}\ \text{cm}^3\ \text{molecule}^{-1}\ \text{s}^{-1}.$$

The relative rate constants of Lloyd et al. (1976), Barnes et al. (1982) and Ohta (1983) are in very good agreement with this recommendation. By analogy with the alkenes such as ethene, propene and the butenes, this rate

constant (for the OH radical addition pathway) will be very close to the high-pressure limit, at least up to around 500 K. The recommendation (Atkinson, 1989) provides a good fit to the rate constants measured by Liu et at. (1988) in one atmosphere of argon diluent up to around 700 K.

Using the rate constants cited above for the gas-phase reactions of OH radicals with 1,3-butadiene combined with an estimated ambient atmospheric concentration of 1.5×10^6 molecule/cm^3 during a 12-hour daytime period (Prinn et al., 1987), the calculated atmospheric lifetime of 1,3-butadiene with respect to reaction with OH radicals ranges from 2.3 to 2.8 hours.

b. Reaction with Nitrate Radicals

Kinetics data for the reaction of NO_3 radicals with 1,3-butadiene have been obtained by Canosa-Mas et al. (1988), Rahman et al. (1988) and Atkinson et al. (1988). These data are summarized in Table V-2. The recommended rate constant of:

$$k(1,3\text{-butadiene}) = (1.0 \pm 0.2) \times 10^{-13}\ cm^3\ molecule^{-1}\ s^{-1} \text{ at } 298\ K$$

for the reaction of NO_3 radicals with 1,3-butadiene is based upon the study of Atkinson et al. (1988).

Table V-2
Room Temperature Rate Constants for the Gas-Phase Reaction of the NO_3 Radical with 1,3-Butadiene

$k(cm^3\ molecule^{-1}\ s^{-1})$	Reference
$(2.2 \pm 0.6) \times 10^{-13}$	Canosa-Mas et al. (1988)
$(1.7 \pm 0.3) \times 10^{-13}$	Rahman et al. (1988)
$(9.79 \pm 1.17) \times 10^{-14}$	Atkinson et al. (1988)

Using a tropospheric concentration for NO_3 radicals during a 12-hour nighttime period of 2.4×10^8 molecule/cm^3 (Platt et al. 1984; Atkinson et al. 1986) and the reaction rate constant for NO_3 radicals listed above, the atmospheric lifetime of 1,3-butadiene with respect to gas-phase reactions with NO_3 radicals ranges from 9 to 13 hours.

c. Reaction with Ozone

Kinetic data for the gas-phase reaction of ozone with 1,3-butadiene have been obtained by Hanst et al. (1958), Japar et al. (1974), Becker et al. (1974), Toby and Toby (1975) and Bahta et al. (1984), and given in Table V-3.

Table V-3

Reported Rate Constants k and Arrhenius Parameters, $k = Ae^{-B/T}$, for the Reaction of O_3 with 1,3-Butadiene

A (cm^3 $molecule^{-1}$ s^{-1})	B (K)	$10^{18} \times k$ (cm^3 $molecule^{-1}$ s^{-1})	At T (K)	Technique	Reference	Temperature Range Covered (K)
		8.1	a	F/S-IR	Hanst et al. (1958)	
		8.4 ± 0.2	299 ± 2	S-CL	Japar et al. (1974)	
5.45×10^{-14}	2682 ± 101	6.7[b]	298	S-UV	Becker et al. (1974)	~286-358[b]
1.05×10^{-13}	2919 ± 403	7.5 ± 1.3[d]	303	S-UV	Toby and Toby (1975)	273-343
2.20×10^{-14}	2430 ± 55	6.3[b]	298	S-UV	Bahta et al. (1984)	254-299

[a]Room temperature.

[b]Calculated from the Arrhenius expression.

[c]Derived from graphical tabulations given.

[d]Value cited by Toby et al. (1976).

The data from all these studies are in reasonable agreement. Since the individual rate constants at the temperatures studied were not given by Becker et al. (1974) or Toby and Toby (1975), a least-squares analysis of the entire data set cannot be carried out.

A unit-weighted average of the room temperature rate constants of Hanst et al. (1958), Japar et al. (1974), Becker et al. (1974) and Bahta et al. (1984) [neglecting the rate constant study of Toby and Toby (1975) because of the magnitude of the error limits associated with it] leads to:

$$k(O_3 + \text{1,3-butadiene}) = 7.4 \times 10^{-18}\ cm^3\ molecule^{-1} s^{-1} \text{ at } 298\ K.$$

Combining this 298 K rate constant with a temperature dependence of B=2500 K derived from the temperature dependencies of Becker et al. (1974) and Bahta et al. (1984) leads to the recommendation of:

$$k(O_3 + \text{1,3-butadiene}) = 3.26 \times 10^{-14}\ e^{-2500/T}\ cm^3\ molecule^{-1}\ s^{-1}.$$

At 265 K (the average temperature of the troposphere), the rate constant is $2.6 \times 10^{-18}\ cm^3\ molecule^{-1}\ s^{-1}$.

Using a tropospheric concentration for O_3 during a complete 24-hour day of 7×10^{11} molecule/cm^3 (Logan, 1985) and the rate constants listed above, the calculated atmospheric lifetime of 1,3-butadiene due to reaction with O_3 ranges from 2.2 to 6.4 days.

d. Reactions with Other Chemical Species

None of the other reactive species in the troposphere are expected to react with 1,3-butadiene, at least not at rates which are significant relative to its rate of reaction with OH and NO_3 radicals or with O_3.

e. Photolysis

1,3-Butadiene will not undergo photolysis in the actinic region (Calvert and Pitts, 1966).

2. Physical Removal Mechanisms

No data concerning the physical removal processes for 1,3-butadiene are

available. However, neither wet nor dry deposition is expected to be important as a loss process compared with the chemical reactions with OH and NO_3 radicals and O_3.

3. Atmospheric Formation Processes

No atmospheric formation processes for 1,3-butadiene have been identified.

B. FATE OF 1,3-BUTADIENE

1. OH Radical Reaction

Under atmospheric conditions, the reaction of the OH radical with 1,3-butadiene proceeds essentially entirely (greater than 99 percent) by initial OH radical addition (Atkinson, 1988):

$$OH + CH_2{=}CHCH{=}CH_2 \longrightarrow HOCH_2\dot{C}HCH{=}CH_2 \text{ and } \dot{C}H_2\dot{C}HOHCH{=}CH_2$$

These initially formed radicals will rapidly (usually faster than 1 microsecond) add O_2 to yield the peroxy radicals

$$HOCH_2\underset{\underset{OO^{\bullet}}{|}}{C}HCH{=}CH_2 \text{ and } \dot{O}OCH_2CHOHCH{=}CH_2$$

In the presence of NO, these peroxy radicals are expected to yield NO_2 and the corresponding alkoxy radical,

$$HOCH_2\underset{\underset{OO^{\bullet}}{|}}{C}HCH{=}CH_2 + NO \longrightarrow HOCH_2\underset{\underset{O^{\bullet}}{|}}{C}HCH{=}CH_2 + NO_2$$

$$\dot{O}OCH_2CHOHCH{=}CH_2 + NO \longrightarrow \dot{O}CH_2CHOHCH{=}CH_2 + NO_2$$

although it is possible that a small amount (less-than/about 5 percent) of an organic nitrate may be formed. For example:

$$HOCH_2\underset{\underset{OO^{\bullet}}{|}}{C}HCH{=}CH_2 + NO \longrightarrow HOCH_2\underset{\underset{ONO_2}{|}}{C}HCH{=}CH_2$$

When the NO mixing ratios are less-than/about 30 parts per trillion, the above peroxy radicals will react with HO_2 to form ROOH (hydroperoxides) and/or other

peroxy radicals (to form, at least in part, the above alkoxy radicals) in competition with the reactions with NO given above.

The alkoxy radicals shown above are expected to decompose rapidly.

$$HOCH_2\overset{O^{\bullet}}{C}HCH=CH_2 \longrightarrow CH_2OH + CH_2=CHCHO$$

$$\downarrow O_2$$

$$HCHO + HO_2$$

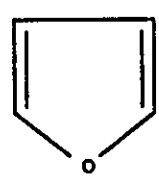
$$OCH_2CHOHCH=CH_2 \longrightarrow HCHO + CH_2=\overset{\bullet}{C}HCHOH$$

$$\downarrow O_2$$

$$CH_2=CHCHO + HO_2$$

Thus, in the presence of NO the major products are expected to be HCHO and CH_2=CHCHO, and this is observed.

Heuss and Glasson (1968) obtained approximately 30-40 percent yields of HCHO and CH_2=CHCHO (uncorrected for losses) in an irradiated NO-1,3-butadiene-air mixture. However, furan



has been observed as a minor product (approximately 4-6 percent) from the OH radical reaction with 1,3-butadiene, both in the presence and absence of NO (Ohta 1984, Atkinson et al. 1989).

2. <u>Nitrate Radical Reaction</u>

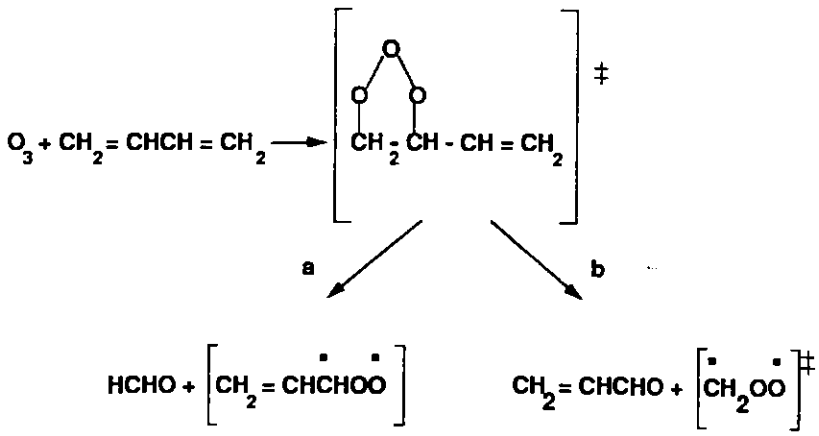
As for the OH radical reaction, the NO_3 radical reaction proceeds by initial addition

$$NO_3 + CH_2=CHCH=CH_2 \longrightarrow O_2NOCH_2\overset{\bullet}{C}HCH=CH_2 \text{ and } \overset{\bullet}{C}H_2\overset{ONO_2}{C}HCH=CH_2$$

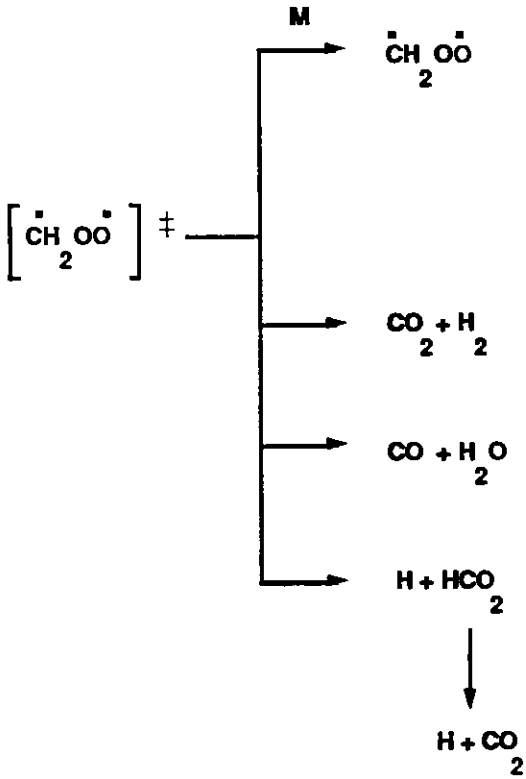
which will rapidly add O_2, and the resulting peroxy radicals are then expected to react to HO_2 radicals and/or other peroxy radicals. The reaction mechanisms and products formed from these reactions are as yet unknown.

3. Reaction with Ozone

As discussed by Atkinson and Carter (1984), the gas-phase reaction of O_3 with 1,3-butadiene involves initial addition to form an energy-rich "molozonide",



which is then expected to decompose rapidly, with the relative importance of pathways (a) and (b) not being known. The initially energy-rich $[\dot{C}H_2O\dot{O}]^{\ddagger}$ biradical can be collisionally thermalized or decomposed (Atkinson and Carter, 1984):



with the thermalized biradical $\dot{C}H_2\dot{O}O$ reacting predominantly with water vapor under atmospheric conditions:

$$\dot{C}H_2\dot{O}O + H_2O \longrightarrow HCOOH + H_2O \text{ (formic acid).}$$

The fate of the $\left[CH_2{=}C\dot{H}C\dot{H}OO\right]^{\ddagger}$ biradical is not presently known.

The available data suggest that the major products of the O_3 reaction with 1,3-butadiene will be formaldehyde (HCHO) and acrolein ($CH_2 = CHCHO$), together with products including formic acid (HCOOH), CO, CO_2, H_2, and HO_2 radicals, which arise from subsequent reactions of the "Criegee" biradicals.

1,3-Butadiene will be removed from the atmosphere both by gas-phase reaction with OH radicals during the day and with NO_3 radicals at night, with calculated atmospheric lifetimes of a few hours and half a day, respectively, for the two processes. It has a half-life of approximately 1.6 to 9 hours in the atmosphere. Thus, 1,3-butadiene will be confined to a local radius of impact largely within the airshed in which it is emitted.

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Appendix A

Methods for Estimating Usage and Emissions of 1,3-Butadiene in California

Appendix A

Methods for Estimating Usage and Emissions
of 1,3-Butadiene in California

A. ON-ROAD MOTOR VEHICLES

The following table summarizes the 1,3-butadiene emissions for individual classes of on-road motor vehicles for inventory year 1990. The estimates are based on the total organic gas (TOG) from on-road vehicular exhaust and the 1,3-butadiene emission factors developed by the ARB's Mobile Source Division (MSD). The emission factors include an adjustment for the chemical stability of 1,3-butadiene in the exhaust (ARB, 1991a). Approximately 2,600 tons per year of 1,3-butadiene are emitted by this source category.

Summary of 1,3-Butadiene Emissions from On-road Vehicular Exhaust for Inventory Year 1990

	Daily Vehicle Miles Traveled[a]	TOG[a]	1,3-Butadiene	
	(1,000 miles)	(tons/day)	TOG Wt %[b]	tons/year
Light Duty Passenger				
Non-Catalyst	26,127	165.23	0.97	585
Catalyst	425,545	387.67	0.59	835
Diesel	11,799	4.21	0.90	14
Light Duty Truck				
Non-Catalyst	6,745	40.88	0.97	145
Catalyst	114,681	131.88	0.59	284
Diesel	3,254	1.2	0.90	4
Medium Duty Truck				
Non-Catalyst	3,369	22.65	0.97	80
Catalyst	32,782	43.22	0.59	93
Diesel	--	--	0.90	--
Heavy Duty Truck				
Non-Catalyst	12,717	38.22	0.97	135
Catalyst	7,708	5.32	0.59	11
Diesel	26,788	77.21	1.36	383
Buses	794	3.17	1.36	16
Motorcycles	4,107	13.34	0.97	47
Total		934.20		2,632

(continued on next page)

Table Notes:

a. ARB, 1990. Data retrieved from EMFAC7E/BURDEN7C for inventory year 1990 (1987 baseline). Emission Inventory Branch, Technical Support Division, Sacramento, CA.

b. ARB, 1991a. Internal ARB memorandum from K. D. Drachand to Terry McGuire regarding butadiene emission factors, dated July 17, 1991. Mobile Source Division, El Monte, CA.

"For the light-duty passenger (LDP) non-catalyst class, emission factors were developed using test data from 16 pre-1974 non-catalyst vehicles. For the LDP catalyst class, a total of 62 vehicles were tested including 55 three-way catalyst and 7 oxidation catalyst vehicles. For the LDP diesel class, two LDP diesel vehicles were tested. Only one heavy-duty (HD) diesel prototype vehicle was tested. The emission factor developed from this HD diesel vehicle was used for both the HD diesel trucks and urban bus classes.

The emission factor for the LDP non-catalyst vehicle class is assumed to be applicable to non-catalyst light-duty (LD), medium-duty (MD), HD gasoline trucks, and motorcycles. Similarly, the emission factor for the LDP catalyst vehicle class is assumed to be applicable to catalyst-equipped LD, MD, and HD gasoline trucks. The emission factor for the LDP diesel vehicle class is assumed to be applicable to LD and MD diesel trucks."

B. OTHER MOBILE SOURCES

Other mobile sources include off-road vehicles, boats, trains, ships, aircraft, mobile equipment and utility equipment. Emissions of 1,3-butadiene from this category are based on total organic gas (TOG) emissions for each of three types of combustion fuels (ARB, 1991b), speciation profile (distillate oil), and emission rates (diesel and gasoline) of 1,3-butadiene. The emissions are separately estimated and then added together for the statewide estimate. The staff estimates statewide emissions from this category to be approximately 1,100 tons of 1,3-butadiene per year.

1. Distillate Oil Combustion

The Air Resources Board's Speciation Manual (ARB, 1989b), contains species profiles by processes. The Speciation Manual lists 1,3-butadiene as a compound contained in Profile #9: Industrial Internal Combustion Engine-Distillate Oil. The weight fraction of 1,3-butadiene is listed as .0590 in this profile. Because there are no profiles for distillate oil combustion from other mobile sources available, the staff applied profile #9 to other mobile sources.

There are an estimated 779.36 tons per year of total organic gas (TOG) emissions statewide from distillate oil combustion of other mobile sources (ARB, 1989a). Emissions from residual oil combustion are also included in this statewide estimate. The staff estimated 1,3-butadiene emissions statewide from distillate oil combustion of other mobile sources as follows:

$$EMS_{Dis} = TOG_{Dis} \times W_{Dis}$$

Where:

EMS_{Dis}: Statewide 1,3-butadiene emissions from distillate oil combustion of other mobile sources (tons per year).

TOG_{Dis}: Statewide emissions of Total Organic Gas from distillate oil combustion of other mobile sources (tons per year).

W_{Dis}: Weight fraction of 1,3-butadiene to Total Organic Gas from distillate oil combustion of other mobile sources.

Substituting the values of TOG_{Dis} and W_{Dis}, the equation becomes:

$$EMS_{Dis} = \frac{779.36 \text{ Tons TOG}}{\text{Year}} \times \frac{.0590 \text{ Tons of 1,3-butadiene}}{\text{Ton of TOG}}$$

$$EMS_{Dis} = \frac{45.98 \text{ Tons of 1,3-butadiene}}{\text{Year}}$$

2. <u>Gasoline Combustion</u>

There is an estimated 83,580 tons per year of total organic gas (TOG) emissions statewide from gasoline combustion of other mobile sources (ARB, 1991b). Because there is no species profile for this source, the staff applied an emission rate for non-catalyst, gasoline fueled, on-road motor vehicles to gasoline combustion sources. A 1,3-butadiene emission factor of 0.97 weight percent of total organic gas (TOG) (or 9.7 tons of 1,3-butadiene per thousand tons of TOG) for non-catalyst, gasoline-fueled vehicles was developed by the MSD (ARB, 1991a). The staff estimates 1,3-butadiene emissions statewide from gasoline combustion of other mobile sources as follows:

$$EMS_{Gas} = TOG_{Gas} \times ER_{Gas}$$

Where:

EMS_{Gas}: Statewide 1,3-butadiene emissions from gasoline combustion of other mobile sources (tons per year).

TOG_{Gas}: Statewide estimate of Total Organic Gas from gasoline combustion of other mobile sources (tons per year).

ER_{Gas}: Emission rate of 1,3-butadiene relative to TOG (expressed as 9.7 tons of 1,3-butadiene per thousand tons of TOG).

Substituting the values of TOG_{Gas} and ER_{Gas} the equation becomes:

$$EMS_{Gas} = \frac{83{,}580 \text{ Tons of TOG}}{\text{Year}} \times \frac{9.7 \text{ Tons 1,3-butadiene}}{1000 \text{ Tons TOG}}$$

$$EMS_{Gas} = \frac{811 \text{ Tons 1,3-butadiene}}{\text{Year}}$$

3. <u>Diesel Combustion</u>

There are an estimated 32,081 tons per year of total organic gas (TOG) emitted statewide from diesel combustion of other mobile sources (ARB, 1991b). Because there is no speciation profile for this source, the staff applied an emission rate for diesel on-road motor vehicles to diesel combustion sources. An emission rate of 9.0 mg of butadiene per gram of TOG from diesel vehicular exhaust was estimated (ARB, 1991a). Using this emission rate, the staff estimates 1,3-butadiene emissions statewide from diesel combustion of other mobile sources as follows:

$$EMS_{Die} = TOG_{Die} \times ER_{Die}$$

Where:

EMS_{Die}: Statewide 1,3-butadiene emissions from diesel combustion of other mobile sources (tons per year).

TOG_{Die}: Statewide emissions of Total Organic Gas from diesel combustion of other mobile sources (tons per year).

ER_{Die}: Emission rate of 1,3-butadiene relative to TOG (expressed as 9.0 tons of 1,3-butadiene per thousand tons of TOG).

Substituting the value of TOG_{Die} and ER_{Die} the equation becomes:

$$EMS_{Die} = \frac{32{,}081 \text{ Tons TOG}}{\text{Year}} \times \frac{9.0 \text{ Tons 1,3-butadiene}}{1000 \text{ Tons TOG}}$$

$$EMS_{Die} = \frac{289 \text{ Tons 1,3-butadiene}}{\text{Year}}$$

4. <u>Total Emissions Statewide for Other Mobile Sources</u>

$$EMS_{Tot} = EMS_{Dis} + EMS_{Gas} + EMS_{Die}$$

Where:

EMS_{Tot}: Statewide 1,3-butadiene emissions from all other mobile sources (tons per year).

EMS_{Dis}: Statewide 1,3-butadiene emissions from distillate oil combustion of other mobile sources (tons per year).

EMS_{Gas}: Statewide 1,3-butadiene emissions from gasoline combustion of other mobile sources (tons per year).

EMS_{Die}: Statewide 1,3-butadiene emissions from diesel combustion of other mobile sources (tons per year).

Substituting the value of EMS_{Dis}, EMS_{Gas}, and EMS_{Die} the equation becomes:

$$EMS_{Tot} = \frac{45.98 \text{ Tons}}{\text{Year}} + \frac{811 \text{ Tons}}{\text{Year}} + \frac{289 \text{ Tons}}{\text{Year}}$$

$$EMS_{Tot} = \frac{1{,}146 \text{ Tons 1,3-butadiene}}{\text{Year}}$$ or approximately 1,100 tons per year.

C. FUEL COMBUSTION (STATIONARY POINT SOURCES)

Sources of fuel combustion include boilers, heaters, internal combustion engines, and turbines. Emissions of 1,3-butadiene from fuel combustion sources are based on total organic gas (TOG) emissions from each of three types of fuels (ARB, 1989d), speciation profile (distillate oil), and emission rates (diesel and gasoline) of 1,3-butadiene. The emissions are separately estimated and then added together for the statewide estimate. The staff estimates statewide emissions from fuel combustion sources to be approximately 35 tons of 1,3-butadiene per year.

1. Distillate Oil Combustion

The Air Resources Board's Speciation Manual (ARB, 1989b), contains species profiles by processes. The Speciation Manual lists 1,3-butadiene as a compound contained in Profile #9: Industrial Combustion Engine-Distillate Oil. The weight fraction of 1,3-butadiene is listed as .0590 in this profile. In the absence of a specific profile for boilers, heaters, and turbines using distillate oil, the staff applied profile #9 for internal combustion engines to all the distillate oil combustion sources.

There are an estimated 518.9 tons per year of total organic gas (TOG) emissions statewide from distillate oil combustion (ARB, 1989d). Emissions from residual oil combustion are also included in this statewide estimate. The staff estimated 1,3-butadiene emissions statewide from distillate oil combustion as follows:

$$EMS_{Dis} = TOG_{Dis} \times W_{Dis}$$

Where:

EMS_{Dis}: Statewide 1,3-butadiene emissions from distillate oil combustion sources (tons per year).

TOG_{Dis}: Statewide emissions of Total Organic Gas from distillate oil combustion sources (tons per year).

W_{Dis}: Weight fraction of 1,3-butadiene to Total Organic Gas from distillate oil combustion sources.

Substituting the values of TOG_{Dis} and W_{Dis}, the equation becomes:

$$EMS_{Dis} = \frac{518.9 \text{ Tons TOG}}{\text{Year}} \times \frac{.0590 \text{ Tons of 1,3-butadiene}}{\text{Ton of TOG}}$$

$$EMS_{Dis} = \frac{30.6 \text{ Tons of 1,3-butadiene}}{\text{Year}}$$

2. <u>Gasoline Combustion Sources</u>

There is an estimated 395.5 tons per year of total organic gas (TOG) emissions statewide from gasoline combustion sources (ARB, 1989d). Because there is no species profile for this source, the staff applied an emission rate for non-catalyst, gasoline-fueled, on-road motor vehicles to gasoline combustion sources. An emission rate of 9.7 mg of butadiene per gram of TOG from gasoline vehicular exhaust was estimated (ARB, 1991a). Using this emission rate, the staff estimates 1,3-butadiene emissions statewide from gasoline combustion sources as follows:

$$EMS_{Gas} = TOG_{Gas} \times ER_{Gas}$$

Where:

EMS_{Gas}: Statewide 1,3-butadiene emissions from gasoline combustion sources (tons per year).

TOG_{Gas}: Statewide estimate of Total Organic Gas for gasoline combustion sources (tons per year).

ER_{Gas}: Emission rate of 1,3-butadiene relative to TOG (expressed as 9.7 tons of 1,3-butadiene per thousand tons of TOG).

Substituting the values of TOG_{Gas} and ER_{Gas} the equation becomes:

$$EMS_{Gas} = \frac{395.5 \text{ Tons of TOG}}{\text{Year}} \times \frac{9.7 \text{ Tons 1,3-butadiene}}{1000 \text{ Tons TOG}}$$

$$EMS_{Gas} = \frac{3.84 \text{ Tons 1,3-butadiene}}{\text{Year}}$$

3. <u>Diesel Combustion Sources</u>

There are an estimated 23.9 tons per year of total organic gas (TOG) emissions statewide from diesel combustion sources (ARB, 1989d). Because there is no species profile for this source, the staff applied an emission rate for diesel on-road motor vehicles to diesel combustion sources. An emission rate of 9.0 mg of butadiene per gram of TOG from diesel vehicular exhaust was estimated (ARB, 1991a). Using this emission rate, the staff estimates 1,3-butadiene emissions statewide from diesel combustion sources as follows:

$$EMS_{Die} = TOG_{Die} \times ER_{Die}$$

Where:

EMS_{Die}: Statewide 1,3-butadiene emissions from diesel combustion sources (tons per year).

TOG_{Die}: Statewide Total Organic Gas emissions from diesel combustion sources (tons per year).

ER_{Die}: Emission rate of 1,3-butadiene relative to TOG (expressed as 9.0 tons of 1,3-butadiene per thousand tons of TOG).

Substituting the value of TOG_{Die} and ER_{Die} the equation becomes:

$$EMS_{Die} = \frac{23.9 \text{ Tons TOG}}{\text{Year}} \times \frac{9.0 \text{ Tons 1,3-butadiene}}{1000 \text{ Tons TOG}}$$

$$EMS_{Die} = \frac{.22 \text{ Tons 1,3-butadiene}}{\text{Year}}$$

4. <u>Total Emissions Statewide for Combustion Sources</u>

$$EMS_{Tot} = EMS_{Dis} + EMS_{Gas} + EMS_{Die}$$

Where:

EMS_{Tot}: Statewide 1,3-butadiene emissions from all combustion sources (tons per year).

EMS_{Dis}: Statewide 1,3-butadiene emissions from distillate oil combustion sources (tons per year).

EMS_{Gas}: Statewide 1,3-butadiene emissions from gasoline combustion sources (tons per year).

EMS_{Die}: Statewide 1,3-butadiene emissions from diesel combustion sources (tons per year).

Substituting the value of EMS_{Dis}, EMS_{GAs}, and EMS_{Die} the equation becomes:

$$EMS_{Tot} = \frac{30.6 \text{ Tons}}{\text{Year}} + \frac{3.84 \text{ Tons}}{\text{Year}} + \frac{.22 \text{ Tons}}{\text{Year}}$$

$$EMS_{Tot} = \frac{34.66 \text{ Ton 1,3-butadiene}}{\text{Year}}$$ or approximately 35 tons per year.

D. PETROLEUM REFINING

The Bay Area Air Quality Management District (BAAQMD) reports 6,200 lbs (3.1 tons) of 1,3-butadiene emissions from the five refineries in their jurisdiction (BAAQMD, 19B9a). These five refineries have a total design capacity of 807,900 barrels (bbl) of crude oil per stream day (Oil & Gas, 1989). Total design capacities for all 31 refineries in the state is 2,416,930 bbl per stream day.

Assuming the refineries in other parts of the state would emit 1,3-butadiene similar to the Bay Area facilities, the staff estimates 1,3-butadiene emissions statewide as follows:

$$EMS = EMS_{BA} \times R$$

Where:

EMS: Statewide 1,3-butadiene emissions from refineries (tons per year).

EMS_{BA}: 1,3-butadiene emissions from refineries in the Bay Area (tons per year).

R: Ratio of design capacities of statewide refineries to design capacities of Bay Area refineries.

Substituting the values of EMS_{BA} and R, the equation becomes:

$$EMS = \frac{3.1 \text{ tons of 1,3 Butadiene}}{\text{Year}} \times \frac{2{,}416{,}930 \text{ bbl crude oil/day}}{807{,}900 \text{ bbl crude oil/day}}$$

EMS = 9.27 or approximately 9 tons per year.

E. FUEL COMBUSTION (STATIONARY AREA SOURCE)

The area source of fuel combustion includes agricultural sources, oil and gas production, manufacturing and industrial, services and commerce, and residential area sources. Emissions of 1,3-butadiene from fuel combustion area sources are based on total organic gas (TOG) emissions for each of two types of combustion fuels (ARB, 1989a), speciation profile (distillate oil), and emission rate (diesel) of 1,3-butadiene. The emissions are separately estimated and then added together for the statewide estimate. The staff estimates statewide emissions from fuel combustion area sources to be approximately 110 tons of 1,3-butadiene per year.

1. Distillate Oil Combustion

The Air Resources Board's Speciation Manual (ARB, 1989b), contains species profiles by processes. The Speciation Manual lists 1,3-butadiene as a compound contained in Profile #9: Industrial Internal Combustion Engine-Distillate Oil. The weight fraction of 1,3-butadiene is listed as .0590 in this profile. In the absence of a specific profile for the sources listed above using distillate oil, the staff applied profile #9 for internal combustion engines to all the distillate oil combustion sources.

There is an estimated 1,473.90 tons per year of total organic gas (TOG) emissions statewide for distillate oil combustion area sources (ARB, 1989a). Emissions from residual oil combustion are also included in this statewide estimate. The staff estimated 1,3-butadiene emissions statewide for distillate oil combustion area sources as follows:

$$EMS_{Dis} = TOG_{Dis} \times W_{Dis}$$

Where:

EMS_{Dis}: Statewide 1,3-butadiene emissions from distillate oil combustion area sources (tons per year).

TOG_{Dis}: Statewide emissions of Total Organic Gas from distillate oil combustion area sources (tons per year).

W_{Dis}: Weight fraction of 1,3-butadiene to Total Organic Gas from distillate oil combustion area sources.

Substituting the values of TOG_{Dis} and W_{Dis}, the equation becomes:

$$EMS_{Dis} = \frac{1{,}473.90 \text{ Tons TOG}}{\text{Year}} \times \frac{.0590 \text{ Tons of 1,3-butadiene}}{\text{Ton of TOG}}$$

$$EMS_{Dis} = \frac{86.96 \text{ Tons of 1,3-butadiene}}{\text{Year}}$$

2. Diesel Combustion

There are an estimated 2,417.61 tons per year of total organic gas (TOG) area emissions statewide from diesel combustion (ARB, 1989a). Because there is no species profile for this source, the staff will apply an emission rate for diesel on-road motor vehicles to diesel combustion sources. An emission rate of 9.0 mg of butadiene per gram of TOG from diesel vehicular exhaust was estimated (ARB, 1991a). Using this emission rate, the staff estimates 1,3-butadiene area emissions statewide from diesel combustion as follows:

$$EMS_{Die} = TOG_{Die} \times ER_{Die}$$

Where:

EMS_{Die}: Statewide 1,3-butadiene emissions from diesel combustion area sources (tons per year).

TOG_{Die}: Statewide estimate of Total Organic Gas from diesel combustion area sources (tons per year).

ER_{Die}: Emission rate of 1,3-butadiene relative to TOG (expressed as 9.0 tons of 1,3-butadiene per thousand tons of TOG).

Substituting the value of TOG_{Die} and ER_{Die} the equation becomes:

$$EMS_{Die} = \frac{2{,}417.61 \text{ Tons TOG}}{\text{Year}} \times \frac{9.0 \text{ Tons 1,3-butadiene}}{1000 \text{ Tons TOG}}$$

$$EMS_{Die} = \frac{21.76 \text{ Tons 1,3-butadiene}}{\text{Year}}$$

3. Total Emissions Statewide for Fuel Combustion Area Sources

$$EMS_{Tot} = EMS_{Dis} + EMS_{Die}$$

Where:

EMS_{Tot}: Statewide 1,3-butadiene emissions from all area sources involving fuel combustion (tons per year).

EMS_{Dis}: Statewide 1,3-butadiene emissions from area sources involving distillate oil combustion (tons per year).

EMS_{Die}: Statewide 1,3-butadiene emissions from area sources involving diesel combustion (tons per year).

Substituting the value of EMS_{Dis} and EMS_{Die} the equation becomes:

$$EMS_{Tot} = \frac{86.96 \text{ Tons}}{\text{Year}} + \frac{21.76 \text{ Tons}}{\text{Year}}$$

$$EMS_{Tot} = \frac{108.72 \text{ Ton 1,3-butadiene}}{\text{Year}}$$ or approximately 110 tons per year.

References for Appendix A

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BAAQMD, 1989b. Telephone conversation between Steve Hill of BAAQMD and Frederick Medina of ARB (Technical Support Division). Regarding: 1,3-butadiene emissions from Difolatan production at Chevron Chemical in Richmond, CA. Date of conversation: 12/21/89. (December 21)

Oil & Gas (Oil & Gas Journal), 1989. "Annual Refining Survey." (March 20).

U.S. Environmental Protection Agency (U.S. EPA), 1989. "Revised Draft Final Report, Locating and Estimating Air Emissions From Sources of 1,3-Butadiene." Noncriteria Pollutant Programs Branch, Air Quality Management Division, U.S. Environmental Protection Agency, Research Triangle Park, North Carolina 2771 (August 10).

Appendix B

Standard Operating Procedure for the Determination of 1,3-Butadiene in Ambient Air

SUMMA Polished Canister Method (Current SOP) ..... B-1 to B-14

Tedlar Bag Method (Previous SOP) ..... B-15 to B-27

CALIFORNIA AIR RESOURCES BOARD
MONITORING AND LABORATORY DIVISION

S.O.P. No. MLD 013

STANDARD OPERATING PROCEDURE FOR THE DETERMINATION OF 1,3-BUTADIENE IN AMBIENT AIR

1. SCOPE

This document describes a procedure for the sampling and analysis of 1,3-butadiene at concentrations normally found in ambient air. This procedure was developed by NLB staff.

2. SUMMARY OF METHOD

Ambient air is continuously sampled into a stainless steel SUMMA polished canister and immediately sent to the NLB organics laboratory for analysis. A 200ml sample is drawn from the canister through a Carbotrap C preconcentration system to trap the hydrocarbons with subsequent thermal desorption to the gas chromatograph for analysis. The C_2 to C_4 saturated and unsaturated hydrocarbons are separated by a packed column and temperature programming. The components eluting from the column are detected by photoionization (PID) and flame ionization detectors (FID) and quantitated by comparisons to standards. The 1,3-butadiene is identified based on retention times and confirmation is accomplished by the use of a second detector, FID.

3. INTERFERENCES/LIMITATIONS

3.01 Compounds having similar GC retention times may interfere, causing misidentification or faulty quantitation.

3.02 Although preliminary studies have shown that 1,3-butadiene can be considered stable in stainless steel canisters, every effort must be made to analyze the sample as soon as possible. Extreme care should be taken to prevent contamination during sample collection, transportation and subsequent analysis.

3.03 Although the analytical system will separate and detect saturated and other unsaturated hydrocarbons, 1,3-butadiene is the only compound addressed by this procedure.

3.04 The FID is used as a confirmation detector for results greater than 10X the PID detection limit. No results are reported from this detector. It should be monitored for possible coeluting compounds and ambiguous results from the PID.

3.05 Not more than 10 samples should be run consecutively without recalibration due to PID lamp window hazing causing decreased sensitivity. The lamp should be cleaned when the signal decreases substantially.

3.06 Butadiene standards are unstable and have been found to degrade. NIST standards should be ordered frequently to monitor older standards. (Butadiene stability is estimated at approximately six months) A comparison of butadiene response to another stable compound in the calibration cylinder should be made. A change in the butadiene/isoprene ratio in the cylinder will indicate degradation of butadiene in the calibration standard.

3.07 When running dual method using the DS 654 data system the WI should be the same for the two methods or it will cause the data system to lockup, and loss of data will occur.

3.08 Increased background noise could be caused by a buildup of high boiling compounds in the detector. To reduce this noise the detector temperature can be increased to 280°C followed by lamp cleaning or disassembly of the PID chamber and cleaning with an erasure and methanol.

3.09 Daily baseline shifting may be caused from high boiling compounds not eluting from the column due to the low column limit. The column should be baked out after each set of analytical runs to remove the higher boiling compounds from the column.

3.10 Vinyl chloride is monitored by this method but not reported due to the low breakthrough volume and low ambient concentration.

3.11 Isoprene is monitored by this method but not reported due to poor baseline and interfering peaks in the later portion of the chromatogram.

4. <u>APPARATUS</u>

4.01 Varian Model 6000 Gas Chromatograph equipped with a 10.2 ev PID and FID, a 10' x 2 mm i.d. glass column packed with 0.19 percent picric acid on 80/100 Carbopack C, and a Vista 654 Data Station.

4.02 Varian Vista 654 data system used for GC and autosampler control.

4.03 Nelson 2600 data station. IBM PS-2 model 30 based. Used for data collection and integration.

4.04 A preconcentration system equipped with a Carbotrap and automated sampling valve.

4.05 Porter mass flow controller 50 or 100cc/min calibrated in the 50 cc/minute range.

4.06 House vacuum system capable of drawing greater than 50cc/min.

4.07 16-port autosampling valve equipped with a low volume regulator.

4.08 3-way automated valve capable of being controlled externally and equipped with a particulate filter.

5. REAGENTS

NIST standard with 1,3-Butadiene and Isoprene. A control cylinder containing 1,3-Butadiene at a concentration determined using the NIST cylinder. The Isoprene will be used as an internal standard to monitor the degradation of 1,3-butadiene in the NIST standard.

6. PROCEDURE

6.01 Initial Set Up

6.01.01 Connect samples to the autosampler and purge the autosampler lines.

6.01.02 Set up appropriate number of samples in the Varian 654 data system to sequence through the samples and methods. (See Appendix II.)

6.01.03 Set up Nelson 2600 data system to receive data for integration. (See Appendix III.)

6.02 Sample Preconcentration

6.02.01 The Carbotrap preconcentration system is shown in Figure I.

6.02.02 Standards and ambient samples are introduced to the GC from the automated preconcentration system. The sample is selected by the autosampler and passes through the pressure regulator set at zero (with flow).

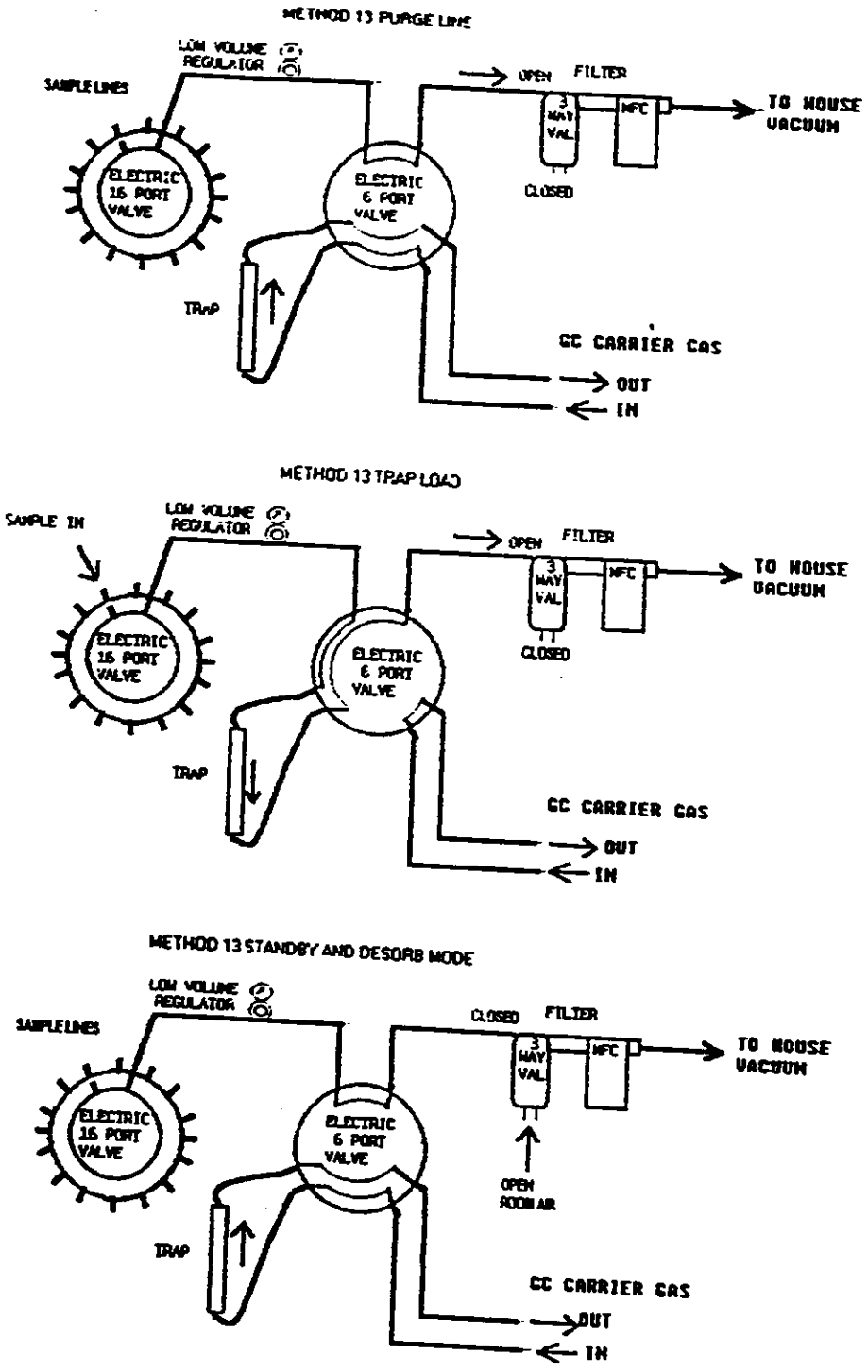
6.02.03 A three way valve is automatically opened 1 min before loading the trap to purge the lines and regulator with the current sample. The flow is controlled by a mass flow controller connected to house vacuum.

6.02.04 The sampling valve is then cycled to divert the flow of sample through the trap. After 200 cc (4 min) of sample has been trapped the valve cycles back to divert the column flow through the trap. The carrier gas travels in a direction opposite of the sample flow.

6.02.05 The desorption of the trapped 1,3-butadiene and other sample components is automatically initiated for a 4 min period at 180 C. The compounds are transferred to the head of the GC column for subsequent analysis.

Figure 1

PRECONCENTRATION SYSTEM



6.02.06 The 16-port valve is advanced at the end of the sampling period to the next sample. The three way valve is closed to reduce loss of the next sample.

6.03 Analysis

6.03.01 The preconcentrated sample is separated under the chromatographic conditions listed below. The resulting chromatogram (Figure 2) is then integrated and quantitated by reference to calibration standard gases.

6.03.02 Instrument Conditions:

GC: Column: 10' x 2 mm i.d. glass column, packed with 80/100 Carbopack C/0.19 percent picric acid

Temperatures: Injector: 150°C
Detector: 250°C
Oven: 40°C, hold for 9 min.,
3°C /min ramp to 70°C, hold for 0 min
20°C/min ramp to 115°C, hold for 13.75 min.

Flow Rate: Carrier: N_2, 30 cc/min.
H_2, 30 cc/min.
Air, 240 cc/min

Detector: PID: Range X1, 10.2 ev lamp

FID: Range X 10^{-12}

Concentrator: Carbotrap: Sampling: 4 min. at 50 cc/min. (25°C)
Desorb: 4 min. at 30 cc/min. (180°C)

Time Relays:

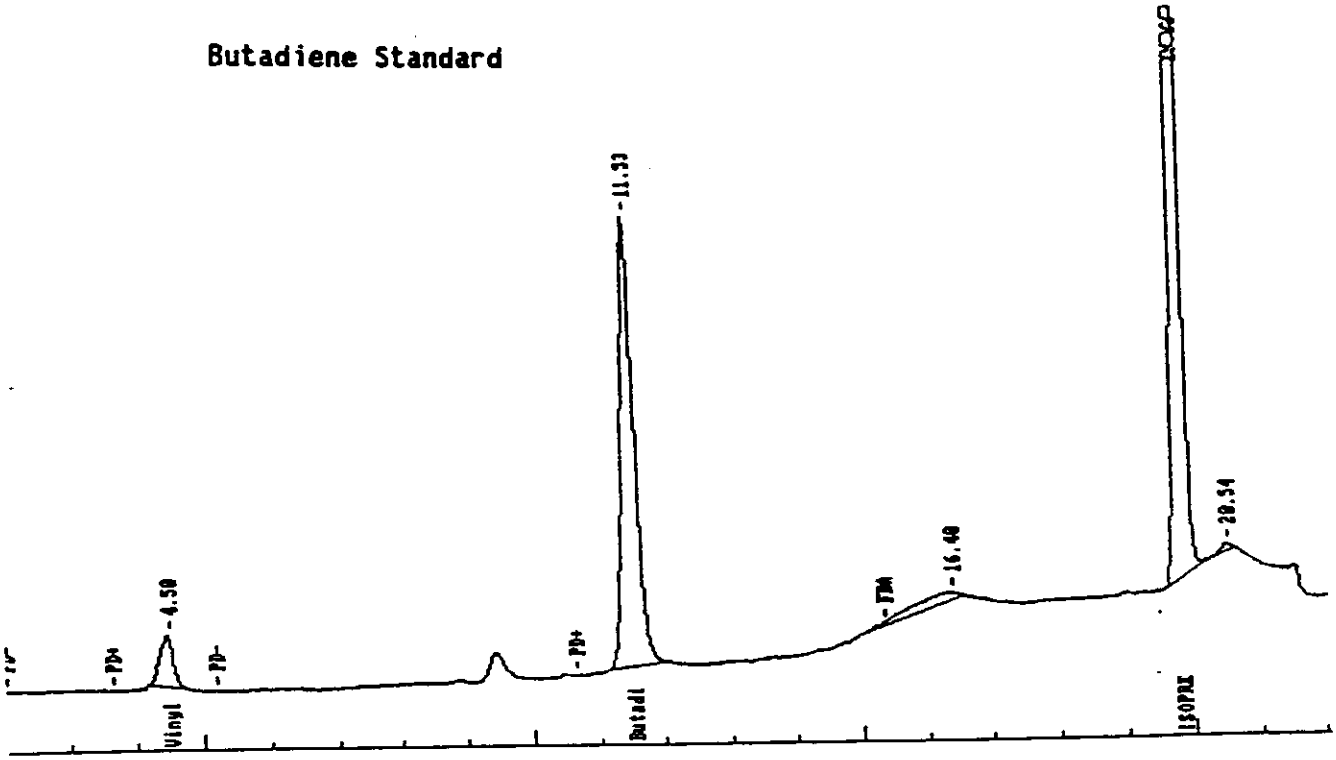
Line#	Time(min)	On	Off
1	0.00		23458
2	0.01	5	
3	1.00	2	
4	5.00	348	25
5	5.08		38
6	9.00		4

Time Relay Functions

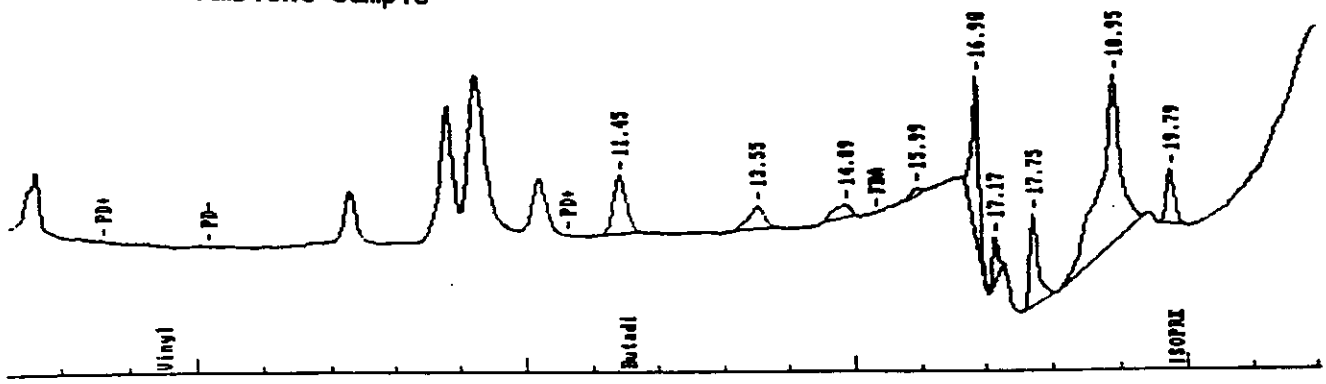
relay 2 advances valve to load trap
relay 3 (a) returns valve for desorption of trapped compounds
(b) advances autosampler to next sample
(c) sends start signal to 2600 data system
relay 4 controls trap thermal desorption
relay 5 controls three way solenoid valve for line purging and sampling
relay 8 alarm

Figure 2

Butadiene Standard



Ambient Sample



6.03.03 Prior to instrument calibration, a zero air must be analyzed to detect instrument or sampling contamination. The zero air analysis must be free of interfering peaks. System blanks must also be run after any sample which contains high concentrations (>4 ppb) of 1,3-butadiene to detect possible carry-over.

6.03.04 System calibration is performed daily by passing 200cc of a primary or secondary NIST traceable standard of a concentration not greater than 2ppb. Attention must be given to trends in response factor values which may indicate instrument, standard or detector sensitivity changes.

6.03.05 A control cylinder is then analysed to assess the calibration and first sample analysis. The results of the control cylinder must fall within 3 standard deviations of the assigned value determined from the standard.

6.03.06 Ambient samples are introduced in the same manner as the calibration and control standards. The resultant peaks are identified by retention times and quantified relative to the calibration standard.

6.03.07 Multipoint calibrations are conducted every three months. Each multipoint calibration consists of a system blank and at least three different concentrations of the standard performed in triplicate. If data indicate that the analyses are consistently unacceptable, more frequent multipoint calibrations may be required.

7. PERFORMANCE

7.01 Duplicate analyses are performed on 10 percent of all ambient samples received from the field. The relative percent difference of the duplicate analyses are recorded and included in the monthly Quality Control report. Only those data for concentrations in excess of the quantitation limits (0.04 ppb for 1,3-butadiene) are reported.

7.02 The results of the daily control standard analyses are plotted on the Method Control Chart. Warning and control limits are established from historical data to track analytical trends. The warning limit is two times the method standard deviation and the control limit is three times the method standard deviation.

7.03 If the control results continuously fall outside the warning limit the instrumentation should be checked thoroughly for any instrumentation problems.

7.04 If the results from the control cylinder are out of the control limits no data shall be reported for that run. The standard and control cylinder should be reanalysed prior to ambient sample analysis.

7.05 The FID which is connected second in series to the PID will be used as a confirmation check for butadiene.

8. METHOD SENSITIVITY, PRECISION AND ACCURACY

8.01 Linearity is determined by multipoint analysis. The detection limit and correlation are derived from this analysis and listed in table 1.

Table 1.

Vinyl Chloride
R squared = 0.6326 (Breakthrough volume)
LOD = Not calculated
Amount = 1.4004E-03 * Area + -2.994

Butadiene
R squared = 1.0000
LOD = 0.044 ppb
Amount = 3.0656E-05 * Area + 4.0699E-02

Isoprene
R squared = 0.9998
LOD = 0.15 ppb
Amount = 3.2687E-05 * Area + 1.0606E-01

The method used to calculate the limit of detection is
LOD = Intercept + 3(RSD of lowest std.)*Intercept

8.02 The breakthrough volume for vinyl chloride is less than 200 cc. For butadiene and isoprene the breakthrough volume is greater than 400 cc.

APPENDIX I

BUTADIENE STANDARD
NIST 9005

Cylinder NIST 9005 is the current cylinder being used for the calibration of method 013. This is the latest cylinder available for butadiene. This cylinder has not been certified for butadiene due to the known instability, however this represents the best source available for instrument calibration.

COMPOUND	CONCENTRATION
Vinyl Chloride	2.0 +/- 0.1 ppb
Butadiene	1.2 ppb (not certified)
Isoprene	2.1 +/- 0.2 ppb

APPENDIX II
VARIAN 654 DATA SYSTEM

The Varian 654 data system is currently being used as method sequencer, sample sequencer and backup data system. It is equipped with the interactive graphics integration software. The 654 is used because of the lack of control of the instrumentation in the Nelson 2600 software.

AUTOMATION

The Varian 654 controls the automation system. This includes the GC parameters, the automation events, and the different methods used for the butadiene analysis.

In the automodify (AM) mode yes is selected for self start. The current methods used are Bakeout, Buta, and Autoend.

Bakeout is a ten minute run that bakes out the trap for five minutes and the GC for ten minutes. This is used prior to the analysis each day.

Buta is a dual method that synchronizes the PID and FID methods. M1A is the current PID primary method. M1B is the FID secondary method. In method M1A be sure in section 9 that the sample list contains the correct number of samples for the day. Be sure method M1B has the correct number of samples or more. The automation will only run as many times as the least number of samples in the method lists.

Autoend is the last method in the method list. This is a bakeout method that begins after all of the samples have been run.

To initiate an automated run with the 654 use the autostart (AS) command. The sequence line determines which method is selected, 1 is bakeout, 2 is buta, and 3 is autoend. Method sample list line is which sample in the sample list is selected within the current method. Inject should usually be 1 when started, this is related to where the method is started. For more information on the data system use the DS-650 Series Data System Operator's Reference Manual.

INTERACTIVE GRAPHICS

Interactive graphics can be used to backup the Nelson system in case the Nelson system is inoperable for any reason. A brief description of some of the vague topics in the manual will be covered below.

To use the interactive graphics (IG), first a file known as a graphics data set must be saved from a raw data file. This can be done in two ways. In section 7 of the method save file must be chosen, GDS type. Now a GDS file can be saved during a normal run or by performing a recalculation of a raw data file (RF) and selecting yes for the save file per method option. Either of these methods may be used to generate a GDS file.

To select items in the interactive graphics use the arrow keys and the spacebar.

For more information on the interactive graphics use the DS-650 Series Data System Software Options Manual.

MULTIPOINTS

Multipoint analysis can be performed more easily using the Varian 654 to sequence through methods that change the sampling time. The methods currently used are MULTI 50, MULTI 100, MULTI 200, MULTI 300, AND MULTI 400. The methods reduce the sampling time by opening the sampling valve at a later time and holding the thermal desorption at a constant time to maintain the same retention time. Connect the standard that will be used for the multipoint analysis to the displayed port. Change the AUTO ADVANCE switch on the autosampler to the OFF position to hold the autosampler in one position. Perform auto modify (AM) and put the multi methods (listed above) in the desired sequence. Set up the Nelson 2600 to receive the data for integration and calculation. Initiate an autostart (AS) to begin the multipoint analysis.

REFERENCES:

DS-650 Series Data System Operators Manual, Volume 1, Varian Associates, Inc., 1988, Publication Number 03-914182-00

DS-650 Series Data System Software Options Manual, Volume 2, Varian Associates, Inc., 1988, Publication Number 03-914183-00

APPENDIX III
NELSON 2600 DATA SYSTEM

NELSON DATA SYSTEM: The Nelson 2600 is a software based menu driven system. The Nelson set-up information is presented below:

Method BUTA and CUTA
Sequence File Name BUTA
Interface Number 1
The file naming procedure for the Nelson 2600 system is presented below.

BASE NAME

The users of the Nelson 2600 system are given 5 characters to form the base name. Data is stored using this base name. The cycle of injection number is appended to the selected base name and the data files are stored on the specified drive. The same base name is used for storing raw, area and header files, but the three character extension differentiates one file from another. If a duplicate file name exists the system will rename the current sample. This can and will cause problems with LIMS transfer procedures. To prevent this and to allow easy identification of data, the following convention will be used for naming Nelson files.

1. Use only a 5 character base name for data runs.
2. The first two characters will be a month code.
3. The third and fourth characters will be a date code.
4. The fifth character will be an instrument code.
5. The sixth through eighth character is automatically assigned by the Nelson system and represents the sample number for the base name.

MONTH CODES

JAN = JA	JUL = JU
FEB = FB	AUG = AU
MAR = MR	SEP = SE
APR = AP	OCT = OC
MAY = MA	NOV = NO
JUN = JN	DEC = DE

INSTRUMENT CODES

METHOD 002 = A
METHOD 002A = B
METHOD 012 = C
METHOD 013 = D

EXAMPLE: MA08A WOULD BE MAY 8TH ON METHOD 002A

SAMPLE NAME FORMAT:

Within a sequence or during a manual download you must enter a sample name. The format for this is as follows.

Generally a system blank or sample of zero air is run as the first sample of the day to insure that the system is free of contamination.

Enter a zero air sample as: Zero Air.

Enter a system blank as: System Blank .

After the zero air, a calibration standard is run. If you wish to automatically update the calibration factors put a ^ symbol in the Nelson sample list at the end of the sample name. For example:

NIST 9005^1AV (for a level 1 average of calibration factors)

or

NIST 9005^1RE (for a level 1 replacement of calibration factors)

Samples that have been logged into LIMS and that will be transferred will use the first 6 characters for the LIMS number. The LIMS number itself will be limited to 5 characters and the 6th character is used as a replicate code. If this is left blank it represents the first replicate. A-J represents the 2nd through 10th replicates. The first character must be 0, 1 or 2 for LIMS to recognize this as a LIMS number. If the LIMS number is 90003458 use 03458 as the Nelson code. After the first 6 characters, enter in a space and then type in the sample location or other information. For example the first replicate of a Modesto sample having a LIMS number of 02345 would be entered as:

02345A Modesto

If the sixth character is not used insert a space after the LIMS number.

The BUTA method is used to call both PID and FID methods. Normally only one GC will be running in the autosample mode. The Nelson system will then sequence the samples and append a number to the root (default file name)

MULTIPOINTS

The combination of the autosampler and the Nelson 2600 data system reduces the task of multipoints from a time consuming ordeal to a easy analysis and calculation. Use method BMPTS in the 2600and connect multipoint standard and select auto advance on the autosampler to the off position. Use the multipoint methods in the varian 654 to control the valve switching. Enter the standards in the 2600 as different level standards.(ex. Nist9005^1AV for the first level; Nist9005^2AV for the second level; Nist9005^3AV for the third level; etc.) Perform a calibration factor update and a plot calibration curve from the 2600 main menu. Calculate LOD from this data.

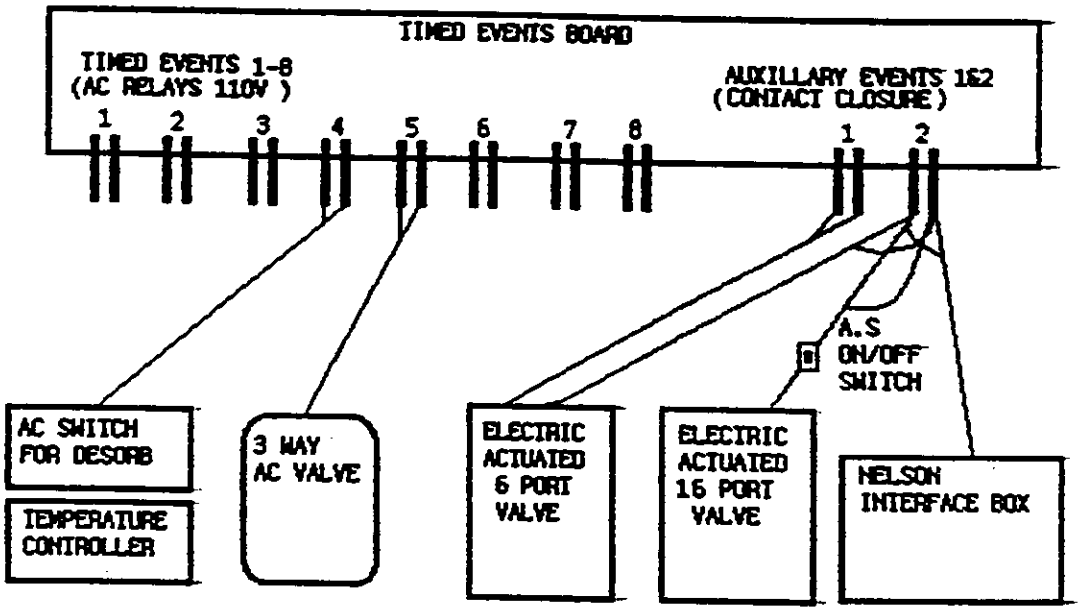
REFERENCES:

S.O.P No. MLD 002, Revision 4.0, 03/01/90

PE NELSON 3000 Series Chromatography Data System Users Manual, 1987, Part Number 68-2600-00

APPENDIX IV
TIMED EVENTS BOARD DIAGRAM

This diagram shows connection of the relays and auxiliary events to the automated devices used for Method 13.



NOTE: AUXILLARY 1 IS ACTUATED WITH EVENT 2
AUXILLARY 2 IS ACTUATED WITH EVENT 3

State of California
Air Resources Board
Monitoring and Laboratory Division

S.O.P. No. NLB013

Standard Operating Procedure for the Determination of 1,3-Butadiene and Vinyl Chloride in Ambient Air

1.0 SCOPE

This document describes a procedure for the sampling and analysis of 1,3-butadiene and vinyl chloride at concentrations normally found in ambient air.

2.0 SUMMARY OF METHOD

After being continuously sampled into a Tedlar bag over a period of 24 hours, ambient air is immediately sent to the laboratory for analysis. A subsample (300 cc) from the bag is passed through a Carbotrap preconcentration system to trap the hydrocarbons with subsequent thermal desorption to the gas chromatograph for analysis. The C_2 to C_4 saturated and unsaturated hydrocarbons are separated by a packed column and temperature programming. The components eluting from the column are detected by photoionization (PID) and flame ionization detectors (FID) and quantitated by comparisons to standards. The 1,3-butadiene and vinyl chloride are identified based on retention times and confirmation is accomplished by the use of a second detector, FID.

3.0 INTERFERENCES/LIMITATIONS

a. Compounds having similar GC retention times may interfere, causing misidentification or faulty quantitation.

b. Although preliminary studies have shown that 1,3-butadiene can be considered stable in Tedlar bags if protected from sunlight, every effort must be made to analyze the sample as soon as possible. Extreme care should be taken to prevent contamination during sample collection, transportation and subsequent analysis. Stability of vinyl chloride in tedlar bags has not been studied in the lab as yet.

c. Although the analytical system will separate and detect saturated and other unsaturated hydrocarbons, 1,3-butadiene and vinyl chloride are the only compounds addressed by this procedure.

d. The FID detects almost all compounds,and may not give accurate values for confirmation results. For now, only those values which are at least 10X the PID LOD and <54% relative error (RE) as obtained from Table 1 will be reported as confirmation values. Other columns and/or GC/MS methods will be investigated in the future.

4.0 APPARATUS

a. Varian Model 6000 Gas Chromatograph equipped with a 10.2 ev PID and FID, a 10' x 2 mm i.d. glass column packed with 0.19 percent picric acid on 80/100 Carbopack C, and a Vista 604 Data Station.

b. A preconcentration system equipped with a Carbotrap and sampling valves.

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

d. Laboratory timer, accurate to 0.05 minute.

e. Gas-tight microliter syringes; 5, 10, and 25 ul sizes.

f. Vacuum pump, GAST, with by-pass valving.

5.0 REAGENTS

a. Scott-Marin, Inc. specialty gas tank, 5.21 ppb and 0.94 ppb 1,3-butadiene in high purity nitrogen used as a working standard and a control standard, respectively.

b. Scott-Marin, Inc. Specialty gas mixture containing 10.0 ppb vinyl chloride used as a working standard.

6.0 PROCEDURE

a. Sample Preconcentration

1) The Carbotrap preconcentration system is shown in Figure 1.

Table 1

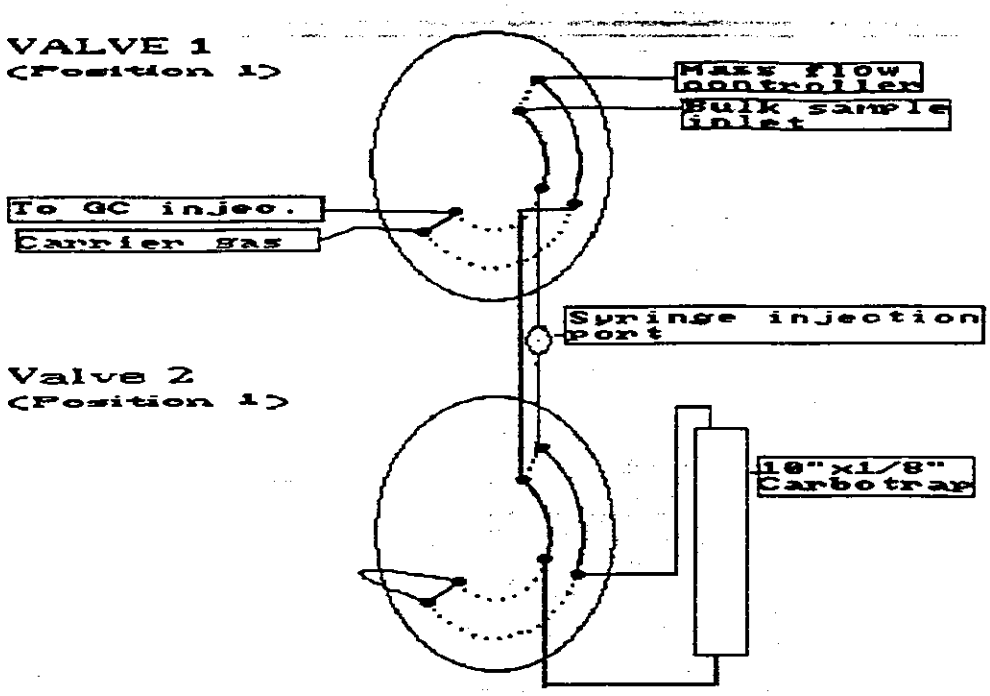
ANALYSIS VALUES OF CYLINDER AAL1728 (0.94 ppb 1,3-BUTADIENE USING PID AND FID

ANALYSIS DATE	PPB 1,3-BUTADIENE PID	FID
10/05/88	0.97	1.03
10/06/88	0.93	1.14
10/07/88	0.90	0.70
10/11/88	0.90	1.18
10/12/88	0.95	0.87
10/13/88	1.0	0.49
10/14/88	0.95	0.61
10/18/88	0.95	0.92
10/27/88	0.82	0.73
10/28/88	0.87	0.88
11/02/88	0.90	1.04
11/03/88	1.02	1.05
11/07/88	0.99	0.80
11/15/88	0.99	0.62
11/17/88	0.73	1.00
11/18/88	0.75	1.29
11/22/88	0.70	1.05
11/23/88	0.94	0.91
11/30/88	0.84	0.60
12/01/88	0.94	0.88
12/06/88	1.05	0.92
12/07/88	0.88	1.16
12/08/88	1.01	0.88
AVERAGE:	0.91	0.90
STD. DEV.:	0.093	0.21
% RSD:	10.2	23.0

AVERAGE BIAS BETWEEN DETECTORS = 1%

Figure 1

PRECONCENTRATION SYSTEM



OPERATIONAL VALVE POSITIONS

Valve 1 Position	Valve 2 Position	Sampling Mode
1	1	Bulk Sampling
2	1	Purge/Desorb
2	2	Trap Isolate

2) High concentration samples and standards are introduced into the preconcentration system through the in-line septum using a gas-tight microliter syringe. The injected sample is swept into the Carbotrap at 25 C with 300 cc of high purity nitrogen (50 cc/minute for six minutes).

3) Ambient samples are introduced from Tedlar bags using a vacuum pump and mass flow controller. The sample is drawn through the Carbotrap at 50 cc/minute for six minutes (300 cc total volume). The 1,3-butadiene and vinyl chloride are concentrated on the trap for later desorption. Similary, standards are introduced directly from the gas cylinders or canisters. Fifty cc of the 5.21 ppb working standard and 100cc of 0.94 ppb standard are used for calibration and control standards. respectively.

4) The carrier gas from the chromatograph is then diverted to the Carbotrap. The carrier gas flows in a direction opposite to the sample flow. The gas chromatograph/data system is started and the Carbotrap heated ballistically to 180 C. The collected hydrocarbons are thermally desorbed from the trap and are swept into the gas chromatographic column.

b. Analysis

1) The preconcentrated sample is separated under the chromatographic conditions listed below. The resulting chromatogram (Figure 2) is then integrated and quantitated by reference to calibration standard gases.

PID and FID chromatograms of vinyl chloride and 1,3-butadiene are shown in Figures 3A, 3B, 4A, and 4B.

2) Instrument Conditions:

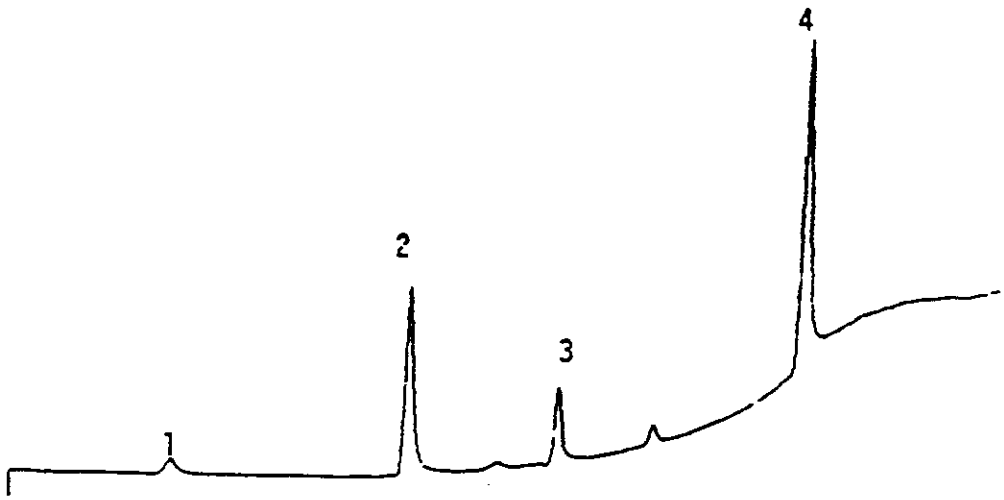
GC: Column: 10' x 2 mm i.d. glass column, packed with 80/100 Carbopack C/0.19 percent picric acid

Temperatures:
- Injector: 150 C
- Detector: 250 C
- Oven: 50 C, hold for 1 min., 4 C/min. ramp to 85 C, hold for 1 min.,10 C/min. ramp to 110 C, hold for 5 min.

Figure 2

CHROMATOGRAM OF AN OLEFIN STANDARD

DETECTOR: 10.2 ev PID
ATTENUATION: 32



LIST OF COMPONENTS

1.	1-Propene	10ppb
2.	1-Butene	10ppb
3.	1,3-Butadiene	2.5ppb
4.	1-Pentene	10ppb

Figure 3A

PID Chromatogram of Vinyl Chloride (0.83 ppb)

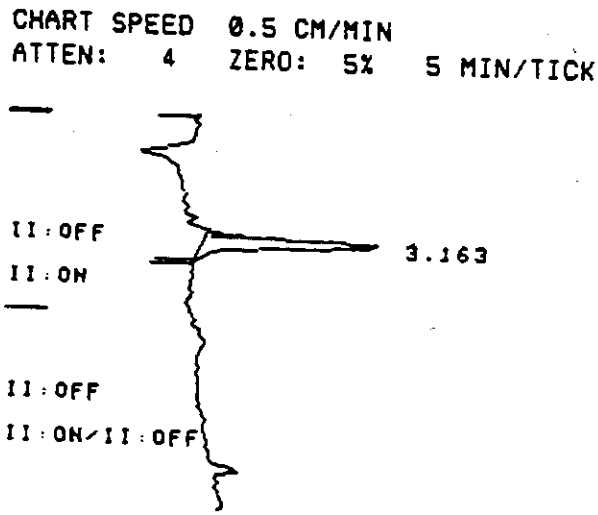


Figure 3B

FID Chromatogram of Vinyl Chloride (0.83 ppb)

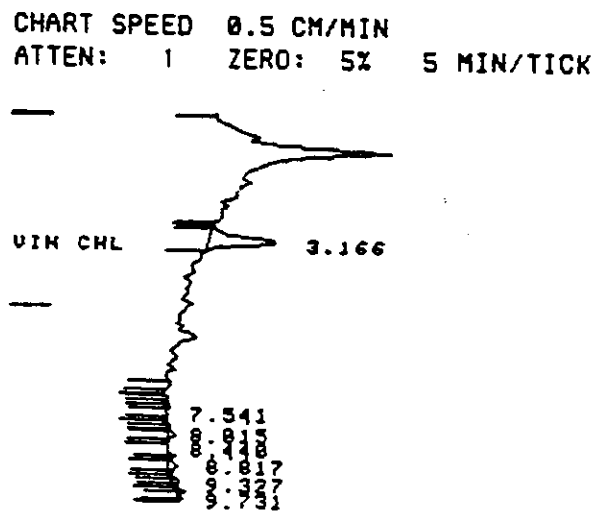


Figure 4A

PID Chromatogram of 1,3-Butadiene (0.31 ppb)

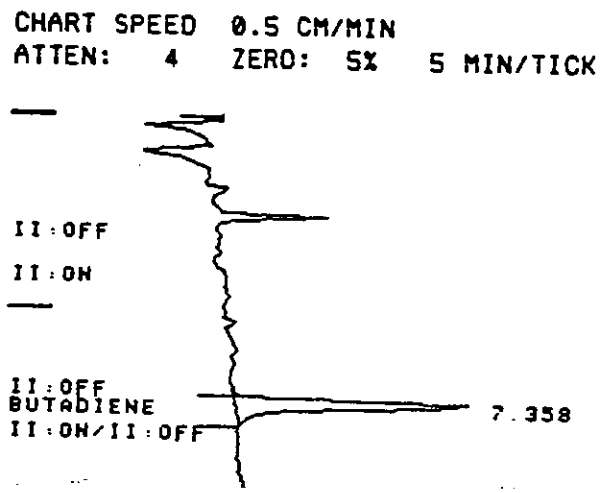
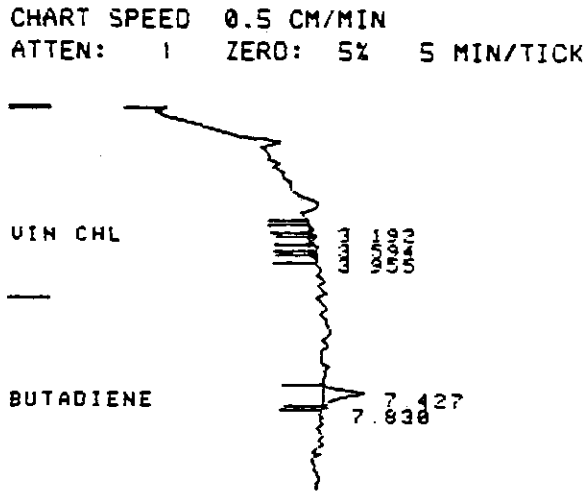


Figure 4B

FID Chromatogram of 1,3-Butadiene (0.87 ppb)



Flow Rate: Carrier: N_2, 20 cc/min.
H_2, 20 cc/min.
Air, 240 cc/min

Detector: PID: Range X1, 10.2 ev lamp

FID: Range X 10^{-12}

Concentrator: Carbotrap: Purge: 0.5 min. at 20 cc/min. (25 C)
Sampling: 6 min. at 50 cc/min. (25 C)
Desorb: 4 min. at 20 cc/min. (180 C)

3) Prior to instrument calibration, a system blank must be analyzed. This is done by trapping 300 cc of purge gas with subsequent desorption and analysis. The system blank analysis must be free of interfering peaks. System blanks must also be run after any sample which contains high concentrations (>4 ppb) of 1,3-butadiene and vinyl chloride to detect possible carry-over.

4) System calibration is performed daily by passing 50 cc (effective concentration of 0.87 ppb) of 5.21 ppb 1,3-butadiene standard through the trap assembly. For vinyl chloride calibration, 25 cc (effective concentration of 0.83 ppb) of 10 ppb standard is passed through the trap assembly. The trapped standards are then thermally desorbed into the chromatographic system for analysis. The calculated concentrations resulting from this analysis are compared to the "assigned" concentrations to insure consistency with previous calibrations. The stored data are then used to generate response factors used to calculate the concentrations of the analytes in subsequent samples. The instrument data system will not accept a response factor which differs from historic data by more than 15 percent. The response factor generated is tabulated in the instrument laboratory workbook. Attention must be given to trends in response factor values which may indicate instrument or detector sensitivity changes.

5) Multipoint calibrations are conducted every three months. Each multipoint calibration consists of a system blank and three standard concentration levels performed in triplicate. If data indicate that the analyses are consistently unacceptable, more frequent multipoint calibrations may be required.

6) Ambient samples are analyzed by drawing 300 cc (50 cc/minute, six minutes) through the Carbotrap preconcentrator, then desorbing the trapped hydrocarbons into the chromatograph as in the calibration step above. The resultant peaks are identified by retention times and quantitated relative to the calibration standards.

7.0 PERFORMANCE

a. Duplicate analyses are performed on 10 percent of all ambient samples received from the field. The relative percent difference of the duplicate analyses are recorded and included in the monthly Quality Control report. Only those data for concentrations in excess of the quantitation limits (0.40 ppb for 1,3-butadiene and 1.20 for vinyl chloride) are reported.

b. Cylinder AAL1728 (0.94 ppb 1,3-butadiene) is used as a control standard. The results of the daily control standard analyses are plotted on the Method Control Chart. If the result of any individual analysis falls outside the warning limits ($\pm 2\sigma$), the Control Standard shall be re-analyzed prior to the analysis of ambient samples. If subsequent data falls outside the method warning limits, or if an individual result falls outside the control limits ($\pm 3\sigma$), complete method validation must be performed. Method control chart for vinyl chloride shall be established as soon as a NBS traceable standard becomes available.

c. The FID which is connected in series to the PID will be used as a confirmation check for both compounds (see section 3.0 d).

8.0 METHOD SENSITIVITY, PRECISION AND ACCURACY

The method sensitivity, precision and accuracy are outlined in Tables 2, 3, and 4. Multipoint analyses of a NBS traceable standard (AAL1728,0.94 ppb 1,3-butadiene) using both the PID and FID are presented in Tables 2A and 2B. Multipoint analyses of cylinder 62359 for vinyl chloride (10.0 ppb) using the same detectors are presented in Tables 3A and 3B.

Analyses of NBS traceable butadiene standards (AAL17249 and AAL1728) were carried out using our working standard, CC62053 for calibration and Nitrogen carrier gas as the sample matrix. Results are shown in Table 4. Analysis values for both standards are within 8% of their assigned values.

EPA audit cylinders (Groups IV and V) were also analysed using the NBS traceable standard, AAL17249 for calibration. Results as shown in Table 4 are within 4% of their assigned values.

Accuracy of the method for vinyl chloride will be determined upon availability of a NBS traceable standard.

Table 2A

METHOD SENSITIVITY AND PRECISION
FOR 1,3-BUTADIENE USING PID (Primary Detector)
(AAL1728, 0.94 ppb)

Sample Vol. (cc)	Estimated Conc., ppb	Mean Area Counts, n=3	Standard Deviation	RSD (%)
25	0.078	3027	243	8.0
50	0.156	6908	390	5.6
100	0.313	16776	934	5.6

Y-intercept = 1878
X-intercept = -0.032
Slope = 59134

Correlation Coefficient = 0.99851
Limit of Detection (LOD, ppb) = 0.040

Table 2B.

METHOD SENSITIVITY AND PRECISION
FOR 1,3-BUTADIENE USING FID (Confirmation Detector)
(AAL1728, 0.94 PPB)

Sample Vol. (cc)	Estimated Conc., ppb	Mean Area Counts, n=3	Standard Deviation	RSD (%)
150	0.47	202	28.6	14.2
300	0.94	593	95.7	16.1
600	1.88	1533	84.9	5.5

Y-intercept = -268
X-intercept = 0.28
Slope = 952

Correlation Coefficient = 0.99905
Limit of Detection (ppb) = 0.40

Table 3A

METHOD SENSITIVITY AND PRECISION
FOR VINYL CHLORIDE USING PID (Primary Detector)
(CC62359, 10 PPB)

Sample Vol. (cc)	Estimated Conc., ppb	Mean Area Counts, n=3	Standard Deviation	RSD (%)
25	0.833	14280	1450	10.2
50	1.67	29205	3182	10.9
100	3.34	61238	4464	7.2

Y-Intercept = -1699
X-Intercept = 0.090
Slope = 18795

Correlation Coefficient = 0.99984
LOD, ppb = 0.12

Table 3B

METHOD SENSITIVITY AND PRECISION
FOR VINYL CHLORIDE USING FID (Confirmation Detector)
(CC62359, 10.0 ppb)

Sample Vol. (cc)	Estimated Conc., ppb	Mean Area Counts, n=3	Standard Deviation	RSD (%)
25	0.833	897	200	22
50	1.67	2130	144	6.8
100	3.34	4284	164	3.8

Y-Intercept = -177
X-Intercept = 0.13
Slope = 1342

Correlation Coefficient = 0.99943
LOD, ppb = 0.22

Table 4

ANALYSIS OF NBS TRACEABLE STANDARDS FOR 1,3-BUTADIENE
(Based Upon Laboratory Working Standards Shown)

ANALYSED CYLINDER	CALIBRATION CYLINDER	ASSIGNED CONC.,ppb	AVERAGE CONC. FOUND,ppb	RSD (%)	BIAS (%)
17249	62053	1.85 +/-10%	1.85	3.1 (n=7)	0
1728	62053	0.94 +/-10%	0.87	2.9 (n=6)	-7.4
8184 (Grp.V)	17249	10.1	10.0	10.6 (n=11)	-1.0
16577 (Grp.IV)	17249	12.6	13.0	7.7 (n=3)	+3.2

Appendix C

Standard Operating Procedure for the Determination of 1,3-Butadiene in Motor Vehicle Exhaust

April 1990

Air Resources Board

PROCEDURE FOR THE ANALYSIS OF 1,3-BUTADIENE IN AUTOMOTIVE EXHAUST

DRAFT S.O.P. No. MLD 114

Southern Laboratory Branch
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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 reflect the views and policies of the Air Resources Board, nor does mention of trade names or commercial products constitute endorsement or recommendation for use.

PROCEDURE FOR THE ANALYSIS OF 1,3-BUTADIENE IN AUTOMOTIVE EXHAUST

I. Introduction

1.1 This procedure describes a method of analyzing 1,3-butadiene in automotive exhaust in the range of 0.2 to 50 parts per billion concentrations by gas chromatography (GC) with photo-ionization detector (PID).

1.2 Lower concentrations may be analyzed by increasing the sample volume.

1.3 Higher concentrations may be analyzed by direct injection of a diluted sample or a smaller volume of sample through the cryogenic trap.

II. Method

2.1 A portion of the sample from a Tedlar bag is transferred by syringe to the heated volumetric sample loop or the cryogenic freeze-out trap of a gas chromatograph.

2.2 The sample is introduced into the chromatograph by means of a gas injection valve and analyzed by a photo-ionization detector.

2.3 The GC data system quantifies the 1,3-butadiene by integrating the peak area and calculating concentration from a factor determined during calibration with the 1,3-butadiene standard.

III. Apparatus

3.1 Tedlar bags, 2 mil thick, nominally of 0 to 100 liter capacity, and equipped with Quick-Connect fittings are used to contain the sample.

3.2 A gas chromatograph equipped with a gas injection valve,loop/freeze-trap inlet system, and a photo-ionization detector is used.

3.3 A stainless steel column (6' x 1/8 inch O.D.) packed with 0.19% picric acid on 80/100 mesh Carbopack-C coupled with a second column (6' x 1/8 inch O.D.) of N-octane on Porasil-C are used. These columns can be substituted by other columns which are capable of resolving 1,3-butadiene from other organic compounds with similar physical and retention properties.

3.4 An analog recorder and an electronic integrator are used to quantify peak areas.

3.5 Ground glass syringes (20, 50, 100, 250 ml) or other suitable devices are needed to transfer gas samples from Tedlar bags to the sample inlet of the gas chromatograph.

IV. Gases

4.1 All gases used to support the GC analysis shall be of the highest commercial purity available.

4.2 Helium shall have a minimum purity of 99.995%.

4.3 A Scott-Marin 1,3-butadiene cylinder is used as the control standard.

4.4 A NBS prepared 1,3-butadiene cylinder is used as the primary standard for daily calibration.

V. Procedure

5.1 The air sample is analyzed for 1,3-butadiene by using either the loop method or the cryogenic freeze-out method. The freeze-out method is used for measurement of low concentrations of 1,3-butadiene.

5.2 The procedure for the loop method follows:

5.2.1 The sample is transferred from the sample bag to sample inlet and injected into the sample loop of the gas chromatograph by means of a syringe fitted with Luer-Lok to a Quik-Connect adaptor.

5.2.2 The gas sampling valve is equipped with a 1 ml loop.

5.2.3 After the gas sampling valve is rotated, the sample enters the GC for analysis.

5.2.4 The 1,3-butadiene is separated from other organic compounds under the following GC operating conditions:

40 ml/min. helium carrier gas flow
50 deg C sample valve temperature
200 deg C detector temperature
50 deg C injector temperature
60 deg C isothermal column temperature

5.2.5 Concentrations of 1,3-butadiene are calculated by a Perkin Elmer LIMS system or similar system.

5.3 The procedure for the freeze-out method follows:

5.3.1 Immerse the sample trap in liquid nitrogen (LN_2) and allow the trap to cool down to liquid nitrogen temperature.

5.3.2 After discarding 50 ml of the sample, withdraw exactly 100 ml sample from the Tedlar bag with a 100 ml syringe and transfer the sample into the trap.

5.3.3 Back fill the syringe with another 20 ml of helium and flush the 20 ml through the trap; then flush helium through the trap for 3 minutes at 100 ml/min.

5.3.4 Rotate the valve to isolate the cryogenic trap and to allow the carrier gas to by-pass the trap.

5.3.5 Replace the liquid nitrogen Dewar with a Dewar containing hot boiling water (approx. 95 degrees C).

5.3.6 Allow the trap to warm up.

5.3.7 Rotate the valve and allow the carrier gas stream to flush the sample into the gas chromatograph.

5.3.8 The instrument operating conditions are the same as those described in Section 5.2.4 above.

VI. Calculations

6.1 The 1,3-butadiene concentration in ppb is calculated by the data system using the external standard method:

concentration = Area x Calibration Factor

6.2 The calibration factor (CF) is calculated during calibration by the equation

$$CF = \frac{\text{concentration}}{\text{area}}$$

The replicate calibrations are averaged and the arithmetic mean is stored as the CF to be used in subsequent analyses.

VII. Quality Control

7.1 Calibrations are performed daily with working and quality control standards. Accuracy of the method is determined with reference material.

7.1.1 A NBS reference material of 520 ppb and 6.4 ppb (parts per billion) 1,3-butadiene in nitrogen is used as the calibration standards.

7.1.2 A control standard is used daily to check the GC performance.

7.1.3 An assurance audit of the standards is performed quarterly.

7.2 Calibrations are performed on a daily schedule.

7.2.1 The daily calibration of GC performance consists of at least two for automotive exhaust analysis.

7.2.2 The calibrations are repeated on both quality control calibration standards if the response of the quality control check changes by more than 2 sigma standard deviation. If the calibrations fail on both standards, the GC system will be completely checked and a corrective action will be taken to bring the system under the control before any sample analysis.

7.2.3 If the lamp voltage is adjusted, allow time for the lamp to stabilize and repeat the calibrations.

7.2.4 A record is kept for the lamp voltage settings and all preventative maintenance procedures, i.e. lamp replacement, cleaning of lamp window.

7.2.5 Blank samples are run daily between calibrations and sample analyses as necessary.

7.3 Linearity is checked annually as time allows.

7.3.1 A gas chromatographic linearity check is performed with standards of at least four different concentrations. The concentrations must cover the anticipated range of sample concentrations.

7.3.2 Multipoint calibration

7.3.3 Any region of concentration that deviates more than 5% from the least square line is considered non-linear.

7.3.4 Samples are analyzed only in the linear range. (Figure 1).

7.4 Limits of detection are established.

7.4.1 The limit of detection (LOD) is calculated by the following equations:

(i) if $b>o$, $LOD = 3\ s/m$

(ii) if $b<o$, $LOD = \frac{|b|}{m} + \frac{3\ s}{m}$

LOD = 0.2 ppb

b,m = intercept, slope of calibration - curve near the LOD.

S = standard deviation of replicates.

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

7.4.3 If the calibration factor changes by more than 10%, the instrument must be checked and the LOD re-determined.

7.5 GC column condition parameters should be checked and documented.

7.5.1 All GC parameters should be checked daily and recorded.

7.5.2 The efficiency and resolution of the column should be checked frequently. If there is more than 10% change, the column needs replacement.

7.5.3 If the headpressure required to maintain a specified flow through the column increases by more than 100%, the column needs replacement.

7.5.4 If the drift of retention times of the peaks results in peak misidentification, all instrument parameters need to be checked.

7.6 Replicate analyses are performed daily.

7.6.1 A duplicate analysis is performed on at least one ambient sample. A duplicate analysis is not performed with exhaust samples due to the high reactivity and decay of 1,3-butadiene.

7.6.2 If the duplicate analysis differs by more than 20% and if the concentration of the sample is higher than 3 x LOD, then an additional analysis is needed.

7.6.3 If the relative standard deviation (RSD) of the replicate analyses is greater than 15% and if the concentration of the sample is greater than 3 x LOD, none of the analyses for that day are acceptable.

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

7.7 Compound confirmation is required in quality control procedures.

7.7.1 At least 10% of the analyses are to be confirmed by a different analytical system. e.g. different columns or detector (GC/MS).

VIII. Critique and Comments

8.1 The minimum measurable concentration of 1,3-butadiene has been determined to be 0.2. ppb using this method with 100 ml freeze-out.

8.2 Any organic compound present in the sample having a retention time similar to that of 1,3-butadiene under operating conditions described in this method may interfere with the quantification.

8.3 Proof of chemical identity for 1,3-butadiene requires confirmation by other means.

8.4 1,3-butadiene can be positively identified by a gas chromatograph/mass spectrometer.

8.5 This method has been proven to be useful in analyzing ambient air or vehicle emission exhaust samples.

8.6 Exhaust samples must be analyzed as soon as received because 1,3-butadiene is extremely reactive and decays in the Tedlar bag. Most of the 1,3-butadiene is in Mode 1 of the dyno CVS sample. That sample is to be run within 5 minutes after received. The time when the sample is analyzed must be recorded.

Appendix D

Glossary

GLOSSARY

Actinic Region: The region of the radiation spectrum that can cause photochemical activity.

Adiponitrile: A chemical intermediate used in the manufacture of nylon.

Alkene: A class of unsaturated aliphatic hydrocarbons having one or more double bonds obtained by thermal cracking of petroleum fractions at high temperature (e.g., 1,3-butadiene).

Arrhenius Equation: A mathematical equation used to determine the rate of a chemical reaction with rise in temperature.

Biradical: Paired radicals, as in the case of the $\left[\dot{C}H_2O\dot{O}\right]^{\ddagger}$ radical.

Copolymer: An elastomer produced by the simultaneous polymerization of two or more dissimilar monomers (e.g., rubber from styrene and butadiene).

Criegee Reaction: Cleavage (division) of a compound into two compounds.

Dialkene: An unsaturated aliphatic hydrocarbon having two double bonds (e.g., 1,3-butadiene).

Elastomer: Synthetic polymers having properties similar to those of natural rubber, namely, the ability to be stretched to at least twice their original length and retract very rapidly to (approximately) their original length upon release.

Emission Factor: The rate of emission of a particular substance from an emission source.

Limit of Detection (LOD): The quantifiable limit (threshold) at and above which a substance can be accurately and precisely given a concentration value.

Monomer: A simple molecule or compound usually containing carbon and capable of conversion to polymers, elastomers, or synthetic resins by combination with itself or other similar molecules or compounds (e.g., styrene, the monomer from which polystyrene resins are produced).

Oxidative Dehydrogenation: The process whereby hydrogen is removed from compounds by chemical means, considered a form of oxidation.

Partially Observed Data (POD): An expression used to indicate (for a series of environmental measurements) that at least one of the measurements was found to be below the detection limit of the measurement method.

Pharmacokinetic Data: Data derived from a model that considers multiple routes of exposure to a substance (total body burden) and the substance's metabolism/deposition/excretion to and from various organs in an organism's body.

Polymer: A macromolecule formed by the chemical union of five or more identical combining units called monomers.

Population-weighted Exposure Estimate: An estimate of exposure to a substance that adds more weight (emphasis) to a site that represents a large population than it does to a site representing a smaller population.

Radical: A highly reactive substance having a charge (either positive or negative), that can act as an initiator or intermediate in combustion, oxidation, or polymerization.

Troposphere: The portion of the atmosphere (extending from ground level to around six kilometers above the ground) where weather occurs and pollutants are concentrated.

Unsaturated Aliphatic Hydrocarbon: An open-chain compound containing only carbon and hydrogen, capable of forming addition products because of its unsatisfied valence state (e.g., 1,3-butadiene).

California Environmental Protection Agency

Air Resources Board

Technical Support Document

Benzene

as a Toxic Air Contaminant

Part B
Health Assessment

Stationary Source Division

May 1992

PART B

HEALTH EFFECTS OF 1,3-BUTADIENE

OFFICE OF ENVIRONMENTAL HEALTH HAZARD ASSESSMENT

MARCH, 1992

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1.0 SUMMARY

1,3-Butadiene (BD) is an important industrial chemical produced as a by-product of ethylene. About three billion pounds were produced domestically in 1987 and an additional half-billion pounds were imported. BD is used in the production of styrene-butadiene copolymers, polybutadiene, and chloroprene/neoprene. The U.S. Environmental Protection Agency (EPA) has estimated total emissions of BD from industrial production and major uses at 10 million pounds per year in 1987 with approximately 70% attributable to equipment leaks. BD has also been found in automobile exhaust, gasoline vapor, incineration products of fossil fuels, cigarette smoke, and drinking water. The California Air Resources Board (ARB) has measured urban ambient concentrations of BD ranging from <0.1 to 1.8 ppb in a dozen California communities.

Although there are no human data on the metabolism of butadiene, studies in rodents indicate that BD is taken up rapidly by the inhalation route and is distributed to all tissues. At low concentrations (75 ppb) in air about 16-17% of BD was retained following inhalation. BD is metabolized, via cytochrome P450 monooxygenase mediated epoxidation, to 1,2-epoxy-3-butene or butene monoxide (BMO). This key metabolite is further metabolized to 3-butene-1,2-diol and to 1,2:3,4-diepoxybutane. Further conversion of the diol to 3,4-epoxy-1,2 butanediol by monooxygenase has also been observed.

1,3-Butadiene has only mild acute toxicity and produces neurological effects at high concentrations. The lowest concentration associated with neurological effects following subchronic exposure in animals is 13.5 ppm. Only minor noncarcinogenic adverse effects have been observed following subchronic exposures of 0.5 ppm. This level is more than 200-fold greater than ambient levels, thus significant adverse noncarcinogenic effects are not expected to occur at the ambient levels of up to approximately 0.002 ppm (2 ppb). However, due to the unavailability of data, hotspots have not been evaluated.

While a No Observed Adverse Effect Level (NOAEL) for reproductive effects has not been determined for BD, a chronic exposure Lowest Observed Adverse Effect Level (LOAEL) for reproductive toxicity is 6.25 ppm. This level produced gonadal atrophy in female mice. In developmental toxicity studies BD has been shown to be fetotoxic in the absence of producing maternal toxicity. At 40 ppm, BD exposure resulted in reduced fetal weight and in reduced ossifications in the offspring. The LOAEL is approximately 3000-fold greater than the upper range of ambient levels. Thus, these adverse reproductive effects are not expected to occur at ambient levels. However, the findings of a dominant lethal assay in mice may signal a mutagenic effect in male germ cells that might not be characterized by a dose threshold. Thus, reproductive or developmental effects cannot be ruled out at any level of BD exposure, although at ambient levels the incidence of such effects (which in humans might include undetected or very early-term spontaneous abortion) would be expected to be extremely low. Due to the unavailability of data, hot spots have not been evaluated.

A number of lines of evidence indicate that the mechanism of the oncogenic activity shown by BD in rodent bioassays is related to its genetic toxicity. These include: 1) the mutagenicity of BD in Salmonella in vitro with exogenous activation; 2) clastogenic/DNA damaging activities in mammals in vivo; 3) the

direct mutagenic action in Salmonella of the two principal metabolites of BD, namely BMO and 1,2:3,4-diexpoxybutane (DEB), in vitro and their detection in blood of experimental animals exposed to BD via inhalation; 4) the binding of BD (metabolites) to DNA; and 5) the isolation and identification of a DNA adduct from the liver DNA of mice exposed to BD, namely 7-(1-hydroxy-3-buten-2-yl)guanine. These studies clearly indicate that BD is genotoxic. In view of BD's probable mode of action in carcinogenicity (genetic toxicity), the effective dose of the carcinogen may be so low as to be indistinguishable from zero. No threshold mechanisms have been shown to specifically affect the action of BD. No practical threshold is thought to exist.

Epidemiological studies of the effects of BD on human populations have been reviewed and evaluated by the EPA, the International Agency for Research on Cancer (IARC) and the U.S. Occupational Safety and Health Administration (OSHA). EPA (1985) and IARC (1987) concluded that the evidence of human carcinogenicity was inadequate. However, more recent evaluations have considered more recently published studies. Based on a more recent review of epidemiologic studies, OSHA (1990) is of the opinion that exposure to BD is associated with an increased risk of lymphohematopoietic cancer. An IARC committee has concluded that there is limited evidence of human carcinogenicity of BD based on the studies of Downs et al. (1987), Divine (1990) and Matanoski et al (1990). OEHHA staff expect that IARC will soon formally concur with this conclusion. Among the recent epidemiologic studies are two that were published in 1990.

Divine (1990) found a significantly elevated Standardized Mortality Ratio (SMR) for lymphosarcoma among 2,582 workers in a butadiene manufacturing facility. The SMR for lymphosarcoma was even higher in those with routine exposure to butadiene. Matanoski et al. (1990) observed an excess of leukemia and lymphatic and hematopoietic cancers in black production workers and an elevated SMR for residual cancers of the lymphohematopoietic system for all production workers in several styrene-butadiene rubber (SBR) plants in the United States and Canada. Presumably, in SBR plants, production workers had the highest likelihood of exposure to butadiene, although they may have been exposed to other substances that are, or may be, linked to some of these cancers. While it is difficult to establish a causal relation with BD exposure, the fact that cancers of the lymphohematopoietic system were reported in mice suggests that the association deserves close attention in future studies. With respect to quantitative risk assessment, the epidemiological data base is still considered inadequate for predicting risks of community exposure to BD. Thus, the quantitative risk assessment presented in this document relies on data from animal bioassays rather than epidemiologic studies.

Butadiene was identified as a chemical known to the State of California to cause cancer on April 1, 1988. OSHA (1990) has classified 1,3-butadiene as a "potential occupational carcinogen." EPA (1985) and IARC (1987) have concluded that the evidence for carcinogenicity of BD in animals is sufficient. These organizations have classified the chemical as Group B2 and 2B respectively in their schemes of ranking potential human carcinogens. Inhalation of BD induced tumors in mice and rats. The animal bioassays for carcinogenicity indicate that BD can induce cancer in multiple sites. These sites include the heart, lung, mammary gland, ovaries, forestomach, liver, pancreas, Zymbal gland, thyroid, testes, and hematopoietic system. BD is only

one of two chemicals known to induce cancer of the heart in laboratory animals.

The most detailed evaluations of the carcinogenicity of BD are the mouse inhalation studies sponsored by the National Toxicology Program, mouse I - NTP 1984, and mouse II - Melnick et al., 1990. The nominal doses of study I were 0, 625 or 1,250 ppm administered 6 hours/day, 5 days/week for either 60 weeks (males) or 61 weeks (females). Fifty animals per sex/dose were used. Although the study was designed for 103 weeks, early deaths resulted largely from malignant neoplasms involving multiple organs (heart, hematopoietic lymphomas, lung, mammary gland, ovaries, forestomach, and liver). The incidences of total significant tumor bearing animals at control, middle, and high doses were 2/50, 43/49, and 40/45 in the males and 4/48, 31/48, and 45/49 in the females.

In study II, lower exposure concentrations of 1,3-butadiene (i.e., 0, 6.25, 20, 62.5, 200, and 625 ppm) were used than had been employed in the first study. Interim sacrifices at 40 and 65 weeks of exposure were also added to the original study design in order to follow progression of lesions. As in the previous study, hemangiosarcomas of the heart, hematopoietic lymphomas, squamous cell neoplasms of the forestomach, alveolar-bronchiolar neoplasms, and/or adenocarcinomas of the mammary gland were frequently observed in mice which died between weeks 40 and 65 of the study. In female mice exposed to 6.25 ppm BD, the incidence of alveolar-bronchiolar neoplasms was increased (15/60, 25%) vs. control (4/70, 6%). Also as in the previous study, gonadal atrophy was observed in both sexes at 200 ppm and 625 ppm for males and 6.25 ppm and higher for females. Bone marrow toxicity was evident as a poorly regenerative anemia at 62.5 ppm and higher. Thus this study did not establish a no effect level for reproductive endpoints. The 6.25 ppm nominal dose level might be considered a chronic LOAEL for reproductive toxicity. The Melnick et al. (1990) report is reproduced in the Appendix.

A two-year rat inhalation toxicity/carcinogenicity study (Hazleton Europe, 1981) was also evaluated. Groups of 100 per sex/dose were exposed to 0, 1,000, or 8,000 ppm BD for 6 hours/day, 5-days/week for 105-111 weeks. Unlike the mouse I study, survival of treated animals was not adversely affected in the first year of the study, but during the second year there was a statistically significant relationship between mortality and air concentration of BD. The published incidences are slightly different from those given by EPA (1985). The total significant tumor incidences (number of animals bearing one or more significant tumors) in males based on EPA criteria and 1987 published incidences are 4/100, 5/100, and 20/100 for the control, low, and high dose groups (Leydig cell tumors, pancreatic exocrine tumors, and Zymbal gland tumors). Total female significant tumor incidences in the control, low, and high dose groups were: 18/100, 19/100, and 41/100 (mammary carcinoma, thyroid follicular cell tumors, and zymbal gland tumors).

Cancer potency estimates were made for mice and rats using total significant tumor incidences and individual site incidences, three measures of dose, and the linearized multistage model of low dose extrapolation. The most sensitive tumor site was the lung alveolar and bronchiolar neoplasms in female mice (mouse II). The continuous internal dose was considered to be the best measure of dose available. When interspecies equivalent units of mg/m^2 surface area were used the resulting upper range of human cancer potency based

on all rodent assays was 9.8×10^{-6} to 8×10^{-4} $(ppb)^{-1}$ (4.4×10^{-6} to 3.6×10^{-4} $(\mu g/m^3)^{-1}$). The current EPA cancer risk estimate is 6.4×10^{-4} per ppb. OSHA (1990) has estimated a cancer risk for occupational exposure to 1 ppm BD of 0.0027, corresponding to a lifetime exposure risk of 1.4×10^{-5} $(ppb)^{-1}$ or 6.1×10^{-6} $(\mu g/m^3)^{-1}$, with an upper bound corresponding to 1.9×10^{-5} $(ppb)^{-1}$ or 8.4×10^{-6} $(\mu g/m^3)^{-1}$. More recently, staff of the National Institute for Occupational Safety and Health (NIOSH, 1991) have recommended to OSHA potency values based on data from the NTP "mouse II" study. The NIOSH-recommended cancer risk value for occupational exposure to 1 ppm BD is 0.0305, corresponding to a lifetime exposure risk of 1.5×10^{-4} $(ppb)^{-1}$ or 6.9×10^{-5} $(\mu g/m^3)^{-1}$, with an upper bound approximately corresponding to 2.5×10^{-4} $(ppb)^{-1}$ or 1.1×10^{-4} $(\mu g/m^3)^{-1}$.

The range of upper bound risk is based on the two orders of magnitude difference between potency figures for the mouse and the rat. This difference has been the subject of much additional metabolic and kinetic investigation. In addition to a higher metabolic rate for BD in the mouse, limited detoxification and accumulation of the primary reactive genotoxic metabolite (BMO) may be a significant factor in the increased susceptibility of mice to BD-induced carcinogenesis. Clearly work should continue to determine whether the mouse is the most appropriate model for prediction of human susceptibility to BD-induced carcinogenesis. The most detailed evaluation of the carcinogenicity of BD has been conducted in the mouse. The staff of the Office of Environmental Health Hazard Assessment (OEHHA) concludes that, for use in risk assessment, the quality of the mouse II bioassay data is superior to that of the rat data. The primary reasons for this conclusion are: 1) the use of lower, more relevant dose levels in the mouse II study; 2) the use of five dose levels in the mouse II study, compared to two in the rat study; 3) the presence of two mouse studies; 4) the fact that the rat study has not been replicated; 5) the consistency in sites of carcinogenicity between the two mouse studies; 6) the greater detail in the available mouse data which allows in-depth analysis; and 7) suggestions from limited epidemiological observations that BD exposure may be associated in humans with lymphatic and hematopoetic cancers, effects that were seen in mice.

The best value for the upper bound excess cancer risk is based on the mouse II bioassay data of Melnick et al. (1990). The present analysis gives a mouse based cancer potency of 3.4 $(mg/kg\text{-}d)^{-1}$ [0.37 $(ppm)^{-1}$, or 3.7×10^{-4} (ppb)-1], and a unit risk of 1.7×10^{-4} $(\mu g/m^3)^{-1}$ for the best value. Exposure to 6×10^{-3} $\mu g/m^3$ is associated with a lifetime excess cancer risk of 10^{-6}. The difference between the EPA estimate (6.4×10^{-4} $(ppb)^{-1}$) and the OEHHA best value (3.7×10^{-4} $(ppb)^{-1}$) is largely due to the use of more recent butadiene inhalation absorption data and the mouse II data in the current report. The unadjusted experimental dose and the pharmacokinetic dose were considered inappropriate for establishing the range of risks, although most of these estimates are within the range. In particular, the pharmacokinetic dose was not used since that approach was preliminary and the results did not help explain tumor frequencies in target tissues or species differences. Also for key data sets the multistage model gave relatively poor fits to the pharmacokinetic doses. For comparison purposes, cancer potency values obtained using physiologically based pharmacokinetic modeling (PBPK) of dose are provided. The PBPK values and their assumptions and limitations are presented in Section 4.2. However, in view of our limited understanding of the toxicokinetics of BD and its metabolites and the lack of any direct human

metabolic data on butadiene, it is not possible at present to rely on the pharmacokinetic estimates of metabolized dose for estimating human health risks.

The best potency value of 3.7×10^{-4} $(ppb)^{-1}$ (1.7×10^{-4} $(\mu g/m3)^{-1}$) indicates that community exposure to BD at the statewide population-weighted average ambient (outdoor) level (0.37 ppb) could be associated with an upper limit of 140 additional lifetime cancers per million exposed individuals. The EPA has estimated lifetime extra cancer risks for individuals living near hot spot facilities in California to be in the range of 1 in 10 to 1 in 1000. These calculations are for the upper range of plausible excess cancer risks; the actual risk, which cannot be calculated, may be much lower.

Based on the findings of 1,3-butadiene-induced carcinogenicity and the results of the risk assessment, the OEHHA staff find that, at ambient concentrations, 1,3-butadiene 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.

1.1 Butadiene Highlights

1.1.1 National and International Evaluation of Carcinogenicity

1.1.1.1 International Agency for Research on Cancer (IARC)

Evidence for carcinogenicity to animals is sufficient. Evidence for carcinogenicity to humans is inadequate. IARC has classified 1,3-butadiene as "possibly carcinogenic to humans," Group 2B.

An IARC committee has recommended that the Agency's evaluation be changed to report limited evidence of carcinogenicity in humans; it is expected that IARC will make this change in the near future. This would change IARC's classification of BD to Group 2A, "probably carcinogenic to humans."

1.1.1.2 U.S. Environmental Protection Agency (EPA)

There is sufficient evidence of carcinogenicity in two species of rodents. There is inadequate evidence for carcinogenicity in humans. According to EPA guidelines, 1,3-butadiene would be classified as a "probable human carcinogen," Group B2.

1.1.1.3 U.S. Occupational Safety and Health Administration (OSHA)

On the basis of consistency of results from several epidemiologic studies, "OSHA is of the opinion that exposure to BD is associated with an increased risk of death from cancer of the lymphohematopoietic system" (OSHA 1990, Section V.B.2(v)). OSHA has classified 1,3-butadiene as a "potential occupational carcinogen," based primarily on positive findings of chronic inhalation studies in rodents. OSHA has proposed to lower its permissible exposure limit (PEL) for BD from 1000 ppm to 2 ppm (OSHA 1990).

1.1.2 Exposure Sources

1.1.2.1 Air

Butadiene is a common contaminant of urban air largely arising from vehicle emissions. Ambient air levels of butadiene were measured at 20 locations in California by the Air Resources Board in 1988 and 1989. Concentrations varied from less than 0.00004 ppm (0.04 ppb, limit of detection) to 0.0018 ppm (1.8 ppb).

1.1.2.1 Water

While butadiene has been detected in U.S. drinking water, human exposure is primarily through the inhalation route.

1.1.3 Quantitative Risk Assessment

1.1.3.1 Range of Risks

The theoretical human risks associated with a continuous lifetime exposure to butadiene in ambient air have been estimated from animal carcinogenicity bioassay data using the linearized multistage model. Unit risks for humans ranged from 9.8×10^{-6} $(ppb)^{-1}$ [0.0098 $(ppm)^{-1}$, 4.5×10^{-6} $(\mu g/m^3)^{-1}$] to 8.0×10^{-4} $(ppb)^{-1}$ [0.8 $(ppm)^{-1}$, 3.5×10^{-4} $(\mu g/m^3)^{-1}$] based on rat and mouse inhalation studies respectively. The best value is that based on the mouse II inhalation study, 3.7×10^{-4} $(ppb)^{-1}$ [0.37 $(ppm)^{-1}$, 1.7×10^{-4} $(\mu g/m^3)^{-1}$].

1.1.3.2 Extrapolations

1.1.3.2.1 Route

The animal bioassays were conducted by the inhalation route. Quantitative risk estimates assume that 16-17% of a low dose is absorbed and retained (based on uptake studies with radiolabeled butadiene) and that dose units of mg/m^2 surface area/d are equivalent in different species.

1.1.3.2.2 Dose

The doses in the recent NTP mouse carcinogenicity bioassay (Melnick et al., 1990) ranged from 6.25 to 625 ppm (6 hours/day, 5 days/week). There was a significant increase in lung neoplasms in female mice at the 6.25 ppm level. This experimental dose is equivalent to a continuous exposure of approximately 1 ppm. The estimated population-weighted average ambient exposure is approximately 0.4 ppb in California. Thus, the range of extrapolation of animal data to human exposure is about 4 orders of magnitude.

2.0 TOXICOKINETICS

2.1 Absorption and Distribution

Male Sprague-Dawley rats exposed to BD concentrations of ≤ 1000 ppm in a closed inhalation chamber exhibited 'linear' pharmacokinetics with respect to 1,3-butadiene disappearance and exposure concentration; however, at concentrations >1000 ppm, the elimination rate was constant and was indicative of saturation of the metabolic capacity (Bolt, Filser and Stormer, 1984). Kreiling et al. (1986) reported similar results for B6C3F1 mice, as well as for Sprague-Dawley rats, and further determined that mice had a much greater uptake rate than rats. The metabolic V_{max} at saturation was 400 μmol/h-kg for mice and 220 μmol/h-kg for rats, which was in concurrence with the *in vitro* results observed by Schmidt and Loeser (1985). Since metabolic elimination constants were approximately equal for both species up to 1000 ppm, it can be shown that, under conditions where first-order elimination applies, the steady state concentration of 1,3-butadiene in the mouse is approximately two times that in the rat.

Induction of rats with Aroclor 1254, a cytochrome P450 inducer, increased their metabolic capacity to the extent that saturation was not observed at 1,3-butadiene exposure concentrations as high as 12000 ppm (Bolt, Filser and Stormer, 1984). The inducibility of BD metabolism in the rat agrees with the results of *in vitro* studies. Expired air from Sprague-Dawley rats exposed to ≈7000 ppm 1,3-butadiene in a closed inhalation chamber contained 1,2-epoxy-3-butene (butene monoxide, BMO) (Bolt et al., 1983), thus confirming another *in vitro* observation.

Bond et al. (1986) conducted experiments in adult male Sprague-Dawley rats and B6C3F1 mice to: 1) determine the uptake and retention of 1,3[^{14}C]-butadiene over a wide range of exposure concentrations, 2) to identify routes and half-times for excretion of parent compound and metabolites, and 3) to identify BD metabolites in blood. The animals were exposed for a single 6-hour exposure period to 1,3[^{14}C]-butadiene concentrations (nose-only exposures) ranging from 0.14 to 13,000 μg/L (0.08 to 7100 ppm). Mice were not exposed to the highest concentration. The percentage of ^{14}C absorbed and retained at 6 hours ranged from 1.5 to 17% in rats and from 4 to 20% in mice (Table 2-1 and Table 2-2). During the exposure period, respiratory measurements were made and blood samples were obtained for determination of BD, BMO, and 1,2:3,4-diepoxybutane (DEB) concentrations. Immediately post-exposure four animals of each species were placed in metabolism cages and urine and feces samples were collected at suitable time points for up to 65 hours. Expired air was also pulled from the metabolism cages and analyzed for parent compound and metabolites.

Analysis of blood for metabolites revealed an increase in metabolite concentration with increasing exposure concentration for both species; however, the increases in metabolite concentrations were not directly proportional to the increases in exposure concentration (Table 2-3). Mice had higher blood levels of the metabolite BMO than rats at equivalent exposure concentrations, although rats had higher concentrations of $^{14}CO_2$. Levels of 1,2:3,4-diepoxybutane were similar in the two species.

Bond et al. (1987) further examined species differences in the distribution of 1,3-butadiene between rats and mice by exposing young adult male Sprague-

Table 2-1 Respiratory Tract Retention of Inhaled Butadiene in Rats and Mice[a]

Exposure Concentration (ug/l)	Total Volume Inhaled/Animal (l)[b]		Butadiene Inhaled/Animal (umol)		[14C]Butadiene Equivalents Retained at 6 hr/Animal (umol)[c]		Percentage of Inhaled [14C]Butadiene Equivalents Retained at 6 hr	
	Rats	Mice	Rats	Mice	Rats	Mice	Rats	Mice
0.14	73 ± 5	14 ± 2	0.2 ± 0.001	0.04 ± 0.0002	0.03 ± 0.0002	0.006 ± 0.001	17 ± 2[h]	16 ± 1[h]
1.4	90 ± 4	12 ± 2	2 ± 0.1	0.3 ± 0.06	0.13 ± 0.003	0.06 ± 0.01	6 ± 0.3[g]	20 ± 5
13	83 ± 7	12 ± 1[d]	19 ± 2	3 ± 0.4[d]	0.8 ± 0.1	0.4 ± 0.09[d]	4 ± 0.5[g]	20 ± 5[d]
130	70 ± 8[e]	13 ± 2	170 ± 20[e]	35 ± 5	17 ± 2[e]	3.2 ± 0.1	8 ± 1[e]	8 ± 0.7
1800	100 ± 10	12 ± 2	3,100 ± 360	440 ± 80	65 ± 5	19 ± 0.5	2.5 ± 0.2[g]	4 ± 0.3
13,000	70 ± 15	*f*	17,000 ± 3100	*f*	240 ± 40	*f*	1.5 ± 01	*f*

a Values are the mean ± SE.

b Values corrected for rat mouse body temperature, barometric pressure, inspired gas temperature, and relative humidity.

c Data obtained from radioanalysis of rats and mice which were maintained in plethysmograph tubes during the 6 hr exposure.

d Individual values pooled from three separate experiments.

e Individual values pooled from two separate experiments.

f Mice were not exposed to this concentration of butadiene.

g Significantly different ($p < 0.05$) from mice exposed to the same butadiene concentration using Student's t test.

h a linear regression analysis of the log transformed data vs the log of the exposure concentration indicated that there was a significant ($p < 0.001$) concentration-related decrease in the percent retained at 6 hr with increasing exposure concentration.

(Table taken directly from Bond et al. 1986)

Table 2-2 Inhaled "Dose" of Butadiene in Rats and Mice[a]

Exposure Concentration (μg/l)	Dose (μmol/kg)[b]		Dose (nmol/cm^2)	
	Rats[c]	Mice[c]	Rats[d]	Mice[d]
0.14	0.08 ± 0.01[h]	0.2 ± 0.02	0.6 ± 0.04	0.6 ± 0.07
1.4	0.3 ± 0.01	2 ± 0.2	3 ± 0.1[h]	6 ± 0.8
13	2 ± 0.1[h]	22 ± 4[e]	14 ± 1[h]	49 ± 10[e]
130	40 ± 3[fh]	110 ± 11	310 ± 20[f]	320 ± 40
1800	160 ± 10[h]	650 ± 50	1200 ± 80[h]	2000 ± 150
13000	680 ± 40	g	4900 ± 290	g

[a] Values are the mean ± SE.

[b] Calculated using μmol butadiene retained at 6 hr (Table 2, Col. 4).

[c] the mean body weights (±SE) for rats and mice for all exposures were 398 ± 4 and 28 ± 0.3 g, respectively.

[d] Mean body surface areas (±SE) for rats and mice for all exposures were 54 ± 0.3 and 9.2 ± 0.07 cm^2, respectively.

Body surface areas were calculated as follows:

surface area (cm^2) = body weight (g)$^{2/3}$.

[e] Individual values pooled from three separate experiments.

[f] Individual values pooled from two separate experiments.

[g] Mice were not exposed to the concentration of butadiene.

[h] Significantly different ($p < 0.05$) from mice exposed to the same butadiene concentration using Student's test.

(Table taken directly from Bond et al., 1986)

Table 2-3 Distribution of ^{14}C in Blood of Rats and Mice Exposed to Different Concentrations of Butadiene[a]

	Exposure Concentration (ug/l)	Residue[b]			Butadiene diepoxide · 45°C trap[c]			1,2-Epoxy-3-butene · 95°C trap[d]			Butadiene · 130°C trap[e]			CO_2 ·195°C trap[f]		
		2[g]	4	6	2	4	6	2	4	6	2	4	6	2	4	6
Rats	130	5±0.2 (87)	7±0.4 (86)	8±1[h] (77)	0.04±0.02[h] (0.7)	0.1±0.02 (1)	0.1±0.04 (1)	0.4±0.04[h] (6)	0.4±0.08[h] (5)	0.4±0.04 (3)	0.1±0.02 (3)	0.2±0.4 (2)	0.1±0.02[h] (1)	0.3±0.02[h] (5)	0.5±0.02[h] (6)	0.6±0.04[h] (5)
	1800	14±3[h] (66)	40±7[h] (77)	51±8 (82)	0.4±0.02 (2)	1±0.3 (2)	1±0.04 (2)	2±0.4 (12)	4±1[h] (8)	4±0.7[h] (7)	1±0.08[h] (6)	4±0.6 (7)	4±0.3 (6)	2±0.02 (11)	3±0.5 (6)	4±0.2[h] (6)
Mice	13	2±0.4 (56)	3±0.6 (70)	6±0.4 (83)	0.06±0.01 (2)	0.06±0.02 (1)	0.08±0.001 (1)	0.6±0.1 (19)	0.3±0.2 (8)	0.7±0.1 (11)	0.6±0.02 (19)	0.9±0.5 (21)	0.2±0.06 (3)	0.09±0.01 (3)	0.1±0.01 (3)	0.08±0.01 (1)
	130	10±2 (82)	13±3 (86)	17±1 (89)	0.2±0.03 (1)	0.2±0.03 (1)	0.01±0.01 (0.5)	2±0.05 (14)	2±0.2 (10)	0.9±0.4 (5)	0.3±0.1 (2)	0.2±0.1 (1)	0.8±0.1 (4)	0.2±0.04 (2)	0.2±0.0.1 (1)	0.2±0.02 (1)
	1600	71±12 (32)	110±13 (79)	100±20 (70)	3±1 (3)	3±0.2 (2)	1±0.2 (0.8)	4±0.8 (4)	21±1 (14)	15±1 (10)	7±0.3 (8)	8±2 (5)	3±1 (2)	2±0.4 (2)	3±0.8 (2)	2±0.05 (2)

[a]Data are expressed as the mean nmol/ml blood ±SE; n = 3; numbers in parentheses are present of total ^{14}C/ml blood.

[b]Represents nonvolatile butadiene metabolites.

[c]Diepoxybutane codistills in this trap.

[d]1,2-Epoxy-3-butene codistills in this trap.

[e]Butadiene codistills in this trap.

[f]CO_2 codistills in this trap.

[g]Time (hr) after start of exposure.

[h] Significantly different ($p < 0.05$) from mice exposed to the same concentration of butadiene.

Table taken directly from Bond, et al. 1986.

Dawley rats and B6C3F1 mice (nose-only) to 670 and 65 ppm 1,3[^{14}C-] butadiene, respectively, for a single 3.4 hour period. These concentrations were chosen because: 1) they resulted in similar concentrations of BD and its metabolites in the two species, and 2) BD excretion and metabolism rates for both species are linear with respect to exposure concentration in this range (Bond et al., 1986).

Results of these studies indicated that 1,3-butadiene and its metabolites were widely distributed to all tissues in both species within one hour after the start of exposure (Table 2-4). Several tissues in both species contained high concentrations of ^{14}C within 1 hour postexposure. Tissues with the three highest ^{14}C-concentrations at 1 hour postexposure were, in rats, the bladder, the thyroid, and the kidney, and, in mice, the bladder, the small intestine, and the kidney. The concentration of ^{14}C in the blood was low compared to other tissues in both species. Although rats absorbed a greater amount of BD on a μmol/kg body weight basis, the concentrations of ^{14}C radiolabel/μmol 1,3-butadiene inhaled were 15 to 100 times greater in the tissues of mice than in rats.

2.2. Metabolism and Excretion

BD was shown to be metabolized to 1,2-epoxy-3-butene (BMO) in the presence of rat liver microsomes and NADPH (Malvoisin et al. 1979; Bolt et al., 1983). The participation of cytochrome P450 in this epoxidation reaction was indicated by 1) the enhancement of the epoxidation reaction following pretreatment of the rats with the P450-inducer, phenobarbital, and 2) the inhibition of the formation of BMO with the known cytochrome P450 inhibitors, SKF-525A and dithiocarb. 3-Methylcholanthrene (3-MC) was also reported by Bolt et al. (1983) to induce the epoxidation of 1,3-butadiene; however, Malvoisin et al. (1979) reported that pretreatment of rats with 3-MC did not result in induction. Bolt et al. (1983) further confirmed the occurrence of this metabolic pathway *in vivo* by identifying BMO in the expired air of rats exposed to BD.

Malvoisin and Roberfroid (1982) showed that BMO was metabolized to both 3-butene-1,2-diol and to 1,2:3,4-diepoxybutane (DEB) by rat liver microsomes, and that the 3-butene-1,2-diol was further oxidized to 3,4-epoxy-1,2-butanediol (EBD) (Figure 1). The metabolism of BMO to 3-butene-1,2-diol presumably occurred via epoxide hydrolase since inhibition of the epoxide hydrolase with 1,1,1-trichloropropene oxide increased the levels of BMO (Bolt et al., 1983). The next metabolic step, formation of two isomers of EBD, occurs via the cytochrome P450-dependent monooxygenase system. This latter reaction is inducible by pretreatment with phenobarbital, and is inhibited by the presence of SKF-525A. Since the P450-system has a greater affinity for BMO than epoxide hydrolase does, the balance lies in favor of the formation of DEB (Malvoisin and Roberfroid, 1982). Bolt et al. (1983) also demonstrated that the presence of glutathione in the medium causes a decrease in the BMO concentration. This decrease probably occurs when the diol, formed by the epoxide hydrolase, is conjugated with glutathione. Removal of the diol as it is formed would shift the reaction equilibrium to the right and decrease the steady state concentration of BMO.

Jelitto et al. (1989) exposed male Sprague-Dawley rats and male B6C3F1 mice for 7 h to 250, 500, or 1000 ppm BD. After exposure cell nuclei of liver and lung tissues were isolated and subjected to alkaline elution. Analysis of the

Table 2-4 Concentration of ^{14}C (nmol/g) in Tissues of Rats and Mice Exposed to ^{14}C-Butadiene[a] at Selected Time Points

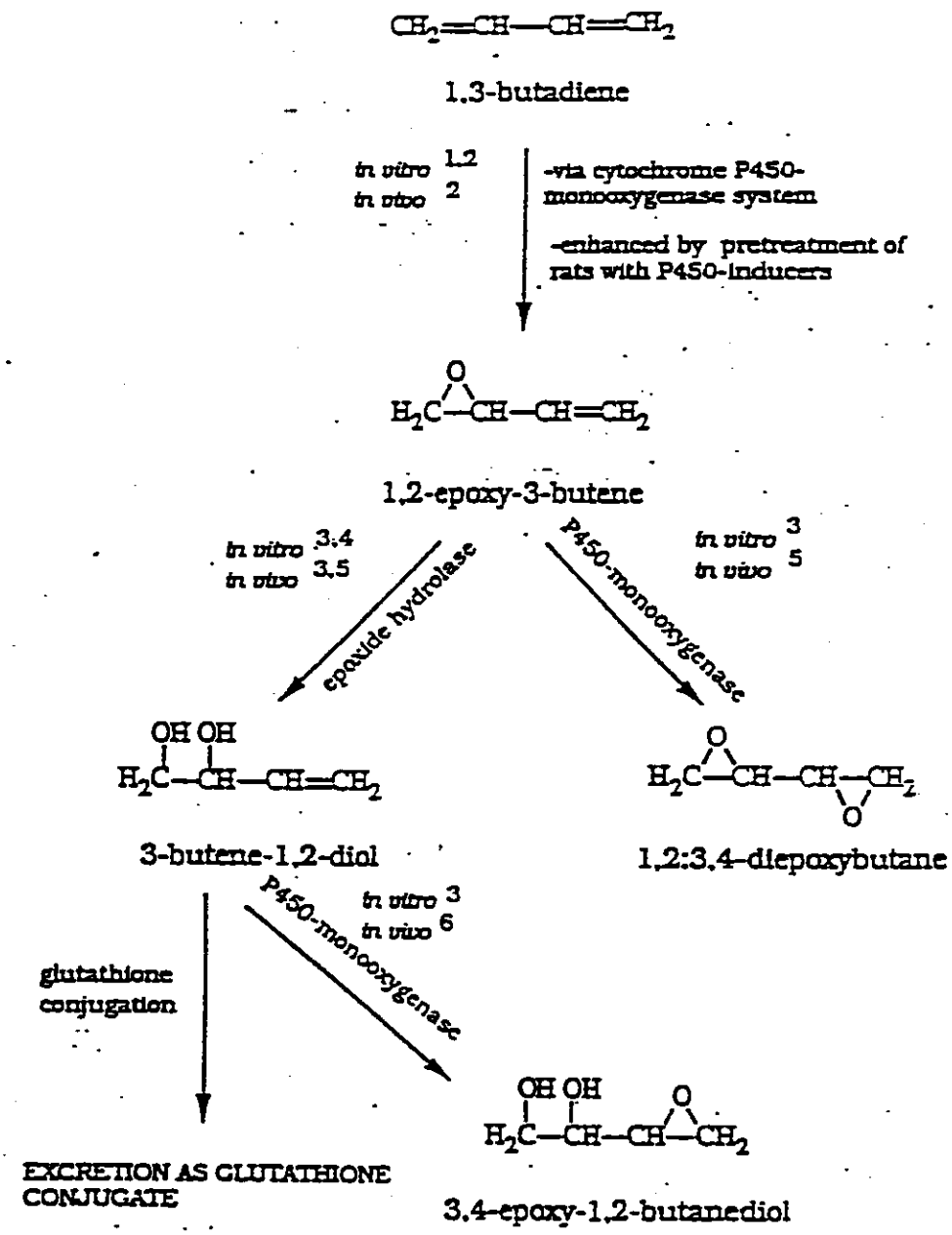
	1 hr[b]		8 hr		67 hr	
	Rats	Mice	Rats	Mice	Rats	Mice
Adrenal Glands	210 ± 24 (0.1)	76 + 6 (6)	120 + 11 (0.07)	204 + 133 (17)	45 + 10 (0.03)	9 + 2 (0.8)
Bladder	950 ± 380 (0.6)	1300 + 660 (19)	63 ± 5 (0.04)	1900 ± 1200 (1)	22 ± 6 (0.0-1)	4 ± 0.6 (0.3)
Blood	124 ± 24 (0.07)	49 ± 3 (4)	116 ± 20 (0.7)	43 ± 17 (4)	20 ± 4 (0.01)	4 ± .06 (0.3)
Heart	120 ± 8 (0.07)	47 ± 1 (4)	83 ± 4 (0.05)	43 ± 17 (4)	20 ± 5 (0.01)	6 ± 0.3 (0.5)
Kidney	390 ± 25 (0.2)	180 ± 18 (15)	190 ± 5 (0.)	110 ± 34 (9)	39 ± 8 (0.02)	8 ± 0.5 (0.7)
Large Intestine	170 ± 20 (0.1)	130 ± 70 (11)	150 ± 20 (0.08)	160 ± 53 (13)	24 ± 6 (0.01)	5 ± 0.3 (0.4)
Liver	330 ± 55 (0.2)	120 ± 9 (10)	240 ± 7 (0.1)	120 ± 45 (10)	47 ± 8 (0.03)	9 ± 0.6 (0.8)
Lung	200 ± 18 (0.1)	96 ± 7 (8)	100 ± 1 (0.06)	95 ± 45 (8)	23 ± 5 (0.01)	4 ± 1 (0.3)
Mammary Glands	140 ± 29 (0.08)	30 ± 7 (2)	48 ± 11 (0.03)	150 ± 82 (13)	12 ± 4 (0.007)	4 ± 0.3 (0.3)
Turbinates	300 ± 35 (0.2)	67 ± 4 (6)	180 ± 7 (0.1)	23 ± 4 (2)1	56 ± 12 (0.03)	9 ± 0.8 (0.7)
Pancreas	320 ± 31 (0.2)	160 ± 25 (13)	200 ± 18 (0.1)	220 ± 110 (18)	20 ± 5 (0.01)	4 ± 0.5 (0.4)
Small Intestine	320 ± 67 (0.2)	300 ± 120 (24)	180 ± 7 (0.1)	130 ± 52 (10)	21 ± 3 (0.01)	4 ± 0.2 (0.3)
Subcutaneous Fat	220 ± 97 (0.1)	31 ± 4 (3)	41 ± 9 (0.01)	170 ± 83 (14)	9 ± 2 (0.005)	4 ± 0.6 (0.3)
Thyroid Gland	490 ± 190 (0.3)	60 ± 11 (5)	86 ± 0.8 (0.05)	62 ± 22 (5)	37 ± 10 (0.02)	12 ± 6 (1)
Trachea	260 ± 130 (0.2)	59 ± 17 (5)	77 ± 6 (0.04)	67 ± 37 (6)	24 ± 6 (0.01)	6 ± 0.6 (0.5)
Bone Marrow	10 ± 2	0.5 ± 0.2	10 ± 3	0.2 ± 0.02	3 ± 0.8	0.05 ± 0.0086

a Values are the mean nmole ^{14}C-butadiene equivalents/g±SE. Values in parentheses are the mean nmole ^{14}C-butadiene equivalents/g tissue/ umole butadiene inhaled. Values for blood are per mL.

b Time after end of exposure.

Values are mean nmole ± SE

Table taken directly from Bond et al. 1987.



1. Malvoisin et al. 1979
2. Bolt et al. 1983
3. Malvoisin and Roberfroid 1982
4. Schmidt and Loeser 1985
5. Bond et al.1986
6. Kreiling et al. 1987

Figure 1. Metabolism scheme for 1,3-butadiene.

mouse liver DNA showed the formation of 7-N-(1-hydroxy-3-buten-2-yl)guanine and 7-N-(2,3,4-trihydroxybutyl)guanine the reaction products of BMO and DEB with guanosine. Analysis of rat liver DNA did not show the trihydroxy butyl-guanine adduct. At exposure concentrations of 250 ppm and above, mouse tissues showed the occurrence of protein-DNA and DNA-DNA crosslinks. These crosslinks were not observed in rat tissues. Contrary to this latter finding in mice Ristau et al. (1990) found no evidence of DEB-induced DNA-DNA crosslinks fomation in purified DNA from the livers of BD-treated mice or rats.

Schmidt and Loeser (1985; 1986) compared the ability of liver microsomes from several species to metabolize BD to BMO. Microsomes from male and female B6C3F1 mice, Sprague-Dawley rats, human (one sex only, not specified), and the rhesus monkey were all capable of forming the epoxide. However, the rates differed considerably. Hepatic microsomes from female mice had the highest activity, followed by male mice, male rats, female rats, human (n=1), and the rhesus monkey. (Male and female activities were the same for the monkey.) The ratio of mice to monkeys was approximately 7:1. The ability of lung tissue microsomes from these species to form the epoxide was also tested, but no measurable activity was found for either human or monkeys and the rodent values were very low (mouse lung was 1/30 mouse liver).

Elfarra et al. (1991) have observed that NADPH - dependent oxidation of 1,3-butadiene by mouse liver microsomes or H_2O_2-dependent oxidation by chloro peroxidase produced both butadiene monoxide and crotonaldehyde. The ratio of BMO to crotonaldehyde was about 50 in microsomal preparations.

Bond et al., (1988) examined the effect of repeated inhalation exposure of rats and mice to 1,3-butadiene on the metabolism of BD by lung and liver microsomes in these species. Male Sprague-Dawley rats and $B6C3F_1$ mice were exposed by nose-only to 0 (air) or 7600 ppm BD and 740 ppm BD respectively for 6 hours/day for 5 days after which the tissues were removed from the animals and microsomes prepared. The authors observed a statistically significant ($p<.05$) depression relative to control preparations in the rate of 1,3-butadiene metabolism in microsomes from lungs of both rats and mice. There was no effect noted on liver or nasal tissue (rat only) metabolism of BD. The data indicate that interspecies differences in carcinogenic susceptability are unlikely to be the result of an inductive or inhibitory effect of BD on its own metabolism, at least in the tissues studied.

The total amount of ^{14}C accumulated on a normalized body-weight or surface-area basis (Bond et al., 1986) indicated that mice accumulated a larger amount of BD and metabolites than rats, in agreement with the predictions of Kreiling et al. (1986). Urine and exhaled air were the major elimination routes for ^{14}C in both species. In mice, exhalation of CO_2 accounted for ≈20% of the total ^{14}C eliminated or retained in the carcass, and about 12% remained in the carcass 65 hours postexposure. At lower concentrations analysis of bubbler traps indicated that all ^{14}C present in traps was accounted for as metabolites including BMO and DEB. In rats as in mice, urine and exhaled air were the major routes of elimination although the percentage of exhaled radioactivity as CO_2 increased considerably at 13,000 μg/L (7100 ppm) (Table 2-5).

Table 2-5 Distribution of ^{14}C in Urine, Feces, Exchaled Air, and Carcass of Rats and Mice Expposed to Different Concentrations of Butadiene at 65 hr after a 6-hr Expcsure[a]

	Exposure concentration (ug/l)	Urine	Feces	Exhaled Air		Carcass
				CO_2	Butadiene and Other Volatile Metabolites	
Rats	130	8 ± 0.4 (49)	0.8 ± 0.2 (5)	2 ± 0.5 (12)	3 ± 0.3 (15)	3 ± 0.4 (20)
	1,800	21 ± 4 (31)	1 ± 0.2 (2)	16 ± 0.2 (24)	15 ± 1 (23)	13 ± 0.4 (19)
	13,000	19 ± 2 (8)	4 ± 0.8 (1)	124 ± 4 (51)	62 ± 12 (26)	35 ± 1 (14)
Mice	13	0.4 ± 0.1 (39)	0.06 ± 0.01 (7)	0.2 ± 0.01 (22)	0.2 ± 0.02 (22)	0.09 ± 0.02 (10)
	130	1.6 ± 0.3 (50)	0.4 ± 0.05 (11)	0.1 ± 0.01 (4)	0.6 ± 0.2 (19)	0.5 ± 0.05 (16)
	1,800	7 ± 0.4 (38)	0.6 ± 0.2 (3)	3 ± 0.1 (17)	6 ± 1 (31)	2 ± 0.1 (11)

[a] Values are the mean umol ± SE. Numbers in parentheses are percentage of total ^{14}C absorbed and retained at the end of the 6-hr exposure.

Table taken directly from bond et al. 1986.

There were no differences in the $t_{1/2}$'s for urinary or fecal excretion regardless of exposure concentration for either species. The $t_{1/2}$ for urinary excretion in rats and mice was similar, 5.6 ± 1.6 hours and 4.6 ± 1.5 hours, respectively; however, the $t_{1/2}$ for fecal excretion was much longer in rats than mice, 22 ± 2.6 hours vs 8.6 ± 2.6 hours, respectively.

Sun et al. (1989) and Dahl et al. (1990) have reported on the comparative metabolism and disposition of BD in monkeys (Macaca fasciculanis) and rodents. The normalized uptake and retention values for BD were 3.3, 0.46, and 0.52 µmole/hr/10 ppm/kg body weight in mouse, rat, and monkey respectively. When these values were expressed in terms of body weight to the 2/3 power, a typical method of interspecies scaling, the resulting values were 0.99, 0.35, and 0.94 respectively.

In summary, ^{14}C radiolabel was widely and rapidly distributed to all tissues examined following exposure of rats or mice to [^{14}C] BD and species differences in specific tissue accumulations of ^{14}C were not observed. Consequently, these data do not explain the differences between rats and mice with respect to the target tissues of 1,3-butadiene induced carcinogenicity, or in its carcinogenic potency. (See Carcinogenicity Section 3.5 below for details.)

A first-order elimination pattern was observed for BMO in Sprague-Dawley rats following exposure to the epoxide in a closed inhalation chamber at concentrations up to 5000 ppm; thus saturation of BMO metabolism was not observed (Filser and Bolt, 1984; Kreiling et al., 1987). Mice were found to reach metabolic saturation at a much lower concentration of BMO, ≈500ppm (Kreiling et al., 1987). The estimated metabolic V_{max} at saturation levels of BMO was 350 µmol/h-kg for mice and was > 2600 µmol/h-kg for rats. At the lower levels where first order elimination kinetics applied, mice had a lower metabolic elimination rate than rats by approximately a factor of five. The key finding in their study was that the steady state concentration of BMO in the mouse was about 10-fold greater than in the rat.

When BD metabolism was saturated, BMO was exhaled by both species (Bolt et al., 1983; Kreiling et al., 1987). In addition, when exposed to concentrations of butadiene above 2000 ppm, the hepatic, non-protein sulfhydryl compounds (NPSH, e.g. glutathione) rapidly became depleted in the mouse falling to 20% after 7 h and 4% after 15 h. In the rat depletion was much less, 65-80% of control value after 7 h, and appeared to stabilize (Kreiling et al., 1987, 1988). A further camparison of both species at several concentrations and tissues showed that a dose dependent NPSH depletion was observed in mouse lung, heart, and liver. In rats NPSH depletion is only seen at high exposure concentrations (Deutschmann and Laib, 1989).

The two main routes for detoxification of BMO *in vivo* were conjugation with glutathione-S-transferase and cleavage of the epoxide with epoxide hydrolase to form 3-butene-1,2-diol. (Both pathways have also been shown to occur *in vitro*.) Although these enzyme systems were present in both species, the major route of epoxide detoxification may differ between rats and mice. Mouse liver glutathione-S-transferase had a high affinity for BMO while mouse epoxide hydrolase levels were low and rats had high epoxide hydrolase levels (Schmidt and Loeser, 1985). These differences may have accounted for the fact that BD and BMO metabolic capacities were saturable at lower exposure concentrations

in the mouse than in the rat, and that glutathione depletion was observed in the mouse.

The rat appeared to readily metabolize BMO as was shown by Bolt et al. (1984). This assumption was based on the fact that the actual production rate of the epoxide metabolite (63 μmol/h-kg) was only 29% of the predicted production rate based on the V_{max} for BD metabolism (220 μmol/h-kg). However, since no reference is made to other BD metabolites in this study, the rapid disappearance of BMO may not be due solely to a detoxification reaction.

3.0 HAZARD EVALUATION

3.1 Acute Toxicity

1,3- Butadiene is only mildly acutely toxic. For rats and mice, the median lethal concentrations (LC_{50}) of 1,3-butadiene inhalation are above 100,000 ppm for 2 to 4 hours inhalation. The oral LD_{50} values for rats and mice are 5.48 g/kg and 3.21 g/kg, respectively. Acutely toxic effects of exposure in animals progressed from light anesthesia, to running movements and tremors, to deep anesthesia and death (NIOSH, 1984). Acute toxicity is not expected to occur at ambient levels of butadiene.

3.2 Subchronic and Chronic Toxicity

The subchronic and chronic studies indicate that minor adverse effects can result from prolonged exposure to 0.45 ppm (1 mg/m^3). This concentration is more than 200-fold greater than the ambient levels. Thus, noncarcinogenic effects are not expected to occur at ambient levels of approximately 0.002 ppm.

The following information is taken directly from EPA's 1985 review of butadiene: "A 3-month toxicity study in rats preceded the 2-year chronic inhalation toxicity study conducted at Hazleton Laboratories Europe, Ltd. in England (1981a), and sponsored by the International Institute of Synthetic Rubber Producers, Inc. (IISRP). Further details of the chronic investigation as well as the results with regard to the carcinogenicity of BD are presented in the carcinogenicity section (3.5) of this document. The airborne concentrations of 1,3-butadiene used in the 3-month study were 1,000, 2,000, 4,000, and 8,000 ppm; a group exposed to filtered air (0 ppm) served as controls. The authors considered that there were no effects attributable to exposure to the test chemical on growth rate, food consumption, hemograms, blood biochemical investigations, or pathological evaluation. The only effect the investigators considered to be related to BD exposure was a moderate increase in salivation, particularly among female rats during the last 6 to 8 weeks of exposure at the higher airborne concentrations (Crouch et al., 1979). These results are consistent with an earlier study by Carpenter et al. (1944) in which the investigators found only a slight reduction in body weight gain among rats and guinea pigs exposed for 8 months, 7.5 hours/day, 6 days/week, to a airborne concentration of 8,000 ppm. These authors noted no effects among animals exposed at 600 and 2,300 ppm.

"Preliminary inhalation toxicity studies in mice were used as a basis for dose selection for the chronic studies of B6C3F1 mice, conducted at Battelle Pacific Northwest Laboratories and sponsored by the NTP (1984). Further details of the chronic exposure and the results with regard to the carcinogenicity of 1,3-butadiene are presented in the section 3.5 of this document. Two range-finding studies, a 15-day and a 14-week study, were conducted at International Bio-Test Laboratories. In the 15-day study, weight loss at airborne concentrations of 1,250 ppm was observed. Even the mice exposed to 8,000 ppm, the highest airborne concentration, survived the exposure period. In the 14-week study, reduced body weight and death were observed among mice treated at 2,500 ppm or more. Necropsy findings were not reported (NTP 1984).

"Miller (1978) reviewed a series of papers from Russian investigators, particularly Ripp (1967), and summarized the subchronic toxic effects in rats. Ripp (1967) exposed rats to airborne 1,3-butadiene concentrations of 0.45 ppm, 1.4 ppm, 13.5 ppm (1, 3, and 30 mg/m^3 where 1 mg/m^3 = 0.45 ppm). The highest concentration in this study (equivalent to 13.5 ppm) is only about 1/50 of the lowest concentration (600 ppm) in any of the other studies reported in this section. At 13.5 ppm, blood cholinesterase was elevated, blood pressure was lowered, and motor activity was decreased to 60% of the pre-exposure rate. Histopathological evaluation revealed no changes at 0.45 ppm except for congestion in the spleen and hyperemia and leukocyte infiltration in the cardiac tissue. The changes in the cardiac tissues were more marked at the higher levels with hemorrhage and reduced cellular RNA reported at the highest concentration. At 1.4 and 13.5 ppm, atelectasis, interstitial pneumonia, and emphysema were noted in the lung tissue. These results, showing an adverse response at such low levels, may indicate the continuous exposure is more hazardous than intermittent exposure, the regimen used in all other investigations. An alternative explanation for these findings at such low levels is that Ripp (1967) examined more sensitive indicators of toxicity than other investigators.

"A 2-year chronic inhalation toxicity study of the effects of airborne concentrations (1,000 and 8,000 ppm) of 1,3 butadiene on rats was conducted at Hazleton Laboratories Europe, Ltd. in England (HLE, 1981a) and was sponsored by the IISRP. Among rats exposed to 8,000 ppm, clinical signs, consisting of excessive secretion from the eyes and nose plus slight ataxia, were observed between months 2 and 5 of the study. Variations in mean body weight suggested no consistent adverse effect. Review of the hemograms, blood chemistry, urine analysis, and behavioral testing likewise did not indicate an adverse effect. In female rats exposed to either 1,000 or 8,000 ppm, subcutaneous masses appeared earlier and at higher incidences than in the control. A dose-related increase in liver weights was observed among rats at the necropsy performed at 52 weeks and among those killed at the termination of the study. This could indicate that the chemical induces liver enzymes. Otherwise, no significant changes were noted at the 52-week kill. Increased alveolar metaplasia and nephropathy were observed among males of the 8,000 ppm treatment groups at the termination of the study. Marked or severe nephropathy occurred in 27% of the male rats in the high-dose group as compared with 9% to 10% in the control and the low-dose groups. The author considered nephropathy to be the cause of some of the early deaths in this study.

"A lifetime chronic inhalation study in B6C3F1 mice at 1,3-butadiene concentrations of 625 and 1,250 ppm administered for 6 hours/day, 5 days per week, was sponsored by the NTP (1984). The exposures were prematurely terminated after 61 weeks due to deaths resulting largely from the development of cancer. No increases in clinical signs could be associated with exposure to the test chemical. In addition to the neoplastic changes described in the carcinogenicity chapter, testicular atrophy (control-0/50, 625 ppm-19/47, 1,250 ppm-11/48) and ovarian atrophy (2/49, 40/45, 40/48) were elevated among mice at both doses. Furthermore, among male mice, there was a significant increase in liver necrosis at both doses. In female mice, liver necrosis was significantly elevated only at the higher concentration. While neoplastic lesions of the nasal cavity were not found at any dose, there was an increase in non-neoplastic changes at the high-dose. At 1,250 ppm, chronic inflammation of the nasal cavity (male, 33/50; female, 2/49), fibrosis (male

35/50; female, 2/44), cartilaginous metaplasia (male, 16/50; female 1/49), osseous metaplasia (male, 11/50; female, 2/49), and atrophy of the sensory epithelium (male, 32/50) were observed. No non-neoplastic lesions of the nasal cavity were found in the controls (NTP, 1984). Huff et al. (1985) have suggested that the lack of neoplasms in the nasal cavity as compared to the lungs may reflect a requirement for biotransformation of 1,3-butadiene to a reactive epoxide metabolite. The nasal cavity changes then suggest that the intact molecule may have some adverse effects at 1,250 ppm. However, as discussed in the metabolism and carcinogenicity chapters, exposure to 1,3-butadiene did not decrease minute volume, as occurs with other respiratory irritants.

"In summary, since a no-effect dose has not been established for the non-carcinogenic chronic toxicity, further investigations are warranted. These investigations should focus on the liver, testes, and ovaries because in mice these tissues are adversely altered at the lowest concentrations. In addition, the minimum effect dose for cardiac and respiratory tract changes needs to be further explored. For a complete understanding of the toxicity, non-rodent species might be used."

3.3 Reproductive and Developmental Toxicity

3.3.1 Reproductive Effects

An NTP-sponsored sperm morphology study was conducted in B6C3F1 mice following a 5-day inhalation exposure to 1,3-butadiene at 0, 200, 1000, or 5000 ppm, 6 hours/day (Hackett et al., 1988b; Morrissey et al., 1990). Mice were sacrificed 5 weeks after the exposure period and sperm suspensions obtained from the cauda epididymis. The 5-day exposure period to 1,3-butadiene increased the percentage of abnormal sperm heads in a dose-related fashion: 0 ppm, 1.61 $\pm$ 0.38; 200 ppm, 1.95 $\pm$ 0.11; 1000 ppm, 2.79 $\pm$ 0.18; and 5000 ppm, 3.69 $\pm$ 0.42. The values were significantly greater than the control group for both the 1000 and 5000 ppm groups ($p<0.05$). These abnormalities occurred in the absence of any effect on body weight during the 5-week post-exposure period. The collection of sperm at only one post-exposure time period precludes any statements regarding the temporal effects of 1,3-butadiene on sperm development. However, results at 5-weeks post-exposure indicate that late spermatogonia and/or early spermatocytes were sensitive to the effects of 1,3-butadiene. A conclusion as to the reproductive consequences of these abnormalities cannot be made from this study.

The NTP also sponsored a dominant lethal assay in Swiss (CD-1) mice (Hackett et al., 1988a; Morrissey et al., 1990). Males were exposed to 0, 200, 1000 or 5000 ppm BD for 5 days, 6 h/day, then mated to several pairs of females, a new pair each week for 8 weeks. No mortality or changes in body weight were observed in the males. Statistically significant adverse effects were seen in matings from the first 2 weeks postexposure; none were seen thereafter. With the week 1 matings, there was a significant increase in the ratio of dead implants (resorptions) to total implantations in the 1000 ppm group; increases in the other dose groups were not statistically significant. The number of females with 2 or more dead implants was significantly higher in the 200 and 1000 ppm groups, and the number of dead implants per pregnancy was significantly elevated in the 1,000 and 5,000 ppm groups. With the week 2 matings, the number of dead implants per pregnancy was significantly elevated in the 200 and 1000 ppm groups.

BD's effects in this assay were most pronounced with 1000 ppm exposure; no steadily increasing dose-response relationship was observed. This may reflect a biological phenomenon, such as induction of detoxifying enzymes at the high dose level, or random variation within the experiment. These findings signal that BD may have a mutagenic effect in male germ cells, particularly more mature cells (spermatozoa and spermatids) (Morrissey et al., 1990). It is important to note that such a mutagenic effect might not be characterized by a dose threshold below which we would not expect the effect to occur. No NOEL was established by this assay.

In 2-year inhalation studies mice were exposed to 0, 6.25, 20, 62.5, 200, and 625 ppm BD (Huff et al., 1985; Melnick et al., 1988, 1989; Melnick, Huff and Miller, 1989; Miller, 1989; Miller, Melnick and Boorman, 1989). The studies were designed as cancer bioassays and are reported in greater detail in section 3.5. Gonadal atrophy was observed at a high incidence in exposed animals of both sexes at levels of 200 ppm and above, but not in any of the control animals. In the later study, using the entire dose range, levels of 6.25 ppm and higher also produced gonadal atrophy in females. Thus, a NOAEL was not established in these studies, but a LOAEL of 6.25 ppm was observed. In contrast, the Hazleton rat bioassay (HLE, 1981a) did not report any reproductive effects even at the 8000 ppm level.

3.3.2 Developmental Toxicity

The earliest reproductive study reported on BD was conducted by Carpenter et al. (1944). In this study male and female rats were exposed to 600, 2300, or 6700 ppm 1,3-butadiene, 7.5 hours/day, 6 days/week, for an 8-month period. Although this study was not specifically designed as a reproductive study, the fertility and the number of progeny were recorded for the rats. No significant effects due to BD exposure were noted on either the number of litters per female or on the number of pups per litter.

In a teratology study conducted by Hazleton Laboratories, Europe (1981), female Sprague-Dawley rats were exposed for 6 hours/day to 0, 200, 1000, and 8000 ppm 1,3-butadiene from 6 to 15 days of gestation (dg). Dams exhibited a significant exposure-correlated reduction in weight gain during the exposure period, and reductions were significant for the two highest exposure concentrations. The number of fetuses per litter and the number of live fetuses per litter were not affected. Post-implantation loss was increased slightly, but not significantly, and not in an exposure-related fashion. There was a significant reduction in fetal body weight for the 8000 ppm group, and a significant increase in the incidence of fetal variations in the 200 ppm group, while exposure to 8000 ppm increased the incidence of hematomas and major skeletal defects. The authors concluded that the fetal response was not indicative of a teratogenic effect, but was a result of maternal toxicity.

The potential for the inhalation of BD during gestation to cause developmental toxicity was further assessed in Sprague-Dawley rats (Hackett et al. 1987a) and Swiss (CD-1) mice (Hackett et al. 1987b). Dams were exposed to 0, 40, 200, or 1000-ppm 1,3-butadiene on 6-15 dg, 6 hours/day, and killed prior to parturition (e.g., rats on 20 dg and mice on 18 dg). Exposure to these concentrations of BD did not result in significant maternal toxicity in the rat, with the exception of a reduction in the extra-gestational weight gain for the 1000 ppm dams ($p<0.05$). There were no effects on the reproduction

indices (e.g., number of live fetuses per litter or the number of intrauterine deaths), on fetal body weights, or on the incidence of malformations, variations, and reduced ossifications. Under these exposure conditions there was no evidence of developmental toxicity due to BD exposures in the Sprague-Dawley rat. The results of this study are basically in agreement with those reported by Hazleton Laboratories, Europe (1981).

Exposure to these concentrations of BD did not result in significant maternal toxicity in the mouse, with the exception of a reduction in the extra-gestational weight gain for the 200 and 1000 ppm dams ($p<0.05$). There were no effects on the reproduction indices (e.g. number of live fetuses per litter or the number of intrauterine deaths), although there was a concentration-related, statistically significant reduction in fetal body weights for both sexes. There was no exposure-related increase in the incidence of fetal malformations; however, the incidence of reduced ossifications was increased in the 200- and 1000-ppm groups ($p<0.05$). The increased incidence of reduced ossifications and the fetal weight reductions in the absence of apparent maternal toxicity in the 40- and 200-ppm groups is evidence of fetotoxicity, but not teratogenicity, in the Swiss (CD-1) mouse.

3.4 Genetic Toxicity

3.4.1 Mutagenicity

1,3-Butadiene was originally reported to be a direct-acting mutagen to *Salmonella typhimurium* strains TA1530 and TA1535 which had been grown in an atmosphere of 70% BD (de Meester et al., 1978). However, later reports by the same group did not confirm their earlier efforts. BD was later found to be mutagenic to strains TA1530 and TA1538 (strains susceptible to base-pair substitution mutations), but only in the presence of the supernatant (S-9) fraction of rat liver homogenate (de Meester et al,. 1980; de Meester, Mercier and Poncelet, 1981). Furthermore, mutagenic activity was only significant when the S-9 mix was obtained from rats previously exposed to phenobarbital or Arcolor 1254, both cytochrome P450 monooxygenase inducers. BD was not mutagenic in the presence of S-9 mix from uninduced rats, nor was it mutagenic to strains TA100, TA98, or TA1538 (strains susceptible to frame-shift mutations) either in the presence or the absence of S-9 mix. Interestingly, the addition of cytochrome P450-dependent mixed-function-oxidase system inhibitors did not alter the mutagenic activity of BD to TA1530 (de Meester, Mercier, and Poncelet, 1981). This is in contrast to the results of Malvoisin et al. (1979) who found SKF 525A, a cytochrome-P450 inhibitor, to strongly inhibit the formation of BMO, the putative proximate mutagen (see below). The addition of glutathione or N-acetyl cysteine, deactivators of electrophilic intermediates, strongly decreased the mutagenic activity of BD, presumably due to the removal of BMO. Butadiene was also mutagenic when the S-9 preparation was placed in a separate petri dish within the same closed exposure container, thus indicating that the S-9 mix metabolized BD to a volatile, mutagenic metabolite. 1,2-Epoxy-3-butene (BMO), the first metabolite of BD (Malvoisin et al., 1979), was found to be a direct-acting mutagen. *S. typhimurium* strains TA1530 and TA1535 were the most sensitive, followed by strain TA100 (de Meester et al., 1980). The activity of 1,2-epoxy-3-butene in these strains, and the absence of activity in strains TA1537, TA1538 and TA98, indicated that DNA base-pair substitutions were probably involved in the mutagenic action.

Another butadiene metabolite, 1,2:3,4-diepoxybutane (DEB), has been shown to be a potent mutagen in several systems: the *S. typhimurium* mammalian microsome assay (McCann et al., 1975; Gervasi et al., 1985), assays employing the fungi *Neurospora crassa* and *Saccharomyces cerevisiae* (Olzewska and Kilbey, 1975; Polakowska and Putrament, 1979; Zuk et al., 1980), and in Luria and Delbruck's fluctuation test with *Klebsiella pneumoniae* (Voogd, Stel and Jacobs, 1981). Interestingly, in *Saccharomyces cerevisiae* nuclear, but not mitochondrial, DNA was affected (Polakowska and Putrament, 1979). A suitable explanation of this phenomenon was not presented.

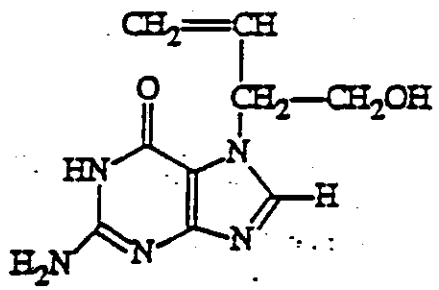
In addition to its mutagenicity, DEB has been shown to activate the enzyme guanylate cyclase which catalyzes the formation of the nucleotide guanosine 3'5'-monophosphate (cyclic GMP) (Vesely and Levey, 1978). Experiments were performed on the supernatant of a centrifuged (37,000 g) homogenate of various male Sprague-Dawley rat tissues and an increase in guanylate cyclase activity was observed in all tissues examined: bladder > kidney > heart > liver > spleen > stomach > pancreas > colon. The activation of guanylate cyclase may play a role in the regulation of cell growth and differentiation.

3.4.2 DNA Alkylation

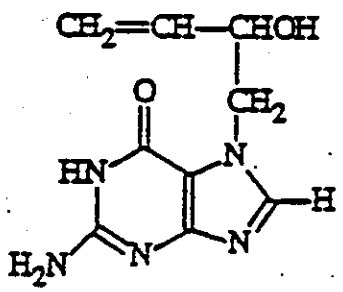
A considerable amount of research has gone into identifying the details of the interaction of these direct-acting mutagenic epoxides with DNA, particularly with respect to the alkylation of the N-7 site of deoxyguanosine. The capabilities of the two BD metabolites, BMO and DEB, to alkylate the N-7 site of the guanine moiety of DNA have long been known and have been studied extensively (Brooks and Lawley, 1961a, 1961b).

In a recent account, Gervasi et al. (1985) examined the correlation between the direct-acting mutagenic activity and the alkylating ability toward the N-heteroatom of nicotinamide of known and possible epoxide intermediates of BD metabolism. Of the three epoxides studied, BMO, DEB, and 1,2-epoxybutane, DEB was the strongest mutagen toward *S. typhimurium* strain TA100 followed by BMO, and finally 1,2-epoxybutane. 1,2-Epoxybutane has not been reported as a metabolite of 1,3-butadiene either *in vivo* or *in vitro*. None of the epoxides demonstrated mutagenic activity in strain TA98, used to detect frame-shift mutations. The relative N-alkylation rates of these compounds toward nicotinamide closely followed the order of their mutagenic activities. These compounds have been shown to alkylate the N-heteroatom through a mixed SN_2/SN_1 nucleophilic substitution mechanism, primarily SN_2 (Ehrenberg and Hussain, 1981).

The *in vitro* interactions of BMO with the N-heteroatom of guanosine (GUO), deoxyguanosine (d-GUO), or DNA were studied by Citti et al. (1983). When BMO was reacted with GUO in acetic acid at 50°C, followed by acid catalyzed hydrolysis to break the N-9 glycosyl bonds, two adducts were identified, 7-(2-hydroxy-3-buten-1-yl) guanine (I) and 7-(1-hydroxy-3-buten-2-yl) guanine (II); the latter was the major product (Figure 2). Reaction of BMO with deoxyguanosine (d-GUO) or with the d-GUO moiety of intact DNA resulted in the formation of the same two adducts; adduct II was the major product in each case. Formation of the adducts appeared to proceed via an SN_2-type mechanism where attack by the nucleophilic N-7 heteroatom occurred at either the C-1 or C-2 carbon. Since a pure SN_2-type mechanism should have resulted in the formation of approximately equal amounts of each adduct, the production



ADDUCT I



ADDUCT II

Figure 2. Guanosine adducts.

of greater amounts of II than I led to the proposal of a mixed SN_1/SN_2-type mechanism (Citti et al., 1984). Studies on the depurination rates of the adducted DNA formed from reaction with 1,2-epoxy-3-butene determined that the rate of spontaneous depurination obeyed first order kinetics with adducts I and II being released at equal rates (Citti et al., 1984). The $t_{1/2}$ for this reaction was ≈50 hours. Thus, the mutagenicity of 1,2-epoxy-3-butene may result from the relative ease of depurination which could subsequently lead to the formation of apurinic sites in the DNA and eventually to strand breaks. These strand breaks are capable of causing *in vivo* replication blocks and provoking error prone repair which could result in mutations (Schaaper, Glickman, and Loeb, 1982).

The *in vivo* alkylation of DNA following inhalation exposure of male Wistar rats or B6C3F1 mice to 1,3-[1,4-^{14}C]-butadiene was shown by Kreiling, Laib, and Bolt (1986). Four rats or 24 mice were exposed to 1,3-butadiene (≈700 ppm maximum concentration) in a closed system until 98% of the radioactivity had been taken up (6.6 hours for rats; 4 hours for mice) and then killed 30 min later. Nucleoproteins and DNA were obtained from purified hepatic nuclei and analyzed for ^{14}C-content. The nucleoproteins of the mice were found to contain approximately two times as much radioactivity as the rat nucleoproteins, values which correlated with the relative metabolic rates for 1,3-butadiene previously observed in the two species (see above). Hepatic DNA was alkylated in both species as shown by the presence of ^{14}C in the DNA fractions; however, unlike alkylation of the nucleoproteins, alkylation levels for DNA were equivalent in both rats and mice. In a subsequent report 7-(1-hydroxy-3-buten-2-yl)guanine, a product of reaction of BMO with guanine, was identified in liver DNA of mice exposed to BD (Laib and Kreiling, 1987).

3.4.3 Sister-Chromatid Exchange and Chromosomal Aberrations

BMO was found to be a powerful inducer of *in vivo* chromosome damage in the C57BL/6 male mouse (Sharief et al., 1986). Thirty minutes after subcutaneous implantation of a 5-bromodeoxyuridine (BrdUrd) tablet to label DNA, mice were administered a single IP injection of 1,2-epoxy-3-butene in corn oil at the following doses: 0 (vehicle control), 25, 50, 100, or 150 mg/kg. Colchicine (2.2 mg/kg) was administered 22 hours after BrdUrd and animals were sacrificed 2 hours later. Three of the four animals in the highest dose group died; however, all animals in the lower dose groups survived and apparently did not exhibit overt symptoms of toxicity. (The 25, 50, and 100 mg/kg doses were referred to as "nontoxic" by the authors.) Bone marrow cells from exposed animals were examined for sister chromatid exchange (SCE) frequency in second division cells and for chromosomal aberrations (CA) in first division cells, and the mitotic indices (MI) were determined. Statistically significant and dose-related increases in the SCE and CA frequencies ($p<0.001$) were observed. The MI was found to be increased for the 25 and 50 mg/kg doses, approximately equal to controls for the 100 mg/kg dose, and extremely low for the one remaining animal in the 150 mg/kg group. Chromosome damage was evident at the lowest dose, 25 mg/kg, as an increase in both CA and SCE frequencies.

BMO has also been shown to be a direct-acting mutagen (see above); however, it cannot be determined from Sharief et al. (1986) whether it was the proximate mutagen, or whether it may have first been metabolized to DEB. DEB has been

reported to induce high levels of SCEs in mice following *in vivo* exposure to this bifunctional alkylating agent (Conner, Luo, and de Gotera 1983), and to induce SCEs and CAs in hamster cells *in vitro* (Dean and Hodson-Walker, 1979; Perry and Evans, 1975).

Marx et al. (1983) studied the ability of DEB to induce chromosomal breakage in cultured human bone marrow cells or lymphocytes. This capability has been used as a diagnostic tool to identify patients either homo- or heterozygous for Fanconi's anemia (FA). Diepoxybutane induces a significant and dose-related increase in the incidence of chromosomal breakage in primary cultures of both human bone marrow cells and lymphocytes (Marx et al., 1983) as well as DNA cross-linking (Lawley and Brooks, 1967). The degree of induction of chromosome breakage by DEB in lymphocytes from patients homozygous for FA was approximately three times greater than it was in control lymphocytes ($p<0.001$). It was also possible to identify patients that were heterozygous for FA with this assay, a finding in contrast to Cohen et al. (1982) who were unable to distinguish between heterozygous FA lymphocytes and control patients in long term lymphoid cell lines. Interestingly, it has been reported that diepoxybutane is ineffective in increasing the SCE frequency in peripheral lymphocytes from patients either homo- or heterozygous for FA, although it did increase SCE frequency in cells from control patients (Porfiro et al., 1983).

Diepoxybutane has also been shown to be genotoxic in the mei-9^a Drosophila chromosome breakage test as evidenced by induced chromosomal loss and partial chromosomal loss in the offspring of both excision repair deficient mei-9^a females and repair proficient females (Zimmering, 1983).

Diepoxybutane tested positive in three in vitro mammalian cell-transformation assays using Balb/3T3 cells, Syrian hamster embryo cells (SHEM), and Fischer 344 rat embryo cells infected with Rauscher murine leukemia virus (Dunkel et al., 1981). No exogenous enzyme-activating systems were added to the cultures and any activation of procarcinogens resulted from the innate metabolic capabilities of the cell lines. The lowest concentration of DEB in the culture medium that induced transformation was 0.008 μg/ml for Balb/3T3 cells, 0.05 μg/ml for SHEM cells, and 0.17 μg/ml for R-MuLV-RE cells. Induction of cell transformation at these low concentrations, plus the fact that it was positive in all three assays, made DEB one of the more potent carcinogens of the ≈45 compounds tested.

3.4.4 In Vivo Sudies Including Hematotoxicity

The potential role of DEB induced DNA cross-links and monoadducts in the induction of SCE in murine bone-marrow and alveolar macrophage cells *in vivo* was investigated by Conner, Luo, and de Gotera (1983). Swiss-Webster mice or C57B16 X DBA/2J (BDF_1) mice were administered 10 to 291 μmol/kg DEB by i.p. injection either just prior to, during, or after infusion with BrdUrd. BrdUrd infusion was followed by administration of 3.3 mg/kg colchicine and bone marrow cells and alveolar macrophages were harvested in 4 hours. The timing of administration of the DEB and BrdUrd was varied in order to evaluate the persistence of SCEs over successive cell cycles.

The dose-response studies, conducted in Swiss Webster mice, produced steep log dose-SCE response curves with significant increases in SCE frequency at all doses for both bone marrow cells and alveolar macrophages (Conner, Luo, and de Gotera, 1983). There were no significant differences between the responses of

hepatectomized and intact mice. Cytotoxicity, evident by a noticeable shift in the distribution of first, second, and third division treated cells relative to controls, was observed in only the two highest dose groups, Cellular SCE responses were similar in Swiss Webster and the BDF_1 mice. Studies on the persistence of SCEs over three cell cycles indicated that repair was rapid and that complete repair of SCE-inducing lesions (e.g. DNA adducts) occurred between the first and second post-treatment cell cycles. The presence or absence of DNA interstrand cross-links could not be determined from this study due to the rapid repair of the DEB induced lesions.

The effects on the immune response of male B6C3F1 mice following inhalation exposure to 1250 ppm 1,3-butadiene, 6 hours/day, 5 days/week for 6, 12, or 24 weeks were reported by Thurmond et al. (1986). Immune function assessment included immunopathology, antibody plaque-forming cell (PFC) response, cell surface marker determination, mitogen-stimulated lymphocyte proliferation, lymphocyte proliferation and cytotoxicity to alloantigens, and spontaneous cytotoxicity. The dose chosen did not cause changes in body weight with respect to controls during the exposure period; however, the same exposure regimen had been shown to result in macrocytic-megaloblastic anemia (Irons et al., 1986a) and in alterations in hematopoietic stem cell development (Liederman et al., 1986).

Thurmond et al. (1986) reported that after 6 weeks of exposure there was a decrease in the spleen to body weight ratio relative to control animals (20%; $p<0.05$), and assumed that the decrease was due to a 29% decrease in spleen cellularity ($p<0.01$). There was also evidence of significant extramedullary hematopoiesis in the spleens of BD-exposed mice, but the number of IgM antibody PFC/10^6 splenocytes remained unchanged. The number of T lymphocytes was reduced, and the numbers of cytotoxic and suppressor T cells appeared to be marginally reduced, although there was no reduction in the percentage of T-helper lymphocytes. These changes may not be biologically significant, but may only reflect a shift in the proportion of mature lymphocytes relative to hematopoietic precursor cells in the spleen. Extramedullary hematopoiesis may also be encountered following exposure to compounds that induce either hemolytic or megaloblastic changes and may have no direct correlation with leukemogenesis. The Ig-bearing B-lymphocyte population was unaffected by BD exposure.

There was an increase in spontaneous lymphocyte proliferation in both the mitogen assay and in mixed lymphocyte cultures, but mitogen responsiveness was reduced at both 6 and 12 weeks. These changes may have resulted from increased hematopoiesis since unpurified splenocytes were used in the assays, thereby reducing the relative proportion of mitogen-responsive lymphocytes. B-lymphocyte proliferation in response to lipopolysaccharide was also decreased. After 12 weeks of exposure there was a slight decrease in bone marrow cellularity which recovered to normal levels after 18 weeks of exposure. BD exposure did not alter the expression of natural killer (NK) cell activity.

Moderate histopathological changes were observed in the spleen and thymus after 24 weeks of exposure, the only time point examined. In the spleen there was evidence of erythroid hyperplasia and extramedullary hematopoiesis, and in the thymus a decrease in the number of cortical lymphocytes.

In summary, Thurmond et al. (1986) state that 1,3-butadiene does not appear to exert a direct or persistent immunomodulatory effect on functional end-stage cells, but may affect early hematopoietic or lymphoid precursor cells. No important functional deficits were detected in the immunological defense of exposed mice.

The ability of 1,3-butadiene exposure to induce genotoxic and cytotoxic damage in mouse bone marrow was evaluated in male B6C3F1 mice (4 to 6 weeks old) following inhalation exposure to 6.25, 62.5, and 625 ppm 1,3-butadiene, 6 hours/day, 5 days/week, for a total of 10 exposure days, 15 days total elapsed time (Tice et al., 1987). The endpoints examined and their respective responses were as follows: CA (chromosomal aberrations), increased; SCE (sister-chromatid exchanged frequencies), increased; AGT (average cell generation time), increased; MI (mitotic index), decreased; %PCE (percent polychromatic erythrocytes), increased; MN-PCE (number of micronucleated PCE), increased; MN-NCE (micronucleated normochromatic erythrocytes), increased.

All endpoints, with the exception of MI, were significantly affected by 625 ppm BD. The AGT and MN-PCE were significantly altered at 62.5 ppm BD. Longer term exposure (13 weeks) led to significant induction of MN-NCE in both male and female mice exposed to 6.25 ppm BD (Jauhar et al., 1988). Trend tests with increasing exposure concentration were statistically significant for alterations in all endpoints. The induction of SCE and the type of CA observed (predominantly chromatid-type breaks and exchanges) were consistent with the induction of DNA damage by an S-phase-dependent clastogen. The presence of micronuclei associated with the chromosomal aberrations suggests that the micronuclei result from chromosomal breakage; however, one of the BD metabolites could also be inducing lagging chromosomes in addition to, or instead of, clastogenic damage.

An early depression in the %PCE in bone marrow (after only 2 days of exposure) followed by a significant elevation in circulating %PCE after 15 days (10 days total exposure) suggests a compensatory response in the rate of erythropoiesis, probably extramedullary (Thurmond et al., 1986). However, the number of proliferating cells in the marrow as well as their rate of division remained significantly depressed. There was also a high correlation between the frequency of SCE and the number of MN-PCE.

The most sensitive endpoint for detecting the ability of BD to induce bone marrow toxicity in male B6C3F1 mice was the increase in SCE frequency, which was significantly greater at 6.25 ppm than in the controls. (Note that the current workplace TLV (threshold limit value) for 1,3-butadiene is 10 ppm [ACGIH, 1987]). The next most sensitive indicator was in the number of circulating MN-PCE, which were significantly increased at 62.5 ppm. In summary, Tice et al. (1987) report that 1,3-butadiene is genotoxic and cytotoxic to the bone marrow cells of male B6C3F1 mice subjected to multiple inhalation exposures.

Irons, Oshimura, and Barrett (1987) examined genotoxic endpoints in bone marrow, similar to those described above after exposing male B6C3F1 mice or NIH Swiss mice to a single 6-hour exposure of 1250 ppm BD. In concurrence with Tice et al. (1987), they found BD caused a high frequency of chromosomal aberrations and chromatid breaks as well as increased chromatid and iso-

chromatid gaps in both species. Both mouse strains tested were subject to BD-induced CAs.

The comparison between these two strains of mice with respect to bone marrow toxicity was significant since B6C3F1 male mice exhibited a high incidence of leukemia/lymphoma following 12 months of exposure to 1250 ppm BD (65%) while NIH Swiss mice exhibited a much lower incidence (14%) (Irons et al., 1988). One important difference between the strains is that the B6C3F1 strain, but not the NIH Swiss mouse, has an endogenous ecotropic retrovirus (MuLVL). This retrovirus may play a role in murine leukemogenesis.

Both strains of mice exhibit macrocytic anemia as a result of exposure to BD, a particularly striking feature of which is a large increase in the frequency of micronuclei in circulating peripheral erythrocytes (RBC) and their prominence in precursor cells in bone marrow. These results indicate that BD induces cytogenetic damage in murine bone marrow cells, and that this damage is independent of both endogenous retroviral background and leukemogenesis.

Altered stem cell development was assessed in 6 to 8 week old male B6C3F1 mice following exposure to 1250 ppm BD, 6 hours/day, 5 days/week, for either 6 or 30 weeks (Leiderman et al., 1986). Quantitative assessment of pluripotent stem cells was made using the spleen colony-forming assay (CFU-S). The differentiation of committed myeloid cells was made by enumerating the CFU of granulocyte/macrophage (CFU-GM), and the effects on hematopoiesis were assessed by long-term bone marrow culture. Neither the number of CFU-S nor CFU-GM were altered following 6 weeks of exposure to BD, although the colonies derived from treated animals were smaller in size. Large colonies were assumed to have arisen from mature pluripotent stem cells which differentiated upon stimulus, while the smaller colonies originated from the more primitive stem cells. There were no changes in bone marrow cellularity after 6 weeks of exposure.

The number of both CFU-S and CFU-GM were significantly decreased after 30 weeks of exposure. There was also a significant suppression in the number of CFU-GM in long-term bone marrow cultures after 14 days in culture relative to cultures from control animals; after 28 days of culture the situation was reversed and there was a significant increase in CFU-GM relative to control cultures. This alteration in the kinetics of stem cell proliferation in long-term cultures suggests a profound change in stem cell regulation, a shift in maturation or a delay in differentiation to the granulocyte/macrophage committed cell (Leiderman et al., 1986). A similar delay in stem cell maturation has been described for bone marrow cells cultured from preleukemic patients (Golde and Cline, 1973; Koeffler and Golde, 1978).

Exposure of male B6C3F1 mice to 1250 ppm BD, 6 hours/day, 6 days/week for 3, 6, 12, 18, or 24 weeks resulted in an increased incidence of macrocytic-megaloblastic anemia (Irons et al., 1986a). Peripheral hematology was examined after 6, 12, 18, and 24 weeks of exposure. The numbers of micronuclei and reticulocytes were assayed after 6 and 24 weeks, bone marrow cellularity after 3, 6, 12, and 18 weeks, and bone marrow cell cycle kinetics after 6 weeks of exposure.

Exposure to 1250 ppm BD caused no alteration in body weight gain during the exposure period. No biologically significant effects were observed on bone

marrow cellularity after 3, 6, 12, or 18 weeks of exposure, although there was a 58% increase in the proportion of cells in S phase, and an increase in the overall proliferative index after 6 weeks. Bone marrow cells did exhibit mild megaloblastic changes and numerous micronuclei. Significant differences in peripheral hematology were observed at all time points examined; the number of leukocytes and erythrocytes, the hemoglobin content, and the hematocrit were all reduced. There was no evidence of accelerated erythropoiesis, nor any increase in the number of polychromatic cells or of nucleated erythrocytes. However, there was a marked increase in the number of circulating micronucleated cells. The platelet count was unaffected.

These findings were considered consistent with megaloblastic anemia and were reported to be similar to those encountered in human preleukemic syndrome. The results suggest that 1,3-butadiene exposure interferes with normal bone marrow cell differentiation and/or DNA synthesis in replicating bone marrow cells of the B6C3F1 mouse. Irons and colleagues suggest that the observed changes in bone marrow indicate that, although the thymus may not represent the primary target organ for BD toxicity, alterations in thymocyte precursor cells in the bone marrow may play an essential role in the development of 1,3-butadiene-induced murine thymic lymphoma/leukemia.

An experiment to assess the potential for BD to cause macrocytic-megaloblastic anemia, similar to the study described above, was conducted in NIH Swiss mice (Irons et al., 1986b). The NIH Swiss mouse does not carry the endogenous type-C murine leukemia retrovirus (MuLV1) which may play a role in the induction of murine thymic lymphoma/leukemia in B6C3F1 mice by BD. Male Swiss mice, 4 to 6 weeks old, were exposed to 1250 ppm BD 6 hours/day, 5 days/week, for 6 weeks. This exposure regimen did not result in body weight changes in treated animals during the course of the experiment. Peripheral hematology reports after 6 weeks of exposure demonstrated treatment-related reductions in the number of erythrocytes and decreases in the hemoglobin and hematocrit parameters. There was no evidence of accelerated erythropoiesis nor an increase in the number of polychromatic or nucleated erythrocytes; however, there was a striking increase in the number of micronucleated erythrocytes. Evaluation of the bone marrow indicated a significant decrease in bone marrow cellularity with only mild megaloblastic changes.

The results obtained from NIH Swiss mice (Irons et al., 1986b) are entirely consistent with those described above for B6C3F1 mice (Irons et al., 1986a), thereby indicating that BD-induced bone marrow toxicity is independent of the endogenous ecotropic MuLV.

The potential for BD to induce sister-chromatid exchanges (SCEs) and micronuclei in bone marrow polychromatic erythrocytes (MN-PCEs) was assessed in male B6C3F1 mice and Sprague-Dawley rats exposed to 1,3-butadiene vapor at concentrations of 10 to 10,000 ppm, 6 hours/day on 2 consecutive days (Cunningham et al., 1986). Bone marrow was obtained 24 hours after the last exposure and examined for MN-PCEs and SCEs. Evidence of bone marrow toxicity, present as a decrease in the ratio of polychromatic erythrocytes to normochromatic erythrocytes, was observed in both rats and mice and was related to increasing exposure concentrations. Mortality was present in the 10,000 ppm group for mice, but no mortality was observed in rats. A significant concentration-related increase was observed in MN-PCEs in the mice starting at 100 ppm 1,3-butadiene. The percent MN-PCEs ranged from 0.44% at

100 ppm to 3.0% at 10,000 ppm while the value for control animals was 0.08%. No increases in MN-PCEs were observed at or below 50 ppm. No significant increase in MN-PCEs were found in the rats.

SCE frequencies in bone marrow cells were increased in mice, in a dose-related fashion, from 10 to 100 ppm, with the first significant increase observed at 100 ppm. However, in a second experiment where BD concentrations ranged from 200 to 10,000 ppm, sufficient second-division metaphases were not obtained for adequate analyses although there was evidence of elevated frequencies. SCE frequencies in rats were slightly, but not significantly elevated at 500 ppm. In the second set of experiments at 200 to 10,000 ppm no significant increases were observed in rats.

Results of these studies (Cunningham et al., 1986) indicate that 1,3-butadiene does not cause bone marrow toxicity in the rat, at least when exposures are of a short duration. However, it is curious that the SCE frequencies observed in the mice in this study did not exhibit a better dose-response relationship, especially since this has been demonstrated by others (Tice et al., 1987).

3.5 Carcinogenicity

The earliest reported study on the inhalation toxicology of 1,3-butadiene was conducted by Carpenter et al. (1944). In this study, rats, rabbits, and guinea pigs were exposed to 0, 600, 2300, and 6700 ppm 1,3-butadiene, 7.5 hours/day, 6 days/week, for 8 months. Although the data obtained from this study were limited with respect to blood cytology, fertility evaluation, and gross pathology, the authors reported that these BD exposures did not result in "significant progressive injury to small animals during the 8-month exposure period."

In a more recent inhalation study (Huff et al., 1985) male and female B6C3F1 mice (8 to 9 weeks old) were exposed to 0, 625, or 1250 ppm BD, 6 hours/day, 5 days/week, for 60 or 61 weeks. Although this study was intended to last 103 weeks, it was terminated at 60 or 61 weeks because of reduced survival in the BD exposed groups due to fatal tumors. Data from this publication are provided in Table 3-1.

The early deaths were primarily due to malignant neoplasms involving multiple organs. The total numbers of primary malignant and benign neoplasms per animal were much greater in the BD-exposed groups than in the control group. Primary tumors caused by exposure to BD at both exposure levels in both sexes included malignant lymphoma, hemangiosarcoma of heart, alveolar-bronchial neoplasms, and squamous cell carcinoma of the forestomach. Tumors observed in females only (in both exposure groups) were acinar cell carcinoma of mammary gland, granulosa cell neoplasm of the ovary, and hepatocellular neoplasms. Malignant lymphomas were observed in both sexes as early as week 20 and appeared to originate in the thymus; however, involvement of the spleen, lymph nodes, liver, lung, kidney, heart, pancreas, and stomach was common. Hemangiosarcomas of the heart were also a major cause of death in both exposure groups and both sexes. A significant dose-response relationship was observed for the lesions in females, but not in males. The absence of a dose-response in males was attributed to a lower survival rate for the high-dose males than for the low-dose males. Most heart lesions were encountered in

Table 3-1 Incidence of Primary Tumors in Mice Exposed to Butadiene for 60-61 weeks[a]

Site/Lesion	Sex	Nominal Dose (ppm) in Air		
		0	625	1250
Heart/Hemangiosarcoma	M	0/50[d]	16/49[c]	7/49[c]
	F	0/49[b]	11/48[c]	18/49[c]
Hematopoietic System Malignant Lymphoma	M	0/50[b]	23/50[c]	29/50[c]
	F	1/50[b]	10/49[b]	10/49[c]
Lung/Alveolar and Bronchiolar Adenoma	M	2/50	12/49	11/49
	F	3/49	9/48	20/49
Lung/Alveolar and Bronchiolar Neoplasm	M	2/50[b]	14/49[c]	15/49[c]
	F	3/49[b]	12/48[c]	23/49[c]
Mammary/Acinar Cell Carcinoma	F	0/50[b]	2/49	6/49[e]
Ovary/Granulosa Cell Neoplasm	F	0/49[b]	6/45[c]	13/48[c]
Forestomach/Papilloma and Carcinoma	M	0/49	7/40[c]	1/44
	F	0/49[b]	5/42[e]	10/49[c]
Liver/Adenoma	F	0/50	1/47	4/49
Liver/Adenoma and Carcinoma	F	0/50[d]	2/47	5/49[e]

[a] Incidences based on EPA evaluation of NTP (1984) study.
[b] Increasing trend ($p < 0.01$).
[c] Increase compared to control ($p < 0.01$).
[d] Increasing trend ($p < 0.05$).
[e] Increase compared to control ($p < 0.05$).

early death animals after 40 weeks of exposure or in animals killed at the termination of the study. However, one hemangiosarcoma was diagnosed in a low-dose male after only 32 weeks of exposure. 1,3-Butadiene is one of two known animal carcinogens that induce vascular cardiac neoplasms; the other is the fungicide captafol (Ito et al., 1984). A high incidence of these rare neoplasms (23% in males and 30% in females) occurred at a relatively young age in BD exposed mice. The heart was regarded to be the primary site of the lesion and the lesions in liver, lung, and kidney were regarded as metastatic foci (Solleveld et al., 1988). Gonadal atrophy was also observed at a high incidence in exposed animals of both sexes, but not in any of the control animals. The sites of neoplastic development in these B6C3F1 mice were different from those observed for Sprague-Dawley rats (Owen et al., 1987).

Interim and final results have been published on a second 2-year inhalation study in B6C3F1 mice (Melnick et al., 1988, 1989; Melnick, Huff and Miller, 1989; Miller, Melnick and Boorman, 1989; Melnick et al., 1990). In this study, lower exposure concentrations of 1,3-butadiene (i.e. 0, 6.25, 20, 62.5, 200, and 625 ppm) were used than had been employed in the first study. Interim sacrifices at 40 and 65 weeks of exposure were also added to the original study design in order to follow progression of lesions. Four stop-exposure groups were also added; 50 male mice were exposed to 625 ppm for 13 or 26 weeks, 312 ppm for 52 weeks, or 200 ppm for 40 weeks, and then held for the remainder of the 2-year period. Lymphocytic lymphoma was the major cause of death in the 625 ppm group during the first 50 weeks of the study and appeared to limit the expression of neoplasms at other sites. For example, in male mice exposed to 625 ppm BD, the evidence of lymphocytic lymphomas was 70%, whereas, in male mice exposed to 200 ppm BD, the incidence of lymphocytic lymphomas was 3% and of hemangiosarcomas of the heart was 30%. In the 625 ppm, 13-week, stop-exposure group, lymphocytic lymphomas were induced (17/50) and the incidence was doubled (30/50) in mice exposed to 625 ppm for 26 weeks. However, if the exposure concentration was reduced by one-half to 312 ppm and the exposure duration doubled to 52 weeks, the incidence of lymphocytic lymphoma was less than for the 625 ppm, 26-week group. Thus, the dose rate appears to have a significant effect on carcinogenesis of butadiene. This may be important for occupational exposures, and interpretation of epidemiology based on occupational exposures. As in the previous study, hemangiosarcomas of the heart, squamous cell neoplasms of the forestomach, alveolar-bronchiolar neoplasms, and/or adenocarcinomas of the mammary gland were frequently observed in mice which died between weeks 40 and 65 of the study. In female mice exposed to 6.25 ppm BD, the incidence of alveolar-bronchiolar neoplasms was increased (15/60, 25%) vs. control (4/70, 6%). Also as in the previous study, gonadal atrophy was observed in both sexes at 200 ppm and 625 ppm for males and 6.25 ppm and higher for females. Bone marrow toxicity was evident as a poorly regenerative anemia at 62.5 ppm and higher. Thus this study did not establish a no effect level for reproductive endpoints. The 6.25 ppm nominal dose level might be considered a chronic LOAEL for reproductive toxicity. The published Melnick et al. (1990) study (Mouse II) is reproduced in the Appendix. The tumor incidence used in the quantitative assessment are given in Table 3-2.

Irons et al. (1986c) characterized BD-induced thymic lymphomas in B6C3F1 mice exposed to 1250 ppm BD for 28 to 45 weeks. The lymphomas consisted of a uniform proliferation of poorly differentiated lymphoblastic cells which

Table 3-2 Incidence of Primary Tumors in Mice Exposed to Butadiene for 2-Years (Melnick et al. 1990)[a]

Site/Lesion	Sex	Nominal Dose (ppm) in Air					
		0	6.25	20	62.5	200	625
Heart/hemangio-sarcroma	M	0/70	1/49	1/50	5/38	20/35	6/11
	F	0/70	0/50	0/50	1/33	20/31	26/31
Hematopoietic system/ All malignant lymphomas	M	4/50	3/50	8/42	11/44	9/33	69/71
	F	10/50	14/47	18/44	10/38	19/33	43/48
Lymphocytic lymphomas	M	2/50	1/50	2/40	4/40	2/29	62/65
	F	2/50	4/44	6/43	3/38	11/27	36/42
Lung/alveolar and bronchiolar neoplasm	M	22/48	23/48	20/44	33/46	42/48	12/16
	F	4/50	15/44	19/43	27/44	32/40	25/30
Forestomach/papilloma and carcinoma	M	1/70	0/50	1/60	5/38	12/33	13/17
	F	2/70	2/50	3/38	4/33	7/23	28/33
Ovary/granulosa cell neoplasm	F	1/69	0/59	0/59	9/38	11/25	6/14

[a]Figures adjusted for intercurrent mortality.

possessed surface markers indicative of early T lymphocytes and demonstrated variable, but elevated, levels of murine leukemia virus.

In an attempt to define the possible role of the endogenous retrovirus in BD toxicity, Irons et al. (1987) examined the expression and behavior of the retroviruses in male B6C3F1 and NIH Swiss mice during the preleukemic phase of BD exposure. Animals were exposed to 1250 ppm BD, 6 hours/day, 5 days/week, for 3 to 21 weeks. The spleen, thymus, and bone marrow tissues were evaluated for ecotropic murine leukemia virus (eMuLV) content after the first three weeks of exposure.

The results indicated that BD exposure did not qualitatively alter the pattern or types of retroviruses detectable in mouse tissues. However, there was an increase in the frequency of ecotropic virus-producing cells isolated from the bone marrow, thymus, and spleen of the B6C3F1 mouse. The greatest relative increase occurred in the spleen where virus-producing cells were quantifiable following only three weeks of exposure. The frequency of virus-producing cells in the spleen increased logarithmically over the 21-week exposure period. In contrast, no viruses were detected in the control of BD exposed NIH Swiss mice over the 21-week exposure period.

An attempt was then made to characterize the mechanism by which 1,3-butadiene enhanced the eMuLV present in the B6C3F1 mice by: 1) testing for generation of NB-tropic viruses which would allow escape of the virus from the *Fv-1* restriction gene which governs the spread and infection of murine leukemia viruses, and 2) by evaluating the possibility of abrogation of host *Fv-1* restriction (Irons et al., 1987). The authors concluded that the increases in eMuLV expression were probably not associated with intrinsic alterations in the host *Fv-1* restriction, and were not due to the emergence of an eMuLV which exhibited an altered host range. Therefore, the most likely conclusion is the *de novo* activation of endogenous sequences in many individual cells. The activation of the eMuLV may be due to alteration (e.g., methylation) of some gene regulatory site by one of the epoxide metabolites of 1,3-butadiene. Although the role of eMuLV activation in the etiology of BD-induced murine leukemogenesis is not clearly understood, these results provide presumptive evidence for eMuLV involvement. This presumption is supported by the fact that, although target organ toxicity is virtually identical during the preleukemic phase in both mouse strains, NIH Swiss mice, which lack the retrovirus, are not nearly as susceptible to BD-induced lymphomas as are the B6C3F1 mice, who carry the retrovirus (Irons et al., 1986a and 1986b).

Goodrow et al. (1990) examined the tumors generated in mice by inhalation of 6.25 to 625 ppm BD for 1-2 years for the presence of activated oncogenes. Activated K-ras genes were detected in 2 of the 11 lymphomas assayed. These authors concluded that the K-ras activation probably occurred as a result of genotoxic effects of BD or its metabolites. The oncogenes most frequently detected in human lung adenocarcinomas are K-ras genes. Activated K-ras genes have also been detected in some human lymphomas.

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Table 3-3 Incidence of Primary Tumors in Rats Exposed to Butadiene for 2-Years[a]

Site/Lesion	Sex	Nominal Dose (ppm) in Air		
		0	1,000	8,000
Mammary/Fibroadenoma	F	32	64	55
Carcinoma		18	15	26
TOTAL		50[b]	79[c]	81[c]
Thyroid/Follicular	F			
cell-Adenoma		0	2	10
carcinoma		0	2	1
TOTAL		0[b]	4	11[c]
Uterus/Cervical Stromal Sarcoma	F	1	4	5
Testis/Leydig Cell Adenoma/Carcinoma	M	0[d]	3	8[c]
Pancreas/Exocrine Adenoma	M	3[d]	1	10[e]
Zymbal Gland	M	1	1	1
Adenoma	F	0	0	0
Carcinoma	M	0	0	1
	F	0	0	4
TOTAL	M	1	1	2
	F	0[d]	0	4
Number of rats	M	100	100	100
examined	F	100	100	100

[a] Tumor incidences from Owen et al, 1987.
[b] Increasing trend ($p < 0.01$).
[c] Increase compared to control ($p < 0.01$).
[d] Increasing trend ($p < 0.05$).
[e] Increase compared to control ($p < 0.05$).

detected in human lung adeno carcinomas are K-ras genes. Activated K-ras genes have also been detected in some human lypphomas.

In addition to the mouse studies described above, several inhalation studies on 1,3-butadiene have been conducted in rats. In a study performed at Hazleton Laboratories in Europe, Sprague-Dawley rats (5 weeks old) were exposed to 0, 1000, or 8000 ppm BD, 6 hours/day, 5 days/week for 105 weeks for females and 111 weeks for males (Owen et al., 1987). Tables of data from this publication are summarized in Table 3-3.

A statistically significant relationship was observed between increased mortality and increasing exposure concentration during the second year of the study. There was a significant increase in liver to body weight ratios for low- and high-dose males during both years of the study, for low-dose females during the first year of the study, and for low- and high-dose females during the second year of the study. High-dose males had increased heart, lung, spleen, and kidney weight to body weight ratios during the second year of the study.

Treated rats also exhibited some types of tumors in larger numbers than did the controls animals. For males there was an increased incidence of pancreatic exocrine adenoma and Leydig-cell tumors in the high-dose group, and for females an exposure- related trend in uterine sarcoma and mammary gland tumors. Zymbal's gland carcinoma, confined to the high-dose females, exhibited a significant treatment-related trend. The incidence of mammary gland adenoma and carcinoma and of thyroid follicular-cell adenoma were increased to a similar extent in both treatment groups.

The total number of tumor bearing animals in this study was not greater for exposed animals, male or female, than it was for the 0 ppm group, nor were the number of treated males with multiple tumors greater than for control males. However, both exposure groups of females had a greater proportion (not statistically significant) of animals with multiple tumors than did the control group. A strong dose related trend in fatal multiple mammary tumors was noted for both adenomas ($p<.001$) and carcinomas ($p<.001$). This relationship of tumor incidence to BD exposure was expressed by the authors (Owen et al., 1987) as follows: "although the biological interpretation of the significance of some of the tumor types is equivocal, the evidence suggests that the test article is a weak oncogen to the rat."

Crouch et al. (1979) conducted another relatively short-term study in male and female Sprague-Dawley rats. The rats were exposed to 0, 1000, 2000, 4000, or 8000 ppm BD, 6 hours/day, 5 days/week, for up to 12 weeks. No statistically significant effects on body weight were observed during the course of the study. The authors reported that rats exposed to these concentrations of BD showed no treatment related untoward effects.

3.6 Cancer Epidemiology

Several studies have examined cancer mortality rates among industrial workers who were likely to have been exposed to butadiene. However, these studies generally considered workers likely to have had contemporaneous exposure to other potential carcinogens (most notably styrene). Nevertheless, studies of two work environments are sufficiently specific to butadiene exposure to

provide limited supporting evidence for the carcinogenic effects observed in animal bioassays. These studies are a case-control study of rubber workers by Matanoski et al. (1989) and cohort studies of a butadiene manufacturing plant by Downs et al. (1987) and Divine et al. (1990). In addition, Checkoway and Williams (1982) observed statistical associations of blood parameters with butadiene exposure at a facility where excess leukemia and lymphoma had been reported. Although the review below discusses these studies, the quantitative risk assessment presented in this document is based on bioassays in laboratory animals.

Investigators studying the relationship of 1,3-butadiene and cancer in humans face certain limitations. First, exposure must be measured indirectly for most of the cohort since personal monitoring data of workers did not begin until the mid-1970's. Second, changes in processing in styrene-butadiene rubber (SBR) plants after World War II (WWII) substantially reduced worker exposure such that workers from 1943 to 1945 (the earliest workers) were exposed to much higher levels of 1,3-butadiene than other workers. Studies of the petrochemical industry have found that WWII workers remained at their jobs for a much shorter time than other workers. Although earlier (WWII) butadiene-exposed workers were more heavily exposed, they are likely to have stayed at their jobs for less time than later workers. Thus, studies of earlier workers may be less likely than studies of later workers to detect a dose-response association if the studies use duration of employment as the measure of dose. In such studies, WWII workers should be examined separately. These factors should be considered when reviewing the epidemiological studies presented in this document.

3.6.1 Studies of Cancer Mortality

The first epidemiological study evaluating the possibility of an increased risk of carcinogenicity following occupational exposure to 1,3-butadiene and other compounds was conducted by the National Institute of Occupational Safety and Health (NIOSH). NIOSH was informed of five leukemia cases among workers in a Port Neches, Texas SBR plant in March 1976. That information and a study conducted at the University of North Carolina (Spirtas, 1976) prompted NIOSH to hold a meeting addressing the issue of styrene-butadiene exposures and a possible link to leukemia (NIOSH, 1976). Leukemia rates in the area surrounding the Port Neches plants were found to be above national rates. While the leukemia rate for males per 100,000 from 1950-1969 for the United States was 8.8, it was 9.2 for Texas, 10.7 for Orange County, Texas, 11.1 for Jefferson County, Texas, and 13.2 for Chambers County, Texas (Wagoner, 1976). The Port Neches plant mentioned above as well as several other SBR facilities are located in Orange County, Texas.

At the time of the NIOSH symposium in March, 1976, elevated leukemia rates were reported at two SBR plants in Texas, and elevated leukemia and lymphoma rates were reported at an Ohio SBR plant. There was also another plant where the rate appeared to be in excess of the total U.S. male population (Lloyd, 1976). SBR facilities do not actually produce 1,3-butadiene, but use it as a monomer in the styrene-butadiene polymerization process. Deaths at an adjacent 1,3-butadiene manufacturing facility were reviewed and no cases of leukemia were found (n = 122).

McMichael et al. (1976) examined deaths occurring from 1964 through 1973 in a male population which had been employed at a large tire manufacturing plant in

Akron, Ohio. Standardized mortality ratios (SMRs) for the study population indicated that deaths due to several types of cancer exceeded rates for the 1968 U.S. male population. Statistically significantly elevated SMRs were found for stomach cancers (171) and lymphatic and hematopoietic cancers (136). In order to obtain more detail, worker histories were reviewed and a series of occupational title groups (OTGs) were identified. A study design was used that required work histories for ≈25% of the entire cohort; then each case group of interest (e.g., a specific cancer type) was compared to an age-stratified random sample equivalent to 22% of the total study population. An odds ratio was computed relating risk of disease to work in a particular OTG for more than two or five years. This odds ratio was then used to estimate a relative risk. Results from workers in the synthetic plant OTG, where elastomers of SBR and other rubbers are synthesized, are reviewed here. Other solvents and monomers to which these workers were exposed included styrene, benzene, and toluene. Work for five years or more in the synthetic plant was associated with significantly elevated risk ratios for lymphatic and hematopoietic neoplasms (6.2), lymphatic leukemia (3.9), and stomach cancer (2.2). McMichael et al. also compared odds ratios for those with 2 versus 5 years work in a given OTG. The authors found an effect of job duration in many OTGs including the synthetic plant. The synthetic plant workers used in this analysis represent 2% of the study population.

Andjelkovich et al. (1976) and Andjelkovich et al. (1977) reported the mortality experience of 8,418 white male workers in a large rubber manufacturing plant, also in Akron, Ohio. They included in the analysis any man who was an active worker or a living retiree of the plant as of January 1, 1964, and at least 40 years old. Deaths from January 1964 to December 1973 were included in the analysis. The cohort was initially divided into two age groups, those under 65 and those 65 or older so that the cohort could be compared to the one analyzed by McMichael et al. (1976). SMRs were elevated (but not necessarily significantly) in both age groups in both cohorts for cancers of the stomach, large intestine, and prostate as well as for lymphosarcoma. The SMR for monocytic leukemia (311) was significantly elevated for this entire cohort. An OTG was then determined for each worker, and the data reanalyzed. SMRs (compared to U.S. population for all workers combined across OTGs) were recalculated for each OTG. Workers from the compounding and mixing (479) and milling (369) OTGs accounted for the observed excess in stomach cancer. Excess leukemia was found only in the general service OTG (246). Meinhardt et al. (1982) point out that this OTG probably has little 1,3-butadiene contact. The SMR for lung cancer was high (434) among synthetic latex workers, but not among the cohort as a whole. As in the McMichael cohort, synthetic latex workers had the highest likelihood of butadiene exposure.

Another cohort of 13,570 white males who had worked for ≥ 5 years in a Goodrich plant in Akron, Ohio was examined for mortality outcome from 1940-1976 (Monson and Fine, 1978). External comparisons (SMR) of mortality, based on U.S. white males, and internal comparisons of incidence were performed in this study. Leukemia and lymphatic cancers were elevated in a number of job categories as was the incidence of gastrointestinal cancer. Solvents were suggested by the authors to have been responsible for the increased incidences; the elevation cannot be specifically attributed to 1,3-butadiene. The authors did not divide this cohort into those who worked during World War II and those who did not. However, the report on the Akron cohort makes

reference to the fact that workers employed after 1945 did not have an excess incidence of leukemia.

In an investigation of the health effects of styrene exposure, Ott et al. (1980) studied 2,904 employees of Dow Chemical plants who had worked for at least one year over the years from 1937 to 1970. Plants in four different states were included in this study: Michigan, Texas, Connecticut, and California. SMRs for leukemia (176) and for lymphatic and hematopoietic neoplasms (132) were elevated but not significantly. In this analysis, only 391 of the workers studied were employed in styrene/butadiene latex manufacturing and there were no cases of leukemia observed in this group. OEHHA staff were not able to confirm that this OTG was the only one in which butadiene exposure occurred. Therefore, as with the previous studies (McMichael et al., 1976; Andjelkovich et al., 1976; Andjelkovich et al., 1977; Monson and Fine, 1978), this report is suggestive of an increase in incidence of lymphatic and hematopoietic cancers in a cohort associated with multiple types of chemical exposures. However, the increase cannot be definitely attributed to 1,3-butadiene exposure.

White male workers who had been employed for at least six months in two SBR plants in eastern Texas were studied for an excess of leukemia (Meinhardt et al., 1982) and an attempt was made to correlate the results to occupational chemical exposures. There were 1,662 study subjects in plant A and 1,094 in plant B. Workers were followed from 1943-1976 at plant A and from 1950-1976 at plant B. Since plant B did not include WWII workers, one might expect the SMRs from this cohort to be lower. In fact, there were no significantly elevated SMRs observed from workers in plant B. In plant A, SMRs were elevated but not significantly for lymphatic and hematopoietic neoplasms (155) and for several subcategories within that classification including leukemia (203). Among workers first employed from 1943 to 1945 (all from plant A), there were excesses of leukemia (278) as well as all lymphatic and hematopoietic tumors combined (212). The authors note that the excesses for early workers would have been significant using a one-tailed test. A nonsignificant elevation of lymphopoietic tumors was also observed among all workers when both plants were included in the analysis. All 5 of the qualifying cases and 3 of 6 nonqualifying cases had first been employed between 1943 and 1945, pointing to the possible increased risk of these workers. According to the authors, this study had the power to detect only a 4-fold or greater increase in risk. In a subsequent analysis, mortality was followed up to 1982 in plant A and 1981 in plant B. The same pattern of results was observed (Lemen et al., 1990).

In more recent studies, Matanoski and Schwartz (1987) and Matanoski et al. (1990) examined a cohort which consisted of 12,110 males with at least one year working in the SBR industry in the U.S. or 10 years working in an SBR plant in Canada. Deaths from each plant were originally followed until 1979 (Matanoski and Schwartz, 1987) but were subsequently followed to 1982 (Matanoski et al., 1990) The results from this most recent analysis are presented below. Follow-up began in different years for different plants. Only four plants had complete records back to 1943 and follow-up for one plant did not begin until 1970. All plants were included in the analysis. In this study, 75% of the cohort were white males. An additional nineteen percent of the cohort, in which data on race were missing, were assumed to be white. No SMR, calculated on the basis of U.S. males for specific causes of death for

all males, all plants, or all job types in the cohort, was significantly elevated.

The cohort was also subdivided by major work area and the SMRs recalculated based on the U.S. male population and race-specific rates where appropriate. In black production workers, SMRs for cancers of the hematologic system (507) and leukemia (656) were significantly elevated. There was also a significantly elevated SMR of 260 for other lymphomas among all production workers.

The authors offer two caveats to the findings among production workers. (1) Elevated SMRs in black production workers and the lack of significant SMRs among white production workers could have resulted from the bias of having racial information on all deaths but misclassification of race among living workers. This type of bias would inflate the white expected and deflate the black expected, resulting in higher SMRs for black workers and lower SMRs for white workers. (2) More than 2000 workers with incomplete work history were excluded from the analysis by job areas. Many of these were active workers in 1976 and therefore likely to be alive at the end of the follow-up period. The authors note that the higher percentage of deceased workers in this analysis could result in higher cause-specific ratios by work area than those seen in the total population.

The following factors should also be considered in interpreting the results: (1) there may have been as much as 32 years difference in the length of employment periods among workers (a mean employment period was not given); (2) the authors note that much of the population was still relatively young; (3) the probability of the study to find an effect may have been hampered by the exclusion of employees who worked for less than one year which may have excluded a greater proportion of war time workers. Follow up from four of eight plants did not include WWII workers.

To elucidate the role of 1,3-butadiene versus styrene in the cancers observed in the cohort study, Matanoski et al. (1989) conducted a nested case-control study. Lymphopoietic cancer cases were compared to workers who did not have cancer. A log transformation of exposure data was used to classify workers into high- and low-exposure categories. The leukemia cases were associated with exposure specifically to 1,3-butadiene (OR=9.4, 95% CI=2.1-22.9) but not to styrene. This study's results appear sensitive to the choice of exposure classification scheme. In addition, Acquavella (1989), in a review of butadiene epidemiology studies, notes that, since the original analysis of the cohort did not reveal a leukemia excess overall, the high odds ratio may have resulted from an exceptionally low leukemia rate among unexposed workers. However, since elevated rates of leukemia and other lymphatic and hematopoietic cancers have been observed consistently in other studies of SBR plants (Meinhardt et al., 1982) and in those parts of tire manufacturing plants in which SBR is synthesized, these results cannot be easily dismissed. This case-control study is also important in that it was able to assign different odds ratios for butadiene and styrene exposure.

Cause-specific mortality in a cohort of male workers employed from 1943 for at least six months in a 1,3-butadiene plant (Neches Butane, Port Neches, Texas) was assessed by Downs et al. (1987) and Divine (1990). Deaths were followed initially to 1979 and later to 1985. The results from the most recent studies

are presented below. Since work histories and historical industrial hygiene data were not available for this cohort, a qualitative scale for 1,3-butadiene exposure was constructed based on department codes. Four exposure groups (I-IV) based on the estimated frequency of exposure to 1,3-butadiene were defined for the cohort (n=2582): I - low exposure (very rare; n=433), II - routine exposure (daily; n=715), III - nonroutine exposure (intermittent; n=993), and IV - unknown exposure (varied; n=451).

This cohort was initially analyzed on the basis of race (black or white); however, it was later decided to treat all subjects as white and use death rates for U.S. white males in the final analyses. In the most recent study (Divine, 1990) the SMR for lympho- and reticulosarcoma (229) was significantly greater for the cohort than for U.S. white males. When the cohort was partitioned into a wartime cohort (>6 months employment prior to the end of 1945) and a postwar cohort (>6 months employment after 1945), the SMRs for lympho-hematopoietic cancers for those hired before 1946 were significantly elevated (SMR=269). SMRs were also calculated for the cohort after it had been subdivided into exposure groups I through IV. These calculations showed a significant increase in deaths from lymphosarcoma among those with routine exposure (SMR=561). All of the lymphosarcomas occurred in persons employed for less than ten years. No effect of latency was observed. The SMR for leukemia (SMR=102) in the total cohort was not elevated.

The authors note that the cohort has certain shortcomings, mainly an unreliable designation of race, a lack of worker histories, and a lack of industrial hygiene data; furthermore nearly half of the total cohort worked at the plant for less than 5 years. In addition, many individuals spent significant time working at the neighboring SBR plants, thus confounding the duration and type of chemical exposures. The cohort is also relatively small, especially when split into groups on the basis of exposure category. However, this cohort was employed specifically for the manufacture of butadiene, unlike cohorts in SBR plants and tire manufacturing plants where exposures to styrene were encountered. It is important to note that in this cohort associations with cancer were observed that are similar to those observed in cohorts with mixed exposures.

3.6.2 Cancer-Related Studies

Checkoway and Williams (1982) measured hematologic parameters of male workers employed in the SBR synthesizing plant at the Akron, Ohio, facility studied by McMichael et al. (1976). This study was part of a health and industrial hygiene survey initiated after McMichael et al. reported excess leukemia and lymphoma (see Section 3.6.1, above). Checkoway and Williams sought to quantify workplace exposures to styrene, butadiene, benzene, and toluene, and to relate these exposures to measurable hematologic parameters which may be indicative of bone marrow toxicity. Personal breathing zones of 163 workers were sampled, and blood was drawn from 154 workers. Based on medical history questionnaires, one worker who reported a history of leukemia was excluded from this study. The mean 8-hour time-weighted average concentrations of butadiene, styrene, benzene, and toluene were less than 2 ppm in all departments, with the exception of the "Tank Farm" area where the values were 20.03 ppm and 13.67 ppm for butadiene and styrene, respectively. There were statistically significant correlations of butadiene and styrene levels (considered separately) with red blood cell (negative correlation) and basophil (positive correlation) counts. After stepwise linear regression to

control for age and medical status, the positive association of basophil count with butadiene exposure and the negative association of red blood cell count with styrene exposure persisted, and there were significant findings regarding the two chemicals and corpuscular hemoglobin concentration (positive association for butadiene, negative for styrene). The reported associations explained very little of the variance in the blood parameters, however.

Checkoway and Williams (1982) divided their cohort into two populations, Tank Farm workers (n=8) and all others, in order to compare blood parameters between the subgroups with the greatest exposure differences. The investigators reported that the small number of Tank Farm workers precluded reliable statistical tests of significance. Nevertheless, increased band neutrophil and monocyte counts were apparent in the Tank Farm workers. Checkoway and Williams noted other differences, which they characterized as "small" or "slight." They found their results to be suggestive of possible biological effects of butadiene and styrene exposure (including cellular immaturity), with clinical consequences that are not readily apparent. Firm conclusions cannot be drawn from this study, however, as it did not control for smoking or other behavioral factors that may affect blood parameters, and was hampered by a small sample size and limited sampling protocol. It is important to note, however, that Checkoway and Williams (1982) found differing effects of styrene and butadiene exposure.

3.6.3 Interpretation of Human Butadiene Studies

Results from selected studies are presented in Table 3-4. In evaluating epidemiological studies of 1,3-butadiene, the following factors must be considered: (1) Misclassification of exposure; (2) Exclusion of most highly exposed workers; (3) No dose-response effect; (4) Varying health endpoints.

(1) Misclassification of exposure: Personal monitoring data for exposed workers did not begin until the mid-1970's. Therefore exposure must be inferred from job title, function, and duration. Jobs are categorized in different ways in each study. None of the cohort studies reviewed tested their exposure estimate methods against actual exposure data. Misclassification of unexposed as exposed would bias results toward the null.

In those studies with significant results, however, it is possible that the cancers are a result of some other compound which covaries with butadiene. The strongest evidence that the lymphopoietic and hematopoietic cancer excesses observed are from butadiene exposure is provided by the nested case-control study of Matanoski et al. (1989). In this study leukemia was significantly associated with butadiene and not styrene exposure.

(2) Exclusion of most highly exposed workers: Changes in SBR processing after World War II reduced worker exposure such that workers from 1943 to 1945 (the earliest workers) were exposed to much higher levels of 1,3-butadiene than other workers. Some cohorts have excluded WWII workers because of lack of records, or because plants were not yet in operation. If WWII workers are at greater risk, then those studies in which these workers were not included may be less likely to see significant effects. This was observed in the study conducted by Meinhardt et al. (1982) in which SMRs were only elevated in the plant with WWII workers, and it may have affected the results of Matanoski and Schwartz (1987) and Matanoski et al. (1990) where four of eight plants studied did not have WWII workers.

Table 3-4. Human Cancer Epidemiology Cohort Studies Related to Butadiene Occupational Exposures

Study, Size (n)	Cancer Sites	# Observations / population	Measure Used	Value	Notes
McMichael et al., 1976 (n=1,500)	Lymphatic and hematopoietic neoplasms	–	Risk Ratio	6.2 *	Values only reported for those in the synthetic latex plant, 2% of the study population.
	Lymphatic leukemia	–	Risk Ratio	3.9 *	Increases with duration worked.
	Stomach cancer	–	Risk Ratio	2.2 *	No excess of other cancers in this OTG.
Andjelkovich et al. 1977 (n=8,416)	Lung	3	Risk Ratio	4.3 *	Finding in synthetic latex workers.
Meinhardt et al, 1982 (n=1,662 Plant A n= 1,094 Plant B)	Lymphatic and hematopoietic neoplasms	9 (Plant A) 9 (A, early workers)	SMR SMR	155 212	Risks in early workers in plant A are significant when one-tail test is used.
	Leukemia	5 (A) 5 (A, early workers)	SMR SMR	203 278	
Matanoski et al., 1990 (n=12,110)	Lymphatic and hematopoietic neoplasms	48 (all) 7 (black) 6 (black production)	SMR SMR SMR	92 146 507 *	None of the cancer ratios was significant when the total cohort was analyzed nor when it was analyzed by race.
	Leukemia	3 (black production)	SMR	656 *	
	Other lymphomas	9 (all production)	SMR	260 *	
Divine et al, 1990 (n=2,582)	Lymphosarcoma	9	SMR	229 *	Butadiene manufacturing facility.
		7 (early workers) 5 (routine exposure)	SMR SMR	269 * 561 *	No effect of latency or duration.

* $p<0.05$

(3) No dose-response effect: Recent studies conducted at SBR or butadiene manufacturing plants have not detected an effect of duration of butadiene exposure (as measured by job or employment duration). Studies of workers in the petrochemical industry show that WWII workers remained at their jobs for a much shorter time than other workers (Wen et al., 1986). Since earlier workers were more heavily exposed, if (as in the petrochemical industry) their stay at work was much shorter, studies would be less likely to detect a dose-response effect by using duration of employment. There might be a subset of the worker population that quickly develops certain kinds of cancers when exposed to moderate levels of butadiene. This also might represent a real effect. Stop-exposure studies conducted by Melnick et al. (1990) in mice indicated that short-term high exposures to butadiene may result in greater tumor incidences than longer-term, lower exposures. In view of the data in animal studies, it is difficult to conclude that cancer among short-term exposed workers is due to confounding factors. McMichael et al. (1976) did note an increased risk in workers who worked in the synthetic plant for five versus two years.
(4) Varying health endpoints: Divine (1990) questioned the significance of an association of butadiene exposure with different and sometimes inconsistent subtypes of lymphatic and hematopoietic neoplasms. Matanoski et al. (1989) note that this apparent inconsistency may not be real since there are close interrelationships between many of these tumors. Landrigan (1990) observed that the diagnostic categories are overlapping and are also complicated by changes in nomenclature over time. Therefore in his review of the major 1,3-butadiene epidemiological studies, he states that the observed increases in all subtypes are probably related. Furthermore, 1,3-butadiene exposure has consistently caused cancers of the hematopoietic and lymphatic system in mice (although not in rats), thus lending support to the evidence that the lymphatic system may be a target organ for 1,3-butadiene in humans.

The evidence for an association between butadiene exposure and lymphatic and hematopoietic neoplasms is strengthened by several considerations. First, a strong effect was observed in the routinely exposed workers of the butadiene production plant cohort which presumably has the likeliest exposure to butadiene (and not styrene) of all cohorts studied. Second, the cancers observed in cohorts from SBR plants and the synthetic plants of tire manufacturing facilities are consistent with the findings from the cohort in the butadiene production facility. Third, the case-control study conducted by Matanoski et al. (1987) and the cellular study of Checkoway and Williams (1982) both attribute effects specifically to butadiene, independent of styrene exposure. Fourth, the cancers observed are consistent with those seen in mice in butadiene exposure studies.

In conclusion, the epidemiological studies reported to date give limited evidence for increased incidences of leukemia and/or lympho-hematopoietic neoplasms resulting from exposure to vapors in styrene-butadiene rubber plants or butadiene production plants. Butadiene production workers and those with routine exposure to butadiene have been at higher risk for these cancers. High levels of butadiene during WWII in certain facilities may have contributed to this excess risk, although elevated cancer rates specific to WWII workers have not been conclusively demonstrated. The evidence for elevated rates of stomach and lung cancer is not conclusive. Elevated stomach cancer and lung cancer rates were observed in some studies but not

consistently. Further studies could clarify the relationship between butadiene and these types of cancer.

4.0 QUANTITATIVE RISK ASSESSMENT

Bioassays of 1,3-butadiene (BD) in laboratory animals have provided data that may be used in quantitative assessment of BD's cancer risk to humans. Three bioassays have provided satisfactory data: two in mice and one in rats. The bioassay that provided the best data is the most recent of the two mouse assays (Melnick et al. 1990, or "mouse II"). Epidemiologic data adequate for use in quantitative risk assessment are not available.

4.1 Objectives and Limitations

The chief objective of this section is to derive a numerical value for the incremental cancer risk associated with anticipated lifetime exposures to ambient concentrations of BD. Initially an individual lifetime extra risk will be estimated. A unit risk value associated with lifetime exposure to 1 $\mu g/m^3$ of BD will also be derived.

Several subordinate objectives include:

a) defining a rationale for the mechanism of carcinogenicity;

b) evaluating the potency of BD and its metabolites at anticipated exposures in terms of response per unit dose with an appropriate high to low dose extrapolation model(s);

c) evaluating experimental applied doses in terms of the dose which was probably retained by target tissues and organs (internal dose), the dose which was metabolized to genotoxic metabolites (metabolized dose), and the dose which may have reacted with cellular DNA in selected target tissues (molecular dose);

d) extrapolating animal potency values to human values with appropriate scaling factors and/or other appropriate assumptions.

The limitations of these procedures include uncertainties regarding the applicability of the low dose extrapolation model, particularly where individual tumor site data do not readily fit the model parameters. Further uncertainties may be associated with the possibility of low dose thresholds for toxic metabolites, large intercurrent mortality corrections for excessive early deaths on study, excessively high doses in the mouse study which probably saturated metabolic systems, large interspecies differences in toxicokinetics of BD and metabolites (Laib et al., 1988), and possible cooperative or synergistic effects resulting from concurrent exposure to other air toxicants.

4.2 DOSE-RESPONSE RELATIONSHIP

4.2.1 Mechanism of Oncogenicity

A number of findings noted in Section 3 above bear on the mode of action of BD: the mutagenicity of BD in Salmonella *in vitro* with exogenous metabolic activation; clastogenic/DNA damaging activities in mammals *in vivo*; the direct mutagenic action of the two principal metabolites of BD, namely 1,2-epoxybutene (BMO) and 1,2:3,4-diepoxybutane (DEB), *in vitro*, and their detection in blood of experimental animals exposed to BD; the binding of BD (metabolites) to DNA; and the isolation and identification of DNA adducts.

These findings strongly suggest that genetic toxicity via gene mutation, chromosomal aberrations, and less specific damage to DNA represent at least one `mechanism' by which BD exerts its oncogenic action.

4.2.2 Dose Threshold

There are no data suggesting a practical threshold to the carcinogenic action of BD. In view of the probable mode of action (genetic toxicity) the effective dose for this carcinogen may be so low as to be indistinguishable from zero. While threshold mechanisms based on saturation of detoxification enzyme(s), DNA repair, cytotoxicity, and multiple stages in the carcinogenic process have been proposed, none has been convincingly shown to specifically affect the action of BD. For the purposes of this risk assessment it is assumed that no practical threshold exists.

4.2.3 Evaluation of Biological Data for Quantitative Risk Assessment

Quantitative estimations of human risk are usually based on lifetime animal studies and/or human studies showing excess cancer risk resulting from exposure to the agent in question. In the case of BD, while the data base of human cancer epidemiology, largely from occupational exposures, is indicative of possible carcinogenic impact on the lymphatic system, the data are insufficient for quantitative estimation. Therefore, this risk assessment will rely on lifetime cancer bioassays in experimental animals.

The NTP (1984) mouse inhalation study showed significant early increases in hemangiosarcomas of the heart and malignant lymphomas in both sexes (Huff et al., 1985). The Hazleton (1981) (Owen et al., 1987) rat inhalation study showed Leydig cell tumors, pancreatic exocrine tumors, and Zymbal gland carcinomas in males, and mammary gland carcinomas, thyroid follicular tumors, and Zymbal gland carcinomas in females. Both of these studies will be evaluated in the quantitative risk assessment presented below.

4.2.4 Estimation of Delivered Dose

4.2.4.1 Continuous Internal Dose

Both rodent bioassays employed a repetitive chronic exposure regimen of 6 hours/day x 5 days/week at measured external BD concentrations. In this subsection continuous internal dose estimates are derived for the various treatment levels in terms of mg per kg body weight per day. For the purposes of this risk assessment a 6 hours/day exposure results in a daily dose and is not averaged over 24 hours. The most relevant data currently available to estimate internal dose from applied external BD is that of Bond et al. (1986). These investigators exposed rats and mice to [^{14}C]-butadiene at concentrations ranging from 0.14 μg/L (75 ppb) to 13,000 μg/L (6964 ppm) and measured the quantities of inhaled [^{14}C]-BD equivalents retained after 6 hours exposure. These data are reproduced in Table 4-1 and show that mice retained approximately 5 to 10% of inhaled BD whereas rats retained approximately 1.5 to 3% at the doses tested. To estimate doses in mg/kg-d the higher applied doses ($\log_{10}$ ppm) are linearly regressed against retained BD ($\log_{10}$ μg/kg-d). For the mouse data the following relation was obtained:

Table 4-1 Respiratory Tract Retention of Inhaled Butadiene in Rats and Mice[a]

Exposure[b] Concentration (ppm)	Total Volume Inhaled/Animal(L)[c]		Butadiene Inhaled/Animal (umol)		[^{14}C]Butadiene Equivalents Retained at 6 hr/Animal (umol)[d]		Percentage of Inhaled [^{14}C]Butadiene Equivalents Retained at 6 hr	
	Rats	Mice	Rats	Mice	Rats	Mice	Rats	Mice
0.075	73 ± 5	14 ± 2	0.2 ± 0.01	0.04 ± 0.002	0.03 ± 0.002	0.006 ± 0.001	17 ± 2[i]	16 ± 1[i]
0.75	90 ± 4	12 ± 2	2 ± 0.1	0.3 ± 0.06	0.13 ± 0.003	0.06 ± 0.01	6 ± 0.3[h]	20 ± 5
7	83 ± 7	12 ± 1[e]	19 ± 2	3 ± 0.4[e]	0.8 ± 0.1	0.4 ± 0.009[e]	4 ± 0.5	20 ± 5[e]
70	70 ± 8[f]	13 ± 2	170 ± 20[f]	35 ± 5	17 ± 2[f]	3.2 ± 0.1	8 ± 1[f]	8 ± 0.7
1000	100 ± 10	12 ± 2	3100 ± 360	440 ± 80	65 ± 5	19 ± 0.5	2.5 ± 0.2[h]	4 ± 0.3
7000	70 ± 15	g	17000 ± 3100	g	240 ± 40	g	1.5 ± 0.1	g

[a]Values are the mean ± SE.
[b]Concentration expressed in ppm were calculated from reported exposures of 0.14, 1.4, 13, 130, 1800 and 13,000 ug/L.
[c]Values corrected for rat and mouse body temperature, barometric pressure, inspired gas temperature, and relative humidity.
[d]Data obtained from radioanalysis of rats and mice which were maintained in plethysmograph tubes during the 6 hour exposure.
[e]Individual values pooled from three separate experiments.
[f]Individual values pooled from two separate experiments.
[g]Mice were not exposed to this concentration of butadiene.
[h]Significantly different ($p < 0.05$) from mice exposed to the same butadiene concentration using Student's t test.
[i]A linear regression analysis of the log transformed data vs the log of the exposure concentration indicated that there was a significant ($p < 0.001$) concentration-related decrease in the precent retained at 6 hour with increasing exposure concentration.

Source: Bond et al., 1986

$$\log_{10}(\mu g/kg\text{-}d) = 0.68 \log_{10} (ppm) + 2.51, R = 1.00$$

Using this relation the bioassay measured applied doses are converted to internal doses:

627 ppm = 25800 μg/kg-d = 25.8 mg/kg-d
1236 ppm = 41000 μg/kg-d = 41.0 mg/kg-d

These doses were then adjusted from 5 days/week to a continuous (7 days/week) scenario.

$$25.8 \text{ mg/kg-d} \times \frac{5 \text{ days}}{7 \text{ days}} = 18.4 \ \mu g/kg\text{-}d$$

$$41.0 \text{ mg/kg-d} \times \frac{5 \text{ days}}{7 \text{ days}} = 29.3 \ \mu g/kg\text{-}d$$

Similarly for the rat:

$$\log_{10}(\mu g/kg\text{-}d) = 0.60 \log_{10} (ppm) + 2.22, R = 1.00$$

999 ppm = 10470 μg/kg-d = 10.47 mg/kg-d
7886 ppm = 36150 μg/kg-d = 36.15 mg/kg-d

$$10.47 \text{ mg/kg-d} \times \frac{5 \text{ days}}{7 \text{ days}} = 7.5 \text{ mg/kg-d}$$

$$36.15 \text{ mg/kg-d} \times \frac{5 \text{ days}}{7 \text{ days}} = 25.8 \text{ mg/kg-d}$$

These continuous internal doses, as well as the measured experimental doses, will be employed in low dose extrapolation models to assess the BD potencies or slope factors at environmentally relevant exposures for various oncogenic endpoints.

4.2.4.2 Metabolized Dose

Since the internal doses calculated from the data of Bond et al. (1986) are based only on the amount of ^{14}C-radiolabel retained at the end of the 6-hour exposure and do not take into account any butadiene metabolites that were absorbed and exhaled during the exposure period, Hattis and Wasson (1987) have employed a pharmacokinetic modeling approach to estimate internal exposure to the chief metabolite of butadiene, namely BMO. The modeling, based largely on partition coefficients and Michaelis-Menten enzyme kinetics, including many measured parameters for BD, does take several additional factors into account and estimates delivered or metabolized doses for the applied doses used in the cancer bioassay studies. Of course, only BMO is considered and the possible influence of DEB or other epoxide metabolites is not addressed. Also, it is important to point out that the pharmacokinetic modeling is not extended to predict human doses, since no appropriate human pharmacokinetic data on BD were available to serve as a guide. Of course the model can be used to estimate human exposure if one employs various scaled parameters and makes assumptions about BD metabolism and ambient exposure.

The model employed by Hattis and Wasson (1987) was based somewhat on that of Ramsey and Andersen (1984) and Fiserova-Bergerova (1983). The body is divided into a number of compartments representing organs with similar ratios of blood flow to tissue volume. Each compartment is represented as a well mixed pool. Three compartments are considered:

- o Liver and Vessel-Rich Group (LVRG) containing liver, brain, kidney, heart, adrenal, and thyroid tissues as well as additional small viscera,
- o Muscle Group (MG) containing the lean body tissue: muscle, skin, and tongue; and
- o Fat Group (FG) containing perirenal and subcutaneous fat and the bone marrow.

The concentration of BD in blood leaving each compartment is assumed to have reached equilibrium with the concentration of BD in the compartment. The following expression relates the increase or decrease in BD stored in each compartment:

$$\frac{d(BD)}{dt} \text{ (moles/min)} = Q_{tissue} \; [BD]_{art} - \frac{Q_{tissue} \; [BD]_{tissue}}{V_{tissue} \; L_{tissue/blood}} - M$$

where:

Q_{tissue} = rate of blood flow to the compartment in L/min
$[BD]_{art}$ and $[BD]_{tissue}$ = butadiene concentrations in arterial blood and tissue

e.g., $= Q_{tissue} \; [BD]_{art} - \frac{Q_{tissue} \; [BD]_{tissue}}{V_{tissue} \; L_{tissue/blood}} - M$

$Q_{tissue} \; [BD]_{art}$ = input from arterial circulation

V_{tissue} = volume of the compartment
$L_{tissue/blood}$ = equilibrium ratio of tissue and blood butadiene concentrations
$Q_{tissue} \; [BD]_{tissue}/V_{tissue} \; L_{tissue/blood}$ = output via venous blood
M = metabolism

The values for tissue volumes and flows were based on 400 g rats and 28 g mice. The metabolism term which was included only for LVRG is a simple Michaelis-Menten expression:

$$M = V_{max} \; [BD]_{tissue}/(K_m + [BD]_{tissue}).$$

V_{max} is the maximum reaction velocity in moles/min and K_m, the Michaelis constant, is the BD concentration at which the reaction proceeds at half its maximum velocity. This is the key nonlinear relation that makes the model as a whole respond nonlinearly at high external exposures.

A possible weakness of this pharmacokinetic approach was that the various partition coefficients for BD and BMO (octanol/water, blood/air, oil/air, tissue/blood) were not determined experimentally but rather were estimated

based on regression analyses or the use of empirically derived atomic or group fragment constants and structural factors (Lyman et al., 1982).

Hattis & Wasson (1987) employ several values of blood/air partition coefficient and K_m to determine various "best estimate" and "alternate" model predictions of metabolized dose of BD (BMO) for rats and mice. The results of the model runs are summarized in Tables 4-2 and 4-3. These 'best estimate' model simulations were verified using STELLA 2.10 (High Performance Systems, Inc. 1988) run on an Apple Macintosh computer. Model simulations were also carried out for the measured applied doses and these are also given in Table 4-2 and 4-3. The published doses were used to derive comparative estimates of cancer risk.

The risk estimates from this metabolized dose approach for mice fall within the range of risks presented here. The approach was not considered reliable for risk assessment purposes, primarily due to its consideration of only BMO and not DEB or other epoxide metabolites. Furthermore, numerous input values used to derive the estimates were not validated experimentally. As indicated in Section 2.2, accumulation of ^{14}C labeled BD and the rate of elimination of ^{14}C from tissues are not different for rats and mice. And finally, estimates of metabolites in various tissues (as described below) do not provide an explanation of the tumor frequencies in rats and mice. Consequently, cancer risk estimates using this approach were presented for comparative purposes only.

4.2.4.3 Target Tissue Doses

In this subsection the concentrations of the mutagenic metabolites, BMO and DEB, were estimated in various tissues of rodents at doses similar to those employed in the carcinogenicity bioassays. OEHHA staff consider these estimates to be highly uncertain. The data of Bond et al. (1986, 1987) on [^{14}C]-BD absorption, tissue distribution, and metabolite profile in blood were employed. The blood metabolite data of Bond et al. (1986) is reproduced in Table 4-4. In order to estimate tissue concentrations at the bioassay doses (625 or 1250 ppm) or at doses other than those employed in the tissue distribution studies (65 ppm for mouse and 1200 for rat), it was necessary to assume that these were proportional to the uptake of ^{14}C radiolabel into the animal carcass (Table 4-5). Uptake values were estimated graphically for exposure concentrations employed in the cancer bioassays. Distribution data are available for periods of 1, 8, and 65 to 67 hours after a 3.4 hour exposure to [^{14}C]-BD. The results of converting 1 hour data are shown in Tables 4-6 and 4-7.

The first column in the tables gives the tissue distribution of ^{14}C radiolabel in nmol BD equivalents/g tissue. The second column gives an estimate of tissue distribution at the higher bioassay dose based on carcass uptake. The columns to the right give estimates of how this tissue distribution of ^{14}C radiolabel would break down if the relative proportions of metabolites were the same as observed in blood. The table headings in parentheses give the percentage values of each metabolite as found in blood.

The sum of the estimated concentrations of mutagenic metabolites BMO and DEB are given in the right hand column of these tables. Naturally these

Table 4-2 Predictions of Metabolized Dose (BMO) by Pharmacokinetic Modeling of Mouse Inhalation Data (Hattis and Wasson, 1987)

Blood/Air Partition Coefficient	K_m 5 days	Exposure 6h/d	BD Metabolized 5 days		7days	
		ppm	µmol/animal	µmol/kg	µmol/kg	mg/kg-d
Best Estimate Model 0.35	2×10^{-5}	625	28.1	1003	716	38.8
		1250	45.8	1634	1167	63.2
		627	28.2	1007	719	38.9
		1236	45.5	1625	1161	62.8
Alternate Model 0.2552	2×10^{-5}	625	23.8	850	607	32.8
		1250	41.4	1479	1056	57.2
		627	22.2	793	566	30.6
		1236	38.1	1361	972	52.6
EPA, 1985 Continuous Internal		625	13.3	474	339	18.3
		1250	20.1	719	514	27.8

NOTE:

Alveolar Ventilation = 0.0233 L/min.

$V_{max} = 1.87 \times 10^{-7}$ for all runs.

EPA projections of continuous internal doses based on Bond et al. (1986). Net absorption data were 25.7 mg/kg (474 µmol/kg) at 625 ppm and 38.9 mg/kg (719 µmol/kg) at 1250 ppm. Mouse weight of 0.028 kg assumed.

Table 4-3 Predictions of Metabolized Dose (BMO) by Pharmacokinetic Modeling of Rat Inhalation Data (Hattis and Wasson, 1987)

Blood/Air Partition Coefficient	K_m	Exposure 6h/d 5 days	BD Metabolized 5 days		7days	
		ppm	µmol/animal	µmol/kg	µmol/kg	mg/kg-d
Best Estimate Model 0.35	5×10^{-6}	1000	359.6	899	642	34.7
		8000	607.7	1519	1085	58.7
		999	359	898	641	34.7
		7886	603	1508	1077	58.2
Alternate Model 0.2552	1×10^{-5}	1000	274.7	686.8	490	26.5
		8000	347.1	867.8	620	33.5
		999	263	658	470	25.4
		7886	559	1398	998	54.0
EPA, 1985 Continuous Internal		1000	78	195	139	7.5
		8000	274	685	489	26.5

NOTE:

Alveolar Ventilation = 0.15 L/min.

$V_{max} = 1.47 \times 10^{-6}$ for all runs.

EPA projections of continuous internal doses based on Bond et al (1986), net absorption data were 10.5 mg/kg (195 µmol/kg) at 1000 ppm and 37.1 mg/kg (685 µmol/kg) at 8000 ppm. Rat body weight of 0.4 kg assumed.

Table 4-4 Blood Distribution of BD and Metabolites (Bond et al., 1986)[a]

Species	Dose[b] ppm	Metabolites and Parent nmol/ml				
		nonvolatile[c] (conj)	DEB	BMO	BD	CO_2
Rat	70	8	0.1	0.4	0.1	0.6
	1000	51	1.0	4.0	4.0	4.0
Mouse	7	6	0.08	0.7	0.2	0.08
	70	17	0.1	0.9	0.8	0.2
	1000	100	1.0	15.0	3.0	2.0

[a] Metabolites measured 6 hours after exposure.
[b] Concentrations exposed in ppm were calculated from reported exposure of 13, 130 and 1000 μg/L.
[c] Nonvolatile metabolites thought to be largely conjugates (conj) of diol metabolites.

Table 4-5 Incorporation of ^{14}C Radiolabel from ^{14}C-BD into Carcass[a]

Species	Dose[b] ppm	μmol/kg	mg/kg	μmol ^{14}C/carcass
Rat	70	18.8	1.0	3
	1000	167	9.0	13
	7000	860	46.5	35
	(1000)[c]			(14)
Mouse	7	12.8	0.7	0.09
	70	90.5	4.9	0.5
	1000	845	45.7	2.0
	(625)[c]			(1.7)

[a] Radiolabel measured 65 hours after 6 hours exposure.
[b] Concentrations expressed in ppm were calculated from reported exposures of 13, 130, 1000 and 13,000 μg/L.
[c] Values in parentheses were estimated graphically and correspond to 1000 ppm - rat and 625 ppm - mouse bioassay doses.

Table 4-6 Estimated Concentrations of Metabolites in Mouse Tissues After Inhalation Exposure to [^{14}C]BD

Tissue	65 ppm[1]	625 ppm[2]	nmol BD equiv/g tissue				
	Test Dose	Est. Dose	BD(5)[3]	BMO(14)	DEB(2)	CONJ(79)	BMO+DEB
	Total ^{14}C						
Adrenal	76	258	13.0	36.1	5.2	204	41.3
Blood	49	167	8.4	12.3	3.3	132	26.7
Heart	47	160	8.0	22.4	3.2	126	25.6
Kidney	180	612	30.6	85.7	12.2	483	97.9
Liver	120	408	20.4	57.1	8.2	322	65.3
Lung	96	326	16.3	45.6	6.5	258	52.1
Mammary	30	102	5.1	14.3	2.0	80.6	16.3
Pancreas	160	544	272	76.2	10.9	430	87.1
Thyroid	60	204	10.2	28.6	4.1	161	32.7

[1] 3.6 hour exposure to 65 ppm [^{14}C]BD, values measured at 1 hour after exposure (Bond et al., 1987). Figures are nmol/g tissue.

[2] 625 ppm estimates based on carcass uptake vs. applied dose (Graphical Interpolation Table 4-5). Figures are nmol/g tissue.

[3] Relative metabolite values in percent based on blood values (Table 4-4).

Table 4-7 Estimated Concentrations of Metabolites in Rat Tissues After Inhalation Exposure to [^{14}C]BD

Tissue	1200 ppm[1]	1000 ppm[2]	nmol BD equiv/g tissue				
	Test Dose	Est. Dose	BD(7)[3]	BMO(8)	DEB(2)	CONJ(77)	BMO+DEB
	Total ^{14}C						
Adrenal	210	190	13.0	15.2	3.8	146	19
Blood	124	112	7.8	9.2	2.2	86	11.4
Heart	120	108	7.6	8.6	2.2	83	10.8
Kidney	390	352	24.6	28.2	7.0	271	35.8
Liver	330	269	18.8	21.5	5.4	207	26.9
Lung	200	181	12.7	14.5	3.6	139	18.1
Mammary	140	126	8.8	10.1	2.5	97	12.6
Pancreas	320	289	20.2	23.1	5.8	222	28.9
Thyroid	490	442	30.9	35.4	8.8	340	44.2

1 3.4 hour exposure to 1220 ppm [^{14}C]BD values measured at 1 hour after exposure (Bond et al., 1987). Figures are nmol/g tissue.

2 1000 ppm estimates based on carcass uptake vs. applied dose (Graphical Interpolation Table 4-5). Figures are nmol/g tissue.

3 Relative metabolite values in percent based on blood values (Table 4-4).

figures rest on many assumptions: that carcass ^{14}C concentrations can be used to extrapolate average tissue concentrations with varying dose; that the metabolite pattern of blood reflects that of the various tissues; and that the differences between 3.4 hours exposure and 6 hours exposure are relatively minor. While admittedly crude these estimates may be improved by considering target tissue/blood partition coefficients as calculated by Hattis and Wasson (1987) or Fiserova-Bergerova (1986). Using oil/water partition coefficients estimated by the method of Hansch and Leo (Lyman et al., 1982), the tissue/blood partition coefficients are as shown in Table 4-8. These figures indicate that tissue metabolite patterns are likely to show higher concentrations of BMO and lower concentrations of DEB than blood. BD tissue concentrations would probably have been much higher than blood concentrations. In Table 4-9 are shown the relative proportions of BD and its epoxide metabolites in selected tissues as a result of blood/tissue partitioning.

Since the blood/tissue partition coefficients of the chief toxic epoxide metabolites of BD are close to 1.0 (within a factor of 2), blood patterns of the compounds may give a reasonable estimate of tissue concentrations in most cases. From Tables 4-6 and 4-9 it appears that at 625 ppm applied dose target tissue concentrations of BMO and DEB in the mouse could vary from 3.2 x 0.75 = 2.4 nmol/g for DEB in heart tissue to 45.6 x 9/8 = 51.3 nmol/g for BMO in the lung. In the rat target tissues, at 1000 ppm doses could vary from (5.8 x 0.75 =) 4.35 nmol DEB/g pancreas to (35.4 x 8.5/7 =) 43.0 nmol BMO/g thyroid (Table 4-10). These estimates of concentrations of mutagenic epoxides do not appear unusually high with respect to non-target (e.g., mammary) tissues, with the possible exception of rat thyroid.

The target tissue dose estimates were not considered to be reliable for risk assessment purposes for several reasons. The target tissue dose estimates were qualitative and not quantitative. Tissue concentrations for the bioassay studies had to be considered proportional to those measured in the distribution studies. The tissue dose estimates did not correspond with tumor incidences. For example, the kidney exhibited the greatest concentration of all metabolites in the mouse, but was not a site of increased tumor incidence. Consequently, cancer risk estimates using this approach were presented for comparative purpose only.

4.2.4.4 Molecular Tissue Doses

When B6C3F1 mice or Wistar rats were exposed to 1,3-[1,4-^{14}C] butadiene in a closed system, the mice metabolized the test compound at twice the rate observed in rats based on body weight (Kreiling et al., 1986). Covalent binding of [^{14}C]BD derived radiolabel was observed in both rat and mouse liver DNA at comparable amounts. Covalent binding to mouse-liver nucleoproteins was twice as high as seen in rats (Table 4-11). After exposure of mice to butadiene, 7-(1-hydroxy-3-buten-2-yl)guanine, a product of the reaction of epoxybutene (BMO) with deoxyguanosine and DNA (Citti et al., 1984) was identified in liver DNA (Laib and Kreiling, 1987). The doses applied to the rats and mice (ca. 800 ppm peak) approximate 1 to 2 hours of the low dose bioassay level. For mice, this corresponded to roughly 1,300 times the dose employed for the DNA binding study and resulted in 100% incidence of fatal tumors.

Table 4-8 Estimated Tissue/Blood Partition Coefficients of BD and Epoxide Metabolites

Tissue	Coefficient	BD	BMO	DEB
	Oil/Water (K_{ow})[1]	125.9	27.5	6.02
Lung	Tissue/Blood[2]	1.65	1.20	1.10
Kidney/Vessel Rich Group	Tissue/Blood	5.34	1.22	0.77
Muscle	Tissue/Blood	4.20	1.39	0.77
Liver	Tissue/Blood	5.93	1.74	0.83

NOTE:

1 Determined by the method of Hansch & Leo: $\log K_{ow} = \Sigma f + \Sigma F$ where f are atomic or group fragment constants and F are structural parameters such as molecular flexibility, unsaturation, halogenation, branching, etc.

For BD: $\log_{10} K_{ow} = 4fC + 10fH\ 1 + (3\text{-}1)Fb + 2f =$
$4(.2) + 10(.23) + 2(-.12) + 2(-.38) = 2.10 \pm 0.1$ SD.
$K_{ow} = 125.9(100\text{-}158)$.

2 Tissue/blood partition coefficients estimated by the regressions of Fiserova-Bergerova, 1986: $L_{T/B} = a(L_{F/B} + b)$ where the fat/blood coefficient is assumed to be similar to K_{ow}.

Table 4-9 Estimated BD and Epoxide Metabolite Proportions in Selected Tissues

	Rat				Mouse			
	Blood	Lung	Kid/VRG	Muscle	Blood	Lung	Kid/VRG	Muscle
BD	7	12	37	29	2	3	10	8
BMO	7	8	8.5	10	8	9	10	11
DEB	2	2	1.5	1.5	1	1	0.75	0.75

Table 4-10 Estimate of Mutagenic Epoxide Concentrations in Rodent Target Tissues[1]

	RAT		MOUSE	
Metabolite	Pancreas	Thyroid	Heart	Lung
BMO	28.0[2]	43.0	30.8	51.3
DEB	4.4	6.6	2.4	4.9
Total	32.4	49.6	33.2	56.2

1 Based on tissue distribution data for 3.4 hour exposures to [^{14}C]BD measured 1 hour after exposure.

2 Values in nmol BD equivalent/g tissue at interpolated applied doses of 625 ppm (mouse) and 1000 ppm (rat) and adjusted for blood/tissue partition.

Table 4-11 Radioactivity in Nucleoproteins and DNA of Mouse and Rat Liver After Exposure to [1,4 -^{14}C]1,3-Butadiene[a]

Number of Animals		pmol/mg ($\bar{X}$ ± SD)[d]	
RATS[b]	MICE[c]	RATS[e]	MICE[f]
		(a) Early Eluting Nucleoproteins	
4	4 x 6	15.6 ± 2.6	29.7 ± 3.5
		(b) Late Eluting Nucleoproteins	
4	4 x 6	7.6 ± 0.6	13.2 ± 1.7
		(c) Liver DNA	
4	4 x 6	34.9 ± 10.2	30.4 ± 7.2

[a] Source: Kreiling et al., 1986.

[b] Dose of 2.3 mCi/mmol = 0.205 mmol/4 rats/6.4 L air.

[c] Dose = 0.205 mmol/24 mice/6.4 L air.

[d] Covalent Binding; pmol BD equivalents/mg DNA or nucleoproteins.

[e] Uptake = 5.95 x 10^9 DPM/kg

[f] Uptake = 6.15 x 10^9 DPM/kg.

While the total radiolabel content of rat liver DNA is virtually the same as for the mouse, the pattern of metabolites could be quite different due to the more rapid metabolism of BMO in the rat. Conceivably, mouse DNA and nucleoprotein radiolabel could contain adducts from subsequent alkylating metabolites, such as diepoxybutane and 3,4-epoxy-1,2-butanediol. Since the liver is not a major target for BD carcinogenesis in either species, heavily affected target tissues may have greater covalent binding.

Alternatively, as indicated by nucleoprotein binding in the mouse, molecular doses may need to include more than DNA adducts. Also, measurements based on whole tissues may miss sensitive subpopulations of cells with high affinity for adduct formation, proliferation, etc. (Belinsky et al., 1987). Clearly more work needs to be done on the molecular aspects of BD carcinogenesis before one can relate molecular doses in target tissues with tumor incidences seen in those tissues.

Risk estimates were not derived using molecular tissue doses due to the limited amount of information available. Molecular tissue doses are only available for the liver which is not a major tumor site. Furthermore, the molecular tumor dose results do not shed any light on the different sensitivity of mice and rats to BD, since molecular doses were equivalent in the two species. Consequently, this information was presented only to provide a complete perspective of the risk assessment of BD.

4.2.5 Intercurrent Mortality Correction

For animal carcinogenicity studies where the duration of the experiment Le was less than the experimental lifetime of the animal L, the dose response is corrected according to the assumption that the cumulative tumor incidence would be expected to increase by (at least) the third power of age. In this report, the correction was applied to the carcinogenic potency or 95% upper confidence limit of the slope of the dose response relationship, q_1*:

$$q_1^* \text{ animal} = q_1^*(L/Le)^3 = q_1^*(104/Le)^3; \text{ for } Le < L.$$

4.2.6 Models For Low Dose Extrapolation

4.2.6.1 Linearized Multistage Model

In order to extrapolate from high experimental doses in animal studies to low doses of relevance to human environmental exposures, a mathematical extrapolation model was employed. With the type of data available on BD, the model of greatest relevance and flexibility was the linearized multistage. The program employed was GLOBAL 86 (K.S. Crump & Co., Ruston, LA, May 1986) which fits the multistage model to quantal animal tumor incidence data and calculates maximum likelihood estimates and statistical confidence limits for the extra risk over background at a given dose and for the dose corresponding to a given value of extra risk. The multistage model is given by

$$P(d) = 1 - \exp - (q_0 + q_1 d + q_2 d^2 +, \ldots, + q_k d^k),$$

$q_i \geq 0$, $i = 1, \ldots k$, where d is the dose, P(d) is the lifetime probability of a health effect at a dose d, and k and q_i are parameters. The extra risk is defined as

$$[P(d) - P(0)]/[1 - P(0)].$$

The parameter q_0 represents the background lifetime incidence of tumor(s). The parameter q_1 is the maximum likelihood estimate of the slope or cancer potency, and q_1* the 95% upper confidence limit of that estimate. The GLOBAL 86 algorithm selects the number of stages in the multistage model k, $1 \leq k \leq 6$, that minimizes q_1* subject to the condition that the model fit is adequate ($p \geq 0.01$). When the dose is expressed in units mg/kg-d, the parameters q_1 and q_1* are given in units $(mg/kg\text{-}d)^{-1}$. At low environmental doses, the risk is equal to q_1*d. A variant of the linearized multistage model, Weibull 82 (K.S. Crump and Co., 1985) was also employed. This program fits a model which is multistage in dose and Weibull in time to time-to-tumor dose response data:

$$P(d,t) = 1 - \exp - [(q_0 + q_1 d + \ldots + q_k d^k)(t-t_0)^j]$$

where $q_i \geq 0$, $i = 0, \ldots, k$, $j \geq 1$, t, the time t_0 tumor is $\geq t_0$, the latency period. The value of t_0 can be estimated by the program or set to zero. The value of k, which determines the degree of the polynominal, is chosen by the user. In this case the extra risk is defined as:

$$[P(d,t) - P(0,t)]/[1-P(0,t)].$$

Additional description of the multistage-Weibull model and its comparison with other models can be found in Krewski et al. (1983).

4.2.6.2 Other Models

The BD animal carcinogenicity data and various assay dose estimates were also evaluated using the Tox-Risk program of Clement Associates (Ruston, LA). This program has the ability to fit 10 different models to quantal animal tumor incidence vs. dose data. In addition to the multistage model, the Weibull, Mantel-Bryan and Log-Normal models were fit to the BD experimental data. In general, the multistage model (where k = number of dose groups -1) or the Global 86 (above) gave better fits to the data than the other models did.

4.2.7 Interspecies Extrapolation

For purposes of cancer risk assessment, it was assumed that mg/unit surface area/day is an equivalent dose between species. Since surface area closely correlates with the 2/3 power of body weight (W), the dose units of $mg/W^{2/3}/d$ are also considered equivalent. BD is slightly soluble in water and can be considered a partially soluble gas. The dose in mg/d = m is proportional to O_2 consumption which in turn is proportional to $W^{2/3}$ and to the solubility of BD in body fluids indicated by an absorption fraction r. Expressing O_2 consumption as $kW^{2/3}$, where k is a constant independent of species, it follows that:

$$m = kW^{2/3}vr,$$

where v is the BD concentration in air (mg/m^3), or

$$\frac{m}{W^{2/3}} = kvr.$$

It is assumed that r, the absorbed fraction, is the same for all species. Therefore, for one method of extrapolation, OEHHA staff followed EPA practice (EPA, 1985) and assumed that a certain exposure concentration in ppm or mg/m^3 in experimental animals was equivalent to the same exposure concentration in humans (Anderson et al., 1983).

To convert doses between mg/kg-d internal and ppm external, the following factors were used: (1) a BD conversion factor of 2.21 mg/m^3/ppm at 25°C, 760 mm Hg; (2) the absorption fraction at low concentrations from Bond et al. (1986) of 0.16 for mice and 0.17 for rats; (3) the ventilation rate in cm^3/min. estimated from the relation of Guyton (1947), i.e., rats, 2.10 (body weight in grams)$^{3/4}$; mice, 2.54 (body weight in grams)$^{3/4}$. The resulting values were then converted to m^3/d. For a 700 g rat this conversion would appear as follows (EPA, 1985):

$$2.21\ mg/m^3/ppm \times 0.17 \times 0.41\ m^3/d \times 1/0.7\ kg = 0.22\ mg/kg\text{-}d/ppm.$$

The use of air concentration in ppm or mg/m^3 as an interspecies equivalent dose unit results in potency values about 2.5 fold lower than those based on mg/m^2 surface area (see 4.2.9 below).

4.2.8 Quantitative Estimation

4.2.8.1 NTP (1984) Mouse Inhalation Study (Mouse I)
Presented in Table 4-12 is a sample calculation of a cancer potency estimate for BD based on the mouse total significant tumor incidence data and estimated continuous internal doses.

The quantal data and doses were entered into Global 86 and the q_1 and q_1* values obtained. Intercurrent mortality corrections and the external low dose conversions are then performed to give potency estimates in units of $(ppm)^{-1}$.

Cancer potency estimates for mice using the three measures of dose and individual target tissue lesions as well as total tumor incidence are given in Table 4-13. Individual target tissue potency values ranged from 6.9 x 10^{-4} $(ppm)^{-1}$ for liver neoplasms in females using applied dose to 2.0 x 10^{-1} $(ppm)^{-1}$ for malignant lymphoma in males using continuous internal dose. Usually metabolized dose potencies were midrange between potencies estimated with applied and those estimated with internal doses. When total significant tumor incidence was input, the cancer potency values ranged from 0.016 to 0.32 $(ppm)^{-1}$ for males and from 0.01 to 0.17 $(ppm)^{-1}$ for female mice. These figures compare well with the sums of the individual potencies over tissues of 0.015 to 0.245 $(ppm)^{-1}$ and 0.009 to 0.19 $(ppm)^{-1}$ respectively. Cancer potency estimates from the Weibull 82 time to tumor analysis (Table 4-14) were quite similar to the Global 86 analysis. With total tumors and continuous internal doses, the potency values were 0.292 $(ppm)^{-1}$ for males and 0.162 $(ppm)^{-1}$ for females. For metabolized doses the values were 0.138 and 0.075 $(ppm)^{-1}$ respectively. For these analyses k = 2 and t_0 = 0. In order to compare the ppm^{-1} potency values in Table 4-13 with surface area scaled values in Table 4-15 the values should be multiplied by 2.5 (e.g., 0.32 ppm^{-1} unscaled x 2.5 = 0.80 ppm^{-1} scaled).

Table 4-12 Quantitative Estimation of Butadiene Potency With Mouse Internal Doses.

Nominal Exposure (ppm)	Males: Internal Dose (mg/kg-d)[a]	Males: Tumor Incidence[b]	Females: Internal Dose (mg/kg-d)	Females: Tumor Incidence
0	0	2/50 (4%)	0	4/48 (8%)
625	18.4	43/49 (88%)	18.4	31/48 (65%)
1250	29.0	40/45 (89%)	29.0	45/49 (92%)

[a] Continuous internal dose = daily internal dose x 5/7.
[b] Number of animals with at least one statistically significant tumor/total examined, eliminating animals that died prior to 20 weeks.

Initial Maximum Likelihood Estimates:

Males	Females
$q_0 = 0.042$	$q_0 = 0.087$
$q_1 = 0.091$ (mg/kg-d)$^{-1}$	$q_1 = 0$
	$q_2 = 2.8 \times 10^{-3}$ (mg/kg-d)$^{-2}$

Initial Estimates of 95% Upper Limit:

$q_1^* = 0.114$ (mg/kg-d)$^{-1}$	$q_1^* = 0.066$ (mg/kg-d)$^{-1}$

Intercurrent Mortality Correction:

$(104/60)^3 = 5.21$	$(104/61)^3 = 4.96$

Final Estimate of q_1^* (animal):

0.59 (mg/kg-d)$^{-1}$	0.32 (mg/kg-d)$^{-1}$

Final Estimate of q_1^* in (ppm)$^{-1}$ = q_1^* (human):

2.21 mg/m^3/ppm x 0.16 x 0.053 m^3/d x 1/0.035 kg = 0.54 mg/kg-d/ppm

$q_1^* = 0.32$ (ppm)$^{-1}$	$q_1^* = 0.17$ (ppm)$^{-1}$

Since human low external/internal dose conversion is 9.09 ppm/mg/kg-d,

$q_1^* = 2.91$ (mg/kg-d)$^{-1}$	$q_1^* = 1.58$ (mg/kg-d)$^{-1}$

Table 4-13 Human Cancer Potency Estimates $(ppm)^{-1}$ for Butadiene From Mouse Bioassay Data

Tissue/Lesion	Sex	Experimental Dose q_1^*		Internal Dose q_1^{*b}		Metabolized Dose q_1^{*b}	
		$(ppm)^{-1}$ [a]	$(ppm)^{-1}$ [b]	$(ppm)^{-1}$ [c]	$(mg/kg\text{-}d)^{-1}$ [d]	$(ppm)^{-1}$ [c]	$(mg/kg\text{-}d)^{-1}$ [d]
Heart/hemangiosarcoma	M	9.3×10^{-4}	4.8×10^{-3}	0.073	0.167	0.042	0.078
	F	5.2×10^{-4}	2.5×10^{-3}	0.044	0.099	0.025	0.047
Hematopoietic system malignant lymphoma	M	1.0×10^{-3}	5.2×10^{-3}	0.089	0.203	0.051	0.095
	F	3.2×10^{-4}	1.6×10^{-3}	0.029	0.065	0.016	0.030
Lung/alveolar and bronchiolar adenomma and carcinoma	M	4.6×10^{-4}	2.4×10^{-3}	0.041	0.094	0.024	0.044
	F	5.8×10^{-4}	2.9×10^{-3}	0.046	0.104	0.207	0.050
Mammary/acinar cell carcinoma	F	1.5×10^{-4}	7.4×10^{-4}	0.012	0.028	7.0×10^{-3}	0.013
Ovary/granulosa cell neoplasm	F	3.5×10^{-4}	1.7×10^{-3}	0.028	0.064	0.017	0.031
Forestomach/papilloma and carcinoma	M	5.4×10^{-4}	2.8×10^{-3}	0.041	0.094	0.025	0.046
	F	2.8×10^{-4}	1.4×10^{-3}	0.024	0.055	0.014	0.026
Liver/adenoma and carcinoma	F	1.4×10^{-4}	6.9×10^{-4}	0.012	0.027	7.1×10^{-3}	0.013
All significant tumors (program input)	M	3.0×10^{-3}	0.0156	0.32	0.59	0.150	0.278
	F	2.1×10^{-3}	0.0104	0.18	0.33	0.087	0.161
Sum of individual sites	M	2.9×10^{-3}	0.0152	0.245	0.557	0.142	0.263
	F	1.9×10^{-3}	9.3×10^{-3}	0.194	0.441	0.113	0.210

NOTE: [a]Initial estimate of 95% upper bound on low exposure potency q_1^*.

[b]Final Estimates of q_1^* corrected for length of study Le in weeks by a factor $(104/Le)^3$, i.e. 5.21 for male mice, 4.96 for female mice.

[c]Animal/Human equivalent potencies using external low dose conversion factor of 0.54 mg/kg-d/ppm for the mouse.

[d]Mouse potency estimates derived from low dose extrapolations.

Table 4-14 Weibull Time to Tumor Analysis of NTP 1984 Mouse Data for All Significant Tumors Related to Butadiene Exposure

Sex	Dose	Dose for 10^{-6} Risk (lower bound)	Risk for Unit Dose (upper bound)	$(L/Le)^3$ [a]	q_1^* $(ppm)^{-1}$ [b]
M [c,d]	Continuous[e] Internal	1.1×10^{-5}	0.102	0.53	0.292[c]
M	Metabolized[f] 0, 38.8, 63.2 mg/kg-d	2.3×10^{-5}	4.9×10^{-2}	0.256	0.138
F [c,d]	Continuous[e] Internal	1.9×10^{-5}	0.06	0.30	0.160
F	Metabolized[f]	4.1×10^{-1}	0.028	0.14	0.075

NOTES:

[a] Intercurrent mortality correction: Males $(104/60)^3 = 5.21$; Females $(104/61)^3 = 4.96$.

[b] Internal dose to low dose external conversion = 0.54 mg/kg/d/ppm.

[c] Females include 1 missexed male and males exclude 1 missexed animal.

[d] Males-number of animals evaluated at control, low, high doses: 48,47,50; females: 50,46,45.

[e] 0, 18.4 and 29.3 mg/kg-d continuous internal doses.

[f] 0, 38.8, 63.2 mg/kg-d metabolized doses.

Table 4-15 Human Cancer Potency Estimates in $(ppm)^{-1}$ and $(mg/kg\text{-}d)^{-1}$ For Butadiene From Mouse II Bioassay Data (Melnick et al., 1990)

Tissue/ Lesion	Sex	Experimental Dose q_1^*				Internal Dose q_1^*				Metabolized Dose q_1^*			
		$(mg/kg\text{-}d)^{-1}$ [a]	$(mg/kg\text{-}d)^{-1}$ [b]	$(ppm)^{-1}$ [c]	$(ppm)^{-1}$ [d]	$(mg/kg\text{-}d)^{-1}$ [a]	$(mg/kg\text{-}d)^{-1}$ [b]	$(ppm)^{-1}$ [c]	$(ppm)^{-1}$ [d]	$(mg/kg\text{-}d)^{-1}$ [a]	$(mg/kg\text{-}d)^{-1}$ [b]	$(ppm)^{-1}$ [c]	$(ppm)^{-1}$ [d]
Heart/ hemangio-sarcoma	M	5.1×10^{-3}	0.064	2.8×10^{-3}	7.0×10^{-3}	0.067	0.84	0.04	0.092	0.037	0.47	0.020	0.052
	F	1.2×10^{-3} [f]	0.015	6.5×10^{-4}	1.6×10^{-3}	0.013	0.16	7.0×10^{-3}	1.4×10^{-3}	6.4×10^{-3} [e]	0.08	3.5×10^{-3}	8.8×10
Hematopoietic system/ All malignant lymphomas	M	4.1×10^{-3}	0.052	2.2×10^{-3}	5.7×10^{-3}	0.034	0.43	8.1×10^{-3}	0.047	0.025	0.32	0.014	0.035
	F	5.8×10^{-3}	0.073	3.1×10^{-3}	8.0×10^{-3}	0.093	1.2	0.05	0.13	0.044	0.55	0.024	0.060
Lymphocytic lymphomas	M	1.5×10^{-3}	0.019	8.1×10^{-4}	2.1×10^{-3}	0.014	0.88	7.6×10^{-3}	0.097	7.0×10^{-3}	0.09	3.8×10^{-3}	9.9×10^{-3}
	F	4.5×10^{-3}	0.057	2.4×10^{-3}	6.3×10^{-3}	0.047	0.59	0.025	0.065	0.032	0.40	0.017	0.044
Lung/alveolar and bronchioler adenoma and carcinoma	M	0.017	0.21	9.2×10^{-3}	0.023	0.16	2.0	0.086	0.22	0.125 [e]	1.6	0.068	0.18
	F	0.033	0.42	0.018	0.046	0.27	3.4	0.15	0.37	0.136 [e]	1.7	0.073	0.19
Ovary/ granulosa cell neoplasm	F	5.2×10^{-3} [f]	0.066	2.8×10^{-3}	7.3×10^{-3}	0.018 [e]	0.23	9.7×10^{-3}	0.025	0.034	0.43	0.018	0.047
Forestomach/ papilloma and carcinoma	M	4.0×10^{-3}	0.050	2.2×10^{-3}	5.5×10^{-3}	0.026	0.33	0.014	0.036	0.029	0.36	0.016	.040
	F	4.2×10^{-3}	0.053	2.3×10^{-3}	5.8×10^{-3}	0.040	0.50	0.022	0.055	0.030	0.38	0.016	.042
Sum of individual sites	M	0.030	0.38	0.016	0.042	0.29	3.6	0.16	0.40	0.216	2.7	0.12	0.30
	F	0.049	0.62	0.026	0.068	0.43	5.4	0.23	0.59	0.250	3.2	0.14	0.35

NOTE: [a] q_1^* program output mouse - Experimental Dose - TOX RISK v.1; Internal Dose and Metabolized Dose - GLOBAL 86
[b] q_1^* human, via mouse/human surface area scaling $(70/0.035)^{1/3} = 12.6$
[c] q_1^* (ppm)-1 via mouse low dose external conversion factor 0.54 mg/kg-d/ppm (from a)
[d] q_1^* (ppm)-1 via human low dose external conversion factor 0.11 mg/kg-d/ppm (from b)
[e] high dose group(s) deleted by GLOBAL 86 to achieve p-value of ≥ 0.01 for chi-square goodness of fit statistic
[f] high dose group(s) deleted to obtain p-value of ≥ 0.01 for chi-square goodness of fit statistic

4.2.8.2 Melnick et al. (1990) Mouse Inhalation Study (Mouse II)
Cancer potency estimates for the mouse II study using three measures of dose (experimental, continuous internal, and metabolized) and two mouse/human equivalent dose units (ppm in air, $mg/m^2/day$) are presented in Table 4-15.

The tumor incidence data are located in Table 3-2 and Tables 1, 2, 4, and 5 of Melnick et al. (1990) (see Appendix). The continuous internal doses for the study doses, calculated as described above were: 0; 0.8; 1.75; 3.84; 8.40; and 18.4 mg/kg-d. The metabolized doses, obtained using the PBPK model of Hattis and Wasson (Best Value: Blood/Air = 0.35, alveolar ventilation = 0.023 L min^{-1}, V_{max} = 1.87 x 10^{-7} mols min^{-1}, Km = 2 x 10^{-5}M), were: 0; 0.62; 1.97; 6.12; 17.8; and 54.3 mg/kg-d. In Table 4-15 there are four potency estimates (a through d) under each dose measure heading. The first value (a) represents the unscaled potency value in $(mg/kg\text{-}d)^{-1}$. In the case of the applied or experimental dose this value was obtained by inputting quantal tumor incidence data as a function of experimental dose in ppm into the Tox Risk multistage program (Tox-Risk v3 KS Crump Division Clement International Corp, Ruston, LA). The animal to human conversion method was mg/kg body weight/day. In the cases of continuous internal and metabolized dose, the Global 86 program was used to obtain the respective q_1* values in column (a). The values given in column (b) represent human potency values for each dose measure assuming that the intra-species equivalent dose is mg/m^2 body surface area/day (ie., dose conversion factor = (body weight human/body weight animal)$^{1/3}$). The value given in column (c) assumes that the interspecies equivalent dose is ppm in air and the animal potency in $(mg/kg\text{-}d)^{-1}$ is converted into $(ppm)^{-1}$ units using the mouse conversion factor of 0.54 mg/kg-d/ppm. This is the same approach taken previously in Mouse I (Table 4-13) and by EPA (1985). The fourth column (d) is a separate estimate of human potency in ppm^{-1} obtained from the surface area scaled value in (b) using the human conversion factor of 0.11 mg/kg-d/ppm. Individual target tissue potencies ranged from 6.0 x 10^{-4} ppm^{-1} for heart hemangiosarcomas (experimental dose unscaled) in female mice to 0.37 ppm^{-1} (3.7 x 10^{-4} ppb^{-1}) for alveolar and bronchiolar neoplasms in female lung (continuous internal dose, scaled). Surface area scaled potency values in $(ppm)^{-1}$ were approximately 2.5 fold greater than unscaled values. Potency values obtained using the metabolized dose measure were approximately one-half to three-quarters those obtained with continuous internal dose, whereas those obtained with the experimental dose were about 1/10 those with internal dose.

4.2.8.3 Hazleton (1981, 1987) Rat Inhalation Study
Cancer potency estimates for rats using the three measures of dose and individual target tissue lesions as well as for total tumor incidence are given in Table 4-16. In nearly all cases the tumor incidence figures of EPA (1985) were used with a few minor revisions (Owen et al., 1987). Individual cancer potency values ranged from 5.3 x $10^{-6}(ppm)^{-1}$ for male Zymbal gland neoplasms using applied doses to 1.7 x $10^{-3}(ppm)^{-1}$ for mammary carcinomas using internal doses. Potencies based on metabolized doses were usually intermediate in value compared with experimental or internal doses. When total significant tumor incidence was input, the cancer potency values ranged from 3.6 x $10^{-5}(ppm)^{-1}$ to 1.5 x $10^{-3}(ppm)^{-1}$ for males, and from 9.0 x $10^{-5}(ppm)^{-1}$ to $0.021(ppm)^{-1}$ for females. These figures compare well with the sums of the individual potencies over tissues of 8.9 x $10^{-5}(ppm)^{-1}$

to $1.8 \times 10^{-3}(ppm)^{-1}$ and $1.9 \times 10^{-4}(ppm)^{-1}$ to $4.4 \times 10^{-3}(ppm)^{-1}$ respectively. Cancer potency estimates from the WEIBULL 82 time to tumor analysis (Table 4-16) were in general similar to the GLOBAL 86 analysis. The analysis of mammary adenomas and carcinomas gave the highest potencies: $3.6 \times 10^{-2}(ppm)^{-1}$ by GLOBAL 86 and $1.4 \times 10^{-2}(ppm)^{-1}$ by WEIBULL 82, but these tumors also showed a high incidence in unexposed control rats.

For the purpose of this risk assessment, following the lead of EPA (1985), cancer potency estimates for the rat data will be based on total significant tumor incidence less mammary fibroadenomas and uterine tumors rather than on a single target tissue or organ. The total incidence is the number of animals bearing one or more of the primary lesions in a given dose group divided by the number of animals examined that could have had the tumors in question. For a multi-target carcinogen such as butadiene it is believed that this approach gives a better estimate of potential cancer risk.

From Table 4-16 the best potency value from the rat data would be 3.5×10^{-3} $(ppm)^{-1}$ (3.5×10^{-6} ppb^{-1})based on the continuous internal dose. A comparable value based on an interspecies equivalent dose of $\mu g/m^2$ surface area/d would be 9.8×10^{-3} $(ppm)^{-1}$ (9.8×10^{-6} ppb^{-1}).

4.2.9 Conclusions

Of the various estimates of human cancer potency for 1,3 butadiene given in Tables 4-13 to 4-17, those based on the incidence of multiple significant cancers in female rats or individual sites in mice range from 6.5×10^{-4} $(ppm)^{-1}$ (6.5×10^{-7} ppb^{-1}) to 0.80 $(ppm)^{-1}$ (8.0×10^{-4} ppb^{-1}) with different estimates of the dose of BD. In view of our limited understanding of the toxicokinetics of BD and its metabolites and the lack of any direct human metabolic data on butadiene, it is not possible at present to rely on the current pharmacokinetic estimates of metabolized dose for estimating human health risks. The pharmacokinetic dose approach is preliminary and does not explain tumor frequencies in target tissues or species differences. For key data sets, the multistage model gave relatively poor fits to the pharmacokinetic doses. Unadjusted applied dose estimates may underestimate risk, as the animal exposure regimen resembled occupational exposures more closely than continuous ambient environmental exposures. For comparison purposes, cancer potency values obtained using a variety of dose measures have been presented here.

Since there are large differences in potency values between rats and mice that are not explained by differences in pharmacokinetics and tissue distributions of metabolites, it is still an open question as to which experimental animal is a better indicator of human risk. However, the most detailed evaluations of the carcinogenicity of BD have been conducted in the mouse. For the purpose of risk assessment, the quality of the mouse II bioassay data is superior to that of the rat data. The primary reasons for this conclusion are: 1) the use of the most relevant dose levels in the mouse II study; 2) the use of five dose levels, compared to two in the rat study; 3) the presence of two mouse studies with similar findings; 4) the consistency in sites of carcinogenicity between the two mouse studies; 5) the availability of the mouse data in greater detail, allowing in-depth analysis; 6) the fact that the rat study has not been replicated; and 7)

Table 4-16 Human Cancer Potency Estimates (ppm^{-1}) for Butadiene from Rat Inhalation Study (Hazleton 1981, 1987)

Tissue/Lesion	Sex	Experimental Dose q_1*	nternal Dose q_1*		Metabolized Dose q_1*[b]	
		$(ppm)^{-1}$	$(ppm)^{-1}$[c]	$(mg/kg\text{-}d)^{-1}$[d]	$(ppm)^{-1}$[c]	$(mg/kg\text{-}d)^{-1}$[d]
Mammary/carcinoma	F	1.9×10^{-4}	1.7×10^{-3}	7.9×10^{-3}	4.8×10^{-4}	2.2×10^{-3}
Thyroid follicular cell/ adenoma and carcinoma	F	2.6×10^{-5}	1.5×10^{-3}	7.0×10^{-3}	5.1×10^{-4}	2.3×10^{-3}
Uterus/cervical stromal sarcoma	F	1.1×10^{-5}	8.4×10^{-4}	3.8×10^{-3}	3.2×10^{-4}	1.5×10^{-3}
Testis/Leydig cell adenoma and carcinoma	M	2.0×10^{-5}	1.2×10^{-3}	5.4×10^{-3}	4.0×10^{-4}	1.8×10^{-3}
Pancreas/exocrine adenoma	M	1.6×10^{-5}	2.6×10^{-4}	1.2×10^{-3}	1.4×10^{-4}	6.2×10^{-4}
Zymbal Gland/ adenoma and carcinoma	M F	5.3×10^{-6} 8.4×10^{-6}	3.5×10^{-4} 4.0×10^{-4}	1.6×10^{-3} 1.8×10^{-3}	1.3×10^{-4} 9.2×10^{-5}	5.9×10^{-4} 4.2×10^{-4}
TOTAL SIGNIFICANT TUMORS	M[a] F[b]	8.4×10^{-5} 9.9×10^{-5}	1.8×10^{-3} 3.5×10^{-3}	8.4×10^{-3} 1.6×10^{-2}	3.5×10^{-4} 6.6×10^{-4}	1.6×10^{-3} 3.0×10^{-3}
SUM	M[a] F[b]	4.1×10^{-5} 2.2×10^{-4}	1.8×10^{-3} 3.6×10^{-3}	8.2×10^{-3} 1.6×10^{-2}	6.6×10^{-4} 1.1×10^{-3}	3.0×10^{-3} 4.9×10^{-3}

NOTE: [a]includes pancreatic, Zymbal gland and Leydig cell tumors.
[b]includes mammary carcinoma thyroid and Zymbal gland tumors.
[c]animal/human equivalent potencies using external low dose conversion factor of 0.22 mg/kg-d/ppm for the rat.
[d]rat potency estimates derived from low dose extrapolations.

TABLE 4-17 WEIBULL TIME TO TUMOR ANALYSES OF HAZLETON 1981 RAT DATA

SEX,N	DOSE	SITE	95% LCL DOSE FOR 10-6 Risk	95% UCL Potency $(mg/kg\text{-}d)^{-1}$	$(ppm)^{-1}$
M 106,102,107[a]	Continuous Internal 0,7.5,25.8 mg/kg-d	All[b] SIGNIF	$1.1x10^{-4}$	$9.5x10^{-3}$	$2.1x10^{-3}$
F 110,99,109	Continuous Internal 0,7.5,25.8 mg/kg-d	All[b] SIGNIF	$4.5x10^{-5}$	$2.2x10^{-2}$	$5.0x10^{-3}$
M 96,102,97 93,99,89[c]	Continuous Internal 0,7.5.25.8 mg/kg-d	All[b] SIGNIF	$1.1x10^{-4}$ $1.1x10^{-4}$	$9.5x10^{-3}$ $9.5x10^{-3}$	$2.1x10^{-3}$ $2.1x10^{-3}$
F 100,99,99 99,97,98[c]	Continuous Internal 0,7.5,25.8 mg/kg-d	ALL[b] SIGNIF	$4.7x10^{-5}$ $4.7x10^{-5}$	$2.2x10^{-2}$ $2.2x10^{-2}$	$5.0x10^{-3}$ $4.8x10^{-3}$
F 110,99,109[a]	Continuous Internal 0,7.5,25.8 mg/kg-d	ALL[c] Incl. Uterine Sarcoma	$3.8x10^{-5}$	$2.7x10^{-2}$	$5.9x10^{-3}$
F 100,99,99 99,97,98[c]	Continuous Internal 0,7.5,25.8 mg/kg-d	Mammary Benign and Malignant	$1.9x10^{-5}$ $1.9x10^{-5}$	$6.4x10^{-2}$ $6.4x10^{-2}$	$1.4x10^{-2}$ $1.4x10^{-2}$
M 96,102,97	Metabolized 0,34.7,58.7 mg/kg-d	ALL[c] SIGNIF	$4.2x10^{-4}$	$2.4x10^{-3}$	$5.3x10^{-4}$
F 100,99,99	Metabolized 0,34.7,58.7 mg/kg-d	ALL[c] SIGNIF	$1.4x10^{-4}$	$6.6x10^{-3}$	$1.4x10^{-3}$
F 100,99,99	Metabolized 0,34.7,58.7 mg/kg-d	Mammary Benign and Malignant	$4.4x10^{-4}$	$2.8x10^{-2}$	$6.0x10^{-3}$

a Analysis includes animals from 52 week interim sacrifice.

b All significant tumors; male - pancreatic exocrine adenoma, Zymbal gland adenoma and carcinoma, and Leydig cell tumors; female - mammary carcinoma, thyroid follicular adenoma and carcinoma, and Zymbal gland adenoma and carcinoma.

c Includes animals surviving: 54 weeks or longer (first mammary tumor); 66 weeks or longer (first significant male tumor).

suggestions from limited epidemiological observations that BD exposure may be associated in humans with lymphatic and hematopoetic cancers, effects that were seen in mice. Thus, the staff of OEHHA conclude that the best data for use in risk assessment are the mouse II bioassay data of Melnick et al. (1990).

Among the mouse continuous dose estimates, those based on an interspecies equivalent dose unit of mg/m^2 surface area/day are considered to be the most accurate. The data of Bond et al. (1986) indicate that the basis of the continuous dose measure, namely the ^{14}C BD equivalents absorbed and retained at 6 hours, appear to scale with body surface area (Rat/Mouse = 0.60 ± 0.26 (N = 5)) over 3 orders of magnitude. The corresponding values for body weight and ppm in air, using conversion factors of 0.54/mg/kg-d/ppm for mouse and 0.22 mg/kg-d/ppm for rat, were 0.25 ± 0.11 and 0.54 ± 0.23 respectively.

Assuming that the continuous internal, surface area scaled dose is the best measure currently available, the range of human potencies or slope factors would be 9.8×10^{-6} $(ppb)^{-1}$ to 8.0×10^{-4} $(ppb)^{-1}$ [9.8×10^{-3} $(ppm)^{-1}$ to 0.80 $(ppm)^{-1}$]. The single best estimate is 3.7×10^{-4} $(ppb)^{-1}$ [0.37 $(ppm)^{-1}$] based on lung tumors in female mice. These values can be compared with the current EPA mouse-based geometric mean value of 6.4×10^{-4} $(ppb)^{-1}$. This latter figure is based on multiple significant cancers in mice, a 54% absorption fraction from early unpublished data of the study of Bond et al. (1986), and continuous internal doses of the Mouse I bioassay (NTP, 1984). The unit risk values from the current analyses range from 4.4×10^{-6} to 1.7×10^{-4} $(\mu g/m^3)^{-1}$ and the lifetime BD exposure level associated with an extra risk of cancer of 1 in 10^6 ranges from 6.0×10^{-3} to 0.23 $\mu g/m^3$. These key values are summarized in Table 4-18.

Table 4-18 Human Cancer Potency And Risk Estimates Based on Rodent Data

Measure	Units	Mouse (Melnick et al., 1990)	Rat (Hazleton, 1981)	EPA[a] (NTP, 1984)
Cancer Potency	$(ppm)^{-1}$	0.37	9.8×10^{-3}	0.64
Cancer Potency	$(mg/kg\text{-}d)^{-1}$	3.4	0.42	1.8
Unit Risk	$(\mu g/m^3)^{-1}$	1.7×10^{-4}	4.4×10^{-6}	2.9×10^{-4}
Dose For a 10^{-6} Risk	$\mu g/m^3$	6.0×10^{-3}	0.23	3.5×10^{-3}

[a]EPA (1985) figures based on geometric mean of male and female mouse total significant tumor incidence and a continuous internal dose calculation using absorption fraction of 54%.

5.0 RISK CHARACTERIZATION

5.1 Population Burden

As reported in Part A of this document, the California Air Resources Board has found an overall statewide population-weighted average ambient butadiene concentration of 0.37 ppb. Air basin-specific population-weighted mean concentrations ranged from 0.22 to 0.44 ppb.

Assuming a range of potency values from a rat-based low value of 9.8×10^{-6} $(ppb)^{-1}$ to a mouse-based best value of 3.7×10^{-4} $(ppb)^{-1}$, community exposure to 1 ppb ambient BD could be associated with up to 10 (9.8 rounded) to 370 additional cancers per million individuals exposed for their lifetimes. Acutal exposures in California are generally lower than 1 ppb, however. Using the air basin-specific average BD concentrations observed would yield lower figures, namely up to 2 to 160 additional cancers per million exposed.

5.2 Hot Spots

Since an important source of BD emissions appears to be equipment leaks in industrial plants using or producing BD, the potential for hot spots of community exposure in the vicinity of these facilities exists. The EPA has estimated lifetime extra cancer risks for individuals living near two butadiene production plants in California. A facility in southern California, which emits 48,500 kg BD/yr, would have a lifetime cancer risk at the border of the facility of roughly 10^{-3}. A facility in northern California, which emits 12,600 kg BD/yr, would have a lifetime cancer risk at its fenceline of roughly 10^{-4}. The estimates are based on EPA dispersion modeling and other typical assumptions. The actual risks could be lower (sources: California Air Resources Board, Stationary Source Division and EPA Office of Air Quality Planning and Standards).

5.3 Comparison With Epidemiology

EPA (1985) compared the risk estimates based on the mouse study data with the findings in the epidemiology studies of Meinhardt et al. (1982), Matanoski et al. (1982), and McMichael et al. (1976). They used the estimated exposure of 13.5 ppm from Plant B in Meinhardt et al. to derive a continuous lifetime dose of 0.64 ppm from an average of 10.74 working years. They concluded that there was no evidence that unit risk extrapolation from animal to human was unreasonable or that it seriously over-predicted cancer risk.

A similar comparison can be made between the risk estimates derived in the preceding section and the more recent epidemiological reports of Downs et al. (1987) and Matanoski and Schwartz (1987). Although occupational exposure estimates are not given by these authors, values of 1 and 10 ppm based on the earlier studies seem reasonable (Fajen et al., 1986). These represent 10 and 100% of the current 8 hour weighted average TLV for butadiene (ACGIH, 1987). For the Downs et al. study the average years of employment or exposure were 11.3 and age at entry 30.6. The continuous lifetime equivalent exposure based on 11.3 working years out of about 40 remaining years is:

$$10 \text{ ppm} \times \frac{11.3}{40} \text{ years} \times \frac{240}{365} \text{ days} \times \frac{8}{24} \text{ hours} = 0.62 \text{ ppm}$$

The initial estimate of q_1, the maximum likelihood estimate or MLE, from the incidence of malignant lymphoma in male mice was 3.12×10^{-2} kg-d/mg or 1.68×10^{-2} $(ppm)^{-1}$ while the cancer potency estimate (q_1*) for this site was 2.03×10^{-1} kg-d/mg or 8.9×10^{-2} $(ppm)^{-1}$. The initial estimate is used for comparison with the EPA analysis of the data of Meinhardt et al. (1982) (Table 5.1). Based on the initial MLE of 1.68×10^{-2} $(ppm)^{-1}$ the individual extra lifetime risk would be:

$$R = 1.68 \times 10^{-2} (ppm)^{-1} \times 0.62 \text{ ppm} = 1.04 \times 10^{-2}.$$

Of 2586 workers at risk for 25 years out of their 40 remaining years this risk represents the following extra number of lymphatic cancers:

$$1.04 \times 10^{-2} \times 2586 \times \frac{25}{40} = 16.8$$

Adding 16.8 to the 4.4 cancer deaths expected (Table 5.1) we could expect to observe 21 cancers of the lymphatic system for occupational exposure to 10 ppm BD. For 1 ppm exposure we could expect to observe 1.7 additional cases or 6 total cases. These estimates are remarkably close to 8 cases actually observed in the Downs et al. study. These data are also consistent with predictions obtained using the potency or upper 95% confidence level; namely 13 at 1 ppm and 94 at 10 ppm. If similar estimates are employed with the Matanoski and Schwartz study (i.e. 10 working years average exposure, 36 years observed/50 years lifetime remaining), the mouse data would predict 21.7 to 115 cases at 10 ppm and 2.2 at 1 ppm where the observed excess was only 0.5. This may indicate a lower average continuous exposure for this larger cohort of workers. Alternatively, it may indicate that the potency estimate is too high.

However, the greatest source of uncertainty in this comparison is the assumption of site-specific concordance among species. Since BD is clearly a multi-site carcinogen, BD exposure may result in tumors at different sites in different species. In conclusion, the cancer potency estimates (q_1*), and derived from initial MLE values of the male mouse assay for malignant lymphoma, appear to give a reasonable range of predicted incidence of human lymphatic cancer possibly induced by occupational exposure to BD as described in recent epidemiological studies. This supports EPA's earlier conclusion with regard to their potency estimate (geometric mean of all significant sites) and the earlier epidemiological data. While the recent studies are limited by lack of BD exposure data, the overall conclusion, considering the various uncertainties involved, is that the mouse bioassay data does not greatly over predict the extra cancer risk, at least in those cases where exposures are reasonably close to the bioassay doses (i.e., 2-3 orders of magnitude).

An additional comparison can be made using the mouse-based "best value" unit risk presented in Section 4.2.9 and epidemiologic data from the more recent

Table 5-1 Comparison of Recent (BD) Occupational Epidemology with Incremental Risks Based on Initial MLE and Potency Values of Mouse Data

Study	Sample Size	All Cancers			Lymphatic System Neoplasms			Mouse Data Prediction		Exposure Estimate ppm
		OBS	EXP	SMR	OBS	EXP	SMR	a	b	
Downs et al. (1987)	2586	122	161	76	8	4.4	182	17 1.7	90 9	10 1
Matanoski and Schwartz (1987)[c]	3269	94	106	89	11	10.5	104	21.7[c] 2.2	115 12	10 1
Meinhardt[d] et al. (1982) Plant B	1094	11	20.89	58	2	2.55	78	6.3	-	13.5

a) Male mouse MLE(q_1) for malignant lymphoma = 3.12×10^{-2} kg-d/mg or 1.68×10^{-2} $(ppm)^{-1}$

b) Male mouse potency (q_1*) for malignant lymphoma = 2.03×10^{-1} kg-d/mg or 8.9×10^{-2} $(ppm)^{-1}$

c) Assumed 10 human years average exposure, 36 years observed/50 years lifetime remaining

d) EPA (1985) initial MLE 2.5×10^{-2} $(ppm)^{-1}$ geometric mean of sexes, all significant sites

reports of Matanoski et al. (1990) and Divine (1990). An example is presented here, using subcohorts and tumor sites in which a significantly elevated SMR was observed. This comparison is presented in Table 5-1b.

For consistency with the use of a 95% upper-bound mouse-based potency estimate, an upper-bound 95% confidence limit (95% U.C.L.) on the observed number of cancer cases is calculated for each cohort and site selected. To calculate a U.C.L. for each observed number of cases, a table is used which specifies the U.C.L.s for given expected values of a Poisson distribution. For example, 5 cases of lymphosarcoma or reticulosarcoma were observed in the routinely exposed workers of the Divine (1990) cohort. The 95% U.C.L. on this number, assuming a Poisson distribution, is 11.7; rounded to the nearest integer, this U.C.L. is 12 (Table 5-1b). Since the number of cases observed for a given site is partially dependent on the number of person-years in a study, and the confidence interval around an observed number of cases narrows as the number increases, the bigger the cohort or the greater the number of person-years in a study, the smaller the difference between the observed value and the 95% U.C.L.

In Table 5-1b, 95% U.C.L.s on observed numbers of cancer cases are listed beside cancer incidence counts that would be predicted from the 95% upper-bound mouse-based unit risk estimate. As in the comparisons described above, workplace BD exposures of 1 and 10 ppm are assumed.

Since the epidemiologic studies are based on workers exposed for and followed for only parts of their lifetimes, and the mouse-based upper-bound unit cancer risk value is for lifetime exposure and follow-up, adjustments are applied to the unit risk to generate predictions of cancer cases among the workers. A description of these adjustments follows.

For the Matanoski et al. (1990) study, it is assumed that a time-weighted average exposure would adequately account for the less than lifetime exposure, and that workers were exposed for 10 years. For example, a lifetime time-weighted average (TWA) BD concentration may be estimated for black production workers assumed to be exposed to 1 ppm BD during working hours:

$$1 \text{ ppm} \times \frac{10 \text{ yrs}}{70 \text{ yrs}} \times \frac{50 \text{ wks}}{52 \text{ wks}} \times \frac{5 \text{ days}}{7 \text{ days}} \times \frac{8 \text{ hrs}}{24 \text{ hrs}} = 0.032 \text{ ppm (TWA)}$$

At this exposure concentration, the lifetime excess individual cancer risk would be estimated as:

$$0.032 \text{ ppm BD} \times 0.37 \text{ per ppm BD} = 0.012$$

From this, it may be estimated that approximately 4.5 cancer cases (0.012 x 371) associated with BD exposure would occur among the 371 black production workers over their lifetimes.

Here it is assumed that the proportion of butadiene-associated cancer cases that would have appeared by the end of a study would be the same as the proportion of the study group that had died by the conclusion of the study. Thus, based on the overall mortality of Matanoski et al.'s study group (25%), it may be "predicted" that approximately 1.1 BD-linked cases (4.5 cases x 25%)

Table 5-1b. Comparison of Cancer Incidence in Worker Cohorts with Predictions from Mouse-Based Butadiene Risk Assessment.

			Number of Cancer Cases In Workers			Number of Cases Predicted from 95% U.C.L. of Mouse-Based Potency Estimate[a]	
Study	Cohort	Cancer Type	Exp.	Obs.	95% U.C.L. of Obs.	At 10 ppm BD	At 1 ppm BD
Matanoski et al., 1990	Black Production Workers (N=371)	All	16.5	19	30	28	18
		All Lymphopoietic	1.2	6	13	12	2
Divine, 1990	Routinely Exposed Workers (N=705)	All	46.3	42	57	50 - 80[b]	47 - 50[b]
		Lymphosarcoma & reticulosarcoma	0.9	5	12	5 - 40	1 - 5

Abbreviations: Exp., expected. Obs., observed. U.C.L., upper confidence limit. BD, butadiene. ppm, parts per million.

[a]Based on occupational exposure to the indicated concentrations of BD. These concentrations represent a range of plausible estimates (the actual workplace concentrations were not measured). See text for exposure assumptions and description of calculations.

[b]Ranges given here result from using different numbers of stages in multistage model of human cancer incidence. See text for details.

would appear during the study period. Overall, approximately 18 cancer deaths would be predicted to occur in the cohort (Table 5-1b), based on 1.1 BD-linked cases and 16.5 cases expected due to background incidence.

For the Divine (1990) study, there is sufficient information to apply a Doll-Armitage multistage correction (Brown and Chu, 1983) to account for the less than lifetime exposure and follow-up. The formula used here is:

$$\text{Correction Factor} = (1/L)^{k-1}\,[(T_e - T_a)^{k-1} - (T_e - T_b)^{k-1}]$$

where L = lifespan (70 years)
k = stages of the multistage model
T_e = average age of conclusion of study
T_a = average age at first exposure
T_b = average age at last exposure

Using data reported by Divine (1990), T_e = 57.9 years, T_a = 27.5 years, and T_b = 39.9 years. The k parameter, which represents the number of stages in the multistage model of carcinogenesis, is assumed to be between 4 and 7, in consideration of data reported by Brown and Chu (1983). Using these values, the correction factor was estimated as:

Stages (k)	Correction Factor
4	0.065
5	0.031
6	0.014
7	0.0064

These correction factors are used (in place of the partial lifetime exposure factor and mortality correction used for the Matanoski et al. cohort) to estimate equivalent lifetime exposure concentrations (for the Divine cohort). For example, using 4 stages in the multistage model, the equivalent lifetime exposure is:

$$1 \text{ ppm} \times 0.065 \times \frac{50 \text{ wks}}{52 \text{ wks}} \times \frac{5 \text{ days}}{7 \text{ days}} \times \frac{8 \text{ hrs}}{24 \text{ hrs}} = 0.015 \text{ ppm (TWA)}$$

At this lifetime equivalent exposure concentration, the lifetime excess individual cancer risk is estimated to be:

0.015 ppm BD x 0.37 per ppm BD x 705 workers = 3.9 cases

Thus, approximately 50 cancer deaths are predicted to occur in the cohort at 1 ppm BD (Table 5-1b, far right column), based on 3.9 BD-linked cases plus 46.3 cases expected due to background incidence. This prediction decreases with increasing values of k.

The predictions that use this document's mouse-based "best value" cancer potency estimate for BD are remarkably consistent with epidemiologic observations (Table 5-1b). The concordance is most striking when the workplace concentration of BD is considered to be 10 ppm. Then, the 95% U.C.L.s on the epidemiologic observations are closest to the predictions from the 95% U.C.L. mouse-based potency estimate: 30 vs. 28, 13 vs. 12, 57 vs. 50

to 80, and 12 vs. 5 to 40 (the low end of the ranges corresponds to k=7 and the high end corresponds to k=4).

One cannot draw sweeping conclusions from this comparison, however. Both the mouse and the human studies have their limitations. The degree to which mice are like humans (or vice versa) is still largely unknown. Nevertheless, from the foregoing, the mouse-based "best value" upper-bound unit risk estimate derived in this document appears sufficiently valid for use in human health risk assessment.

5.4 Relative potency

In order to compare the potency of 1,3-butadiene with other toxic air contaminants evaluated by OEHHA, potency slope factors were also expressed in molar units (Table 5-2). The 95% upper confidence limit on the slope of the low dose response of female mice for lung tumors is 0.37 ppm^{-1}. This is converted to $(mg/kg\text{-}d)^{-1}$ for humans as follows, assuming 17% low dose absorption fraction:

$$2.21\ mg/m^3 \times 0.17 \times 20\ m^3/d \times 1/70\ kg =$$

$$0.11\ mg/kg\text{-}d/ppm$$

$$0.37\ (ppm)^{-1} \times \frac{1}{0.11} \quad \frac{ppm}{mg/kg\text{-}d} = 3.36\ (mg/kg\text{-}d)^{-1}$$

By further converting to molar potency using the molecular weight of butadiene (54.1) the following value is obtained:

$$3.36 \left[\frac{mg}{kg\text{-}d}\right]^{-1} \times \left[\frac{1\ \mu mol}{0.0541 mg}\right]^{-1} = 62.2 \left[\frac{\mu mol}{kg\text{-}d}\right]^{-1}$$

$$\log_{10} 62.2 \left[\frac{\mu mol}{kg\text{-}d}\right]^{-1} = 1.8 \left[\frac{\mu mol}{kg\text{-}d}\right]^{-1}$$

In Table 5-2 the molar potencies and their logarithims are listed for ease of comparison. Based on this comparison butadiene ranks fourth in relative potency among this group of toxic air contaminants. In a similar analysis EPA (1985) found butadiene to rank in the third quartile of some 55 carcinogens evaluated by EPA in various environmental media.

5.5 Interspecies Comparisons

Despite the apparent consistency between human cancer epidemiology and the cancer potency values derived from mouse bioassay data, the large differences seen between mouse and rats in BD/BMO metabolism and carcinogenic susceptibility raises some questions about the choice of animal model for extrapolation to humans. Studies of `monooxygenase' and other xenobiotic metabolizing enzymes in human biopsy and in surgical and postmortem tissue specimens, particularly lung, have been quite variable to date. Monooxgenase activities in lung ranged from less than 0.1 (Watanabe and Abe, 1981) to 135 pmol/mg protein/min with benzo[a]pyrene and naphthalene as substrates (Table 5-3).

Table 5-2 Relative Potencies of BD and Selected Toxic Air Contaimnants

Toxic Air Contaminant	Unit Risk $(ug/m^3)^{-1}$	Potency $(mg/kg\text{-}d)^{-1}$	MW	Molar Potency $(umol/kg\text{-}d)^{-1}$	log_{10} Molar Potency
1. TCDD	24 to 38	1.33×10^5	322	4.1×10^5	5.6
2. Chromium (VI) (CrO_4^{-2})	(1.2 to 14.6) x 10-2		100	5.1×10^3	3.7
3. Cadmium (II)	(0.2 to 18) x 10^{-2}	40	112.4	356	2.6
4. Butadiene	(0.04 to 1.6) x 10^{-4}	3.36	54.1	62.2	1.8
5. Ethylene Oxide	(0.6 to 8.8) x 10^{-5}	0.3	44.1	6.8	0.83
6. Benzene	(0.75 to 5.3) x 10-5	0.17	78	2.2	0.34
7. Ethylene Dibromide	(1.3 to 7.1) x 10-5	2.4×10^{-1}	187.9	1.3	0.11
8. Carbon Tetrachloride	(1 to 15) x 10^{-5}	0.15	153.8	0.98	-0.011
9. Ethylene Dichloride	(1.3 to 2.2) x 10-5	7.2×10^{-2}	96.9	.74	-0.13
10 Methylene Chloride	(2.6 to 3.4) x 10^{-6}	1.4×10^{-2}	84.9	0.16	-0.78

Table 5-3 Human Lung Xenobiotic Metobolizing Enzyme Activities

Study	Specimen(N)	Cytochrome P450[a]	BaP monoxygenase[b]	NADPH[c] cytochrome[c] reductase	NADH[d] cytochrome[b5] reductase
Watanabe & Abe (1981)	surgical (16)	<0.01	<0.1 to 6.0	0.04	3.6
Jakobson etal.	post mortem (N)	trace (14)	11.5 ± 4.8(19)	20.9(9)	--
McManus etal. (1980)	surgical (10)	ND	0.23	0.033	0.68
Leboeuf etal.	post mortem (1)	--	0.32	--	--
Devereux etal. (1986)	surgical (5) Alveolar Type II cells	ND	--	0.018-0.029	--
Buckpitt & Bahnson (1986)	surgical (1)	--	42-104	-- --	
	(1)naphthalene	--	11-135	--	--
Mori etal. (1986)	surgical (3)	<.012	0.37	--	--

NOTE: [a] nmol cytochrome P450/mg protein
[b] pmol/min/mg protein
[c] nmol cytochrome c reduced/min/mg protein
[d] pmol ferricyanide reduced/min/mg protein
[e] ND - not determined

According to Lorenz et al. (1984), with the exception of monooxygenase activity in human lung which was two orders of magnitude lower, rodent and human subcellular fractions from liver or lung had specific activity ratios (liver/lung) within one order of magnitude (monooxgenase, epoxide hydrolase, glutathione-S-transferase).

Similar results were obtained by Mori et al. (1986) and are summarized in Table 5-4. Even highly sensitive immunocytochemical techniques using monoclonal antibody to a major form of human hepatic cytochrome P450, P450hA7, have shown only weak immunoreactivity in proximal tubules of the kidney, pancreatic acini, gall bladder epithelium, squamous epithelium and sebaceous glands of the skin, interstitial cells of the testis and luteal cells of the ovary. No activity was detected in adrenal gland, placenta, colonic epithelium and alveolar type II cells and Clara cells of the lung (Murray et al., 1988). Clearly more work is needed to determine whether the mouse is a good model for prediction of human susceptibility to BD induced carcinogenesis or if it is simply a particularly sensitive species due to its enzymatic profile.

Table 5-4 Cytochrome P450 Contents and Aniline Hydroxylase Activities in Animal or Human Lung S-9 (Mori et al., 1986)

Species	N	Sex	P450 Content nmol/mg Protein	AH Activity pmol/mg S9 protein/min
Rat	5	M	0.036 ± 0.009	14 ± 3
	5	F	0.042 ± 0.002	10 ± 3
Hamster	3	M	0.034 ± 0.010	40 ± 8
Mouse	10	M	0.060 ± 0.013	30 ± 2
Rabbit	2	M	0.041 ± 0.004	19 ± 2
Monkey	1	M	0.081	6
	1	F	0.086	10
Human	A	M	<0.012	0.3
	B	M	<0.012	0.5
	C	M	<0.012	0.3

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APPENDIX A

MELNICK ET AL., 1991

[CANCER RESEARCH 50, 6592–6599, October 15, 1990]

Carcinogenicity of 1,3-Butadiene in C57BL/6 × C3H F_1 Mice at Low Exposure Concentrations

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ABSTRACT

The carcinogenicity of inhaled 1,3-butadiene was evaluated in C57BL/6 × C3H F_1 mice exposed to concentrations of this gas ranging from 6.25 to 625 ppm. Butadiene is a high production volume chemical, used mainly in the manufacture of synthetic rubber. In these 2-yr inhalation studies, a potent multisite carcinogenic response was observed, including neoplasms of the lung at concentrations as low as 6.25 ppm. Early occurrence and extensive development of lethal lymphocytic lymphomas in mice exposed to 625 ppm of butadiene reduced the number of animals at risk for the expression of later developing neoplasms at other sites; at lower exposure concentrations, dose responses were demonstrated for hemangiosarcomas of the heart and neoplasms of the lung, forestomach, Harderian gland, preputial gland, liver, mammary gland, and ovary. So far, no long-term studies on butadiene have been conducted at exposure concentrations that have not shown a carcinogenic response. In separate experiments with reduced exposure durations, butadiene induced neoplastic responses at multiple organ sites even after only 13 wk of exposure. Because of the correspondence between these animal data and recent epidemiology findings, there is a worldwide public health need to reevaluate current workplace exposure standards for 1,3-butadiene.

INTRODUCTION

1,3-Butadiene, a colorless gas (bp, −4.4°C) generally produced as a coproduct of ethylene production, is used mainly in the manufacture of synthetic rubber (styrene-butadiene rubber or polybutadiene rubber) and of thermoplastic resins (1). The annual production volume of butadiene is approximately 12 billion pounds worldwide and 3 billion pounds in the United States (2). The United States Environmental Protection Agency estimates that between 5300 and 8200 workers are exposed to butadiene in facilities which produce the monomer or which process the monomer into polymers (3). Occupational exposure to butadiene is generally less than 20 ppm; however, in a recent industrial hygiene survey, it was found that exposures in certain jobs were as high as 375 ppm (4). Butadiene has also been identified in automobile exhaust and cigarette smoke (5).

Butadiene is mutagenic to *Salmonella typhimurium* (strains TA1530 and TA1535) in the presence of S9 metabolic activation systems (6). The mutagenicity of butadiene may be due to its oxidative intermediates, butadiene monoxide and diepoxybutane (7), which are direct acting mutagens in *Salmonella* (8, 9). Butadiene was also genotoxic to bone marrow cells of exposed mice, causing an increase in the frequency of chromosomal aberrations, sister chromatid exchanges, and micronucleated erythrocytes (10).

At exposure concentrations nearly equal to and one-half the Occupational Safety and Health Administration 8-h time-weighted average workroom exposure standard of 1000 ppm, butadiene was carcinogenic in mice (11) and rats (12). A species difference with respect to sites of tumor induction and magnitude of response was demonstrated in these carcinogenicity studies of butadiene (11, 12). The results of those studies prompted the American Conference of Governmental Industrial Hygienists to lower their recommended threshold limit value for butadiene in the work environment from 1000 ppm to 10 ppm (13). Meanwhile, the Occupational Safety and Health Administration has determined that there are significant predicted risks associated with occupational exposure to 100 ppm of butadiene over a working lifetime (14). Most importantly, recent epidemiological findings from the rubber industry indicate an association between occupational exposure to 1,3-butadiene and the development of lymphatic and hematopoietic cancers (15, 16).

This paper reports our 2-yr inhalation studies of butadiene in mice exposed to concentrations ranging from 6.25 to 625 ppm and separate studies using variable exposures and durations. Dose-response relationships are reported for the various neoplastic lesions induced by butadiene, and the relationship between exposure concentrations and durations of exposure on the outcome of butadiene-induced carcinogenicity has been assessed.

MATERIALS AND METHODS

Chemicals. Liquified 1,3-butadiene of 99.7% purity, containing trace amounts of *t*-butyl catechol, a peroxide inhibitor, was obtained from Phillips Chemical Co. (Borner, TX). Concentrations of the dimer, 4-vinyl-1-cyclohexene, in the cylinder headspace were determined by gas chromatographic analysis. Cylinders were not retained for use if the dimer content was greater than 150 ppm. Butadiene gas was metered to Hazelton 2000 chambers (Harford Division of Lab Products, Inc., Aberdeen, MD) via a distribution system and diluted in the fresh air chamber inlets. Chamber concentrations of 1,3-butadiene were monitored continuously during the exposure periods utilizing a Hewlett-Packard 5840 gas chromatograph equipped with a flame ionization detector [oven temperature, 120°C; gas chromatography column, 12- x ⅛-in nickel column packed with 1% SP-1000 on 60/80 Carbopack B (Supelco, Inc., Bellefonte, PA); carrier gas, nitrogen (20 ml/min)]. The daily mean concentrations of 1,3-butadiene, distributed uniformly in the chambers, were within 2% of the target concentrations.

Animals. Five-wk-old male and female C57BL/6 × C3H F_1 (hereafter called B6C3F_1) mice, obtained from the Frederick Cancer Research Facility (Frederick, MD), were quarantined and acclimatized for 10 days prior to the start of the study. Stainless steel Hazelton 2000 chambers were used, and animals were exposed in individual wire mesh cage units, each having a floor area of 106 cm^2. The chamber environment was maintained at 24 ± 2°C and 55 ± 15% relative humidity with a chamber air flow of 15 air changes/h. Fresh softened tap water and NIH-07 diet were available *ad libitum*, except when the feed was removed during the exposure periods and overnight prior to the 40- and 65-wk necropsies.

Exposure Regimen. Groups of 70 to 90 male and female B6C3F_1 mice were exposed to 0, 6.25, 20, 62.5, 200, or 625 ppm of butadiene, 6 h/day, 5 days/wk for up to 2 yr. As many as 10 animals/group were killed and evaluated after 40 and 65 wk of exposure. Additional studies, in which exposure to butadiene was stopped after limited exposure periods, were included to assess the relationship between exposure level

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and duration of exposure on the outcome of butadiene-induced carcinogenicity. In the stop-exposure studies, groups of 50 male mice were exposed to one of the following regimens: (*a*) 625 ppm for 13 wk; (*b*) 200 ppm for 40 wk; (*c*) 625 ppm for 26 wk; or (*d*) 312 ppm for 52 wk. After the exposures were terminated, these groups of animals were placed in control chambers for the remainder of the 104-wk studies. The total exposure to butadiene (concentration × duration of exposure) was approximately equivalent for Groups *a* and *b* and provided about half the total exposure given to Groups *c* and *d*.

Histopathology. All animals that died during the study or that were killed at the end of the exposure period received a complete gross necropsy and histopathological examination. All moribund animals and those remaining at the end of the study were killed by carbon dioxide asphyxiation and necropsied immediately. Tissue samples, preserved in 10% neutral buffered formalin, were embedded in paraffin, sectioned, and stained with hematoxylin and eosin. The following tissues were examined microscopically: gross lesions and tissue masses; bronchial, mediastinal, mandibular, and mesenteric lymph nodes; salivary gland; sternebrae (including marrow); thyroid; parathyroid; small intestine; large intestine; liver; gall bladder; prostate; testes, epididymis, and seminal vesicles; ovaries; lungs and mainstem bronchi; nasal cavity and turbinates; heart; esophagus; stomach; uterus; brain; thymus; larynx; trachea; pancreas; spleen; kidneys; adrenals; urinary bladder; pituitary; and mammary gland.

Statistical Analyses. Differences in survival were analyzed by life table methods (17), and incidences of neoplastic lesions (the ratio of the number of animals bearing such lesions at a specific anatomical site to the number of animals in which that site was examined) including information on time of death were analyzed by life table tests, logistic regression analyses, Fisher exact analyses, and Cochran-Armitage trend analyses (18, 19). Tumor rates were adjusted for intercurrent mortality using the survival-adjusted quantal response test described by Portier and Bailer (20), with a power value equal to 3 (21).

RESULTS

Survival was reduced for male and female mice exposed to 20 ppm of butadiene or higher concentrations, largely due to the development of compound-related malignant tumors (Tables 1 and 2). All of the mice exposed to 625 ppm died by Wk 65. The percentage of animals bearing malignant tumors increased from about 30% in the control groups to nearly 90% in the 625-ppm exposure groups. However, the best approach for detecting potential carcinogenic effects of chemicals is to compare specific tumor incidences between dosed and control groups rather than comparing the overall proportion of animals with malignant tumors.

Lymphocytic lymphomas, of thymic origin, appeared as early as Wk 23 and were the major cause of death for male or female mice exposed to 625 ppm; the incidence of these neoplasms was greater in males than in females (Tables 1 and 2). Malignant lymphomas were classified according to the criteria of Frith and Wiley (22). Most butadiene-induced lymphomas were well differentiated and lymphocytic, and they appeared to originate in the thymus. Furthermore, the thymus was the most frequently and prominently affected organ. The incidence of lymphocytic lymphomas was also increased in females exposed to 200 ppm, but not at lower concentrations. After Wk 65, malignant lymphomas had less thymic involvement, and the other histological types (histocytic and mixed-cell lymphomas) more commonly associated with the spontaneous lymphoma of $B6C3F_1$ mice were observed in all remaining groups.

The incidence of hemangiosarcomas of the heart was increased in male mice exposed to 62.5, 200, or 625 ppm, and in female mice exposed to 200 or 625 ppm. In addition, one animal from both the 20-ppm male group and the 62.5-ppm female group was observed with this endothelial cell tumor. These neoplasms are particularly important because hemangiosarcomas of the heart are uncommon in untreated male or female $B6C3F_1$ mice, occurring at a rate of about 0.04%, or one of every 2500 mice. Interestingly, the incidence of hemangiosarcomas of the heart was greater in male mice exposed to 200 ppm than in those exposed to 625 ppm. Yet, it is evident from Fig. 1 (where the cumulative incidence of lymphocytic lymphoma or hemangiosarcoma of the heart is plotted against the number of weeks on study) that, for male mice exposed to 200 or 625 ppm of butadiene, the early and extensive development of lethal lymphocytic lymphomas at 625 ppm resulted in a reduced number of mice at risk in that exposure group for the later developing hemangiosarcomas. The impact of early mortality on the expression of late developing tumors is largely accounted for in the mortality-adjusted tumor rates shown in Tables 1 and 2. Furthermore, when the rates of hemangiosarcoma of the heart in early death animals were compared over 25-wk intervals for male mice exposed to 200 or 625 ppm of butadiene, it was found that the rates of this neoplastic lesion during these time intervals were nearly equivalent at these two exposure concentrations for the first 75 wk of the study. After that time, a high rate of hemangiosarcoma of the heart (50%) was observed in the 200-ppm exposure group; there were no surviving animals in the 625-ppm exposure group. Thus, for male mice exposed to concentrations of butadiene below 625 ppm, the dose response for butadiene-induced hemangiosarcomas of the heart is more clearly demonstrated. Mortality-adjusted dose-response curves for lymphocytic lymphoma, hemangiosarcoma of the heart, and neoplasms of the lung, forestomach, and Harderian gland in male mice are shown in Fig. 2.

The impact of an early occurring lethal tumor on the detection of later developing neoplasms at other sites is further demonstrated by the finding that, in 15 male mice in the 625-ppm exposure group which did not die early with lymphocytic lymphoma (17% of the animals), the occurrences of hemangiosarcomas of the heart (five), alveolar-bronchiolar neoplasms (six), forestomach neoplasms (eight), and Harderian gland neoplasms (six) accounted for 66% of the total occurrences of these neoplasms in that exposure group.

The incidence of alveolar-bronchiolar neoplasms of the lung in male mice was increased at 62.5 ppm and higher concentrations compared with controls. Statistical significance at 625 ppm was obtained when tumor rates were adjusted for early mortality. In female mice, the incidence of alveolar-bronchiolar neoplasms was increased at all exposure concentrations compared with controls. Thus, even at 6.25 ppm, butadiene is carcinogenic to $B6C3F_1$ mice. The reduced incidence of lung neoplasms at 625 ppm compared with the incidence at 200 ppm is attributed to the high rate of early deaths due to lymphocytic lymphoma in female mice exposed to 625 ppm (Fig. 3). For alveolar-bronchiolar neoplasms, the time to tumor detection was slightly shorter with exposure to 625 ppm; however, because all of the female mice exposed to this concentration of butadiene died by Wk 65, the final incidence of this neoplastic lesion was less than that for female mice exposed to 200 ppm. Furthermore, the rates of alveolar-bronchiolar neoplasms in early death female mice exposed to 200 or 625 ppm of butadiene were nearly equivalent during 25-wk intervals for the first 75 wk of the study. After that time, a high rate of alveolar-bronchiolar neoplasms (78%) was observed in the 200-ppm exposure group. Mortality-adjusted dose-response curves

Table 1 *Survival and incidence of primary tumors in male B6C3F₁ mice exposed to 1,3-butadiene for up to 2 yr*

Incidence is given as the number of animals bearing a neoplastic lesion at a specific anatomical site. Overall rates, based on the number of animals in which that site was examined, are given below the incidence values. Mortality-adjusted tumor rates are given in parentheses.

	Exposure concentration (ppm)					
	0	6.25	20	62.5	200	625
Initial no.[a]	70	70	70	70	70	90
No. of survivors	35	39	24[b]	22[b]	3[b]	0[b]
Target (neoplasm)						
Lymphocytic lymphoma	2 3% (4%)	1 1% (2%)	2 3% (5%)	4 6% (10%)	2 3% (7%)	62[c] 69% (96%)
All lymphomas	4 6% (8%)	3 4% (6%)	8 11% (19%)	11[c] 16% (25%)	9[c] 13% (27%)	69[c] 77% (97%)
Heart (hemangiosarcoma)	0 0% (0%)	0 0% (0%)	1 1% (2%)	5[c] 7% (13%)	20[c] 29% (57%)	6[c] 7% (53%)
Lung (alveolar-bronchiolar neoplasm)	22 31% (46%)	23 38% (48%)	20 33% (45%)	33[c] 48% (72%)	42[c] 60% (87%)	12[c] 13% (73%)
Forestomach (squamous cell neoplasm)	1 1% (2%)	0 0% (0%)	1 1% (2%)	5 7% (13%)	12[c] 17% (36%)	13[c] 14% (75%)
Harderian gland (neoplasm)	6 9% (13%)	7 10% (15%)	11 16% (25%)	24[c] 34% (53%)	33[c] 47% (77%)	7[c] 8% (58%)
Preputial gland (adenoma or carcinoma)	0 0% (0%)	0 0% (0%)	0 0% (0%)	0 0% (0%)	5[c] 7% (17%)	0 0% (0%)
Liver (hepatocellular neoplasm)	31 44% (55%)	27 45% (54%)	35 59% (68%)	32 54% (69%)	40[c] 59% (87%)	12 13% (75%)

[a] Initial number includes animals removed from the study for interim sacrifices at 40 and 65 wk.
[b] Decreased compared with chamber control (0 ppm), $P < 0.05$.
[c] Increased compared with chamber control (0 ppm), $P < 0.05$, based on logistic regression analyses with adjustment for intercurrent mortality.

for lymphocytic lymphoma, hemangiosarcoma of the heart, and neoplasms of the lung, forestomach, and Harderian gland in female mice are shown in Fig. 4. The sharp and rapid concentration-dependent increase in the incidence of alveolar-bronchiolar neoplasms in female mice is particularly noteworthy. The estimated rate of alveolar-bronchiolar neoplasms in female mice after adjustment for intercurrent mortality is 8% at 0 ppm (controls), 32% at 6.25 ppm, 44% at 20 ppm, 61% at 62.5 ppm, 81% at 200 ppm, and 83% at 625 ppm.

The incidences of neoplastic lesions of the forestomach (squamous cell neoplasms), mammary gland (adenocarcinomas), ovary (granulosa cell neoplasms), and liver (hepatocellular neoplasms), the other organ sites identified in the first study (11, 23), were again increased. In addition, the Harderian gland and preputial gland were identified as sites of butadiene-induced neoplasia. The concentrations of butadiene at which these lesions were not significantly increased compared with controls were 62.5 ppm for forestomach squamous cell neoplasms and preputial gland neoplasms, 20 ppm for Harderian gland neoplasms, mammary gland adenocarcinomas, and ovarian granulosa cell neoplasms, and 6.25 ppm for hepatocellular neoplasms.

In the stop-exposure groups, survival was also markedly reduced due to the development of compound-related malignant tumors (Table 3). The tumor incidence profiles in the stop-exposure groups show that lymphocytic lymphomas, hemangiosarcomas of the heart, alveolar-bronchiolar neoplasms, forestomach squamous cell neoplasms, Harderian gland neoplasms, and preputial gland neoplasms were increased compared with controls even after only 13 wk of exposure to 625 ppm of butadiene. Furthermore, at comparable total exposures, the incidence of lymphocytic lymphoma was greater with exposure to a higher concentration of butadiene for a short time compared with exposure to a lower concentration for an extended duration. This is evident by comparing the incidence of lymphocytic lymphoma in the 625-ppm stop-exposure 13-wk group (34%) with that in the 200-ppm stop-exposure 40-wk group (12%), or more notably by comparing the incidence in the 625-ppm stop-exposure 26-wk group (60%) with that in the 312-ppm stop-exposure 52-wk group (6%). Renal tubular cell adenomas, which rarely occur in untreated B6C3F₁ mice (<0.1%), were also observed at increased rates in the stop-exposure groups at the lower butadiene concentrations.

Increased incidences of proliferative, nonneoplastic lesions in the heart (endothelial hyperplasia), lung (alveolar epithelial hyperplasia), forestomach (epithelial hyperplasia), Harderian gland (hyperplasia), and ovary (granulosa cell hyperplasia) probably represent preneoplastic changes caused by butadiene at these target sites, while the distinction between adenoma and carcinoma further reveals the biological progression of chemical-induced neoplastic lesions to malignant neoplasia. The spectrum of these proliferative lesions in mice exposed to butadiene is shown in Tables 4 (males) and 5 (females). At each of these sites, the neoplastic responses were generally supported by accompanying increases in the incidences of related nonneoplastic, proliferative lesions. In the lung of male mice, progression from alveolar-bronchiolar adenoma to carcinoma was evident in the 200-ppm exposure group and in all of the stop-exposure groups. In control female mice, only benign neoplastic lesions were observed in the lung, forestomach, Harderian gland, and ovary; however, in female mice exposed to butadiene, malignant neoplasms were observed in these organs. In partic-

Table 2 *Survival and incidence of primary tumors in female B6C3F₁ mice exposed to 1,3-butadiene for up to 2 yr*

Incidence is given as the number of animals bearing a neoplastic lesion at a specific anatomical site. Overall rates, based on the number of animals in which that site was examined, are given below the incidence values. Mortality-adjusted tumor rates are given in parentheses.

	Exposure concentration (ppm)					
	0	6.25	20	62.5	200	625
Initial no.[a]	70	70	70	70	70	90
No. of survivors	37	33	24[b]	11[b]	0[b]	0[b]
Target (neoplasm)						
Lymphocytic lymphoma	2 3% (4%)	4 6% (9%)	6 9% (14%)	3 4% (8%)	11[c] 16% (41%)	36[c] 40% (86%)
All lymphomas	10 14% (20%)	14 20% (30%)	18[c] 26% (41%)	10 14% (26%)	19[c] 27% (58%)	43[c] 48% (89%)
Heart (hemangiosarcoma)	0 0% (0%)	0 0% (0%)	0 0% (0%)	1 1% (3%)	20[c] 29% (64%)	26[c] 29% (84%)
Lung (alveolar-bronchiolar neoplasm)	4 6% (8%)	15[c] 25% (32%)	19[c] 32% (44%)	27[c] 39% (61%)	32[c] 46% (81%)	25[c] 28% (83%)
Forestomach (squamous cell neoplasm)	2 3% (4%)	2 3% (4%)	3 4% (8%)	4 6% (12%)	7[c] 10% (31%)	28[c] 31% (85%)
Harderian gland (adenoma or adenocarcinoma)	9 13% (18%)	10 14% (21%)	7 10% (17%)	16[c] 23% (40%)	22[c] 31% (67%)	7 8% (48%)
Liver (hepatocellular neoplasm)	17 25% (35%)	20 33% (41%)	23[c] 38% (52%)	24[c] 40% (60%)	20[c] 33% (68%)	3 3% (28%)
Mammary gland (adenocarcinoma)	0 0% (0%)	2 3% (4%)	2 3% (5%)	6[c] 9% (16%)	13[c] 19% (47%)	13[c] 14% (66%)
Ovary (granulosa cell neoplasm)	1 1% (2%)	0 0% (0%)	0 0% (0%)	9[c] 13% (24%)	11[c] 16% (44%)	6 7% (44%)

[a] Initial number includes animals removed from the study for interim sacrifices at 40 and 65 wk.
[b] Decreased compared with chamber control (0 ppm), $P < 0.05$.
[c] Increased compared with chamber control (0 ppm), $P < 0.05$, based on logistic regression analyses with adjustment for intercurrent mortality.

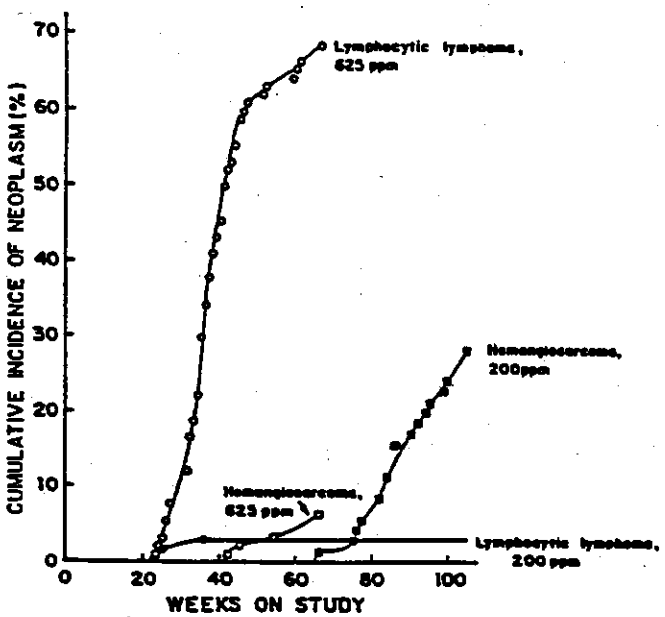

Fig. 1. Cumulative incidence of lymphocytic lymphomas or hemangiosarcomas of the heart versus weeks on study for male B6C3F₁ mice exposed to 200 or 625 ppm of 1,3-butadiene.

ular, high incidences of alveolar-bronchiolar carcinomas were observed in exposed female mice, even at 6.25 ppm of butadiene. Also, the increased incidence of hepatocellular neoplasms in female mice exposed to butadiene compared with controls appears to be largely due to the increase in hepatocellular carcinomas. The greater tendency to malignant neoplasia in mice exposed to butadiene further demonstrates the strong carcinogenic potency of this chemical.

Squamous cell papillomas and squamous cell carcinomas of the forestomach were detected as late as Wk 88 and Wk 105 in male mice that had been exposed to 625 ppm of butadiene for 13 wk and then held in control chambers to allow time for progression or regression of butadiene-induced lesions. The observation of both benign and malignant neoplasms of the forestomach near the end of the 2-yr studies in mice exposed to butadiene for only 13 wk shows that forestomach lesions induced by butadiene may persist and progress to malignant neoplasms in the absence of further exposure to this carcinogenic compound.

The high incidences of neoplastic lesions at multiple organ sites in mice exposed to butadiene also resulted in numerous occurrences of animals with tumors at more than one primary site. The frequencies of multiple organ site neoplasia in male mice and in female mice are shown in Tables 6 and 7. For these evaluations, lung tumors were not considered for male mice and liver tumors were not considered for female mice because of the high incidences of these neoplastic lesions in the respective control groups. Neoplasms were observed in two or more of the designated organ sites in 54% of the female mice and approximately 30% of the male mice exposed to 200 ppm of butadiene. Eight female mice developed primary tumors at 4

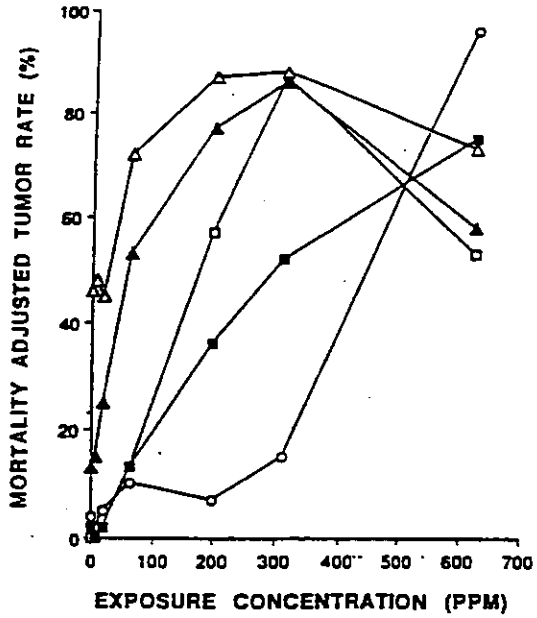

Fig. 2. Dose-response *curves* for lymphocytic lymphomas (○), hemangiosarcomas of the heart (□), alveolar-bronchiolar neoplasms (△), Harderian gland neoplasms (▲), and squamous cell neoplasms of the forestomach (■) in male B6C3F$_1$ mice exposed to 1,3-butadiene. Results from the 312-ppm, 52-wk stop-exposure group are also included, because that was the longest exposure duration of the stop-exposure groups and the incidence of lymphocytic lymphoma was not increased in that group compared with controls. Mortality-adjusted tumor rates were determined by the method of Portier and Bailer (20) using a power value equal to 3 (21).

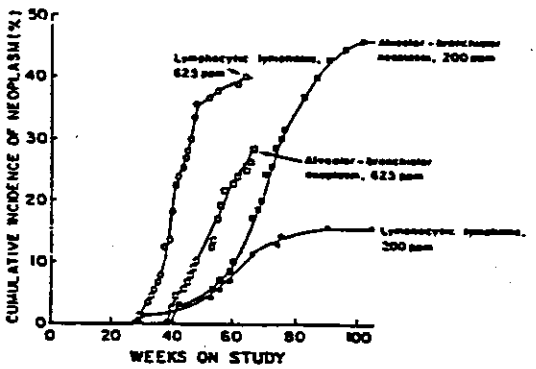

Fig. 3. Cumulative incidence of lymphocytic lymphomas or alveolar-bronchiolar neoplasms versus weeks on study for female B6C3F$_1$ mice exposed to 200 or 625 ppm of 1,3-butadiene.

different organ sites, while one female mouse had 5 different primary sites of tumor development.

DISCUSSION

The present studies demonstrate that 1,3-butadiene is a potent multiple organ carcinogen in mice and is carcinogenic at all concentrations evaluated, ranging from 6.25 to 625 ppm. Because there were no exposure concentrations in these studies that did not result in carcinogenicity, the possibility exists that exposure levels below 6.25 ppm will also cause cancers in laboratory animals. The concentrations of butadiene used in these studies are within the linear range of metabolic elimination of butadiene in B6C3F$_1$ mice (24). Miller and Boorman (25) have provided morphological descriptions and illustrations of neoplastic and nonneoplastic lesions induced by 1,3-butadiene in B6C3F$_1$ mice.

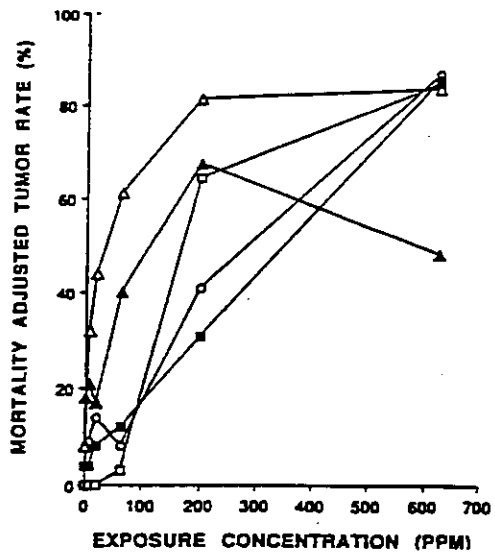

Fig. 4. Dose-response *curves* for lymphocytic lymphomas (○), hemangiosarcomas of the heart (□), alveolar-bronchiolar neoplasms (△), Harderian gland neoplasms (▲), and squamous cell neoplasms of the forestomach (■) in female B6C3F$_1$ mice exposed to 1,3-butadiene. Mortality-adjusted tumor rates were determined by the method of Portier and Bailer (20) using a power value equal to 3 (21).

In male or female mice exposed to 625 ppm of butadiene, or in female mice exposed to 200 ppm, competing risks of early occurring lethal thymic lymphomas limited the detection of later developing neoplasms at other organ sites. However, by adjusting for intercurrent mortality or by comparing tumor rates in early death animals at discrete time intervals of the study, it was possible to account for the influence of reduced survival resulting from induction of lymphocytic lymphoma on the dose responses of the later appearing tumors. At lower exposure concentrations where lymphocytic lymphoma was not a major cause of early death, the incidences of neoplastic lesions in the heart, lung, forestomach, Harderian gland, preputial gland, liver, mammary gland, and ovary were nearly linearly related to the concentration of butadiene. In the lungs of female mice, alveolar-bronchiolar carcinomas were induced at the lowest exposure concentration of 6.25 ppm of butadiene. Malignant lung tumors are uncommon in untreated female B6C3F$_1$ mice (26) and were not observed in control female mice in this study. Thus, butadiene is carcinogenic in mice at concentrations which are less than those to which humans are potentially exposed in occupational settings (4).

The stop-exposure studies show that multiple organ site neoplasia occurs in mice after only 13 wk of exposure to butadiene. It is likely that shorter exposure durations would also produce a positive carcinogenic response. These studies also demonstrate that butadiene-induced forestomach lesions can persist for long periods of time after exposure has ceased and may progress to malignant neoplasms. The development of thymic lymphomas in mice exposed to butadiene is not equivalent under different exposure regimens which result in the same total exposure; the stop-exposure studies show that the concentration of butadiene is a much greater contributing factor than is the duration of exposure. Likewise, time-weighted average exposures to butadiene in the workplace may not accurately reflect the potential human risk of cancer.

The mechanism of butadiene-induced carcinogenicity is not known; however, butadiene monoxide and/or diepoxybutane, oxidative intermediates of butadiene biotransformation (7), is

Table 3 *Survival and incidence of primary tumors in the stop-exposure groups of male B6C3F₁ mice exposed to 1,3-butadiene*

Incidence is given as the number of animals bearing a neoplastic lesion at a specific anatomical site. Overall rates, based on the number of animals in which that site was examined, are given below the incidence values. Mortality-adjusted tumor rates are given in parentheses.

	Exposure concentration (ppm)				
	0 Control	200 Stop-exposure 40 wk (8,000)[a]	625 Stop-exposure 13 wk (8,125)	312 Stop-exposure 52 wk (16,224)	625 Stop-exposure 26 wk (16,250)
Initial no.[b]	70	50	50	50	50
No. of survivors	35	9[c]	5[c]	1[c]	0[c]
Target (neoplasm)					
Lymphocytic lymphoma	2 3% (4%)	6 12% (19%)	17[d] 34% (47%)	3 6% (15%)	30[d] 60% (84%)
All lymphomas	4 6% (8%)	12[d] 24% (35%)	24[d] 48% (61%)	15[d] 30% (55%)	37[d] 74% (90%)
Heart (hemangiosarcoma)	0 0% (0%)	15[d] 30% (47%)	7[d] 14% (31%)	33[d] 66% (87%)	13[d] 26% (76%)
Lung (alveolar-bronchiolar neoplasm)	22 31% (46%)	35[d] 70% (88%)	27[d] 54% (87%)	32[d] 64% (88%)	18[d] 36% (89%)
Forestomach (squamous cell neoplasm)	1 1% (2%)	6[d] 12% (20%)	8[d] 16% (33%)	13[d] 26% (52%)	11[d] 22% (63%)
Harderian gland (adenoma or adenocarcinoma)	6 9% (13%)	27[d] 54% (72%)	23[d] 46% (82%)	28[d] 56% (86%)	11[d] 22% (70%)
Preputial gland (carcinoma)	0 0% (0%)	1 2% (3%)	5[d] 10% (21%)	4[d] 8% (21%)	3[d] 6% (31%)
Kidney (renal tubular adenoma)	0 0% (0%)	5[d] 10% (16%)	1 2% (5%)	3[d] 6% (15%)	1% 2% (11%)

[a] Total exposure expressed as ppm-wk.
[b] Initial number of control mice includes 20 animals removed from the study for interim sacrifices at 40 and 65 wk.
[c] Decreased compared with chamber control (0 ppm), $P < 0.05$.
[d] Increased compared with chamber control (0 ppm), $P < 0.05$, based on logistic regression analyses with adjustment for intercurrent mortality.

Table 4 *Incidence of nonneoplastic lesions and neoplastic lesions in the heart, lung, forestomach, and Harderian gland of male mice exposed to 1,3-butadiene*

	Exposure concentration (ppm)									
Site and lesion	0	6.25	20	62.5	200	625	200 Stop-exposure 40 wk	625 Stop-exposure 13 wk	312 Stop-exposure 52 wk	625 Stop-exposure 26 wk
Heart	(70)[a]	(49)	(60)	(58)	(68)	(90)	(50)	(50)	(50)	(50)
Endothelial hyperplasia	0	1	0	3	8	8	6	7	3	6
Hemangiosarcoma	0	0	1	5[b]	20[b]	6[b]	15[b]	7[b]	33[b]	13[b]
Lung	(70)	(49)	(60)	(69)	(70)	(89)	(50)	(50)	(50)	(50)
Alveolar epithelial hyperplasia	1	5	4	12	19	18	13	11	13	10
Alveolar-bronchiolar adenoma	14	14	11	23[b]	25[b]	10[b]	19[b]	13[b]	24[b]	11[b]
Alveolar-bronchiolar carcinoma	9	13	11	15	24[b]	5	22[b]	20[b]	16[b]	11[b]
Forestomach	(70)	(50)	(60)	(60)	(68)	(89)	(48)	(50)	(48)	(50)
Epithelial hyperplasia	4	3	2	4	3	47	7	8	16	16
Squamous cell papilloma	1	0	1	5	9[b]	9[b]	6[b]	5[b]	8[b]	4[b]
Squamous cell carcinoma	0	0	0	0	3	4[b]	0	4[b]	5[b]	7[b]
Harderian gland	(63)	(59)	(60)	(57)	(60)	(53)	(48)	(42)	(48)	(36)
Hyperplasia	1	3	4	4	9	7	5	2	7	8
Adenoma	6	7	10	23[b]	32[b]	7[b]	26[b]	20[b]	26[b]	11[b]
Adenocarcinoma	0	1	1	2	2	0	2	4[b]	2	0

[a] Numbers in parentheses are the number of animals in which that site was examined microscopically.
[b] Increased compared with chamber control (0 ppm), $P < 0.05$, based on logistic regression analyses with adjustment for intercurrent mortality.

likely involved. These metabolites are direct acting mutagens in *S. typhimurium* (8, 9), whereas the detection of mutagenicity of butadiene requires metabolic activation (6). Furthermore, these epoxides have been shown to induce "local" (application site) neoplasms when applied to the skin of Swiss mice or when administered to Swiss mice or Sprague-Dawley rats by s.c. injection (27, 28). Also, exposure of B6C3F₁ mice to 1250 ppm of butadiene did not result in any persistent defects in humoral or cell-mediated immunity (29).

In groups of 100 Sprague-Dawley rats exposed to 1000 or 8000 ppm of 1,3-butadiene for 2 yr, Owen *et al.* (12) reported that there were increased incidences and dose-response trends

Table 5 *Incidence of nonneoplastic lesions and neoplastic lesions in the heart, lung, forestomach, Harderian gland, liver, and ovary of female mice exposed to 1,3-butadiene*

Site and lesion	Exposure concentration (ppm)					
	0	6.25	20	62.5	200	625
Heart	(70)[a]	(50)	(50)	(59)	(70)	(90)
Endothelial hyperplasia	0	2	1	4	12	10
Hemangiosarcoma	0	0	0	1	20[b]	26[b]
Lung	(70)	(60)	(60)	(70)	(70)	(88)
Alveolar epithelial hyperplasia	6	4	6	11	13	11
Alveolar-bronchiolar adenoma	4	8	10[b]	18[b]	20[b]	14[b]
Alveolar-bronchiolar carcinoma	0	8[b]	13[b]	10[b]	20[b]	12[b]
Forestomach	(70)	(50)	(57)	(68)	(70)	(89)
Epithelial hyperplasia	2	3	4	6	16	48
Squamous cell papilloma	2	1	2	3	6[b]	22[b]
Squamous cell carcinoma	0	1	1	1	1	6[b]
Harderian gland	(63)	(49)	(48)	(59)	(59)	(73)
Hyperplasia	1	3	9	1	4	9
Adenoma	9	9	6	16[b]	22[b]	7
Adenocarcinoma	0	1	1	0	1	0
Liver	(69)	(51)	(51)	(60)	(60)	(90)
Hepatocellular adenoma	12	9	14	15[b]	13[b]	2
Hepatocellular carcinoma	7	13	11	11[b]	12[b]	1
Ovary	(69)	(59)	(59)	(70)	(70)	(89)
Granulosa cell hyperplasia	0	2	1	4	7	4
Granulosa cell tumor, benign	1	0	0	7[b]	8[b]	6
Granulosa cell tumor, malignant	0	0	0	2	3[b]	0

[a] Numbers in parentheses are the number of animals in which that site was examined microscopically.
[b] Increased compared with chamber control (0 ppm), $P < 0.05$, based on logistic regression analyses with adjustment for intercurrent mortality.

Table 6 *Frequency of multiple-site neoplasia in male B6C3F₁ mice exposed to 1,3-butadiene: lymphocytic lymphoma; hemangiosarcoma of the heart; squamous cell neoplasm of the forestomach; and Harderian gland neoplasm*

Exposure group	No. of organ sites/animal			
	0	1	2	3
	No. of animals			
Control	62 (89)[a]	7 (10)	1 (1)	0
6.25 ppm	52 (87)	8 (13)	0	0
20 ppm	47 (78)	12 (20)	1 (2)	0
62.5 ppm	36 (52)	28 (41)	5 (7)	0
200 ppm	27 (39)	23 (33)	16 (23)	4 (6)
625 ppm	15 (17)	65 (72)	7 (8)	3 (3)
200 ppm; stop-exposure, 40 wk	15 (30)	20 (40)	10 (20)	5 (10)
625 ppm; stop-exposure, 13 wk	8 (16)	32 (64)	8 (16)	2 (4)
312 ppm; stop-exposure, 52 wk	4 (8)	22 (44)	17 (34)	7 (14)
625 ppm; stop-exposure, 26 wk	5 (10)	27 (54)	16 (32)	2 (4)

[a] Numbers in parentheses, percentage.

Table 7 *Frequency of multiple-site neoplasia in female B6C3F₁ mice exposed to 1,3-butadiene: lymphocytic lymphoma; hemangiosarcoma of the heart; alveolar-bronchiolar neoplasm; squamous cell neoplasm of the forestomach; Harderian gland neoplasm; adenocarcinoma of the mammary gland; and granulosa cell neoplasm of the ovary*

Exposure group	No. of organ sites/animal					
	0	1	2	3	4	5
	No. of animals					
Control	55 (79)[a]	12 (17)	3 (4)	0	0	0
6.25 ppm	34 (57)	19 (32)	7 (12)	0	0	0
20 ppm	31 (52)	22 (37)	6 (10)	1 (2)	0	0
62.5 ppm	27 (39)	24 (34)	16 (23)	2 (3)	1 (1)	0
200 ppm	13 (19)	19 (27)	20 (29)	15 (21)	3 (4)	0
625 ppm	17 (19)	29 (32)	26 (29)	13 (14)	4 (4)	1 (1)

[a] Numbers in parentheses, percentage.

for pancreatic exocrine adenomas (control, 3; low dose, one; high dose, 10) and Leydig cell neoplasms of the testis (control, 0; low dose, 3; high dose, 8) in males and uterine sarcomas (control, one; low dose, 4; high dose, 5), Zymbal gland carcinomas (control, 0; low dose, 0; high dose, 4), mammary gland neoplasms (control, 50; low dose, 79; high dose, 81), and thyroid follicular cell neoplasms (control, 0; low dose, 4; high dose, 11) in females. The sites of tumor induction and the magnitude of response in rats were different from those reported here for B6C3F₁ mice. The reason for this species difference is not known; however, it is not readily accounted for by differences in rates of butadiene metabolism (23).

Irons and coworkers (30, 31) compared the induction of thymic lymphomas in NIH Swiss mice and B6C3F₁ mice exposed to butadiene and suggested that activation of an endogenous ecotropic retrovirus may play a role in butadiene-induced lymphoma in the B6C3F₁ strain of mice. The NIH Swiss mouse rarely expresses endogenous retrovirus and has a background rate of zero for thymic lymphoma. Thus, the finding that exposure of NIH Swiss mice to 1250 ppm of butadiene for 1 yr caused a 14% incidence of thymic lymphoma (31) indicates that butadiene can induce this neoplasm independently of an activated retrovirus.

The conclusion that the marginally increased incidences of hepatocellular neoplasms in male and female mice were chemically related is strengthened by the detection of an activated K-*ras* oncogene in liver neoplasms obtained from mice exposed to butadiene (32); activated K-*ras* has never been detected in liver tumors from untreated B6C3F₁ mice (33). Exposure to butadiene also caused activation of the K-*ras* oncogene in mouse lymphomas and lung neoplasms. These findings add further relevance to the potential carcinogenicity of butadiene in humans, since K-*ras* is the most commonly detected oncogene in human tumors.

Other toxic effects associated with exposure of mice to butadiene, and relevant to humans, include testicular atrophy in males exposed to 625 ppm, ovarian atrophy in females exposed to 6.25 ppm or higher concentrations, and bone marrow toxicity at 62.5 ppm and higher concentrations (34).

The carcinogenicity of butadiene in laboratory animals at concentrations as low as 6.25 ppm and the activation of the K-*ras* oncogene by butadiene raise further concerns of risk for humans exposed to this chemical. Results of epidemiology studies indicate an association between occupational exposure to 1,3-butadiene and the development of lymphatic and hematopoietic cancers (15, 16); for example, standardized mortality ratios were 500% for lymphopoietic cancers and 660% for leukemia in black production workers (15). In a follow-up nested case-control study of the styrene-butadiene rubber industry, Matanoski *et al.* (35) found that leukemia cases were associated with exposure specifically to butadiene (odds ratio, 9.4; 95% confidence interval, 2.1–22.9), whereas there was not a significant increased risk associated with exposure to styrene. Because of the correspondence between the animal data and the epidemiology findings, there exists an urgent worldwide public health need to reevaluate current workplace exposure standards for 1,3-butadiene.

ACKNOWLEDGMENTS

The authors thank Dr. Joseph Haseman for providing the mortality-adjusted tumor rates and Dr. John Bucher and Dr. Michael Elwell for reviewing this manuscript.

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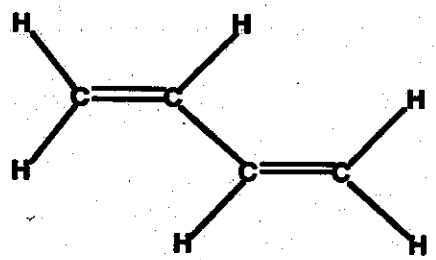
California Environmental Protection Agency

Air Resources Board

Proposed Identification of 1,3-Butadiene



as a Toxic Air Contaminant

Part C and Part C Addendum
Public Comments and ARB/OEHHA Staff Responses

State of California
Air Resources Board
Stationary Source Division

May 1992

PART C

PUBLIC COMMENTS AND ARB/OEHHA STAFF RESPONSES ON THE 1,3-BUTADIENE "IDENTIFICATION" REPORT

Prepared by the staffs of the Air Resources Board
and the Office of Environmental Health Hazard Assessment

October 1991

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I.

Comment Letters Received on the February 1991
Preliminary Draft Version of the 1,3-Butadiene Report

ARCO Products Company
1990 West Crescent Avenue
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Mailing Address: Box 61004
Anaheim, California 92803-6104
Telephone 714 491 6800

March 22, 1991

Ms. Genevieve Shiroma, Chief
Toxic Air Contaminant Identification Branch
Stationary Source Divsion
Air Resources Board
Attn: 1,3-Butadiene
P. O. Box 2815
Sacramento, CA 95812

Dear Ms. Shiroma:

The preliminary draft proposing to identify 1,3-butadiene as a California Toxic Air Contaminant (TAC) does not contain a "best value" unit risk estimate. A similar draft report on Formaldehyde dated February, 1991 does contain such a "best value" estimate along with the range of risks typically provided in such reports. The Department of Health (DHS) Services has been providing such "best values" for Proposition 65, and Hot Spot programs which fall within the range of unit risk estimates for the TAC.

It is recommended that DHS determine a "best value" unit risk estimate for 1,3-butadiene within the current TAC process. Such a value will be needed, and, we believe, would be best set during the process of establishing the range of risk under the TAC program.

Please call me at the above phone number if you wish to discuss our recommendation further.

Very truly yours,

David A. Smith
MANAGER, EH&S SERVICES

das:das

cc: George Alexeef DHS
Kelly Hughes CARB
J. E. Richey ARCO
D. J. Townsend ARCO

APPC-7084 C
(8-89)

ARCO Products Company is a Division of AtlanticRichfieldCompany

3-23

American Petroleum Institute
1220 L Street, Northwest
Washington, D.C. 20005
202-682-8090

Terry F. Yosie
Vice President

March 22, 1991

Genevieve Shiroma, Chief
Toxic Air Contaminant Identification Branch
Stationary Source Division
Air Resources Board
Attn: 1,3,-Butadiene
P.O. Box 2815
Sacramento, CA 95812

Dear Ms. Shiroma:

The American Petroleum Institute is pleased to submit its views on the State of California Air Resources Board (CARB) recently published document, Preliminary Draft Technical Support Document for the Proposed Identification of 1,3-Butadiene as a Toxic Air Contaminant (February 1991). At this time, our comments pertain solely to Part B, the "Draft Health Assessment Document".

API is a trade Association representing over 250 companies involved in all aspects of the oil and gas industry, including exploration, production, transportation, refining and marketing.

API has participated in the development of the extensive comments prepared by the Chemical Manufacturers's Association (CMA). We subscribe to CMA's analysis and suggestions for needed revisions to the Draft Document.

In particular, API finds that the heavy reliance on studies performed on the B6C3F1 mouse leads to an overestimate of human risk. This conclusion is based on data which demonstrate that substantial species differentiation exists among test animals. These data provide evidence that mice retain larger doses of butadiene, metabolize butadiene more rapidly, and detoxify the metabolites more slowly than other species, making the mouse uniquely sensitive to carcinogenic effects of butadiene. Thus, it is appropriate to revise the human risk estimation based upon an alternate species or to adjust the use of the mouse data to account for this hypersensitivity.

Thank you for the opportunity to submit API's views. Should you have any questions, please contact Louise Scott of my staff at (202) 682-8481.

Sincerely,

An equal opportunity employer

CHEMICAL MANUFACTURERS ASSOCIATION

Geraldine V. Cox, Ph.D.
Vice President-Technical Director

March 21, 1991

Ms. Genevieve Shiroma, Chief
Toxic Air Contaminant Identification Branch
Stationary Source Division
Air Resources Board
Attn: 1,3 Butadiene
P.O. Box 2815
Sacramento, CA 95812

Re: California Air Resources Board Draft Technical Support Document for 1,3-Butadiene

Dear Ms. Shiroma:

The Butadiene Panel of the Chemical Manufacturers Association is pleased to submit the enclosed comments on CARB's Draft Technical Support Document for 1,3-Butadiene. The Panel consists of the major U.S. producers and some users of butadiene.

The Panel suggests a number of improvements to the Health Assessment portion of the Document, to enhance its completeness and usefulness. In addition, the Panel believes that the Department of Health Services' quantitative risk assessment for butadiene overestimates the potential human risk by a substantial margin. The Panel recommends adjustments to the potency factor and resulting risk estimates so they reflect more realistically butadiene's potential risk to humans.

Please direct any questions that you may have regarding these comments to Dr. Elizabeth J. Moran, Manager of the Butadiene Panel, at (202)887-1182.

Sincerely yours,

Geraldine V. Cox

000004

2501 M Street, NW, Washington, DC

Telex 89617 (CMA WSH)

STATE OF CALIFORNIA
AIR RESOURCES BOARD
STATIONARY SOURCE DIVISION

COMMENTS OF THE CHEMICAL MANUFACTURERS ASSOCIATION BUTADIENE PANEL ON THE PRELIMINARY DRAFT TECHNICAL SUPPORT DOCUMENT FOR THE PROPOSED IDENTIFICATION OF 1,3-BUTADIENE AS A TOXIC AIR CONTAMINANT

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March 21, 1991

CHEMICAL MANUFACTURERS ASSOCIATION
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EXECUTIVE SUMMARY

The Butadiene Panel of the Chemical Manufacturers Association has reviewed the Preliminary Draft Technical Support Document on the Proposed Identification of 1,3-Butadiene as a Toxic Air Contaminant, prepared for the California Air Resources Board ("CARB"). The Panel has focussed mainly on the draft Health Assessment Document (Part B). The Panel believes that the quantitative risk estimates included in this draft overstate the potential human cancer risks presented by butadiene by a wide margin and should be adjusted so that these risks are portrayed more realistically. In particular, the Panel offers the following recommendations:

- The Health Assessment Document should emphasize that, while butadiene is a potent carcinogen in the B6C3F1 mouse, it is only a weak oncogen in the rat.

- Recent data on species differences in butadiene metabolism and mechanism of action indicate that the mouse is uniquely sensitive to the carcinogenic effects of butadiene. Therefore, the rat, not the mouse, is the more appropriate model for human risk assessment.

- The Hazleton bioassay results for the rat should be used to calculate the "best estimate" of human cancer risk. This risk estimate should be adjusted to reflect recent data on species differences in butadiene metabolism. In particular, adjustments should be made for differences between the rat and primate in blood levels of the reactive monoepoxide. This approach would predict an upper bound lifetime risk of 9.0×10^{-5} at 1 ppm.

- If CARB continues to rely on the mouse bioassay data for risk assessment purposes, it should exclude the lymphomas from that assessment. These malignancies are of questionable relevance to humans. In addition, the risk estimate should be adjusted to reflect species differences in the blood levels of the monoepoxide. These adjustments would result in an upper bound risk estimate of 7.8×10^{-4} at 1 ppm.

- Shell Oil Company has prepared an alternative risk analysis based on the rat study and on preliminary unaudited data from the recent mouse study conducted by the National Toxicology Program. The risk assessment incorporates a simplified time to tumor analysis for the mouse data and takes into account species differences in butadiene

metabolism for both the rat and mouse. It illustrates how such biological data can be incorporated and demonstrates that the predicted risk is much lower when all of the data are used.

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INTRODUCTION

The State of California Air Resources Board ("CARB") recently published a Preliminary Draft of its Technical Support Document for the Proposed Identification of 1,3-Butadiene as a Toxic Air Contaminant (February 1991). This document consists of two parts: Part A, the Exposure Assessment, which was prepared by CARB, and Part B, the Health Assessment, which was prepared by the California Department of Health Services ("DHS"). The Butadiene Panel (the "Panel") of the Chemical Manufacturers Association ("CMA") is pleased to submit these Comments on both portions of the Preliminary Draft.

The Panel includes the major United States producers and importers and some users of butadiene. The Panel member companies are listed in Appendix A. The Panel is sponsoring scientific research on butadiene, and has commented extensively on previous evaluations of the scientific data base for butadiene by the Occupational Safety and Health Administration ("OSHA"), the Environmental Protection Agency ("EPA"), the Agency for Toxic Substances and Disease Registry ("ATSDR"), and other government agencies. Included as appendices to these Comments are five witness statements and an alternative risk assessment by Shell Oil Company which were prepared recently in connection with the ongoing OSHA rulemaking regarding occupational exposure to butadiene.

The Butadiene Panel believes that DHS's quantitative risk assessment for butadiene overstates the potential human cancer risk by a substantial margin. We strongly recommend that

DHS make adjustments in its risk estimates to reflect more realistically butadiene's potential risks to the human population.

DHS has identified a number of studies which demonstrate substantial species differences in butadiene metabolism and mechanism of action. However, the DHS quantitative risk calculations do not fully recognize the limited relevance of the mouse data for human risk assessment. Moreover, DHS does not explore alternative risk modeling approaches which would reflect interspecies differences in response to butadiene. These Comments address the relevant data regarding species differences in butadiene metabolism and mechanism of action and propose modifications in DHS's risk assessment which take these data into account.

Part I of the Comments reviews the studies which form the basis for the DHS butadiene hazard evaluation. The Comments first discuss the major animal carcinogenicity studies on butadiene. These studies demonstrate that, while butadiene is a potent carcinogen in the B6C3F1 mouse, it is only a weak carcinogen in the rat.

The Comments then review the relevant data on species differences in butadiene metabolism and mechanism of action which indicate that the B6C3F1 mouse is uniquely sensitive to the carcinogenic effects of butadiene. These data include recent primate data reported by Dahl *et al*. (1990) and Sun *et al*. (1989) which were omitted from DHS's analysis. These studies

demonstrate that, for equivalent inhaled butadiene concentrations, mice retain larger doses of butadiene, metabolize butadiene to the reactive, carcinogenic monoepoxide more rapidly, detoxify the metabolites more slowly, and attain higher tissue levels of butadiene metabolites than the rat or primate. In addition, cytogenetic and bone marrow effects are seen in the mouse, but not the rat or primate, and the presence of the endogenous murine leukemia virus ("MuLV") in the B6C3F1 mouse (but not the rat or primate) has been implicated in the expression of the malignant lymphoma in that species. These species differences indicate that the incidence of lymphoma in the B6C3F1 mouse has limited relevance to human risk assessment.

Part II of the Comments addresses the implications of the relevant data for human risk assessment and proposes adjustments in DHS's quantitative risk estimates. These recommended adjustments are summarized in Table I.

Based on the available data, the Comments conclude that the rat is the more appropriate model on which to base a quantitative estimation of butadiene's human cancer risk. The Comments also propose several adjustments to DHS's risk assessment based on the rat bioassay results. In particular, the Comments recommend that the DHS quantitative risk estimates be modified to reflect differences in butadiene metabolism between rats and primates and that the mammary carcinomas be excluded from the incidence of total significant tumors. With these

adjustments, the "best estimate" of upper bound cancer risk would be 9.0×10^{-5} at 1 ppm and 3.3×10^{-8} at 0.37 ppb.

If DHS continues to rely on the mouse data from the 1984 study performed by the National Toxicology Program ("NTP") for its best estimate of cancer risk, the Comments recommend that the risk estimates be adjusted to reflect species differences in the blood levels of the monoepoxide or, at a minimum, species differences in butadiene absorption. In addition, since malignant lymphomas in the B6C3F1 mouse are of questionable relevance for human cancer risk, the Comments recommend that incidence of lymphoma be excluded from the risk estimation based on the mouse data from the 1984 NTP study. These adjustments would result in a potency factor ranging from 7.8×10^{-4} to 2.0×10^{-2} at 1 ppm and an upper bound lifetime risk at 0.37 ppb of from 2.9×10^{-7} to 7.4×10^{-6}.

As another approach, the Comments also describe a risk assessment prepared by Shell Oil Company using the rat study and preliminary, unaudited data from the second NTP study in the B6C3F1 mouse. A copy of Shell's Alternative Risk Assessment is provided in Appendix G. The Shell risk assessment was prepared in response to the OSHA proposed 2 ppm workplace standard. The Shell-estimated risk is not linear with exposure and, thus, direct comparisons to the DHS risk estimates are not possible for ambient exposure scenarios. The Shell assessment incorporates available data regarding species differences in butadiene

response and demonstrates that the predicted risks are much lower when all of the data are used.

Part III of the Comments discusses DHS's evaluation of butadiene's potential reproductive and developmental risks. This portion of the Comments takes issue with DHS's conclusion that 6.25 ppm is a LOAEL for reproductive effects in the mouse (based on the second NTP mouse study). Correctly interpreted, this study establishes a NOEL for reproductive effects in the mouse of 20 ppm. DHS should also recognize that, in the Hazleton rat bioassay, no reproductive effects were observed in the rat after lifetime exposure to butadiene at levels as high as 8000 ppm. In light of the unique sensitivity of the mouse to the reproductive effects of butadiene exposure, the absence of reproductive effects in the rat should take precedence in determining butadiene's reproductive risk to humans.

Finally, Part IV of the Comments raises several concerns relating to CARB's procedures for measuring butadiene in ambient air. In particular, the Comments note that it is not clear from the description of CARB's methodology that the analysis adequately separates butadiene from other four carbon hydrocarbons which may also be present in ambient air. In addition, additional information is necessary to evaluate the validity of the sample collection procedures that were used. These issues should be addressed before CARB's measurements of ambient air levels of butadiene are adopted.

I. HAZARD EVALUATION

A. Animal Carcinogenicity Data Indicate That Butadiene is a Potent Carcinogen in the B6C3F1 Mouse But Only a Weak Carcinogen in the Sprague-Dawley Rat

DHS's Draft Health Assessment Document describes three major cancer studies in rodents -- two studies in the B6C3F1 mouse sponsored by the National Toxicology Program ("NTP") and one study in the Sprague-Dawley rat, performed at Hazleton Laboratories. See Draft Health Assessment Document at 3-28 - 3-32. These studies are discussed in detail in the statement of Dr. Hinderer of B.F. Goodrich Company (included in Appendix B).

The two NTP mouse bioassays demonstrate that butadiene is a potent carcinogen in the B6C3F1 mouse. In the first study ("NTP I"), groups of 50 male and 50 female B6C3F1 mice were exposed to 0, 625, and 1250 ppm butadiene six hours per day, five days per week. NTP (1984). The most prominent finding was the high mortality from the malignant (thymic) lymphomas, which resulted in early termination of the study after only 60-61 weeks of exposure. Significant increases in neoplasms were also observed at several other sites, including the heart, lung, and forestomach (all in both sexes) and the mammary gland, ovary, and liver (females only).

The second NTP study ("NTP II") has not been fully audited and reported, but summaries of the unaudited results have been published in several articles. See Melnick et al. (1989a, 1989b, 1990), Melnick (1989). In this study, B6C3F1 mice were

exposed to 0, 6.25, 20, 62.5, 200, and 625 ppm butadiene for six hours per day, five days per week, for periods of up to two years. Results of this second study confirm that butadiene is a potent carcinogen in the B6C3F1 mouse. The effects reported at the two highest doses were similar to those observed in the first study (high incidence of malignant (thymic) lymphomas causing early mortality of the animals). At concentrations below 625 ppm, survival was good and lymphocytic lymphoma no longer played a role as the major cause of early death. However, higher incidences of heart, lung, Harderian gland, liver, preputial gland, and ovarian tumors were noted at these lower concentrations, where animals had better survival. An excess of lung tumors was observed in female mice at the low dose of 6.25 ppm.

In contrast, the results of the Hazleton rat bioassay sponsored by the International Institute of Synthetic Rubber Producers, Inc. ("IISRP") (see Owen et al. 1987, 1990), demonstrate that butadiene is only a weak carcinogen in the rat. Groups of 110 male and female Sprague-Dawley rats were exposed to 0, 1000, and 8000 ppm butadiene for six hours per day, five days per week, for more than two years (105 weeks for females and 111 weeks for males). Statistically increased incidences of tumors were seen at only two sites in each sex: pancreatic exocrine adenomas and testis Leydig-cell tumors in males, and mammary tumors (primarily benign fibroadenomas) and thyroid follicular cell tumors in females. In the low dose group (1000 ppm), the

only significantly elevated tumor response was the benign mammary tumors. Mammary carcinomas were not significantly elevated even at the high dose of 8000 ppm (the incidence of malignant mammary tumors was as follows: 0 ppm-18; 1000 ppm-15; 8000 ppm-26). Several factors have been identified that tend to diminish or challenge the significance of even these limited findings of the rat study. See Hinderer Statement at pp. 3-4.

B. Species Differences in Butadiene Metabolism and Mechanism of Action Demonstrate that the Primate and Rat are Considerably Less Sensitive to Butadiene than the Mouse

1. Species Differences in Butadiene Metabolism

The genotoxicity data discussed in the Draft Health Assessment Document indicate that the reactive mono- and di-epoxide metabolites of butadiene (1,3-epoxy-3-butene and 1,2:3,4-diepoxybutane), and not butadiene itself, are the ultimate genotoxic agents responsible for the carcinogenic effects of butadiene exposure. See Draft Health Assessment Document, Section 3.4. Since butadiene is genotoxic only when metabolically activated, species differences in butadiene uptake and metabolism must be considered in evaluating the results of the rat and mouse bioassays and in assessing their relevance to human risk assessment.

DHS has not evaluated all the relevant data on butadiene pharmacokinetics and metabolism. Most notably, DHS has not included recent metabolism studies in the rat, mouse, and primate by Dahl et al. (1990). The relevant studies are

addressed in detail in the written statements of Drs. Hinderer, Bird, and Bolt (copies included in Appendices B, C, and D, respectively). These data demonstrate that, at equivalent inhaled butadiene concentrations, mice retain larger doses of butadiene, metabolize butadiene to the monoepoxide more rapidly, detoxify the metabolites more slowly, and attain higher blood and tissue levels of butadiene metabolites than rats or primates. These findings are summarized below.

a. Mice retain a larger dose of inhaled butadiene when adjusted for body weight and exposure time than rats or primates. Studies conducted under NTP sponsorship by Dahl *et al*. (1990) show that the rate of uptake of butadiene at 10 ppm in the B6C3F1 mouse is approximately 7.2-fold greater than for the Sprague-Dawley rat, and approximately 6.3-fold greater than for the monkey, when normalized to body weight. Bird Statement at 4.

b. Mice metabolize butadiene to the monoepoxide at about twice the rate seen in rats. The metabolic elimination rates of butadiene in rats and mice have been compared by placing B6C3F1 mice and Sprague-Dawley rats in separate closed chambers with fixed concentrations of butadiene in the air, and then measuring the decline in butadiene concentration over time. Kreiling *et al*. (1986). The decline in concentration indicates that there has been uptake and metabolism of butadiene by the test animals. The results of these studies show that mice metabolize butadiene to the reactive metabolite epoxybutene at

about twice the rate seen in rats. Bird Statement at 6; Bolt Statement at 5-6.

c. Detoxification of the epoxide 1,2-epoxy-3-butene is saturable in mice but not rats. Epoxybutene metabolism has been examined by measuring the exhalation of epoxybutene in a closed chamber using the B6C3F1 mouse and the Sprague-Dawley rat. Kreiling et al. (1987). These studies show that metabolic elimination rates of epoxybutene for rats and mice depend on the atmospheric concentration of the compound. In mice, saturation of epoxybutene metabolism occurs at about 500 ppm. Epoxybutene metabolism in rats is linearly dependent on the atmospheric concentration of the compound up to exposure concentrations of about 5000 ppm. Thus, with increasing exposure concentration, the metabolic capacity for epoxybutene becomes rate limiting in mice at significantly lower exposure levels than in rats. Bird Statement at 7-8; Bolt Statement at 6.

d. Mice attain a higher concentration of epoxide metabolites in the blood than rats or primates when exposed at similar concentrations. In recent studies sponsored by NTP, B6C3F1 mice were exposed to 7.8 and 78 ppm butadiene; Sprague-Dawley rats were exposed to 78 ppm butadiene; and monkeys were exposed to 10 ppm butadiene. Dahl et al. (1990). At 78 ppm butadiene, mice attained approximately 5-fold higher 1,2-epoxybutene-3 levels in their blood than did rats. At 7.8 ppm, mice attained 590-fold higher blood levels of 1,2-epoxybutene-3 than did monkeys exposed to 10 ppm. Rats exposed to 78 ppm

attained 40-fold higher blood epoxide levels than did monkeys exposed to 10 ppm. [Rats were not exposed to 7.8 ppm, preventing direct comparisons between rat and mouse and rat and monkey at this exposure concentration. However, as the airborne concentration of butadiene decreases, the percentage of butadiene retained increases. For example, as the exposure concentration for the mouse was decreased from 78 to 7.8 ppm, the blood level of the monoepoxide (expressed as per ppm butadiene) went up by a factor of approximately 3. Thus, it is reasonable to assume that rats exposed to 7.8 ppm would attain at least a 40-fold greater monoepoxide blood level than monkeys exposed to 10 ppm.] Bird Statement at 8 and 34.

e. Limited human data provide further evidence of species differences in butadiene metabolism. Research conducted by Schmidt and Loeser (1985) provide a comparison of the human, primate, and rodent response to butadiene. Butadiene was incubated with liver and lung in *in vitro* preparations from mice (two strains), rats (two strains), monkey, and human (one surgical sample). The formation of 1,2-epoxy-3-butene in the liver of these species was as follows: mouse > rat > human > monkey. The ratio between mouse and monkey was about 7:1. The mouse lung tissue produced levels of 1,2-epoxy-3-butene equivalent to that of the mouse liver; however, monkey and human lung tissue did not produce any measurable 1,2-epoxy-3-butene. Bird Statement at 8-9.

f. Species differences in enzyme activities relevant to the formation and elimination of butadiene monoepoxide have been demonstrated. The species differences in the formation of 1,2-epoxy-3-butene described in the previous paragraph are consistent with species differences in relative ratios of specific enzyme activities. Lorenz et al. (1984). The mouse has been shown to have higher levels of monooxygenase (enzyme which forms the monoepoxide) and lower levels of epoxide-hydrolase (enzyme which removes the monoepoxide) compared to the rat and humans. For the lung, the ratio of monooxygenase activity in mouse compared to human is 1220 to 1. Bird Statement at 9-10; Hinderer Statement at 19.

g. Species differences have been demonstrated in butadiene-induced depletion of non-protein sulfhydryl (NPSH) content in different tissues. Reactive epoxides, such as epoxybutene, react with intracellular glutathione, the major constituent of NPSH compounds. Interspecies differences in NPSH depletion could therefore be an indication of interspecies differences in the amount of systemically available butadiene epoxide intermediates.

When B6C3F1 mice and Sprague-Dawley rats were exposed to 0, 10, 50, 100, 250, 500, 1000, and 2000 ppm butadiene for seven hours, significant differences in NPSH depletion were observed. Deutschmann and Laib (1989). In rats, minor reduction in liver NPSH content (about 20%) was observed at exposure concentrations of about 250 ppm. There was also a minor

reduction in lung NPSH content in rats between 1000 and 2000 ppm butadiene; NPSH content in the heart was not affected. In mice, reduction of liver NPSH content started at exposure concentrations between 100 and 250 ppm butadiene; at 2000 ppm butadiene, the reduction reached 80 percent. Hence, in the mouse, reserves of NPSH are drastically depleted in the liver (50-70%) and lung (70-90%) and heart (25-40%). The rat, by contrast, shows only some decline and maintains this detoxification pathway throughout butadiene exposure. Bolt Statement at 8-9; Bird Statement at 10-11.

In a study by Kreiling et al. (1988), exposure of mice to concentrations of greater than 2000 ppm 1,3-butadiene resulted in a progressive depletion of hepatic NPSH to about 20 percent after seven hours and almost total depletion after fifteen hours. In rats, the hepatic NPSH content was depleted to between 65 percent (Wistar) and 80 percent (Sprague-Dawley) after seven hours, with little additional change at fifteen hours. At the end of the fifteen-hour exposure, mice showed signs of acute toxicity but rats (of both strains) did not.

h. Species differences have been observed in studies of alkylation of nuclear proteins and DNA by reactive butadiene metabolites. Comparative studies of alkylation of nuclear proteins and of DNA after exposure to radioactive butadiene have been conducted in mice (B6C3F1) and rats (Wistar). Kreiling et al. (1986). Butadiene derived radioactivity was covalently bound to liver nucleoprotein fractions and to total

liver DNA of both species. However, covalent binding of reactive butadiene metabolites to liver nucleoproteins of mice was about twice as high as in rats. Bolt Statement at 10; Bird Statement at 10.

i. Species differences have been demonstrated in the formation of DNA-DNA and DNA-protein crosslinks. When male Sprague-Dawley rats and male B6C3F1 mice were exposed for seven hours to 250, 500, and 1000 ppm butadiene, protein-DNA and DNA-DNA crosslinks were seen in the mouse liver at exposure levels above 250 ppm. Jelitto et al. (1989). No crosslinking activity of butadiene was seen in the rat. Bolt Statement at 11.

2. Species Differences in the Mechanism of Action of Butadiene

The available data also demonstrate significant species differences in the mechanism of action of butadiene. These pertain to (1) cytogenetic and bone marrow changes which have been seen in the mouse, but not the rat or primate; and (2) the presence of the endogenous MuLV retrovirus in the B6C3F1 mouse, which has been shown to play a critical role in the expression of malignant (thymic) lymphoma in this species.

These issues are addressed in detail in the statements of Drs. Bird and Hinderer. A summary of the most relevant data is presented below.

a. Cytogenetic and bone marrow changes have been found in mice exposed to butadiene but not in rats or primates. Tice, et al. (1987) exposed male mice to 6.25, 62.5, and 625 ppm

butadiene for ten exposure days. Five endpoints were evaluated: average generation time (AGT), mitotic index (MI), chromosomal aberrations (CA), sister chromatid exchange (SCE), and micronuclei (MN). Butadiene exposure induced a dose dependent response in all these endpoints. This dose-related response in these multiple cytogenetic endpoints indicates a strong genotoxic and cytotoxic action in the mouse.

Cunningham, et al. (1986) exposed B6C3F1 mice and Sprague-Dawley rats for six hours per day for two days, at butadiene concentrations up to 10,000 ppm, and examined the bone marrow cells for the induction of micronuclei and sister chromatid exchanges. Significant dose dependent increases in micronuclei induction and frequency of SCE were observed in mice at exposures of 100 ppm and greater. In contrast, rat bone marrow cells did not show any significant increases in micronucleated polychromatic erythrocytes nor in the frequency of SCE, even at the highest dose of 10,000 ppm. The lack of response in the rat is consistent with the lack of changes in the bone marrow of rats exposed in the two year cancer bioassay, which involved butadiene exposure as high as 8000 ppm.

Exxon has conducted micronucleus assays on B6C3F1 mice and Syrian hamsters at butadiene exposures of 0 and 1000 ppm for six hours per day for two days. Exxon (1990). Butadiene produced an 11.2-fold increase in micronuclei formation in the exposed mice, and only a 1.4-fold increase in the number of micronuclei in the exposed hamsters.

In primate studies sponsored by NTP, monkeys were exposed to 10, 300, and 8000 ppm butadiene in air for two hours. Sun et al. (1989). At no exposure level was micronuclei induction found to occur nor was there an increase in frequency of SCE.

The above studies indicate that the bone marrow clearly is a target organ of butadiene exposure for the B6C3F1 mouse, may be a target organ in the hamster, but is not a target organ in the Sprague-Dawley rat or primate. These data should be considered when evaluating the relevance of the rat and mouse bioassays for human risk assessment, and, in particular, when evaluating the relevance of the mouse lymphomas to human risk assessment. The bone marrow is known to have an essential role in radiation induced murine T-cell leukemia/lymphomas and also accompanies many instances of chemically induced T-cell lymphoma. The evidence of bone marrow toxicity in the mouse at low exposure levels, the marginal (at most) response in the hamster at 1000 ppm, and the absence of such evidence in the rat and primate at 8000 ppm, indicate that the mouse lymphoma response is of limited relevance to human cancer risk assessment. See Bird Statement at 13-20; Hinderer Statement at 15-18.

b. The presence of the MuLV retrovirus in the B6C3F1 mouse plays a critical role in the expression of thymic lymphoma in this species. Studies by Irons et al. (1986, 1989) show that the presence of the murine leukemia virus (MuLV) affects the incidence of malignant (thymic) lymphomas in the

B6C3F1 mouse. The MuLV retrovirus is endogenous to the B6C3F1 mouse, but is not found in rats or humans.

Irons et al. have demonstrated that exposure to butadiene (1250 ppm) six hours per day, five days per week for 3 to 21 weeks markedly increased the quantity of the esotropic (capable of infecting mouse cells) MuLV retrovirus recoverable from the bone marrow, thymus, and spleen of the B6C3F1 mouse. Irons et al. subsequently conducted a comparative study in the B6C3F1 mouse and the NIH Swiss mouse. The latter strain was chosen because it does not possess intact endogenous proviral sequences and only rarely expresses any type of endogenous retrovirus. B6C3F1 mice and NIH Swiss mice were chronically exposed to 1250 ppm butadiene six hours per day, five days per week for one year. The incidence of thymic lymphoma/leukemia in the B6C3F1 Mouse was 57% at the end of one year. These were all of T-cell origin and exhibited elevated expression of the endogenous esotropic retrovirus (eMuLV). In contrast, NIH Swiss mice similarly exposed to 1250 ppm butadiene had a 14% incidence of thymic lymphoma/leukemia, with no increase in eMuLV, although the same hematologic and cytogenetic abnormalities were observed in the NIH Swiss mouse. The difference in leukemogenic response between the two mouse strains clearly demonstrates that the eMuLV background influences species susceptibility to butadiene-induced leukemogenesis. See Bird Statement at 13-20; Hinderer Statement at 15-18.

II. QUANTITATIVE RISK ASSESSMENT

DHS based its "best estimate" of human cancer risk on the NTP I bioassay results for the male B6C3F1 mouse. The DHS risk assessment relies on internal dose estimates based on data by Bond et al. (1986). These dose estimates reflect intraspecies high to low dose retention differences, but DHS made no adjustments for interspecies differences in butadiene uptake, retention, and metabolism. DHS predicted a cancer potency factor of 0.32 per 1 ppm. The Panel believes that this is an unrealistic estimate that substantially overstates the potential human risk associated with butadiene. We therefore recommend alternative approaches for assessing potential risks and deriving a potency factor for butadiene. The adjusted risk estimates based on these approaches are summarized in Table I.

The following sections propose three risk assessment pathways for consideration by DHS. First, we describe an approach to butadiene risk assessment which relies on the rat bioassay data supplemented by adjustments to DHS's rat risk assessment. It is the Panel's judgment that the rat, and not the mouse, provides the better model for human risk assessment and should be used by DHS to derive its "best estimate" of human risk. Second, we propose a number of adjustments to DHS's mouse risk assessment. If DHS continues to use the mouse data for quantitative risk modelling, it should adopt the recommended adjustments to reflect species differences in butadiene metabolism and mechanism of action. Third, we describe a

quantitative risk estimate prepared by Shell Oil Company which placed primary reliance on the Hazleton rat study, but which also analyzed the NTP II data. Shell's risk assessment demonstrates how data on species differences in butadiene response can be used to derive more meaningful estimates of risk.

A. The Rat, and Not the Mouse, is the More Appropriate Model for Human Risk Assessment and the Rat Should Be Used by DHS for its Best Estimate of Human Cancer Risk

In performing its quantitative risk assessment, DHS acknowledged that "it is still an open question as to which experimental animal [the rat or the mouse] is a better indicator of human risk." Draft Health Assessment Document at 4-33. DHS, however, then stated, without further explanation, that its "staff conclude[d] that the quality of the mouse bioassay data is superior to that of the rat data." Thus, DHS determined that "the mouse provides the best estimate for the upper bound for plausible excess cancer risk to humans." Id.

We believe there is no support for this conclusion. As discussed in detail in the written statement of Dr. Hinderer and the attachments prepared by the authors of the Hazleton study, there are no differences between the quality or reliability of the NTP mouse study and the Hazleton rat bioassay which would warrant assigning greater weight to the mouse than the rat data.

More importantly, when evaluating the relative value of the rat and mouse studies for qualitative and quantitative assessments of human cancer risk, the critical issue is which

species -- B6C3F1 mouse or Sprague-Dawley rat -- provides a better model for human risk assessment. The species differences in butadiene metabolism and mechanism of action described above demonstrate that the B6C3F1 mouse is uniquely sensitive to the carcinogenic effects of butadiene. There are a number of reasons to discount the applicability to humans of the mouse-based risk estimates. When B6C3F1 mice, Sprague-Dawley rats, and primates are similarly exposed to butadiene, the mice achieve higher levels of reactive butadiene metabolites in the blood and tissues than the rats or primates. Additionally, cytogenetic changes and bone marrow effects have been seen in the mouse but not the rat or primate, and the MuLV virus has been detected in the B6C3F1 mouse but not the rat or primate. As primates are more representative of humans than mice, the B6C3F1 mouse should be viewed as an inappropriate model for human risk assessment. The Sprague-Dawley rat provides a more relevant model for human risk assessment. Thus, DHS should use the data from the Hazleton rat bioassay to derive its best estimate of the human cancer risk associated with exposure to low levels of butadiene. Recommended adjustments to the rat risk assessment are discussed in the following section.

B. DHS Should Make a Number of Adjustments to Its Quantitative Risk Assessment Based on the Hazleton Rat Data

1. The Rat Quantitative Risk Estimation Should Exclude Mammary Carcinomas From the Total Tumor Incidence

DHS based its risk estimates in the rat on total significant tumors less mammary fiboadenonas and uterine tumors in the female rat. We support DHS's exclusion of the mammary fibroadenomas from its risk calculation based on the Hazleton rat data. See Draft Health Assessment Document at 4-31. These mammary fibroadenomas are not known to progress to malignancy and were very common among unexposed female rats. See Environ Statement at 4.

The mammary carcinomas should also be excluded from the risk calculation since their incidence in the 1000 and 8000 ppm dose groups is not significantly elevated. Also, the incidence of mammary carcinomas in the low dose group is actually lower than in the control group. Calculating the risk based on the mammary carcinoma response yields an upper bound risk of 6.7×10^{-7} for 0.37 ppb, but the maximum likelihood estimate is 1×10^{-12}, or five orders of magnitude lower, at 0.37 ppb. Additionally, the lower bound is negative, which further demonstrates the high degree of uncertainty in using the mammary gland carcinoma response to extrapolate risk from the rat to human.

Based on the data in Table 4-15, it appears that the risk attributable to the mammary carcinomas (1.7×10^{-3} per ppm)

accounts for approximately fifty percent of the "summed total estimated" risk in female rats of 3.6×10^{-3} per ppm. Considering the uncertainty about the relevance and significance of the mammary tumor response, the risk estimate including the mammary carcinomas appears to overstate risk by a factor of two. By excluding the risk associated with the mammary carcinomas, the human cancer potency estimate based on the rat data would be reduced by fifty percent, from 3.5×10^{-3} to 1.8×10^{-3} at 1 ppm, and the estimate of upper bound risk at 0.37 ppb would be reduced to 6.7×10^{-7}. (The fifty percent contribution to the "total significant tumor risk" is an approximation that could be refined by re-estimating the risk using as input total significant tumors without mammary gland carcinomas.)

2. The Rat Quantitative Risk Estimation Should be Adjusted to Reflect Species Differences in the Blood Levels of Reactive Metabolites of Butadiene

For its quantitative risk estimates based on the Hazleton rat data, DHS used the internal concentration of butadiene as the measure of dose. See Draft Health Assessment Document at 4-4 - 4-6. This approach is certainly more appropriate than using the external concentration of butadiene in inhaled air. However, since DNA-reactive and mutagenic metabolites of butadiene are the probable ultimate carcinogens, it would be more meaningful to use blood levels of 1,2-epoxybutene-3 for the measure of dose. See Environ Statement at 2; Shell Alternative Risk Assessment at 10-12 (copies included in

Appendices E and G, respectively). Although this approach considers only the monoepoxide, and not the diepoxide or other butadiene metabolites, the monoepoxide is the primary metabolite of butadiene and thus provides a more realistic measure of internal or delivered dose than the internal concentration of butadiene.

DHS rejected the use of a risk estimate based on pharmacokinetics modeling for several reasons. The primary reason cited by DHS is that the model employed by Hattis and Wasson (1987) considered only the concentration of the monoepoxide and not levels of diepoxide butadiene ("DEB") or other butadiene metabolites. See Draft Health Assessment Document at 4-9. Crosslinking studies demonstrate that the mouse would have even higher levels of DEB, resulting in further overestimation of human risk. Thus, use of the monoepoxide levels as the measure of dose would not underpredict the actual risk.

While the data currently available may be considered less than optimal for performing a risk assessment based on pharmacokinetics modeling, this does not mean that the available data showing species differences should be ignored completely. These data clearly indicate that the mouse is uniquely sensitive to butadiene and thus provide a basis for reasonable adjustments to the risk estimation derived by DHS. Disregarding species differences in butadiene metabolism and the uniqueness of the

mouse response to butadiene would result in a misleading estimate of risk.

Studies by Dahl et al. (1990) sponsored by NTP demonstrate that, at the low levels of exposure of interest to DHS, rats attain approximately a 40-fold higher blood level of monoepoxide than the primate. Based on the very reasonable assumption (supported by in vitro relative enzyme activity data) that humans metabolize butadiene in the same manner as monkeys, humans should be approximately 40-fold less sensitive to the carcinogenicity of butadiene than are rats. See Environ Statement at 11; Shell Alternative Risk Assessment at 10-12 and Appendix 1.

Thus, the DHS risk assessment based on the internal concentration of 1,3-epoxybutene-3 as the measure of dose in the rat should be adjusted downward to reflect species differences in monoepoxide levels. To avoid underestimating risk, DHS might use only fifty percent of this 40-fold species difference and adjust the risk estimate by a factor of 20. Under this approach, the potency factor based on the rat is further reduced from 1.8×10^{-3} (in female rats, excluding mammary tumors) to 9.0×10^{-5} at 1 ppm. The upper bound lifetime risk at 0.37 ppb would be reduced to 3.3×10^{-8}. We believe that this risk level should be presented by DHS as its "best estimate" of upper bound cancer risk to man.

3. Alternatively, The Rat Quantitative Risk Estimation Should, at a Minimum, be Adjusted to Reflect the Underestimation of the Internal Dose

In performing its risk assessment, DHS relied on the data by Bond et al. (1986) to estimate the internal dose corresponding to a given concentration of inhaled butadiene. These data measure the internal concentration of butadiene which is retained following six hours of exposure to butadiene. See Draft Health Assessment Document at 4-4 - 4-5. By focusing only on the amount of butadiene retained at the end of this exposure interval, this measure ignores both the metabolism and excretion of butadiene during the exposure period, as well as the potential for further metabolism after the six hour period.

Based on pharmacokinetics modeling, Hattis and Wasson (1987) demonstrated that the Bond data underestimate the actual internal dose in rats by a factor of approximately 4.5. See Shell Alternative Risk Assessment at 8-9 and Appendix 1 p. 2. Thus, if DHS bases its estimate of risk on the internal concentration of butadiene which is retained, it should, at a minimum, adjust its risk assessment to reflect this underestimation of dose. This would reduce the estimation of risk based on the rat data from 1.8×10^{-3} (in female rats excluding mammary tumors) to 4.0×10^{-4} at 1 ppm, or 1.5×10^{-7} at 0.37 ppb.

C. If DHS Continues to Rely on the 1984 NTP Mouse Study for Its Best Estimate of Human Cancer Risk, the Risk Estimation Should be Adjusted to Reflect Significant Species Differences in Butadiene Metabolism and Mechanism of Action

While we believe that the rat, and not the mouse, provides the best model for human risk assessment, if DHS continues to rely on the mouse data to estimate risk, several adjustments in that estimate are necessary. These proposed adjustments are discussed below.

1. The Risk Estimation Based on the NTP I Mouse Data Should Exclude the Mouse Lymphomas from the Total Significant Tumor Incidence

DHS should follow the approach taken by OSHA in connection with its recent rulemaking regarding occupational exposure to butadiene and exclude incidences of malignant lymphoma from its risk estimations based upon the B6C3F1 mouse data from NTP I. See Occupational Exposure to 1,3-Butadiene, Proposed Rule 55 Fed. Reg. 32736 (August 10, 1990). Due to the species differences in the mechanism of action of butadiene discussed in Section I.B.2 above, this tumor endpoint is of questionable relevance to potential human cancer risk.

The studies by Irons et al. (1986, 1989) clearly demonstrate that the presence of the MuLV retrovirus, which is endogenous to the B6C3F1 mouse (but not to the NIH Swiss mouse, the rat, or the human), plays a critical role in the expression of malignant lymphoma in that species. In addition, a number of studies have demonstrated cytogenetic and bone marrow

abnormalities in the mouse but not in the rat or the primate. These data indicate that the bone marrow (which plays an essential role in the development of T-cell leukemia and lymphomas) is a target organ of butadiene toxicity for the B6C3F1 mouse, but not the rat or primate. The presence of the MuLV virus and the unique sensitivity of the mouse bone marrow cast doubt on the relevance of the lymphoma response seen in the B6C3F1 mouse for human cancer risk assessment. The fact that, in the NTP II study, leukemias/lymphomas were not seen at the low dose further suggests that the mouse lymphomas are of limited relevance for low dose risk assessment. See Hinderer Statement at 15-18, Bird Statement at 13-24.

Based on the mouse data in Table 4-13 of the Draft Health Assessment Document, DHS estimated the risk for male mice (using internal dose and $(ppm)^{-1}$ scaling) for hematopoietic system malignant lymphoma to be 0.089 $(ppm)^{-1}$. The total risk for male mice, based on the "sum of individual sites," was 0.245 $(ppm)^{-1}$. This suggests that the lymphomas account for approximately 35% or one-third of the total risk (0.089/0.245 x 100). (The total risk for male mice based on "all significant tumors" (the program input) was 0.32 $(ppm)^{-1}$, of which approximately 28% is attributable to the lymphomas (0.089/0.32 x 100). (The 28% contribution to the "total significant tumor risk" is an approximation that could be refined by re-estimating the risk using as input total significant tumors without lymphomas.) The effect of eliminating the mouse lymphomas from

the calculation of total significant tumors can be estimated for the NTP I mouse data by reducing the potency factor by 28%, from 0.32 to 0.23 at 1 ppm.

2. The Mouse Quantitative Risk Estimation Should be Adjusted to Reflect Species Differences in the Blood Levels of Reactive Metabolites of Butadiene

As discussed in Section II.B.2 above, blood levels of the reactive metabolite 1,3-epoxybutene-3, provide a much more meaningful dose estimate than the internal concentration of butadiene as used by DHS. In studies by Dahl et al. (1990), mice developed more than 590-fold higher blood concentrations of the monoepoxide than did monkeys. Thus, humans (assuming that they metabolize butadiene in a manner similar to monkeys) should be 590-fold less sensitive to butadiene's carcinogenicity than are mice. See Environ Statement at 11; Shell Alternative Risk Assessment at 10-12 and Appendix 1.

Using the approach of allowing only fifty percent of this 590-fold difference, the risk estimate would be adjusted downward by a factor of 295. This results in a reduction of the potency factor from 0.23 at 1 ppm (in male mice, excluding lymphomas) to 7.8×10^{-4} at 1 ppm. This corresponds to an upper bound risk of 2.9×10^{-7} at 0.37 ppb.

3. Alternatively, The Mouse Quantitative Risk Estimation Should be Adjusted to Reflect the Underestimation of the Animal Dose and Interspecies Differences in Butadiene Absorption

Even if DHS decides to rely on the internal concentration of butadiene rather than the blood levels of the

monoepoxide for its measure of dose, its risk calculation should be adjusted to reflect underestimation of the retained dose and species differences in butadiene absorption and retention. Thus, reductions in the DHS risk estimates are still needed even if it decides not to use blood levels of the monoepoxide as the best measure of dose.

a. Adjustment for Underestimation of Dose. As discussed in Section II.B.3 above, the pharmacokinetics modeling performed by Hattis and Wasson (1987) demonstrates that the measure of dose based on butadiene retained at the end of six hours, relied on by DHS, underestimates the actual internal dose in mice by a factor of 2. See Shell Alternative Risk Assessment at 8-9 and Appendix 1 p. 2. The estimation of risk based on the internal concentration of butadiene should therefore be reduced by 50%. This results in a reduction of the estimation of risk based on the NTP I mouse study from 0.23 (in male mice, excluding lymphomas) to 0.12 at 1 ppm.

b. Adjustment for Species Differences in Butadiene Absorption. DHS has assumed that the absorbed fraction of butadiene is the same for mice and humans. See Draft Risk Assessment Document at 4-24. This conclusion does not take into account studies in primates by Dahl et al. (1990). As discussed above, Dahl et al. have demonstrated that at 10 ppm the mouse retains approximately 6.3-fold more butadiene than is retained by the monkey. Because the human species is more closely related, both anatomically and physiologically, to the monkey than the

mouse, the primate retention data should be used to estimate butadiene retention by humans. See Environ Statement at 8-9.

On this basis, the DHS risk assessment based on the NTP I mouse data should be further adjusted downward by a factor of approximately six. In conjunction with the prior adjustment for the underestimation of animal dose described above, this would result in a reduction of the potency factor from 0.12 to 0.02 at 1 ppm. This corresponds to an upper bound risk of 7.4×10^{-6} at 0.37 ppb.

D. Alternative Risk Assessment in the Mouse Based on Data from the Second NTP Study

The DHS Health Assessment Document describes the preliminary, unaudited data from the second NTP study reported by Melnick et al. See Draft Health Assessment Document at 3-30. However, DHS does not use these data as a basis for estimating human cancer risk. Shell Oil Company has developed an alternative risk assessment using the preliminary NTP II data. Although this risk assessment cannot be considered definitive because the individual time to tumor data are not yet available, we are providing the Shell analysis to CARB for its consideration as another approach for determining butadiene's potential risk.

Shell's risk assessment placed primary reliance on the rat data because Shell believed the rat to be a considerably more relevant model for man than the mouse. Shell also performed a risk assessment on the NTP II mouse data based on the incidence of pooled malignant tumors and hemangiosarcoma (to correspond to

the OSHA risk analysis). Shell's assessment was conducted using the multistage time-to-tumor model and quantal multistage and one-hit models. Shell also made adjustments to reflect the butadiene retention data of Bond et al. (1986) and metabolism data of Dahl et al. (1990). Shell made additional adjustments to reflect blood levels of the epoxide metabolites in primates as the measure of dose, since the data by Dahl et al. (1990) were developed in the exposure range used in NTP II.

Shell's risk assessment prepared in response to OSHA's proposed workplace standard for butadiene illustrates the reduction in risk estimates based on the NTP II mouse data and the incorporation of appropriate adjustments for interspecies differences, time to tumor considerations, and blood epoxide levels. See Shell Alternative Risk Assessment at 12-18 and Appendix 3. The Shell assessment predicted risks from 10 to 100,000 or more times smaller for the mouse (Shell, Figure 3) and for the rat (Shell, Figure 4) than the comparable OSHA estimates.

* * *

There is a considerable body of data which demonstrate that, as a result of species differences in butadiene metabolism and mechanism of action, the mouse is uniquely susceptible to the carcinogenic effects of butadiene. We therefore believe that the rat, and not the mouse, is the better model for human risk assessment and should be used by DHS to derive its "best estimate" of risk. Moreover, it is misleading to assign no weight to relevant data on species differences in butadiene

metabolism which demonstrate that rodents attain significantly higher blood levels of the reactive butadiene metabolites than primates. When adjustments are made to the DHS risk assessment to reflect these species differences, it becomes apparent that the risk assessment based on the mouse very likely overpredicts the risk to man by 1 to 4 orders of magnitude. The impact of these adjustments is summarized in Table 1.

III. REPRODUCTIVE EFFECTS

The Draft Health Assessment Document states that, in the NTP II mouse study, ovarian atrophy was reported in female mice exposed to 6.25 ppm of butadiene six hours a day, five days a week for two years. DHS therefore concluded that "a NOAEL was not established in these studies, but a LOAEL of 6.25 ppm was observed." Draft Health Assessment Document at 3-6 - 3-7. The data reported for NTP II are preliminary and unaudited, and the histopathology narratives are limited. Therefore, care must be used in interpreting and assessing the significance of the findings. The butadiene reproductive and developmental effects data are discussed in detail in the Statement of Dr. Mildred Christian (copy included in Appendix F). Dr. Christian notes that, in the NTP II mouse study, ovarian atrophy in the 6.25 and 20 ppm groups occurred at the end of the animals' reproductive life and should not be regarded as "reproductive" effects. In addition, the mouse is uniquely sensitive to the effects of butadiene exposure and exhibits effects which are not observed in the rat. Thus, Dr. Christian concludes that 20 ppm should be

considered to be a NOEL for ovarian atrophy in the B3C6F1 mouse. See Christian Statement at 7-13.

The Health Assessment Document should also highlight the absence of reproductive effects in the Hazleton rat bioassay by Owen et al. (1987, 1990). In this study, an absence of ovarian and testicular atrophy was observed in rats following lifetime exposures to butadiene at levels as high as 8000 ppm. See Christian Statement at 4. Given the greater biological relevance of the rat to man, this study should take precedence in determining butadiene's reproductive risks.

IV. EXPOSURE ASSESSMENT

The Panel has reviewed CARB's Standard Operating Procedure for the Determination of 1,3-Butadiene in Ambient Air. See Technical Support Document Part A, Draft Exposure Assessment at Appendix B. We have identified several issues regarding CARB's methodology, which are summarized below.

First, it is common for other four carbon hydrocarbons, in addition to butadiene, to be present in ambient air. The description of CARB's analytical methodology does not demonstrate that adequate steps were taken to separate butadiene from other four carbon components such as butene-1 or isobutane. The failure to differentiate butadiene from these components may result in an overstatement of butadiene levels in ambient air. The presence of butadiene in the samples analyzed by CARB may be confirmed in several ways; the most direct method would be routine GC/MS analysis.

In addition, the CARB protocol does not provide sufficient information to evaluate the validity of the sample collection methodologies which were used. Depending on the details of how the samples were collected and transported, it is possible that the CARB analysis may have either overstated or understated actual butadiene levels.

CONCLUSION

The Butadiene Panel appreciates this opportunity to submit comments on CARB's Draft Technical Support Documents for 1,3-butadiene. We hope these comments will be helpful to the Agency and that the Agency will incorporate them into its Technical Support Documents when they are released in final form.

TABLE I
RECOMMENDED ADJUSTMENTS TO DHS RISK ESTIMATES

	Cancer Potency $(ppm)^{-1}$	Upper Bound Risk Estimate at 0.37 ppb
Rat (Hazleton 1981)		
DHS Risk Estimate	3.5×10^{-3}	1.3×10^{-6}
Exclude Mammary Carcinomas	1.8×10^{-3}	6.7×10^{-7}
Adjustment for Epoxide Dose[a/]	9.0×10^{-5}	3.3×10^{-8}
or		
Adjustment for Dose Underestimation[b/]	4.0×10^{-4}	1.5×10^{-7}
Mouse (NTP, 1984)		
DHS Risk Estimation[c/]	0.32	1.2×10^{-4}
Exclude Lymphomas	0.23	8.5×10^{-5}
Adjustment for Epoxide Dose	7.8×10^{-4}	2.9×10^{-7}
or		
Adjustment for Dose Underestimation[d/] and	0.12	4.4×10^{-5}
Adjustment for Species Differences in Butadiene Absorption[d/]	0.02	7.4×10^{-6}

a/ The Butadiene Panel believes that this calculation represents the "best estimate" of human cancer risk.

b/ If no adjustment is made to reflect species differences in blood levels of the monoepoxide, the risk estimation should, at a minimum, be adjusted to reflect the underestimation of inhaled dose. This estimate also excludes the mammary carcinomas.

c/ This calculation was reported by DHS to be its "best estimate" of upper bound cancer risk.

d/ If no adjustment is made to reflect species differences in blood levels of the monoepoxide, then adjustments are needed for both underestimation of inhaled dose and species differences in butadiene absorption. These estimates also exclude the mouse lymphomas.

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*/ To facilitate the Agency's consideration of these Comments, the CMA Butadiene Panel will, upon request, provide CARB with copies of any of the references which are cited.

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STATE OF CALIFORNIA
AIR RESOURCES BOARD
STATIONARY SOURCE DIVISION

APPENDICES TO THE COMMENTS OF THE CHEMICAL MANUFACTURERS ASSOCIATION BUTADIENE PANEL ON THE PRELIMINARY DRAFT TECHNICAL SUPPORT DOCUMENT FOR THE PROPOSED IDENTIFICATION OF 1,3-BUTADIENE AS A TOXIC AIR CONTAMINANT

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APPENDICES TO THE
COMMENTS OF THE
CHEMICAL MANUFACTURERS ASSOCIATION
BUTADIENE PANEL
ON THE PRELIMINARY DRAFT TECHNICAL SUPPORT DOCUMENT
FOR THE PROPOSED IDENTIFICATION OF 1,3-BUTADIENE
AS A TOXIC AIR CONTAMINANT

A. Member of Companies of the CMA Butadiene Panel

B. Statement of Dr. Robert K. Hinderer

C. Statement of Dr. Michael G. Bird

D. Statement of Prof. Dr. Dr. Hermann M. Bolt

E. Statement of Environ Corporation (Dr. Thomas B. Starr)

F. Statement of Dr. Mildred S. Christian

G. Shell Oil Company Alternative Risk Assessment

ATTACHMENT A

BUTADIENE PANEL
MEMBER COMPANIES

Chevron Chemical Company

Dow Chemical USA

E.I. duPont de Nemours and Company

Eastman Kodak Company

Exxon Chemical Company

GE Plastics

Lyondell Petrochemical Company

Mobil Chemical Company

Oxy Petrochemical, Inc.

Quantum Chemical Corporation

Shell Oil Company

Texaco Chemical Company

Texas Petrochemical Corporation

Union Carbide Corporation

Docket No. H-041

TESTIMONY BEFORE OSHA

ON THE PROPOSED REVISION OF THE WORKPLACE

STANDARD FOR 1,3-BUTADIENE

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DATE: NOVEMBER 9, 1990

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I. INTRODUCTION AND SUMMARY

My name is Dr. Robert K. Hinderer. I am Manager of Health and Toxicology at the BFGoodrich Company and have 15 years of experience in the field of toxicology. This includes the development, monitoring and conduct of toxicology studies, serving as a toxicology consultant to expert groups such as the National Academy of Sciences, and providing expert testimony before Federal and State bodies. Furthermore, I have numerous publications in the field of toxicology.

Over the last 13 years, I have been involved with toxicology research on 1,3-butadiene (BD) primarily through my participation in the International Institute of Synthetic Rubber Producers, Inc. (IISRP). This has included participation in environmental and research steering committee activities. I also have participated in a number of International symposiums and conferences on the health effects of BD.

In my testimony, I will address the following issues:

1) Adequacy, quality, and relevance of the animal bioassays with BD, and;

2) Implications of species differences in metabolism, pharmacokinetics and mechanisms of toxicity in assessing BD cancer risk in man.

Presently, three cancer bioassays in two species indicate that BD is carcinogenic in animals. A long-term inhalation study completed by Hazleton UK in the early 1980's shows that BD is a weak carcinogen in the Sprague-Dawley rat. In contrast, NTP cancer bioassays which were initiated in the early and late 1980's indicate that BD is a potent carcinogen in the $B_6C_3F_1$ mouse. These studies are the culmination of a close technical dialogue between the National Cancer Institute (now NTP) and the IISRP which began in the mid-1970's.

Both the HLE and the first NTP bioassay (NTP-1) have used well-accepted approaches to chronic toxicity and/or carcinogenicity evaluation and have provided quality data. The NTP data has been confirmed by a detailed audit resulting from concerns raised by a preliminary audit and by the second NTP bioassay (NTP-2). Three partial audits of the HLE data also have shown that it is a quality study. Although OSHA has noted a number of issues pertaining to quality and accuracy of the study, HLE has shown that the statements are unfounded or misleading.

Numerous mutagenic, pharmacokinetic, metabolic and mechanistic studies support the results of these bioassays. They show that there are considerable differences in species response to BD and that these differences have a major impact on the assessment of human cancer risk. As a whole, the data do indicate that the risks are different and lower in the rat, the monkey and man than in mice. Ultimately, they indicate that the mouse is not a good model of BD toxicity in man.

Because of species differences in response to BD, there are a number of rodent responses which are not relevant to man. Studies indicate that the thymic leukemia/lymphoma (TL/L) that occurs in the $B_6C_3F_1$ mouse is dependent on bone marrow toxicity and the marine leukemia virus (MuLV) endogenous in this species. Furthermore, the results of pharmacokinetic and epidemiology studies indicate that neither the lung nor kidney are target organs in humans.

II. HLE AND NTP-1 BIOASSAYS

A. Overview Of the Hazleton (UK) Rat Bioassay With BD

In 1978, an inhalation study of the potential chronic toxicity and carcinogenicity of 1,3-butadiene (BD) was initiated, using CD (Sprague-Dawley) rats. Groups of 110 male and 110 female rats were exposed to 0, 1000, or 8000 ppm BD for 6 hrs/day, 5 days/wk. (Owen et al. 1987, Owen and Glaister, 1990). These dose levels were selected based on the results of a three-month study and a determination of the highest non-explosive concentration.

Prior to exposure, the animals were quarantined for 11 days and observed daily for signs of ill health. A sampling of the rats in each batch was examined for pathogenic microorganisms. Furthermore, a number of animals were selected for necropsy and histological examination. Routine laboratory and neuromuscular evaluations were also conducted on 20 pre-selected animals per sex.

During the course of the study, numerous observations and measurements were made to evaluate the vital status of the animals and to assess potential toxic effects. A detailed animal observation and palpation was performed weekly. Body weights were recorded weekly for the first 12 weeks, every 2 weeks up to 52 weeks and thereafter at 4 week intervals. Hematology, clinical chemistry and urine analyses were conducted at various points throughout the study. Neuromuscular function also was evaluated periodically using a rotating conical spiral. Ten rats/sex from each group were killed at week-52 for interim pathological evaluation and selected organs were weighed.

The study was terminated when survival reached 20 to 25% (105 weeks for females and 111 weeks for males). A post-mortem examination was conducted on all animals. Tissues from all animals were examined closely for abnormalities.

A common and well-accepted method was used for the selection of tissues for pathological evaluation. A complete set of tissues from the high dose animals first were examined. Based on the findings in the high dose animals, potential target tissues were identified for examination in the low dose animals.

B. General Findings In the HLE Rat Study

Evidence of the toxicity of BD during the in-life phase of this study was quite limited. Clinical signs and body weight changes were transient. Survival in this study was generally good allowing the animals to be exposed beyond two years. However, the mortality that was observed in this study was the resulted from the humane sacrifice of females with subcutaneous masses and from the occurrence of renal lesions in males. Liver weights were increased in both sexes at both doses without any corresponding pathological changes. Kidney weights were increased and were accompanied by an increase in severity of nephrosis. No compound-related effects on hematology, clinical chemistry, urine analysis, or neuromuscular function was found.

The most significant finding was a weak carcinogenic response to BD (Figure 1). There was an increase in the incidence of pancreatic exocrine adenoma, and testis Leydig-cell tumors in high dose males and of mammary tumors (primarily benign fibroadenomas), and thyroid follicular cell tumors in females. Treatment-related trends were noted for uterine sarcoma and Zymbal gland carcinomas. This overall carcinogenic response was weak in the sense that there were no unusual tumors and that the number of tumors were not markedly increased. In fact, the thyroid and testis tumors at the highest dose were close to the historical control range (0-6%) at HLE. Furthermore, there were a number of factors which tended to diminish or challenge the significance of a number of these findings.

For the pancreatic adenomas, the appropriate diagnosis was not clear. By convention, the tissue changes were classified as tumors. However, the pathologist stated that the small lesions equally could be classified as hyperplasia which would exclude them from any analysis of tumor incidence.

The numbers of uterine sarcomas were close to those expected from the background in untreated rats of the strain used and did not suggest, in isolation, a treatment-related effect. The number of Zymbal gland tumors also was close to that expected as part of the background. Most of the tumors of this type were present in animals killed in a short period (76-90 weeks); none were found at the end of the study. If this early cluster of tumors was due to a direct chemical interaction, others would have been expected to be found later in the experiment.

Although the early appearance of mammary gland tumors in the treated females suggested a relation to treatment, there was some question concerning the interpretation of the numbers. First, mammary carcinomas were not elevated. Secondly, while the incidence of benign fibroadenomas was significantly higher in both treatment groups than in the controls, there was no dose-related increase. Finally, a review of the historical control range for this strain at HLE showed that mammary tumors occurred with a high and variable frequency (14.6-58.1%).

C. Overview Of the NTP Mouse Bioassay (I) With BD

The National Toxicology Program (NTP, 1984) has conducted two mouse bioassays with BD. In the first study, groups of 50 male and 50 female $B_6C_3F_1$ mice were exposed to 0, 625, or 1250 ppm BD 6 hrs/day, 5 days/wk. This study was intended to last 104 weeks, but was terminated after 60/61 weeks (males/females), because of high mortality. The results of earlier studies of shorter duration were used to set these dose levels.

When the animals were received, they were quarantined for 3 weeks. Selected animals were given a complete pathological examination.

During the study, all animals were observed twice daily for signs of moribundity or mortality. Clinical signs were recorded weekly. Body weights were recorded weekly for the first 12 weeks, monthly thereafter. Palpation of mice for masses began 6 months after the study started and continued monthly thereafter. Gross necropsies and pathological evaluations of selected tissues were performed on all animals, except for those excessively autolyzed or cannibalized.

Serological analyses for selected viruses were monitored in the control animals at the end of the study. No analyses for the known endogenous murine leukemia virus (MuLV) were undertaken.

D. General Findings in the NTP Mouse Bioassay I

In the $B_6C_3F_1$ mouse, BD caused a potent carcinogenic response (Figure 1). The most prominent finding in this regard was the high mortality from the malignant lymphomas (TL/L), which resulted in early termination after only 60-61 weeks of exposure. While these lymphomas occurred in both sexes, they were more highly elevated in the males. Significant increases in neoplasms were observed at many different sites. Furthermore, the incidence of these tumors were generally high. Malignant lymphomas and heart, lung and forestomach tumors were observed in both sexes. In addition, mammary gland, ovary and liver tumors were elevated in the females.

Numerous non-neoplastic lesions were also found in mice exposed to BD. Significant increases in chronic changes in the liver and forestomach in both sexes was observed following BD exposure. Testicular atrophy and nasal lesions also were increased in males and the occurrence of ovarian atrophy and uterine changes were elevated in females. However, not all of the increases were dose-related. In contrast to the neoplastic findings, the chronic changes generally were found to occur at a lower incidence.

E. Comparison of the Methodologies Used In the HLE Rat and NTP-1 Mouse Studies With 1,3-Butadiene

1. Purpose:

Both the HLE and NTP studies incorporated state-of-the-art methodologies. They used large numbers of animals; the test animals were species commonly used; and they maximized the potential for seeing an effect by using the highest exposures feasible and by exposing the animals for nearly their complete lifespan.

It is important to note, however, that the primary focus of these two studies is different. The NTP mouse study is basically a cancer bioassay, while the HLE rat study is designed for evaluating carcinogenic potential, but also incorporates elements necessary for a fuller assessment of chronic toxicity. Because of these differences in emphasis and other practical considerations there are, not surprisingly, a number of differences in various aspects of the methodologies used.

2. Chemicals Tested and Impurities

In both HLE and NTP-1 studies, standards and procedures were established to limit the level of 4-vinyl-1-cyclohexene (VCH) and general impurities in the 1,3-butadiene. In the NTP mouse study, a standard of 100 ppm VCH (v/v) of BD in the cylinders was set as the maximum acceptable for use and was exceeded on three occasions when replacement cylinders were not available. Each cylinder was analyzed upon receipt. Generally, the cylinders were used for no longer than 6 weeks in an effort to minimize the amount of dimer formed and delivered. However, no analyses of the actual delivered dose of VCH were conducted, nor were there any standards or procedures to detect and control unacceptable levels actually delivered. Because dimer formation continues with time, there is no way of knowing what levels of VCH were actually delivered to the chamber, nor what the chamber concentration might have been.

In the HLE study, the control process for VCH focused on the VCH levels at the time of delivery of BD to the chamber. HLE measured VCH (v/v) at a minimum of once a week allowing more frequent, sometimes daily, measurements at the discretion of the investigators. A series of action levels were established above the 500 ppm VCH (v/v) acceptance guidelines to trigger activities such as the notification of project monitor and additional analyses by HLE. If a mean value of >800 ppm (v/v) continued for the day, a trap for removal of dimer was incorporated. Although 1000 ppm (v/v) was established as a criteria for terminating exposure, no such excedences were observed because of the trapping procedures that were used.

HLE found that the average weekly measurements of VCH (v/v) was 413 ppm. Based on their calculation that 100 ppm of the dimer (v/v) in the BD supplied to the 8000 ppm chamber would result in a concentration of 0.8 ppm VCH in the chamber (Owen, 1981), the average concentrations of VCH in the chamber would have been around 3 ppm throughout the course of the study.

While VCH raises some similar health concerns as BD, we know that its toxicology profile is somewhat different. VCH, as with BD, is mutagenic in in vitro assays and is metabolized to mono- and di-epoxide forms (Simmons and Baden, 1980; Turche et al. 1981; Smith et al., 1990a; Smith et al., 1990b). Likewise, the mouse has a greater capacity to metabolize VCH that the rat. Although two year cancer bioassays in rats and mice were compromised by poor survival, VCH clearly did not produce the potent

response observed in mice with BD (NTP, 1986). This less potent response indicates that low VCH levels in the chambers would not be expected to have any significant impact on the outcome of the long-term studies with BD.

3. Randomization

Randomization procedures were used in both studies, although they are not described in the NTP-1 report. Randomization was conducted to provide as nearly as possible equal group mean body weight and standard deviations. Issues dealing with randomization are discussed later in Section F.

4. Clinical Chemistry, Hematology and Urinalysis

No clinical chemistry, hematology, or urinalysis tests were conducted in the NTP-1 study.

In the HLE study, blood was withdrawn under light ether anesthesia from the orbital sinus of 20 pre-selected animals of each sex from each group after 3, 6, 12 and 18 months exposure. Before blood tests, the animals were fasted for 24 hrs, but water was available during the last 18 hrs. of the fast. The blood was used to measure mean cell volume and hemoglobin concentration and for counts of erythrocytes, leukocytes (total and differential), platelets and reticulocytes. Packed cell volume, mean cell hemoglobin and mean corpuscular hemoglobin concentration were calculated. In addition, measurements were made of plasma concentrations of glucose (sample taken from caudal vein), blood urea nitrogen, total protein and protein electrophoresis, as well as activities of alkaline phosphatase, glutamic-oxaloacetic transaminase and glutamate-pyruvate transaminase after 3, 6 and 12 months exposure. At 12 months, leucocyte counts (total and differential) were made in an additional ten animals of each sex from each group in order to confirm a possible treatment-related effect noted at the 6-month sampling in the high dose females.

Individual urine samples were obtained after 3, 6 and 12 months exposure. Wherever possible, the same 20 males and 20 females were sampled as were used for the blood sampling. The urine samples were obtained during a 4-hr. period of food and water deprivation beginning approximately 2 hrs. after exposure. During the 2-hr. post exposure period, all animals were given access to water. The volume and specific gravity of the urine were measured and a semi-quantitative assessment of glucose, urobilinogen, ketones, bile pigments, blood, protein and

pH made. The insoluble constituents of the urine were examined microscopically after centrifugation.

5. Neurotoxicity

No special neurotoxicity evaluations were conducted in the NTP-1 study.

In the HLE study, neuromuscular function was evaluated in 40 animals of each sex from each dose group before and at Weeks 1, 4, 15, 26, 52 and 78 of treatment. The time before falling from a rotating cone was recorded as an index of neuromuscular function.

6. Interim Sacrifice

No interim sacrifice group was established in the NTP-1 study.

The study design for the HLE study included 10 rats per sex per dose from each dose group for interim evaluation at 52 weeks.

7. Pathology

Both studies used commonly accepted procedures for pathology evaluations. All animals were subjected to a postmortem examination. Selected tissues and all grossly observable lesions were preserved and processed for histopathological evaluations. In the HLE study, all selected tissues from the high dose and control were examined first. Once the target organs were identified, HLE then examined the corresponding low dose tissues as provided in the protocol. HLE also conducted electron microscopic examination of liver tissue samples from 10 males and 11 females from all dose groups at necropsy. NTP-1 on the other hand, simply chose to evaluate all selected tissues for each animal in each dose group.

8. Quality Assurance

Specific attention was paid to quality assurance in both the HLE and NTP-1 studies. Some of these activities were predetermined, while others were influenced by events subsequent to the termination of the studies.

Throughout the course of the HLE rat study, diverse peer review and quality assurance were maintained. The study design and daily conduct were reviewed, not only by industry scientists, but by scientists from government also. A close liaison was kept with scientists from NCI (NTP). Information, as well as ideas, were exchanged

through meetings, written documents and verbal communications. Additional peer review was obtained from the British Industrial Biological Research Association (BIBRA), through the journal publication process, and by the International Agency for Research on Cancer (IARC).

When the International Institute of Rubber Producers, Inc. (IISRP) decided to sponsor this research project, it recognized the importance of assuring the quality of this work. As a result, the Institute retained BIBRA to oversee the quality and management of this study, as well as to provide additional technical support. BIBRA conducted frequent site visits and data checks. They worked closely with the Hazleton (UK) staff and the Institute to expedite decision-making.

To-date, the Hazleton (UK) rat bioassay data has been reviewed by three organizations - BIBRA, the United Kingdom Health and Safety Executive (UK H&SE) and the Hazleton Quality Assurance Unit. This has included a partial audit of the data.

The NTP-1 Mouse Bioassay has received both peer and quality reviews by Agency, contract and independent personnel. The NTP Board of Scientific Counselors Technical Reports Review Subcommittee and associated Panel of Experts has reviewed the technical report prior to its final publication. Quality assurance audits have been conducted by the NTP using internal and external staff and by the Chemical Manufacturers Association. These assessments identified numerous concerns regarding the methodology, procedures, and accuracy of the data.

While errors in the study have been noted, they do not detract from the basic finding that BD is a potent carcinogen in the mouse. Results from NTP-2 further support this conclusion.

9. <u>Assessments of the HLE and NTP-1 Study Methods, Results and Issues Raised by OSHA</u>

I agree with OSHA "---that both the NTP mouse bioassay and the HLE rat bioassay demonstrate the carcinogenicity of BD and that both provide adequate data on which to base a quantitative risk assessment despite their problems. Both of these studies have qualities which make their data suitable for quantifying risk from occupational exposure. Exposure levels were documented, the routes of exposure were the same as is found in most occupational settings (i.e., inhalation); concurrent controls were used; animals were exposed to two different levels of the test substance and statistically

significant excesses of malignant neoplasms were observed in the exposed groups."

The Agency's decision, however, to base its "best" estimate of risk on the mouse bioassay (52 FR p. 32762) is not based on good science. The factors used and concerns that were raised by OSHA are not convincing. In the following paragraphs, the principle concerns raised by OSHA are addressed. I also refer the Agency to documents prepared by HLE on these subjects (Owen and Brightwell, 1987 and Glaister, J. R. 1990). See Appendix I and II.

F. Specific Issues Raised By OSHA Concerning the HLE Rat Study

1. OSHA is concerned that there was a failure in the randomization process, such that the low dose group is not comparable. OSHA bases its concern on 1) the lower overall tumor incidence in the low dose relative to the control, 2) high survival in the low dose at termination, and 3) a lower occurrence of nephropathy and a higher occurrence of abnormal teeth in the low dose group compared to the controls.

What OSHA fails to note is that the lower tumor incidence in the low dose group (70%) compared with the control is neither alarming, nor surprising. In fact, as OSHA points out, this is likely the result of only selected tissues being examined in this group.

Although OSHA is concerned that the low dose was healthier than the controls, there is no evidence that that is the case. For both males and females, survival was comparable through day 420. During the period of day 420 to 480, survival began to deviate from that of the control group. A dose-related decrease in survival was noted in the low and high dose groups through the 2-year mark (730 days). The only point where survival in the low dose was better than the controls, was in the males beyond the two-year point (>730 days), specifically at 780 days. Furthermore, comparisons of overall trends in body weight, general observations, clinical chemistry, hematology or urinalysis do not support OSHA's contention that the low dose was healthier.

The absence of a dose response for nephropathy, where the low dose shows a lower response than the control (75 and 87% nephropathy), is neither alarming nor unusual. In the NTP-1 study there also are selected instances where the response in the test group is lower than that in the

control (i.e., controls vs. low dose, respectively, in males: lymph node hyperplasia 20% vs. 2%; salivary inflammation 30% vs. 9%; kidney inflammation 84% vs. 17%; in females: lung perivascular cuffing 73% vs. 8%; lymph node hyperplasia 61% vs. 7%; salivary gland inflammation 63% vs. 13%).

OSHA is also incorrect that the low dose animals differed from the other groups in the number of animals with abnormal teeth. Hazleton points out in their comments Glaister, 1990) that in OSHA's own statistics there is no significant difference ($p = 0.125$) between the low dose and control males. The maximum occurrence at any time in surviving animals was 6, 10, 11% for control, low dose, and high dose animals, respectively.

In any event, these data do not indicate that the low dose males were healthier than the controls because of the effect of competing causes of disease and random variations in response. One cannot look at single or a few individual site responses to evaluate the health status or overall effect of the chemical. Again, these data fails to indicate that the low dose group is healthier than the control or is in any way abnormal.

Interestingly, although OSHA is concerned about the failure of the randomization process in the HLE study, it fails to note that the randomization process failed in the NTP study. In the BD report, NTP-1 states that "due to an apparent inadequate randomization, initial weights in dosed males and females were 9-11% higher than those of the controls". Using similar logic, it is possible that OSHA could draw the conclusion that the NTP-1 test group animals were healthier than the controls.

2. <u>The Agency has noted concerns about the quality and accuracy of the data.</u>

Like the NTP-1 bioassay, the HLE rat study has undergone two independent audits, one conducted by BIBRA and the other by the UK H&SE. In addition, HLE conducted its own internal audit. These audits have demonstrated comprehensive data trails and have evidenced that the study is sound.

Hazleton has addressed quality issues raised by OSHA through its comments on the ICF/Clements risk assessment of BD (Owen and Brightwell, 1987) and its recent comments on the proposed standard (Glaister, 1990) (see attachments). They have noted many cases where statements were inaccurate and misleading. These investigators also have identified instances where OSHA's

comments have created false impressions about error rate and have suggested far greater implications for quantitative risk assessment than are real. In general, these responses have shown that OSHA's concerns are not justified and are not supported by fact. For these reasons, HLE has provided additional clarification to assist OSHA.

In Appendix II HLE points out:

1) that the rat study has received three independent audits;

2) that the audits did not reveal any problems, as noted in the NTP-1 study, which would necessitate a 100% review;

3) that the tissue accountability was excellent even by present standards;

4) that the internal QA review showed the final report to be an accurate reflection of the data:

5) that the absence of an "NTP-type" review does not make this a flawed study, and;

6) that OSHA continues to utilize a statistical procedure which is known to introduce bias.

While some of the issues raised about the conduct of these studies are important, none are of sufficient weight or validity to negate the distinct difference in species response in BD or its utility for risk assessment. These findings strongly indicate that the response observed in the Hazleton (UK) study is the result of the biological response of the rat under specified exposure conditions and is not an artifact of the treatment, reporting, or quality of the data. This conclusion is supported by the species differences observed in *in vitro* mutagenic, metabolism and pharmacokinetic studies.

In general, we do not believe that the criticisms are justified, or that they provide a basis for choosing the mouse over the rat for quantitative risk assessment.

3. Finally, the Agency believes that being consistent with other risk assessments is an important reason for using the NTP study for risk assessment.

The use of consistency with other agencies or agency contractors as a basis for selecting data for risk

assessment is inappropriate and irrelevant. Since the CAG and ICF Clements documents were prepared, a considerable amount of information has been developed on species differences in pharmacokinetics, metabolism and mechanism of action. These data do indicate that the mouse is not a good model of human response to BD. This clearly emphasizes that the "best" animal model, not consistency, is the relevant factor.

III. NTP-2 MOUSE BIOASSAY WITH BD

A. Overview of the NTP-2 Mouse Bioassay

A second mouse bioassay with BD has been conducted by the NTP to study the effects of lower concentrations. Although a final NTP report is not available and the data is unaudited, information on this work has been provided by Melnick et al. (1989a), Melnick et al. (1989b), Melnick (1989) and Melnick et al. (1990).

In this study, $B_6C_3F_1$ mice (70 to 90/sex/group) were exposed to 0, 6.25, 20, 62.5, 200, or 625 ppm 6 hrs/day, 5 days/wk for periods up to 2 years. Interim sacrifices were conducted at 40 and 65 weeks on as many as 10 mice/group. While it is not clear what was evaluated during the interim sacrifices, hematological parameters were measured at least at week 40. In an effort to look at the effects of exposure duration, additional groups of 50 male mice were exposed to 625 ppm for 13 or 26 weeks, 312 ppm for 52 weeks or 200 ppm for 40 weeks. All animals in this stop-exposure study were held until the scheduled sacrifice at 104 weeks.

B. General Findings of the NTP-2 Mouse Bioassay

Only a few changes were reported prior to the scheduled termination of the study. At the 40 week sacrifice, hematological changes were found in groups exposed to concentrations of 62.5 and higher. Red blood cell count, hemoglobin concentration and packed red cell volume was decreased, while mean corpuscular volume was increased. Similar hematological changes were noted for females, although no data were reported. No data were reported from the 65 week sacrifice. For those groups where 104 weeks was the intended exposure period, survival was reduced for both sexes exposed to 20 ppm and higher.

The results of this study had many similarities with the first one (Tables 1 and 2). As noted in the first study, fatal tumors were the cause of the decreased survival. Again, the occurrence of malignant lymphomas was the major cause of early

deaths. Also, a similar potent carcinogenic response was observed.

Effects in the lung and lymphopoietic system were the most interesting findings. An analysis of the data revealed an excess of lung tumors in females at the lowest dose, 6.25 ppm. At concentrations below 625 ppm, lymphocytic lymphoma no longer played a role as the major cause of early death. This was evidenced by the low incidence of TL/L and improved survival at lower doses. A higher incidence of heart, lung, Harderian gland, liver, preputial gland and ovarian tumors were noted at these lower concentrations which had a better survival. This indicated that the lymphocytic lymphoma was a competing cause of death at high concentrations (625 and 1250 ppm), precluding full expression of tumors at other sites.

The "stop-exposure" experiments with BD in mice were conducted to study concentration/duration interactions. Conditions were established to compare similar total dose exposures derived under different conditions. Approximately equivalent total doses of 8,000 and 8,125 ppm-weeks were obtained through exposures of 200 ppm for 40 weeks and 625 ppm for 13 weeks while similar total doses of 16,224 and 16,250 ppm-weeks were achieved through exposures of 312 ppm for 52 weeks and 625 ppm for 26 weeks.

OSHA concludes that short-term exposure to BD induces a stronger carcinogenic response than does long-term exposure at a lower equivalent dose (p. 32788), which is apparent for some tumor responses. While OSHA believes that these data support a STEL of 10 ppm, they do not. These studies neither support the concept of a STEL, nor demonstrate a need to establish a STEL for BD at 10 ppm.

The results of these stop-exposure studies are quite variable. The tumor responses in the forestomach (8,000 and 16,000 ppm-weeks), the Harderian gland (8,000 ppm-weeks) and the preputial gland (16,000 ppm-weeks) appear to be dependent on total dose. The incidence of tumors within these total dose groupings for these tissues is similar. For the heart and lung tumors response appears to be more dependent on duration of exposure.

The leukemogenic response of the mouse in these studies is even more complex. At 625 ppm the highest total dose (16,250 ppm-weeks, or 625 ppm for 26 weeks) produce a greater tumor response than the shorter 13 week exposure. However, at lower concentrations (200 and 312 ppm), tumor response does not appear to correlate with either exposure duration, concentration or total dose. Generally, the data do indicate

that at concentrations at or above 625 ppm, concentration is the major determinant of leukemogenic response.

The probable explanation for the apparent absence of a dose response for TL/L at some dose/regimens is the unique involvement of the bone marrow and the MuLV in the $B_6C_3F_1$ mouse. Table 1, 3 and 4 shows that the incidence of TL/L drops off dramatically below 625 ppm rapidly approaching background. These data suggest that there are two mechanisms of carcinogenicity operating in the $B_6C_3F_1$ mouse. At concentrations below 625 ppm, the incidence of TL/L is similar to background levels. The only potential outlier is the 200 ppm X 40 week group.

It appears that once BD concentrations reach 625 ppm, concentration, not total dose, is the most important factor. This suggests that some non-pharmacokinetic/metabolic factor is involved which is likely a threshold phenomena involving the bone marrow and the MuLV.

One could argue that the breakpoint for leukemogenic response is 62.5 ppm BD, because this is the lowest concentration that caused hemopoietic effects. While most of the responses between 62.5 and 625 ppm are probably not significantly different from the control, they are numerically elevated with the exception of the 200 ppm X 104 week group. Still, it is clear that the most demonstrable increased incidence in TL/L occurs at 625 ppm and that this appears to be the results of the unique bone marrow/MuLV mechanism of action that exists in the $B_6C_3F_1$ mouse.

IV. MECHANISM OF BD TOXICITY IN THE B6C3F1 MOUSE

A number of studies provide direct evidence that the mechanism of toxicity of BD in the $B_6C_3F_1$ mouse is different than the rat or primate. Research by Liederman et al. (1986) and Irons et al. (1986), shows that BD affects bone marrow stem cell development and induces macrocytic megaloblastic anemia in the mouse. These results are a striking contrast to the absence of hematopoietic toxicity in the rat or primate (Owen et al. 1987; Owen and Glaister, 1990; Sun et al. 1989).

The fact that the bone marrow is a target organ of BD exposure in the mouse is particularly important, since the TL/L in the $B_6C_3F_1$ mouse provides additional evidence of bone marrow involvement. The role of the bone marrow in radiation induced murine T cell leukemia/lymphomas is well known as it is with instances of chemically induced T cell leukemia/lymphomas. Because bone marrow stem cell depletion frequently occurs prior to radiation or chemically induced thymic neoplasms (Kaplan, 1967 and 1977; Seidel and Bischof, 1983), BD stem

cell depletion in the mouse as evidenced by the work of Leiderman et al. (1986) suggest that this may play a major role in the induction of TL/L by BD in this species. These observations are clearly consistent with the cytogenetic changes observed in the mouse and absent in the rat and primate.

Another factor which makes the mechanism of toxicity unique in the $B_6C_3F_1$ mouse is the presence and action of the endogenous Murine leukemia virus (MuLV). This virus is present in the $B_6C_3F_1$ mouse and most other strains of laboratory mice and is only transferred vertically, from generation to generation. It is of importance because it is known to play a role in the expression of radiation induced leukemia (Gross, 1959). Also, there is evidence that chemicals can interact with mouse leukemogenic viruses (Raikow et al., 1983; Raikow et al, 1985).

In order to examine the role of the virus in BD carcinogenicity, Irons et al. (1986), compared the incidence of TL/L in the $B_6C_3F_1$ and NIH Swiss mice exposed to 1250 ppm BD for one year. The NIH Swiss mouse was chosen because, unlike the $B_6C_3F_1$ mouse, the MuLV proviral sequences are incomplete such that no active viruses can be produced. They found that BD caused a 4-6 times higher incidence of TL/L in the $B_6C_3F_1$ mouse (with the virus) than in the NIH Swiss mouse. TL/L in both stains, was morphologically similar and was confirmed to be of T cell origin. However, only TL/L from the $B_6C_3F_1$ mouse contained the MuLV "env" surface antigen.

These results provide strong evidence that the MuLV plays a role in BD carcinogenicity in the mouse. They also indicate that the occurrence of TL/L in the mouse is dependent on bone marrow toxicity and the mouse virus. This mechanism appears to be unique to the $B_6C_3F_1$ mouse, since no similar mechanism is apparent in rats or primates.

V. ASSESSMENT OF THE FINDINGS

The results of the Hazleton (UK) and NTP bioassays provides clear evidence that BD can cause chronic and carcinogenic effects in rodents. However, these studies also show that there are considerable qualitative and quantitative differences in species response to BD. In the Sprague-Dawley rat, BD is weakly carcinogenic. Lifetime exposure to levels as high as 8000 ppm, causes a relatively low incidence of tumors from organs/tissues of similar type. In contrast, studies with the $B_6C_3F_1$ mouse indicate that BD is a potent carcinogen in this species. This dramatic response is evident from the high early mortality due to the occurrence of

malignant lymphomas and the high incidence of tumors at a variety of different sites.

In the $B_6C_3F_1$ mouse significantly elevated tumor incidences were seen for TL/L, the heart, lung, forestomach in both sexes and mammary gland, ovary and liver in females, while in rats significant elevations were limited to the pancreas and testis in males and the mammary gland and thyroid in females. Furthermore, the benign mammary fibroadenoma was the only tumor in rats which exhibited a significant increase at 1,000 ppm and only when benign tumors were included. This response was in stark contrast to the mouse in NTP-2 where the tumor incidences were significantly elevated at numerous sites and occurred at dose levels 2 to 3 orders of magnitude lower than in the rat (Figure 1 and Tables 1 and 2).

Although OSHA points out in the proposed rule that many experts believe that mammary fibroadenomas represent a carcinogenic response, the real issue is the implications of this observation on human cancer risk assessment.

We know that mammary tumors occur at a high rate and variable frequency (14.6-58.1%) in untreated rats of this strain. Such high background variability suggests that non-genotoxic mechanism may be involved in the occurrence of these benign tumors. The fact that fibroadenomas are estrogen-induced (Lorenz, J., Glatt, H. R., Fleischmann, R., Ferlintz, R., and Oesch, F., 1984), indicates that other factors may effect not only the occurrence, but the risk.

Another important point is that mammary fibroadenomas are of a different histological type and do not progress to a malignant form (IRLG, 1979). For this reason, it is not appropriate to combine mammary fibroadenomas and carcinomas for risk assessment purposes. In fact, because of the likelihood of the involvement of a non-genotoxic mechanism, the HLE mammary fibroadenomas should not be used in traditional risk assessment modeling.

This difference in species response in the animal bioassays is consistent with other data, which both confirm and explain the basis for these results. Mutagenicity studies show that although BD is active in <u>in vitro</u> tests, the response is quite different <u>in vivo</u> (de Meester et al. 1980; Poncelet et al. 1980). Cunningham et al. (1986) and Arce et al. (1990) report that BD is mutagenic to mice in bone marrow microneuclic and sister chromated exchange (SCE) assays, but not mutagenic with rats. Similarly, studies by Sun et al. (1989) indicates that BD does not cause an increase in SCE in primates. These differences are supportive evidence of a divergent species response.

The existing data on BD appears to indicate that there is more than one mechanism involved in BD carcinogenicity in the $B_6C_3F_1$ mouse. Studies by Leiderman, et al. (1986) and Irons, et al. (1986a, 1986b, 1987a, 1987b and 1989), show that the occurrence of the TL/L in the $B_6C_3F_1$ mouse is dependent on the occurrence of bone marrow toxicity and the presence of the murine leukemia virus, endogenous to this species.

These studies provide evidence that BD alters stem cell development and induces macrocytic anemia in the mouse. Stem cell depletion therefore is likely to play a critical role in BD induced TL/L in the mouse because such depletion in the bone marrow is often a precursor of radiation induced thymic neoplasms (Kaplan, 1967, 1977; Seidel and Bischof, 1983). Mechanism studies of leukemogenic action in the $B_6C_3F_1$ mouse with MuLV and in the NIH Swiss mouse without the complete virus show that both species exhibit the same hematological and bone marrow cytogenetic abnormalities (Irons et al. 1986 and 1987). However, there is a four times higher incidence of TL/L in the $B_6C_3F_1$ mouse which is associated with an increase in the amount of MuLV recoverable from the bone marrow. These observations indicate that MuLV background influences the susceptibility of the mouse to BD induced leukemogenesis and that bone marrow damage is a prerequisite for leukemogenesis with the MuLV influencing the degree of susceptibility of the mouse (Irons et al. 1989).

The NTP-2 Bioassay shows that malignant lymphomas cease to be a factor at lower levels. This indicates that at lower levels the prime determinant of the carcinogenic potential of BD in the mouse is its ability to absorb and metabolize BD.

Numerous studies (Malvoisin et al. 1979; Bond et al. 1986, 1987 and 1988; Malvoisin and Roberfroid, 1982; Bolt et al. 1983 and 1984; Kreiling et al. 1986a and 1986b; Bond et al. 1986; Sun et al. 1989; Laib et al. 1990) have shown that BD is metabolized to mono- and diepoxides, the presumed ultimate carcinogen and that there are wide species differences in the metabolism and pharmacokinetics of BD.

These studies show that the amount of BD absorbed and metabolized (activated) is greater in the mouse than rats or primates. Higher metabolic capabilities in some tissues, like the lung, combined with relatively less ability to deactivate and eliminate the epoxides, help explain the occurrence of lung cancer in the mouse and its absence in the rat or man. Variability in incidence rates in the "stop-exposure" are probably the result of the effect of competing causes of death at high levels and differences in body distribution, in the specific pharmacokinetics and metabolism of each tissue, and mechanisms of toxicity.

VI. IMPLICATIONS FOR HUMAN RISK ASSESSMENT

The results of a number of animal studies indicate that BD is a carcinogenic in animals. It is weakly carcinogenic in the Sprague-Dawley rat and a potent carcinogen in the $B_6C_3F_1$ mouse. Mutagenic studies are consistent with these observations. BD is mutagenic in *in vitro* bacterial assays and clastogenic in *in vivo* studies in mice, but it is not clastogenic in rats or in primates. In addition, the metabolism of BD to epoxides in rodents and primates provides a generally accepted mechanism for carcinogenic activity.

The most important question or issue, however, is which animal is the best animal model of human response and therefore, the best model for risk assessment. Both the HLE rat and NTP mouse studies provide adequate data for risk assessment. These studies and numerous others demonstrate that there is a wide difference in carcinogenic and mutagenic response between these species. The existing data do indicate which rodent species is least likely to provide an accurate prediction of human risk.

All of the carcinogenic, mutagenic, pharmacokinetic, and mechanistic studies present consistent evidence that mice are much more sensitive than rats, or primates. The fact that the lowest effect level for BD was 6.25 ppm in the mouse and 1000 ppm in the rat, indicates that the mouse is 2 to 3 orders of magnitude more sensitive than the rat. Although *in vitro* bacterial assays indicate that BD is mutagenic, studies by Cunningham et al. (1986), show that the highest no effect level for sister chromatid exchange and micronuclear induction was 50 ppm in the mouse, while levels as high as 10,000 ppm failed to cause an effect in rats. Again, these studies indicate that the mouse is several orders of magnitude more sensitive than rats. While primates were not exposed under the same conditions, Sun et al. (1989) report that they did not find an increase in SCE in primates at levels as high as 8000 ppm for 2 hours.

The reason for these differences in susceptibility is that both the biological handling and mechanism of toxicity in the $B_6C_3F_1$ mouse is quite different from that of the rat or primates, including man. At 10 ppm the rate of uptake of BD is seven-fold higher in the mouse than the rat or the monkey on a body weight basis. This is consistent with the 15 to 100 times higher concentration of ^{14}C-BD/^{14}C-BD metabolites in the mouse tissues than in the rat.

Once BD is absorbed, the mouse also has a greater capacity for metabolizing BD to the epoxide forms, the presumed ultimate carcinogens. Studies by Dahl et al. (1990), show that the

$B_6C_3F_1$ mouse produces 590 times more BD monoepoxide and 40 times more BD diepoxide at 10 ppm than the monkey.

Likewise, in vitro metabolism studies of BD show that epoxide production is higher in tissues in mice than in rat, monkey, or human tissues. In fact, monkey and human lung did not even produce any measurable epoxide (Schmidt and Loeser, 1985).

The higher blood and tissue levels of BD and BD epoxides in the mouse are not unexpected when one considers the metabolic capacities of the different species. Comparisons of the relative ratios of specific enzyme activities (Table IV) calculated from Lorenz et al. (1984), show that the mouse has over a 1,000 times greater activation capability (monooxygenase specific activity) than man, while the specific activity of deactivation enzymes, such as epoxide hydrolase is lower. What this means is that the mouse can make a lot more epoxide and has relatively less ability to get rid of it. This is even true when one compares lung monooxygenase with glutathione-S-transferase for mice and man.
On the whole, these data show that a generally accepted mechanism exists for BD to cause cancer in rodents and primates, but that it is less likely that cancer will occur in the rat, the monkey and man than in the mouse. Furthermore, the pharmacokinetic, metabolism, mechanistic and epidemiology data do indicate that certain types (sites) of cancer observed in the $B_6C_3F_1$ mice exposed to BD are not likely to be of concern in man.

Lung effects are evident in both the HLE and NTP-1 studies. In 8,000 ppm male rats, lung weights are slightly higher and the incidence of focal metaplasia is increased. While nasal metaplasia is elevated at the high dose NTP-1 study, the predominant respiratory finding of NTP-1 and NTP-2 was the occurrence of lung tumors at doses as low as 6.25 ppm. These findings, however, are of little significance to man. Table 5 shows that the specific activity of the epoxide producing enzyme for different species, monooxygenase in the lung of mice is approximately 10 times higher than the rat and more than 1,000 times higher than in man. These data are consistent with the absence of lung cancer in rats or in BD production, or SBR workers (Meinhardt et al., 1982; Downs et al., 1987; Divine, 1990; Matanoski and Schwartz, 1987; Matanoski et al., 1990). Although the rat is closer to man than the mouse with respect to its metabolic capacity, the limited lung changes in the rat still were only present at BD concentrations 4,000 times higher than the proposed OSHA PEL.

Kidney nephropathy, a common finding in aging male rats, also is present in the HLE study. A higher incidence of the more severe grades of nephropathy is evident only in the high dose group (8,000 ppm). Because there was no difference in the

incidence of nephropathy across groups, this response is simply an exacerbation of an existing condition in senile male rats. Again, while the rat appears to be closer to man than the mouse in its response to BD, this chronic effect occurs only at an exceptionally high concentration which is 4,000 times higher than the proposed OSHA standard. This fact, combined with the absence of any kidney effects in the most sensitive species, the mouse, and the absence of any significant excess in kidney disease in BD/SBR cohort studies, also indicates that the kidney is not a site of concern (Meinhardt et al., 1982; Downs et al., 1987; Divine, 1990; Matanoski and Schwartz, 1987; Matanoski et al., 1990).

Studies by Irons, et al. (1986) and Leiderman, et al. (1986) also demonstrate that the thymic leukemia/lymphoma in the $B_6C_3F_1$ mouse is dependent on the occurrence of bone marrow toxicity and the presence of the endogenous murine leukemia virus. Since the bone marrow is not a target organ in the rat or primates, the occurrence of TL/L in the mouse is of doubtful relevance to man.

In summary, the existing data show that there is a wide difference in the carcinogenic and chronic toxicity response of different species to 1,3-butadiene on both a qualitative and quantitative basis. These data also reveal that these divergent responses are the result of major differences in pharmacokinetic and metabolic handling and in mechanism of toxicity between species. Furthermore, the studies as a whole indicate that the mouse is very sensitive to BD and is not a good model of BD toxicity in man. Although there are some differences between rats and primates in the pharmacokinetic/metabolic handling of BD, the rat is a much better model for predicting human response to BD than the mouse.

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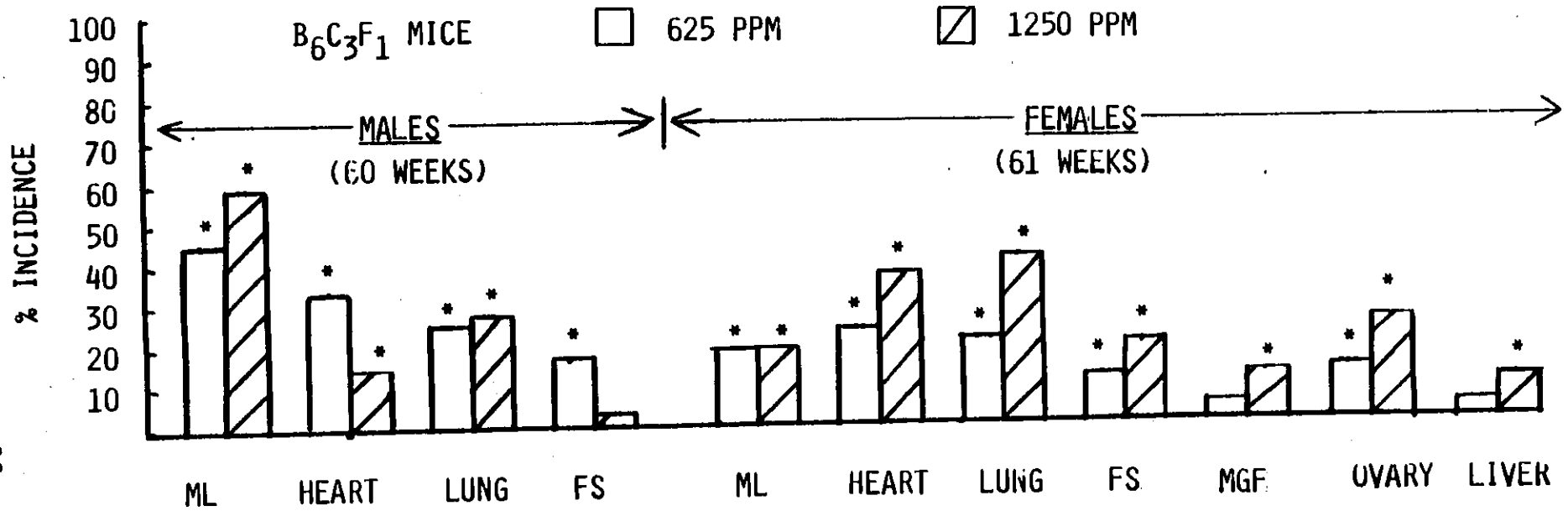
FIGURE 1. COMPARISON OF TUMOR INCIDENCES (PERCENT OVER BACKGROUND) IN SPRAGUE-DAWLEY RATS AND $B_6C_3F_1$ MICE EXPOSED 1,3-BUTADIENE (M.L. = MALIGNANT LYMPHOMA; M.G.F. = MAMMARY GLAND FIBROADENOMAS; Z.G. = ZYMBAL GLAND).

ML = MALIGNANT LYMPHOMA (THYMIC LEUKEMIA/LYMPHOMA) * = SIGNIFICANTLY INCREASED
FS = FORESTOMACH
ZG = ZYMBAL GLAND
MGF = MAMMARY GLAND FIBROADENOMAS



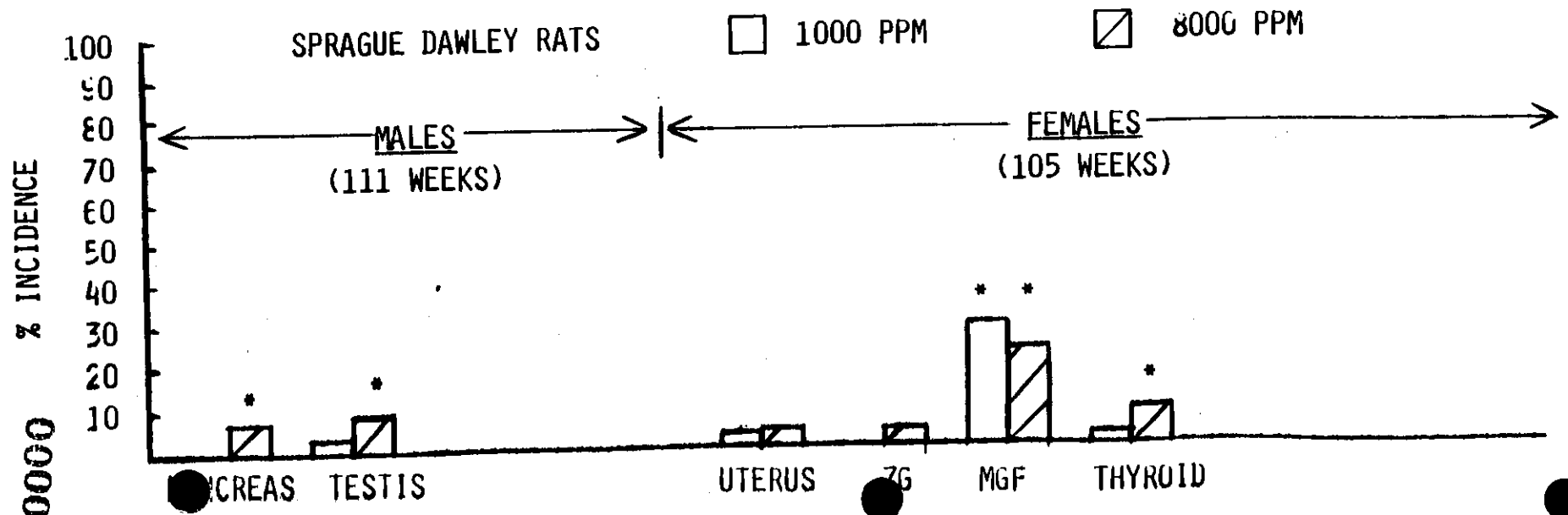


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TABLE 1

INCIDENCE OF PRIMARY TUMORS IN MALE

B6C3F1 MICE EXPOSED TO

1,3-BUTADIENE FOR UP TO 2 YEARS (NTP-2)

TARGET/NEOPLASM	PERCENT INCIDENCE EXPOSURE CONCENTRATION (PPM) 0	6.25	20	62.5	200	625
LYMPHOCYTIC LYMPHOMA	3 (70)[a]	2 (60)	3 (60)	6 (69)	3 (70)	69 (90)
HEART: HEMANGIOSARCOMA	0 (70)	0 (60)	2 (60)	9 (58)	29 (70)	7 (90)
LUNG: ALVEOLAR-BRONCHIOLAR NEOPLASM	31 (70)	38 (60)	33 (60)	48 (69)	60 (70)	14 (88)
FORESTOMACH: SQUAMOUS CELL NEOPLASM	1 (70)	0 (60)	2 (60)	8 (60)	17 (70)	15 (89)
HARDERIAN GLAND: NEOPLASM	9 (70)	12 (60)	18 (60)	35 (69)	47 (70)	8 (90)
LIVER: HEPATOCELLULAR NEOPLASM	44 (70)	45 (60)	59 (59)	54 (59)	56 (70)	13 (89)
PREPUTIAL GLAND: NEOPLASM	0 (70)	0 (60)	0 (60)	0 (69)	7 (70)	0 (90)

a = NUMBER OF ANIMALS OBSERVED.

TABLE 2

INCIDENCE OF PRIMARY TUMORS IN FEMALE B6C3F1 MICE EXPOSED TO 1,3-BUTADIENE FOR UP TO 2 YEARS (NTP-2)

TARGET/NEOPLASM	PERCENT INCIDENCE EXPOSURE CONCENTRATION (PPM) 0	6.25	20	62.5	200	625
LYMPHOCYTIC LYMPHOMA	3 (70)[a]	7 (60)	10 (60)	4 (70)	16 (70)	40 (90)
HEART: HEMANGIOSARCOMA	0 (70)	0 (60)	2 (60)	2 (59)	29 (70)	29 (90)
LUNG: ALVEOLAR-BRONCHIOLAR NEOPLASM	6 (70)	25 (60)	32 (60)	39 (70)	46 (70)	28 (88)
FORESTOMACH: SQUAMOUS CELL NEOPLASM	3 (70)	3 (60)	5 (57)	6 (68)	10 (70)	31 (89)
HARDERIAN GLAND: NEOPLASM	13 (70)	17 (60)	12 (60)	23 (69)	31 (70)	8 (90)
LIVER: HEPATOCELLULAR NEOPLASM	25 (69)	33 (60)	38 (60)	40 (60)	33 (60)	3 (90)
MAMMARY GLAND: ADENOCARCINOMA	0 (70)	3 (60)	3 (60)	9 (70)	19 (70)	14 (90)
OVARY: GRANULOSA CELL NEOPLASM	0 (69)	0 (59)	0 (59)	13 (70)	16 (70)	7 (89)

a = NUMBER OF ANIMALS OBSERVED.

TABLE 3

INCIDENCE OF PRIMARY TUMORS IN THE STOP-EXPOSURE (SE) GROUPS OF MALE B6C3F1 MICE EXPOSED TO 1,3-BUTADIENE (NTP-2)

TARGET/NEOPLASM	PERCENT INCIDENCE				
	EXPOSURE CONCENTRATION (PPM)				
	0 CONTROL	200 SE 40 WK (8,000)[a]	625 SE 13 WK (8,125)	312 SE 52 WK (16,224)	625 SE 26 WK (16,250)
LYMPHOCYTIC LYMPHOMA	3 (70)[b]	12 (50)	34 (50)	6 (50)	60 (50)
HEART: HEMANGIOSARCOMA	0 (70)	30 (50)	14 (50)	66 (50)	26 (50)
LUNG: ALVEOLAR-BRONCHIOLAR NEOPLASM	31 (70)	70 (50)	54 (50)	64 (50)	36 (50)
FORESTOMACH: SQUAMOUS CELL NEOPLASM	1 (70)	12 (50)	16 (50)	26 (50)	22 (50)
HARDERIAN GLAND: NEOPLASM	9 (70)	54 (50)	46 (50)	56 (50)	22 (50)
PREPUTIAL GLAND: NEOPLASM	0 (70)	2 (50)	10 (50)	8 (50)	6 (50)

a = TOTAL EXPOSURE EXPRESSED AS PPM-WEEKS

b = NUMBER OF ANIMALS OBSERVED.

TABLE 4

A COMPARISON OF TOTAL DOSE OF BD AND TL/L INCIDENCE IN THE MALE B6C3F1 MOUSE IN THE NTP-2 STUDIES

DOSE REGIMEN (PPM X WEEKS)	TOTAL DOSE (PPM - WEEKS)	TL/L INCIDENCE (%)
0 X 104	0	3
6.25 X 104	650	2
20 X 104	2,080	3
62.5 X 104	6,500	6
200 X 40	8,000	12
625 X 13	8,125	34
312 X 52	16,224	6
625 X 26	16,250	60
200 X 104	20,800	3
625 X 65	40,625	69

TABLE 5

RATIOS OF SPECIFIC ENZYME ACTIVITIES IN SUBCELLULAR PREPARATIONS OF RODENT AND HUMAN LIVER AND LUNG

	MONOOXYGENASE		EPOXIDE-HYDROLASE		GLUTATHIONE-S TRANSFERASE	
	LIVER	LUNG	LIVER	LUNG	LIVER	LUNG
MOUSE	4	1220	0.2	0.7	3.2	9.3
RAT	2	180	1	0.7	0.8	1
MAN	1	1	1	1	1	1

CALCULATED FROM LORENZ ET AL. (1984)

Butadiene docket

APPENDIX I

PEO/DJH

28 May 1987

Otley Road, Harrogate
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DOCKET OFFICER
DATE JUL 13 1987
TIME

Dr. J. Martonik,
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Hazleton Laboratories Europe Ltd.
Registered in England No. 1171833
Registered Office as above.

Dear Dr. Martonik,

Review of the ICF/Clement risk assessment of 1,3 butadiene

Hazleton has been made aware of the ICF/Clement risk assessment of 1,3 butadiene and in particular the criticisms of our conduct of the 2 year inhalation study in rats.

This document has been prepared to address those criticisms in the hope that this will provide a balanced viewpoint from which OSHA can work.

In order to provide a complete response I have dealt with the comments of ICF/Clement on a point by point basis and where necessary I have appended supportive documentation. Initially, however, I should like to draw your attention to what Hazleton regards as key omissions or errors in the ICF/Clement report.

1. This study has been audited by the United Kingdom Health and Safety Executive. During the audit Hazleton demonstrated comprehensive data trails for all aspects of this study.

2. Rats as a test species neither have the behavioural inclination or physical attributes which make the mouse a master escape artist. The risk of the Hazleton test animals escaping was therefore minimal.

3. ICF/Clement have combined values for 2 distinctly different lesions (angiectasis and telangiectasis) thereby misleading the reader.

4. The Hazleton report is a complete document providing a comprehensive review of the conduct of this study.

5. Hazleton believe that the 3 tier review of
   a) The British Industrial Biological Research Association
   b) UK Health and Safety Executive
   c) Internal Quality Assurance audits
   has confirmed beyond reasonable doubt the soundness of this piece of work.

The following are our comments on a point by point basis.

Continued . . .

ICF/Clement document reference P16 section 2.2

Comment: 'While statistically significant elevations of tumour incidence were observed, the findings were not nearly so striking or unambiguous as in the NTP bioassay'.

Response: I do not believe the Hazleton study can be termed ambiguous as it completed more than 104 weeks of exposure and demonstrated a clear biologically significant response at the high dose level; ref. reduced survival and increased tumour incidence.

ICF/Clement document reference P16 section 2.2.1

Comment: 'Chambers were opened following the exposure period and left open overnight. Additionally, one rack of cages from each chamber was removed and placed in the exposure room overnight'.

Response: Hazleton considered it good practice not to confine the animals within chambers overnight when supervisory staff were not on duty. To increase the airflow around the animals cages one battery from each chamber was pulled out into the exposure room. Since location of the cages on the batteries was randomised at weekly intervals it was considered that no bias was introduced by keeping one battery inside the chamber while the other was pulled clear.

As rats are not prone to escape from their cages this activity did not compromise the study integrity in any way. It should be recognised that rats do not have the physical attributes or behavioural inclination that make mice such good escape artists.

ICF/Clement document reference P17 section 2.2.1

Comment: 'Histopathologic examination was performed on a selected set of tissues from control and high dose animals. Selected target organs (Zymbals' gland, thyroid, lung, kidneys in males, skin, mammary gland, pancreas, brain, uterus, testes) and gross lesions were examined from low-dose animals'.

Response: Hazleton examined the tissues strictly in accordance with protocol content and the sponsors wishes. The rationale for the selection of the tissues from the low dose is clearly that they were those identified as potential targets from the top dose group (see Appendix 1 Gaunt's letter to Bird 29 April 1987).

ICF/Clement document reference P17 section 2.2.2

Comment: 'It is stated that angiectasis is a synonym for telangiectasis'.

Response: ICF/Clement are at fault in combining the 2 lesions. Angiectasis and telangiectasis are 2 different lesions and therefore should not be combined together.

Continued . . .

ICF/Clement document reference P17 section 2.2.2

Comment: 'No consistent exposure-related trends occurred in the body weights of animals, haematology, urinalysis, blood chemistry and neuromuscular function'.

Response: This is the same comment as was made regarding the NTP study. However, the mortality and tumour incidence showed a distinct increase with exposure as opposed to the higher survival rate of the NTP high dose females.

The mortality of the controls in the Hazleton study was consistent with values obtained for controls in a contemporary inhalation study. These data are presented in the Hazleton report pages B121 and B123. This fact further demonstrates that the animal husbandry practices were adequate for a study of this type.

Comment: 'The number of livers examined in terminal sacrifice male rats cannot be determined'.

Response: Table 26 page B73 of the Hazleton report indicates the number of rats by group that were examined at termination. ICF/Clement are correct in stating that the information does not appear in Table 24 or Table 31.

ICF/Clement document reference P18 section 2.2.3

Comment: 'In addition, focal alveolar epithelialization occurred at the following incidences'.

	Male	Female
Control	5/45 (11%)	4/46 (8.7%)
8000 ppm	10/31 (32.3%)	3/24 (12.5%)

The laboratory did not consider the following to be evidence of chronic pulmonary toxicity.

Response: Hazleton does not regard the reported focal alveolar epithelialization as evidence of chronic pulmonary toxicity.

At the time this study was conducted focal alveolar epithelialization was a common finding in untreated 2 year old rats. The animal suppliers were not geared to providing large numbers of barrier reared animals necessary for the conduct of one of these studies and therefore respiratory problems of this type were extremely common.

ICF/Clement document reference P19 section 2.2.4

Comment: 'The laboratory also classified mammary tumours as benign or fatal'.

Continued . . .

Response: When the females with subcutaneous mammary masses either became so debilitated by the mass that they had difficulty moving around the cage and feeding or the surface of the mass became abraded then they were killed on humane grounds. In these cases the masses, although benign, had directly led to the animals death and were considered fatal.

ICF/Clement document reference P19 section 2.2.5

Comment: 'One of the primary problems with use of the HLE rat bioassay for risk estimation is that the study, unlike the NTP mouse study, has not undergone an independent critical audit of toxicology and pathology data.

Response: The audit by ICF/Clement is incorrect is pointing out that this work has not undergone an independent critical audit of toxicology and pathology data; it has in fact undergone 2. During the study The British Industrial Biological Research Association (BIBRA) monitored the work on behalf of the sponsors IISRP (see Appendix 1). Their visits which were always followed by a written report of their findings (see Appendix 2) took place as follows.

Date of visit to HLE	Phase of study
November 1977	Second month of in-life phase
January 1978	Fourth month of in-life phase
March 1978	Sixth month of in-life phase
June 1978	Ninth month of in-life phase
July 1978	Tenth month of in-life phase
October 1978	Thirteenth month of in-life phase
November 1978	Fourteenth month of in-life phase
January 1979	Sixteenth month of in-life phase
March 1979	Eighteenth month of in-life phase
May 1979	Twentieth month of in-life phase
July 1979	Twenty second month of in-life phase
September 1979	Twenty fourth month of in-life phase
October 1979	Terminal data review
November 1980	Report preparation
February 1981	Report preparation
April 1981	Report preparation
June 1981	Report preparation

The outcome of these monitoring visits has been summarised in the letter from Dr. Gaunt (BIBRA) to Dr. Bird (Exxon) dated 29 April 1987 (see Appendix 1). I quote from this letter 'The visits always included the viewing of the stage of the study in progress and reviews of the raw data that had been generated in the meantime. We had complete freedom to demand any of the data and were not able to detect serious errors reflecting problems in the conduct of the study'.

Continued . . .

The second external independent audit was carried out by the United Kingdom Health and Safety Executive who sent 2 auditors to review the data between 6 and 9 December 1983. During the audit a number of minor findings were noted but the overall conclusion of Dr. R. Lowing of the H&SE was, and I quote 'the interpretation of the study by our scientists was not altered due to any of the findings of the audit'. In addition, the thyroid tumours which were identified by various of the sponsors pathologists as the critical tumour type were peer-reviewed by Professor Paul Grasso who concurred with the original diagnosis.

During these independent audits Hazleton has repeatedly demonstrated comprehensive data trails for all aspects of this study. Should any further audits be required the auditors can be furnished with all the detailed study records, SOP's to cover all operations, monitoring reports and operation procedures from the Hazleton QA unit and histological slides with back up wet tissues.

Hazleton is particularly proud of the standard of its own internal QA procedures which were regarded as extremely advanced for the time that this study was conducted.

As evidence of this I cite:

Historical SOP records indicate that during the period of this study the QA used British Standard (BS) 6001 to determine sample size for audit and BS 6001/6000 to determine the SAQL (Set Acceptable Quality Level). In some instances the percentage of data checked was determined by other means see (SOP QAU 41-50 dated 1-12-79 Appendix 3).

In-life audits - the frequency, scheduling and detailed audit and report procedures are also found in the SOP's in place during this period.

Some examples are:

QAU 30 (Appendix 4) details the aspects of the study conduct that are always evaluated. One of these aspects is animal identification, verification of each cage label and animal identity of a selected number of animals.

QAU 34 (Appendix 5) states that 100% audit is carried out on key data e.g. date of death and necropsy findings correlated with clinical findings for incidental deaths.

Hazleton believe that the 3 tier review of:

1) BIBRA
2) H+SE
3) Internal QA

has confirmed beyond reasonable doubt the soundness of this piece of work.

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Continued . . .

ICF/Clement document reference P21 - Lack of Complete Tissue Examination

Comment 'In the low-dose group, only selected organs and tissues (as described in Section 2.2.1) were examined. The rationale for the selection of the tissues to be examined was not fully explained and the exact number of tissues, organs or animals which were examined cannot be determined from the data presented. There is thus the potential for under diagnosis of lesions in the low dose group and for distortions of risk results generated from these data.

Response: The tissues examined by Hazleton were as per protocol and are clearly those identified as potential targets from the high dose. The reason for the choice of tissues, was clearly documented in protocol amendment 24 and presented as page C1435 of the report. It seems very unlikely that there could be toxicologically significant findings in the low dose that were not present in the high dose group.

ICF/Clement document reference P22 - Intercurrent Respiratory Infectious Disease

Comment: A list of clinical signs are presented which indicated that Sendai virus could well have been present in the colony of animals.

Response: At the time this study was conducted Sendai virus was commonly seen in Sprague-Dawley rats at this laboratory. Sendai infection has never been regarded as particularly serious and providing the humidity levels were kept high no lasting damage to the eyes was ever experienced.

Since the overall survival in this study was good and comparable with other contemporary long term inhalation studies it would seem unlikely that the Sendai infection had any major deleterious effect.

ICF/Clement document reference P22 - Post-exposure Chamber Protocol

Comment: 'Chamber doors were opened after the exposure period ended, and one rack of cages from each chamber was removed from the exposure chamber and placed into the exposure room. This action allowed for the possibility of escape of animals into the exposure room as well as switching of entire racks of cages between exposure chambers'.

Response: This question has been dealt with previously under the replies of section 2.2.1. The removal of batteries from the Chambers was done purely on animal welfare grounds and was aimed at providing a better circulation of air to the animals.

The existence of SOP's covering cage and animal identification, the diligence of our staff and close scrutiny by the QA unit did everything reasonable to preclude mixing of the exposure groups. Testimony to this is drawn from Dr. Gaunt's letter to Dr. Bird (see Appendix 1) 'My own viewing of the procedures at the laboratory lead me to believe that the chance of such an event occurring was vanishingly small'.

Continued . . .

ICF/CTement document reference P22 - Pathology-Related Issues

Comment: 'There are several major pathology-related issues which could impact on study results and should be resolved before tumour incidence data are used for risk estimation'.

Response: Since these pathology related issues fall into a discrete unit these will be dealt with en bloc. The responses have been provided by Dr. J.R. Glaister, Head of Pathology:

1. Dr. Glaister read the main study. The other 3 pathologists mentioned did examine health screen animals but this had no material effect on the outcome of the study.

2. Thyroid tumours were identified as the critical tumour type and were peer-reviewed by Professor Paul Grasso. Professor Grasso confirmed the original diagnosis.

   The mammary masses palpated during the in-life phase were accounted for at necropsy and then verified on a histology check list which went to the Pathologist. An extensive and complete data trail exists to track these masses. All masses identified by the Pathologist are accounted for in the final report.

3. Non-standard format such as hand counting was the recognised way of doing things 10 years ago when computer systems were not available. We agree that hand counting is difficult which is why the figures were checked several times including an audit by the QA department. The tissue counts are in the raw data check lists should they be required for review.

   The incidence data on the sporadic deaths was not presented but the Pathologist does not agree that toxic lesions would be masked since the cause of death was given.

4. Over diagnosis - 1) We recorded microscopic lesion as tumours.

   2) Thyroid tumours were reviewed and agreed.

   3) Pancreatic acinar tumours were stated as being of debatable diagnosis.

   4) This study was conducted 10 years ago before publication of reference texts and was state of the art at that time (see FASEB).

Continued . . .

To summarise the feeling within Hazleton about this piece of work I can do no better than quote Dr. Gaunts' letter to Dr. Bird (see Appendix 1) 'My overall impression, and this is supported by Paul Brantom, was that there was an enthusiasm and dedication by the Hazleton staff to do the job well. This extended from the senior management to the technicians in the exposure facility or autopsy room. We did our best to find faults and failed'.

Kind regards,

Yours sincerely,

P.E. Owen,
Study Director,
for Hazleton UK

J. Brightwell,
Director of Toxicology,
for Hazleton UK

APPENDIX II

JRG/sm

HAZLETON UK

11 October 1990

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North Yorkshire HG3 1PY England

Dr. R. K. Hinderer,
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Hazleton Laboratories Europe Ltd.
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Registered Office as above.

Dear Dr. Hinderer,

1, 3 Butadiene: IISRP/Hazleton rat study

Thank you for giving me the opportunity to respond to the criticisms raised by OSHA in their review of the study published in Federal Register 55 (155).

I have related my comments to the following issues:-

- Lack of comparability among male rat groups.
- Study audit and review.
- Study Pathologists and related issues.

A written comment on the randomisation issue by John Alexander (HUK Biostatistician) is also enclosed.

These comments merely reinforce previous statements that the HLE rat study was conducted to very high standards for its time and is an appropriate study for risk assessment.

Yours sincerely,

J. R. Glaister
Director of Pathology
for Hazleton UK

OSHA COMMENT • Lack of comparability among male rat groups

In HLE's opinion, it is usual to expect intergroup differences in long term carcinogenicity studies to occur purely on a random basis because of the numerous intergroup statistical comparisons made. The following comments are offered with specific reference to the OSHA inference that the low dose males were "healthier" than the controls due to a flawed randomisation process.

- Survival: 45 controls vs. 50 low dose survivors at termination was not significantly different. OSHA fail to provide statistical evidence for their assertion that "survival for the low dose males was better than survival for the controls". HLE's analysis showed that the survival curves of the low dose groups of both sexes were not significantly different ($p > 0.05$) from those of their respective controls.

- Nephropathy: If OSHA conclusions about survival cannot be substantiated, then their assertion that one would expect to see "more nephropathy because the low dose males lived longer" is equally invalid. Data of 87% vs. 75% of animals with nephropathy is at best marginally significant (p 0.03) and in HLE's opinion does not rate any biological significance when the vast majority of cases were in the minimal to slight range as would be expected in ageing rats.

- Tumour-bearing animals - the incidence in group 2 was lower than in the controls for the simple reason that tissue evaluation was limited compared with controls. Any conclusions regarding differences between groups that have different sampling protocols is not scientifically valid and should be dismissed out of hand. Attempts to support such a comparison by reference to females is not valid because tumours in females are dominated by pituitary and mammary tumours which are often grossly visible. Such tumours are therefore more likely to be sampled because of the "gross lesions" protocol requirement in low dose animals. Therefore, differences in the number of

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tumour-bearing animals in females will be less marked than in males in which many of the tumours are microscopic entities.

- Abnormal teeth: by their own statistics, OSHA show that there is no significant difference between low dose and control males ($p = 0.125$). The finding of malocclusion in rodents is common and irrelevant (unless the compound is fluoride!), and "approaching statistical significance" is a very weak attempt to support what was already a spurious and scientifically invalid argument that the randomisation procedure was incorrect.

In summary, the assertion by OSHA of the lack of comparability ignores the fact that an approved randomisation procedure was done. The intergroup comparison they present is largely scientifically invalid, statistically insignificant and of dubious or no biological relevance. Their assertion is without foundation.

OSHA COMMENT • Study audit and review

The OSHA concern about the adequacy of the study audit to which the bioassay was subjected has been addressed on several previous occasions. The important points made were:-

- The study was continually reviewed/audited by the British Industrial Biological Research Association (BIBRA) who paid 17 visits to Hazleton during the course of this study. The outcome of these monitoring visits has been summarised in a letter from Dr. Gaunt (BIBRA) to Dr. Bird (Exxon) dated 29 April 1987. We quote from this letter "The visits always included the viewing of the stage of the study in progress and reviews of the raw data that had been generated in the meantime. We had complete freedom to demand any of the data and were not able to detect serious errors reflecting problems in the conduct of the study".

- The second external audit was carried out by the United Kingdom Health and Safety Executive who sent 2 auditors to review the

data between 6 and 9 December 1983. The overall conclusion of their Dr. Lowing was "the interpretation of the study by our scientists was not altered due to any of the findings of the audit".

However, OSHA are correct in their assertion that this old study was not audited according to current NTP methods. This was not a common practice in Europe at that time and the statement is of no relevance unless OSHA intend to invalidate most of the carcinogenicity studies done in Europe in the last decade. With reference to particular points:-

- slide/block review: the universal practice of quality control and quality assurance is built around sampling techniques derived from international sampling standards. The sampling approach was adopted for this study. The adequacy of a 2% sample can be debated by the statisticians, but there are no grounds for invalidating the sampling type of audit just because the mouse study was given a 100% review. This was overkill by quality control standards.

- Tissue accountability: HLE should be congratulated on their high rate of tissue recovery which is excellent even by today's standards. OSHA's implied criticism that a recovery rate of "only" 99% for thyroid samples "has important implications for quantitative risk assessment" would invalidate virtually every carcinogenicity study ever done if taken to the letter of the law. To debate whether the 1% error rate relates to two thyroid lobes in one animal or one lobe in each of two animals is clearly a ludicrous extreme. The impact of such a low error rate on the statistical analysis of thyroid tumour frequencies of 0/100 vs. 11/100 is so small as to be meaningless. Thus, the statement "important implications for quantitative risk assessment" is equally meaningless and the criticism should be revoked.

  Having said that, HLE accept that it is difficult for the reviewer to determine exactly which tissues have been examined

in each individual animal. These data are available in the raw data if OSHA feel the need to know exactly which animal(s) had the missing thyroid lobe.

- Pathology raw data: These are currently defined as the final signed report and the slides/blocks. The slides/blocks are archived and the final signed report was as issued. This study was done in the days before pathology computers were in vogue and the "raw data" were usually the original typescripts with the pathologists corrections on them. Such "draft" data would not be retained by todays standards and only the final report would be archived. The assertion that "it was impossible to verify the study's final report as an accurate reflection of the raw data" thus depends on the definition of raw data - the draft data or the final signed report?

  If the HLE study pathologist's recall is correct, the raw data were available at the internal QA audit stage ie. the issued final signed report was an accurate reflection of the raw data. The "raw data" could not be located at the subsequent external audits some years later. A post-audit mislocation of draft typescripts of debatable value as raw data by today's standards cannot be used as evidence of poor study conduct at the time the study was done.

- Tumour incidence counts: as stated previously, the study was done in the pre-computer days of pathology in HLE and most other companies. Incidence data counts were therefore done manually. Such counting is obviously prone to human error, but we can only state that the counts would be data checked in pathology prior to release of the draft report and 100% checked again by QAU prior to release of the final report. Whilst there can still be no absolute guarantee of total and complete accuracy, the incidence counts by HLE were done to the highest practicable standard available for manually generated data. The criticism levelled by OSHA at the HLE report is therefore unjustified.

HLE cannot comment formally on the incidence counts generated by ICF and Environ. Some of the "problems" generated by manual counts are the result of merging data for presentation in the incidence summary tables and the pooling of data for statistical analysis. It is possible, and perhaps highly likely, that different counts were due to different merge strategies being adopted by the summary data generators. If this was the case, the study itself cannot be criticised by OSHA as the difference lies in the risk assessors approach to data merging for analysis. OSHA are perfectly entitled to adopt their own preferred count and merger methods on the individual animal data records for risk assessment if they do not agree with the strategy adopted by others.

- Pathology peer review: as alluded to earlier, NTP-type reviews were not common practice outside NTP study programs. The scientific review in this study focused on the critical areas.

  The increased number of subcutaneous masses in females was clearly evident from the clinical and necropsy data without any real need to review the histopathology findings. OSHA also accept this position without any apparent hesitation. "There can be <u>no doubt</u> that there was a substantially increased incidence of mammary fibroadenomas in the exposed female rats in the HLE study".

  The significance of increased benign mammary tumours as evidence of carcinogenicity is arguable and therefore the critical peer review focused on the thyroid tumours. Peer review of this critical tumour type was thus undertaken and the thyroid tumour data confirmed.

  The fact that an NTP-type review was not done on this study nor on most European studies at that time is not an acceptable criticism of any study - it is merely the statement of a fact of life. OSHA cannot make any assumptions that this study would have failed a review at that time. However, in view of

the quality problems that did occur in some carcinogenicity studies, HLE sympathises with OSHA's concern that they generally feel more assured with NTP-reviewed studies. Unfortunately, there was no requirement in law at that time to review studies in that manner and criticisms so long after the fact cannot be accepted.

OSHA COMMENT • Study pathologists and related issues

- Study pathologist: HLE agrees that the designation of pathologists to the various aspects of the study was unclear in the report. However, HLE can confirm that the 2 year carcinogenicity study was evaluated by a single pathologist, thus assuring that there was consistency in the diagnosis of neoplastic and non-neoplastic lesions. The conduct of the HLE study therefore cannot be criticised from that point of view. A simple request for information from OSHA would have prevented this item from appearing as a suspect point in the conduct of the study.

- Endocrine diagnoses: HLE sympathises with OSHA over the problems in reviewing old studies in the face of the everchanging nomenclature of the toxicological pathologist. There are 2 main points to consider in response to their comments, the translation of old terminology to the more recent synonyms and the fact that only the terminal kill animals were tabulated for non-neoplastic lesions.

  On synonyms, for example, cortical cell vacuolation would be synonymous today with the altered cell focus or vacuolated focus characteristic of the ageing male rat adrenal cortex. The incidence data clearly support this sex-related disposition. Similarly, angiectasis ("blood cysts") is a common fate of hyperplastic altered cell foci and nodules in the female adrenal cortex and is synonymous with the use of those terms today. Again, the incidence data clearly indicate the classical preponderance of these lesions typical of the

female rat adrenal cortex. Similarly, the term cystic follicle would probably translate today as follicular cystic hyperplasia or an equivalent term. Thus, some of the endocrine proliferations are hidden under other synonyms.

Assessment of other endocrine proliferations is more complex and less clear-cut. Criteria for diagnosis even today are still under review by various societies of Toxicological Pathologists and were very limited over a decade ago when only one appropriate text book was in existence (Pathology of Aging Rats. J. D. Burek (1978) CRC Press). Any retrospective opinions about such old data should be reviewed with this in mind and by definition will be speculative. HLE offers the following comments which hopefully will be more, but certainly no less valid than the comments made by other reviewers.

The minor diffuse C-cell hyperplasia characteristic of ageing rats was probably accepted as normal background pattern and placed under the recording threshold. Attention would be focused on the focal proliferations, the microscopic variants of which would probably be diagnosed as microscopic C-cell adenomas. A similar situation would hold for the adrenal medullary phaeochromocytoma and the qualifier "microscopic" is used throughout the report for the contentious minor variants of these borderline hyperplasias/neoplasias.

The suggestion that some of the minor thyroid C-cell and adrenal medullary cell proliferations may have been overdiagnosed by today's standards may have some merit. However, this would not impact on the conclusions drawn from the study. It is a common practice to pool (merge) focal endocrine hyperplasias and neoplasias for statistical analysis for the very reason that the differential diagnosis of hyperplasia/neoplasia at the microscopic level is so uncertain.

The probability of overdiagnosis of pituitary adenomas would be relatively small. In contrast, to the mainly microscopic

variants of C-cell adenomas and phaeochromocytomas, pituitary tumours are very common, often grossly visible, frequently fatal and undoubtedly neoplasms in the ageing Sprague-Dawley rat. Any focal hyperplasias in young animals quickly give way to gross neoplasms in the ageing animal and a low incidence of hyperplasias would be expected at the end of a 2 year study.

A further comment that may be relevant to the question of overdiagnosis of endocrine proliferations is to compare the incidence of endocrine neoplasia diagnosed in the Butadiene Study control animals with control data from 12 groups generated in the same laboratory in the same strain of rat a decade later. The data fit into the middle of current control ranges and therefore the retrospective suggestion of overdiagnosis in the study appears to be more imagined than real.

	% tumour incidence in control groups			
	Butadiene		control range	
	M	F	M	F
Thyroid C-cell adenoma	15	10	2-26	2-22
Thyroid follicular adenoma	3	0	0-10	0-6
Adrenal phaeochromocytoma	19	4	10-44	2-16
Pituitary adenoma	58	77	40-64	66-82
% tumour-bearers	84	97	75-92	86-98

- Statistics: OSHA focus purely on perceived negative aspects of the pathology to support some of their decisions related to the validity of the study for risk assessment. There are several positive quality indicators in this report. The excellent tissue recovery has already been mentioned. The designation of causes of illness and death, the assignment of fatal context to tumours for PETO analysis is clearly an indicator of the quality of the pathology approach even at that time. On an anecdotal point, several comments were made that the report would be a good model for American companies to use in their

approach to carcinogenicity studies and statistical reports. This approach to the statistical analysis is only now just reaching common practice in the States. It is interesting to note that OSHA still rely on Fisher's Exact Test for analysis even though the bias introduced when this test is used in studies with different survival rates was demonstrated in publications several years ago. Their analyses have yet to reach the level of sophistication that was already evident in a study over 10 years old and their conclusions and criticisms should be judged accordingly.

J. R. Glaister
Director of Pathology
for Hazleton Uk

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HLR STUDY 522/2 - RAT BUTADIENE STUDY

Dr. Glaister has discussed the major issues raised by OSHA in considerable detail. I would like to add the following general comments.

- "failure of randomisation" (p32744)

It is unclear what possible interpretation can be put on this phrase. If the randomisation procedure was followed to the agreed method, then it cannot be said to have failed. In this case there has been no indication from the available data that the agreed procedure was not followed. It may, of course, be argued that the method was inappropriate for the purpose, but then the burden is surely on OSHA to explain how the procedure could lead to incomparability.

It seems, however that OSHA are arguing that randomisation "failed" a posteriori from the data. Their purpose seems to be to establish that there are differences between the control and low-dose groups in males that are so substantial that the likelihood of their having arisen through chance in the randomisation process is negligible, and thus to retrospectively support an inference that non-random allocation was used. To do this requires a sophisticated statistical procedure that takes into account the very great number of parameters that are observed in the study. Using simple significance tests as OSHA have done is wholly incorrect for this purpose. One in twenty parameters can be expected to show differences at the .05 level in a randomised study in the absence of any treatment effect. Hence it will nearly always be possible to find parameters that demonstrates a significant difference at the .05 level (in this case "abnormal teeth" and nephropathy were so identified, although in the former case the significance testing procedure used to support the argument was naive and inappropriate). For this reason the arguments presented using the findings in teeth and nephropathy should be discounted; in no way do they suggest that there was a failure to randomise.

In any case, attempts to demonstrate a failure of randomisation are doomed to failure when study documents exist to show unequivocally that randomisation was performed.

(It should, incidentally, be noted that the variation in survival between groups was taken into account in the derivation of the p-values for nephropathy. Using Peto analysis), the expected numbers of incidences of nephropathy were 88 and 86, or 89 and 91, in the control and low dose groups respectively, depending on the interpretation of the cases of uncertain context. Hence the differential survival can be seen to have little effect on

the expected incidence of this lesion. Thus the adjusted p-value (OSHA's 0.03) has already taken survival differences into account, and the comments in the text on p32744 do not therefore add additional strength to the finding as might be inferred at a casual reading).

- "HLE authors concluded there was no difference in nephropathy incidence between control and low dose males"(p32744)

This is not the case. On page A109 of the report it clearly states that the low dose group has fewer incidences than the controls.

- "incomparable groups" (p32744)

In randomised studies, inferences do not rest on any prior assumption of overall comparability of groups. Where comparability is required in some respects, the study design is modified to control this variable. In this study, comparability in terms of body weight was required, and hence a stratified, or blocked, randomisation scheme was used to ensure this comparability. Hence arguments about overall comparability should only relate to body weight.

The concerns underlying the inappropriate discussion of "incomparability" may be summarised as follows.

In any study there will inevitably be emerging group differences, some larger than others, arising from natural variability and the randomisation process. It is the function of the scientist to identify to which extent any such differences, whether or not they achieve statistical significance, may be correlated with, and hence partially account for, the absence or presence of other significant findings. In the present study, having established a lower incidence of nephropathy in group 2 than in controls it is then valid to ask whether this could be accounted for by some experimental factor which may, in turn, affect other findings, or alternatively whether the chance distribution of slightly fewer animals with a disposition to nephropathy may be correlated with the development of other lesions. In other words the inference issue is not one of the overall comparability of groups but whether nephropathy is a direct or indirect factor in, or marker of, the development of each other lesion analysed. Where this is judged to be the case, statistical techniques could be applied to assess the incidence of other lesions after adjusting for the presence or absence of nephropathy. It is extremely simplistic and scientifically invalid to declare all other comparisons void on the basis of natural group differences in one or two particular parameters.

28/9/90 John Alexander, Biostatistician

APPENDIX III

AUTHOR'S BACKGROUND

Robert K. Hinderer

Date of Birth: February 6, 1946

Place of Birth: Elyria, Ohio

Marital Status: Married, four children

Education:

B.S. In Biology, Kent State University, Kent, Ohio
December, 1969; Major: Biology

M.S. Cleveland State University, Cleveland, Ohio
June, 1971; Major: Biology (Physiological Ecology)

Ph.D. University of Maryland, College Park, Maryland
December, 1974; Major: Entomology (Pesticide toxicology and metabolism)

Doctoral Dissertation Title:

A comparative study of enzyme activities in various tissues of the rat and Japanese quail with respect to the metabolism of a herbicide and two insecticides.

Advisor: Professor Robert E. Menzer, Ph.D.

Toxicology Experience:

April, 1974 - January, 1975

Part-time consultant in insecticide toxicology for Ecosystems, Inc., McLean Virginia.

May, 1974 - June, 1975

Teaching graduate level insecticide toxicology (lecture & laboratory).

July, 1974 - June, 1975

Post-doctoral research, University of Maryland, USDA, Pesticide Degradation Laboratory (Beltsville).

Robert K. Hinderer

Experience (Cont'd):

July, 1975 - June, 1977

Toxicologist, Ethyl Corporation, Toxicology and Industrial Hygiene Department, Baton Rouge, Louisiana.

February, 1976

Toxicology lecturer, NIOSH Industrial Hygiene Training Course, New Orleans, Louisiana.

June, 1977 - September 1971

Sr. Environmental Toxicologist and Manager of Toxicology, BFGoodrich Chemical Group, 6100 Oak Tree Blvd., Cleveland, Ohio.

September, 1981 - January, 1987

Sr. Toxicologist, BFGoodrich Company, Corporate Environmental Health Department, 500 S. Main Street, Akron, Ohio 44318.

)2 - 1983

Guest lecturer on chemical carcinogenicity graduate course, University of Akron, Akron, Ohio.

January, 1987 - Present

Manager of Health and Toxicology, BFGoodrich Company, Corporate Environmental Health Department, 3925 Embassy Parkway, Akron, Ohio 44333-1799.

Professional Activities:

- Society of Toxicology (1974-present) non-member participant at Annual Meetings.

  Poster Sessions Toxicologist 1(1):24 (1981)
  Toxicologist 6(1):52 (1986)
  Toxicologist 7(1):192 and 202 (1987)

- American College of Toxicology - member.

- Toxicology Subcommittee of the Ethylene Dichloride Technical Panel of the Manufacturing Chemists Assoc. (MCA) (1975-present) - member.

Robert K. Hinderer

Professional Activities (Cont'd):

- Toxicology Subcommittee of the Vinyl Chloride Technical panel (MCA) (1975-1977) - member.
- Toxicology Subcommittee of the Allyl Chloride/Epichlorohydrin Technical Panel (MCA) (1975-1977) - member.
- Toxicology Subcommittee of the Trichloroethylene Technical Panel (MCA) (1975-1977) - member.
- Vinyl Chloride Chronic toxicity/Cancer Bioassay Audit Team of MCA Toxicology Subcommittee (1978) - member.
- American Industrial Health Council Task Force on the place of the mouse in carcinogenicity testing (1977-1978) - member.
- National Institute of Environmental Health Sciences (NIEHS), Extrapolation II meeting (1976).
- Third Veterinary Toxicology Symposium (1976).
- NIEHS Conference on comparative metabolism and toxicity of vinyl chloride related compounds (1977) Bethesda, Maryland.
- Inorganic and Nutritional Aspect of Cancer (1977), a conference co-sponsored by NIH and International Assoc. of Bioinorganic Scientists, University of California, San Diego.
- Toxicology Steering Committee, International institute of Synthetic Rubber Producers (IISRP) (1978-1981).
- Chemical Industry Institute of Toxicology, working on strategies for short-term testing for mutagens/carcinogens (1977) Research Triangle Park, North Carolina.
- ACS Polymer Conference on Stabilization, Degradation and Flammability of Polymers (1977) Akron, Ohio.
- Second International symposium on Polynuclear Aromatic Hydrocarbons (1977) Battelle Columbus Laboratories, Columbus, Ohio.
- Environmental Cancer, a Report to the Public (1977), co-sponsored by the League of Women Voters of Texas Education Fund and League of Women Voters of Houston Education Fund in conjunction with Tenneco Chemicals, the Carcinogenesis Center of M.D. Anderson Hospital and Tumor Institute and University of Texas Health Science Center, School of Public Health, Houston, Texas.

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Professional Activities (Cont'd):

- Manuscript Reviewer, Journal of the National Cancer Institute, 1978.
- Testimony, "Health Aspects of Vinyl Chloride Emissions", California Air Resources Board hearings on the proposed ambient vinyl chloride standard, May 24, 1978.
- Testimony, Occupational Safety & Health Administration hearing on the proposed standard for identification, classification and regulation of toxic substances posing a potential carcinogenic risk, June, 1978.
- Chemical Industry Institute of Toxicology, second CIIT conference on toxicology, Scientific considerations in monitoring and evaluating toxicological research, February 28, March 1-2, 1979.
- National Academy of Sciences, Committee on Water Treatment Chemicals, Toxicology Subcommittee, member 1981-1983 (Toxicology Consultant).
- IISRP, committees and subcommittees on toxicology and industrial hygiene 1978-present.
- Manuscript Reviewer, Archives of Environmental Contamination & Toxicology, 1983-1984.
- Manuscript Reviewer, J. Fire Sciences, 1985-1988.
- Testimony before state and local agencies, code groups and judicial bodies on combustion toxicity of PVC (1982-present).
- Workshop on volatile organic chemicals, toxicology group (invited participant) sponsored by EPA under auspices of the American Water Works Assoc. Research Foundation, June, 1982.
- Internationales Kollogrum "Polyurethane in der Medizin-Technik", Stuttgart, W., Germany, January, 1983.
- Gordon Research Conference on Chemistry and Toxicological Aspects of Combustion Products, August, 1983.
- Vinyl Institute Medical Committee Chairman and project monitor (1985-present).
- Research Oversight Committee of the International Institute of Synthetic Rubber Producers, Role of MuLV in BD Carcinogenicity in the $B_6C_3F_1$ Mouse, 1988.

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Robert K. Hinderer

Professional Activities (Cont'd):

- International Symposium on 1,3-Butadiene, April 12-13, 1988, Participant and manuscript reviewer for publication in Environ. Health Perspectives.

- ILSI Conference, "Assessment of Inhalation Hazards: Integration and Extrapolation Using Diverse Data". Hannover, FRG, February 19-24, 1989.

- Chemical Industry Institute for Toxicology - Developmental Committee member, 1990.

Professional Honors:

- National Institute of Environmental Health Post-Doctoral Fellowship, awarded June, 1975.

- Sigma Xi

Publications:

- **R. K. Hinderer** and R. E. Menzer (1976). Comparative enzyme activities and cytochrome P-450 levels of some various rat tissues with respect to their metabolism of several pesticides. Pesticide Biochemistry and Physiology 6:148-160.

- **R. K. Hinderer** and R. E. Menzer (1976). Enzyme activities and cytochrome P-450 levels of some Japanese quail tissues with respect to their metabolism of several pesticides. Pesticide Biochemistry and Physiology 6:161-169.

- H. M. Bolt, J. G. Filser and **R. K. Hinderer** (1978). Rat liver microsomal uptake and irreversible protein binding of 1,2-14_C vinyl bromide. Toxicol. and Applied Pharmacol. 44:481-489.

- **R. K. Hinderer** (1979). Toxicity studies of methylcyclopentadienyl manganese tricarbonyl (MMT). Journal of Amer. Ind. Hygiene Assoc. 40(2):164-167.

- **R. K. Hinderer** (1980). Banbury Report 5, ethylene dichloride: a potential health risk? Summary, Cold Spring Harbor Laboratory, pp, 343-345.

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Publications:

- R. K. Hinderer, M. Knickenbacker and F. J. Koschier (1982). Mutagenic evaluation of two rubber accelerators. Toxicol. Applied Pharmacol. 62(2):335-341.

- R. K. Hinderer, B. Myhr, D. R. Jaggennath, S. M. Galloway, S. W. Mann, J. C. Riddle and D. J. Bursick (1983). Mutagenic evaluations of four rubber accelerators in a battery of in-vitro mutagenic assays. Environ. Mutagenesis 5(2):193-215.

- E. G. Leighty and R. K. Hinderer (1984). N-N^{14}C-dinaphthyl-P-phenylene diamine metabolism in the Rhesus monkey. J. Amer. College Toxicol. 3(1):43-56.

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- R. K. Hinderer and H. L. Kaplan (1986). Assessment of the inhalation toxicity of hydrogen chloride gas to man. Dangerous Properties of Industrial Materials. March/April, pp. 2-4.

- H. L. Kaplan, A. Anzueto, W. G. Switzer, R. K. Hinderer (1986). Respiratory effects of hydrogen chloride in the baboon. The Toxicologist 6(1):52.

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- R. K. Hinderer, B. Y. Cockrell, S. M. Debanne, P. T. Goad (1987). Male reproductive assessment of the rubber accelerator, N-oxydiethylene thiocarbamyl-N-oxydiethylene sulfenamide, in rats. Fundamental and Applied Toxicology 9:763-772.

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Publications (Cont'd):

- H. L. Kaplan, A. Anzueto, W. G. Switzer, **R. K. Hinderer** (1988). Effects of hydrogen chloride on respiratory response and pulmonary function of the baboon. J. Toxicology and Environmental Health 23:473-493.

Papers:

- **R. K. Hinderer**, Implications of Species Differences in Assessing the Toxicity of PVC Combustion Products and Irritants, Such As HCl to Humans. Interwire Conference, November, 1989.

Docket No. H-041

INFORMAL PUBLIC HEARING ON THE PROPOSED OSHA STANDARD ON OCCUPATIONAL EXPOSURE TO 1,3-BUTADIENE

KEY INTERSPECIES DIFFERENCES IN METABOLISM AND CYTOGENICITY OF 1,3-BUTADIENE AND THEIR IMPLICATIONS FOR HUMAN RISK ASSESSMENT

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November 9, 1990

Testimony of Michael G. Bird

KEY INTERSPECIES DIFFERENCES IN METABOLISM 1,3 BUTADIENE AND CYTOGENICITY AND THEIR IMPLICATIONS FOR HUMAN RISK ASSESSMENT

I am a board certified toxicologist with 20 years experience in the field of petrochemical toxicology. I hold a post-doctoral degree in Biochemistry and a Masters Degree in Toxicology. In addition, I am a Fellow of the Royal Society of Chemistry and a Chartered Chemist. As a toxicology Associate of Exxon Biomedical Sciences, Inc., I have extensive involvement in company and industry studies of diolefin toxicity. During my study of the toxicology of 1,3 butadiene and other diolefins over the last ten years, I have been a key participant of the Environmental Health and Butadiene Committees of the International Institute of Synthetic Rubber Producers, and I am currently Chairman of the Chemical Manufacturers Association's Butadiene Toxicology Research Task Group. In 1988, in conjunction with Dr. Melnick, National Toxicology Program, I was co-organizer of the International Symposium on Toxicology, Carcinogenesis, and Human Health Aspects of 1,3 Butadiene.

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A. Bone Marrow Toxicity

B. Metabolism

C. Structure Activity

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I. Introduction

In this testimony, I will address the pharmacokinetic and mechanistic bases which support the different biological responses seen between species exposed to 1,3 butadiene. Specifically, I will show that separate approaches -- consideration of species differences in butadiene uptake and metabolism, cytogenicity, and bone marrow response, and structure-activity information on other chemicals, all lead independently to one conclusion: that the rodent and particularly the mouse, is a poor predictor of butadiene effects in man. This is based on the following observations developed from a comprehensive review of relevant scientific studies: 1) There are major quantitative differences in the uptake and metabolism of butadiene in the mouse, rat, primate, and man that explain greater sensitivity of the mouse and rat to butadiene carcinogenicity. Compared to rat or primate, the mouse retains more inhaled butadiene, metabolizes this more rapidly to toxic epoxide metabolites and is less able to eliminate these metabolites because of saturation. At comparable exposures (10 ppm) the epoxide blood level is 590 fold higher in the mouse than the primate, and 40 fold higher in the mouse than the rat. In vitro studies of butadiene metabolism suggest that man is more like the primate than the rat or mouse; 2) Cytogenetic and bone marrow changes in the mouse exposed to butadiene have not been seen in the rat or primate; 3) The presence of an endogenous retrovirus in the $B_6C_3F_1$ mouse confounds the qualitative evaluation of the murine leukemia response to butadiene exposure; 4) Reactive epoxides which are formed particularly readily in the mouse metabolism are direct acting mutagens and have carcinogenic activity. Butadiene itself, unmetabolized, does not have

mutagenic activity. The diepoxide metabolite of butadiene is known to cause an increased incidence in lung tumors in mice. Species differences in the formation and elimination of this metabolite make this lung response unlikely to occur in man; 5) Structure activity comparison supports the contention that the metabolites rather than the parent compound are toxic and carcinogenic and that there are major species differences in the formation and the biological consequences of these metabolites.

I also offer additional comments on OSHA's discussion in its rulemaking notice pertaining to bone marrow toxicity, metabolism, structure activity and genotoxicity. Specifically for bone marrow toxicity, I include new animal data and dispute OSHA's interpretation of human data. For metabolism, I have provided more recent metabolism data which significantly impact previous assumptions concerning the absorption of butadiene at low concentrations. Structure activity requires the recognition that the butadiene diepoxide causes lung tumors in mice. By reference to our knowledge of butadiene dimer, quantitative differences in formation and detoxification of reactive metabolites can account for significant species differences in biological response.

In conclusion, the projections of cancer mortality based on the rodent test results vastly overpredict the observed worker mortality; this poor predictivity of the rodent (particularly the mouse) can be attributed to the interspecies differences in uptake, metabolism and excretion, and in retroviral mediation.

II. Comparative Pharmacokinetics, Metabolism, and Cytogenetic Response

Before interacting with genetic material, butadiene must be

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converted to oxygenated metabolites which themselves are carcinogenic and can induce mutations in bacteria. In view of this indirect mutagenic action, any cross-species difference in metabolism and cytogenetic response will have an important bearing on butadiene's carcinogenicity. In this section, the uptake and metabolism of retained butadiene are reviewed; pronounced interspecies differences are reflected in the blood levels of the toxic epoxide metabolites of BD. This species difference is further evident in the absence of bone marrow response seen in rat and primate compared to mouse which is discussed subsequently. Finally, interaction of BD with the endogenous murine retrovirus (MuLV) present in the B_6C_3F, mouse identifies that the retrovirus is a confounding factor in this mouse strain and that it is presently impossible to determine the respective contribution of chemical exposure or retrovirus in the mouse leukemogenic response.

A. Uptake of Butadiene

Studies conducted under the National Toxicology Program show major species differences in the uptake, metabolism, and excretion of BD (Melnick et al. 1990). Bond et al. (1986) in studies of inhaled BD, used the same rodent strains as in the rodent cancer bioassays of BD, namely the $B_6C_3F_1$ mouse and the Sprague Dawley rat. Using six hour exposures to [^{14}C] BD, they exposed mice and rats to a range of concentrations, under dynamic flow-through conditions, 0.14 to 1800 μg/l (0.08 to 1040 ppm BD) for mice and 0.14 to 13000 μg/l (0.08 to 7100 ppm BD) for rats. They found that mice retained 4-7 times more of the inhaled butadiene per unit body weight than did rats; this is based on the amount

retained at the end of the six hour exposure and does not account for BD or metabolites that were absorbed and exhaled during exposure. For both species, the uptake (% of inhaled BD retained) decreased with increasing BD exposure concentration. This uptake ranged from 1.5% at the 7100ppm exposure to 17% at the low concentration.

In subsequent studies, this team (Sun et al., 1989, Dahl, et al. 1990) published data that enable the retained doses of BD in rodents and primates to be compared. In this study, three male primates (Macaca fascicularis) were exposed by nose-only inhalation for 2 hours to concentrations ranging from 10-8000 ppm [^{14}C] butadiene. When normalized to body weight, the data from Bond, et al. (1986) show that the rate of uptake of butadiene at approximately 10 ppm [^{14}C] BD in mice was substantially greater than for rats or monkeys. When normalized to body weight for each species and expressed on a μ mole per hour per 10 ppm basis, the measured retention rates were determined to be 3.30, 0.46, and 0.52 μ mole/kg BD for the mouse, rat, and monkey respectively. Thus, for equivalent BD exposures, the rat and primate respectively receive 7.2 and 6.3-fold lower doses of BD than the mouse. Further, when some fifteen different tissues were analyzed, the mouse tissues contained 15 to 100 times higher concentrations of BD-introduced [^{14}C] per μ mole of inhaled BD than did the rat tissues under conditions known to result in retention of similar amounts of BD and its metabolites; the primates were not sacrificed so that comparable data are not available at this time.

For both the mouse and the rat, urine and exhaled air are the major routes of excretion of [^{14}C] BD, approximately 75-85% is thus eliminated. In mice, for the concentrations examined (14-1400 ppm) by Bond et al. (1986), elimination in urine and expired air was increased with increasing BD exposure concentration. Elimination was dose-dependent in the rat. The rat also eliminated a smaller percent of the absorbed [^{14}C] via the urine than the mouse. For monkeys, Dahl et al. (1990) showed that elimination following exposure to 10 ppm BD was mostly by exhalation of carbon dioxide (51%) and urine (33%).

B. Metabolism and Elimination of Retained Butadiene

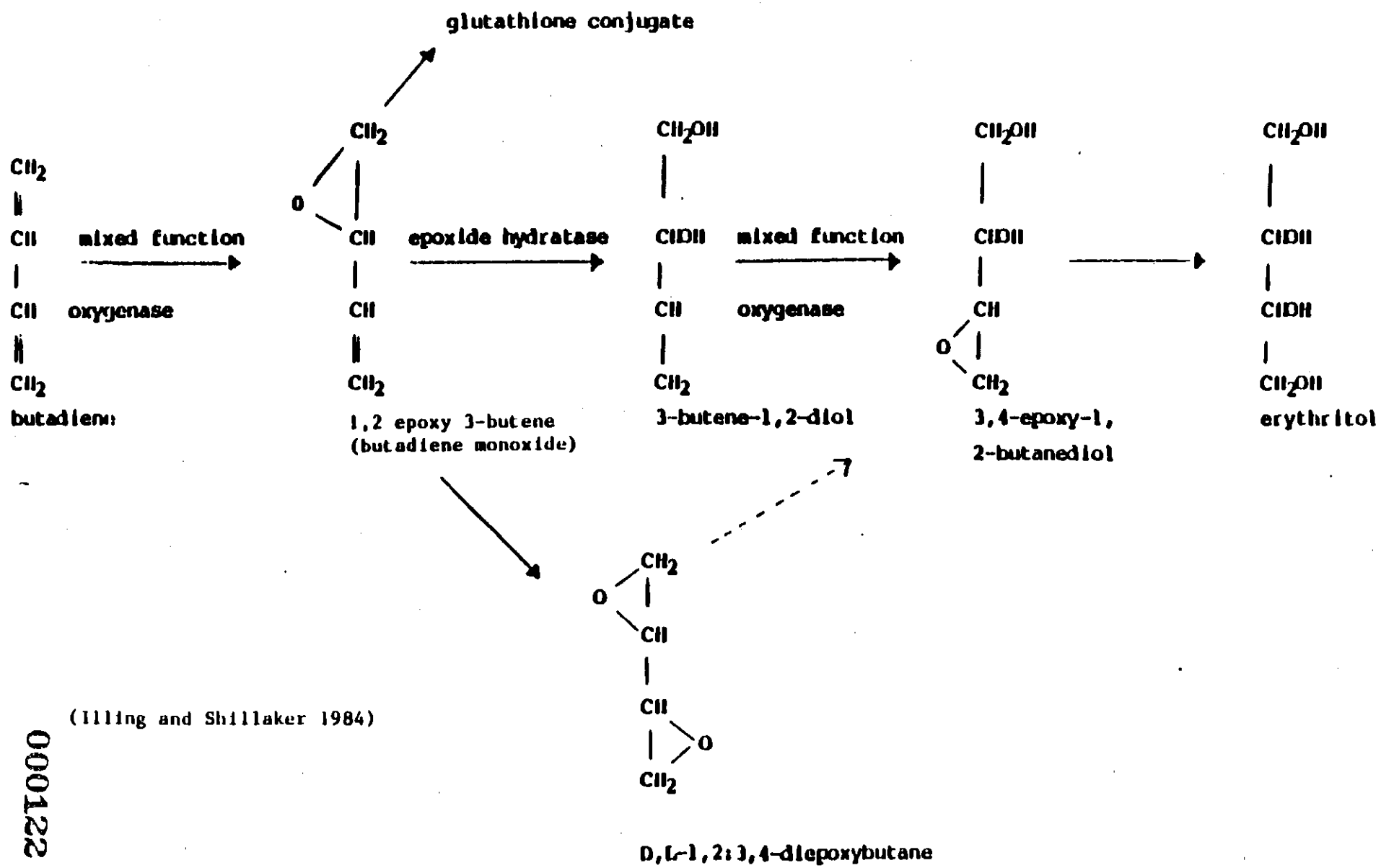
In vitro metabolism studies by Malvoisin between 1979-82 show that BD is first metabolized in the rodent by the cytochrome P450 monooxygenase system (present in the liver, lung, and also other organs to various extents) to the toxic metabolite 1,2 epoxy 3-butene (see Figure 1). This metabolite can be subsequently oxidized by the same system to the toxic diepoxybutane (which can then be detoxified by oxidation to 3,4-epoxy-1,2-butanediol) or, the 1,2 epoxy 3-butene itself can be reduced by epoxide hydrolase activity to the less toxic 3-butene 1,2 diol. The epoxides are also detoxified by conjugation with glutathione through the action of glutathione-S-transferases (Malvoisin and Roberfroid 1982; Bolt, et al. 1983).

Bolt et al. (1984) and Kreiling (1986) have confirmed this metabolism in vivo. Bond, et al. (1986) in studies of rodents exposed to [^{14}C] BD (130-1,800 μg/l), have gone on to show that 1,2

epoxy 3-butene is present at least 2 times greater concentrations in the blood of the mouse than in the rat. This difference is attributed to the metabolic difference between mouse and rat in the formation and elimination of this metabolite. The elimination of BD has been investigated using a closed-chamber method. In this method a fixed amount of BD is placed in the exposure chamber, and the concentration is monitored over time, and any decline is an indication of the uptake and metabolism by the test animal. Up to concentrations of about 1,000 ppm BD, metabolic elimination of BD is proportional to the exposure concentration in mice and rats. Above 1000 ppm, saturation kinetics of BD metabolism occurs. However, under conditions of both low and high exposure concentrations, the metabolic elimination rate of butadiene in mice is about twice that in rats; calculated maximal metabolic elimination rates are 400 μ mol. hr^{-1} kg^{-1} for mice and 220 μ mol. hr^{-1} kg^{-1} for rats (Kreiling 1986).

In addition to the higher production rate of 1,2 epoxy 3-butene, differences also exist in the metabolism of this monoepoxide. When Sprague Dawley rats or $B_6C_3F_1$ mice were exposed to 100-500 ppm of 1,2 epoxy 3-butene at up to 500 ppm exposure, the epoxybutene is metabolized in the mouse liver at twice the rate than that of the rat; 24,900 ml hr^{-1} kg^{-1} for the mouse compared to 13,400 ml hr^{-1} kg^{-1} for the rat. At increasing concentrations above 500 ppm, the metabolic capacity in the mouse becomes rate limiting resulting in accumulation of the monoepoxide and is reflected by the observed higher levels of the monoepoxide in the blood and expired air of the mouse. In contrast,

Figure 1. Possible pathways for metabolism of butadiene in the rat



(Illing and Shillaker 1984)

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epoxybutene metabolism in the rat is linearly dependent on the atmospheric concentration of BD up to exposure concentrations of about 5,000 ppm (Kreiling et al., 1987). Thus, as the atmospheric concentration of butadiene increases, the metabolism of 1,2-epoxy 3-butene becomes rate limited in mice providing for a reduced elimination of this epoxide in this species compared to the rat.

In vivo data are now available in the primate for comparison with the mouse. These data show that while qualitatively the primate may follow the same pathway as in the mouse, quantitative metabolism is much different. In the studies by Sun, et al. 1989 and Dahl, et al. (1990) referred to earlier (see Uptake), male primates were exposed by nose-only inhalation for 2 hours to 10 ppm BD and were found to have 0.13 p mol/ml/ppm 1,2-epoxy-3-butene in the blood. In contrast the level (77 p mol/mL/ppm) of 1,2-epoxy-3-butene found in the blood of mice inhaling 7.8 ppm BD is 590 times higher. After exposures to greater than 130 ppm [^{14}C] BD, mice and monkeys had roughly comparable levels. For rats, Dahl et al. (1990) showed that blood levels of 1,2-epoxy 3-butene are some 40-fold higher in the blood of rats exposed to 78 ppm BD than in the blood of monkeys exposed to 10 ppm BD.

Schmidt and Loeser (1985) have also conducted important research which provides a comparison of the human, primate, and rodent response to BD. BD was incubated with liver and lung in vitro preparations from mice (two strains), rats (two strains), monkey, and human (one surgical sample). The formation of 1,2 epoxy 3-butene in the liver of these species was as follows: mouse > rat > human > monkey. The ratio between mouse and monkey

was about 7:1. The mouse lung tissue produced levels of 1,2 epoxy 3-butene equivalent to that of the mouse liver, however, monkey and human lung did not produce any measurable 1,2-epoxy 3-butene. This is in agreement with the very low ethoxycoumarin diethylase activity (a marker for mono oxygenase activity) in monkey and human lung tissue, and supports the concept that in the whole body response, the different levels of monooxygenase, epoxide hydrolase, and glutathione-S transferase between species are key to the bioavailability of the reactive epoxides.

Although not developed specifically with either BD or its metabolites as substrates, the relative ratios of these specific enzymes in the mouse, rat, and human subcellular preparations of liver and lung have been calculated from Lorenz et al., 1984 and are shown in Table 1. It will be seen that the higher levels of monooxygenase in the mouse liver and lung support the greater formation of the butadiene monoepoxide in this species by this pathway, especially in the lung; likewise, the relatively poor activity of epoxide hydrolase in the mouse would allow for only a slow removal of the epoxide by this pathway.Further in vitro metabolism studies of butadiene and the epoxides in mouse and human tissues are being conducted for the Chemical Manufacturers Association by the Inhalation Toxicology Research Institute (Albuquerque, New Mexico).

TABLE I

RATIOS (RELATIVE TO HUMAN) OF SPECIFIC ENZYME ACTIVITIES IN SUBCELLULAR PREPARATIONS OF RODENT AND HUMAN LIVER AND LUNG

	MONOOXYGENASE		EPOXIDE-HYDROLASE		GLUTATHIONE-S TRANSFERASE	
	LIVER	LUNG	LIVER	LUNG	LIVER	LUNG
MOUSE	4	1220	0.2	0.7	3.2	9.3
RAT	2	185	1	0.7	0.8	1
MAN	1	1	1	1	1	1

CALCULATED FROM LORENZ, ET AL. (1984)

Species differences in detoxification also exist. 1,2 epoxy 3-butene is detoxified by conjugation with glutathione and can be metabolized by glutathione-S-transferase (Malvoisin and Roberfroid, 1982, Bolt 1983). Laib, et al. (1990) determined the effects of BD exposure on liver nonprotein sulfhydryls (NPSH), and the alkylation of DNA and nuclear proteins (Kreiling, et al. 1986, 1988; Deutschmann and Laib 1989). A species difference was noted in the amount of nuclear proteins and DNA alkylated after inhalation exposure to butadiene (Kreiling, et al. 1987, Kreiling, et al. 1988). Exposure of mice to >2,000 ppm BD resulted in a decrease in hepatic NPSH content to about 20% of the corresponding control after 7 hours and to 4% after 15 hours when signs of acute toxicity were observed; yet exposure to rats resulted in a depletion of NPSH to 65 and 80% of control (for Wistar and Sprague Dawley rat respectively) after 7 hours and no change after 15 hours. In a further study by Deutschmann and Laib (1989), the depletion of NPSH content in the liver, lung and heart was also

investigated in $B_6C_3F_1$ mice and Sprague-Dawley rats exposed to 0, 10, 50, 100, 250, 500, 1000 and 2000 ppm BD for 7 hours. In rats, the minor reduction of liver NPSH content (about 20%) was still observed at exposure concentrations of about 250 ppm. There was also a minor reduction in lung NPSH content in rats between 1000 and 2000 ppm BD; NPSH content in the heart was not affected. In mice, reduction of liver NPSH content started at exposure concentrations between 100 and 250 ppm; at 2000 ppm BD, the reduction of 80% was confirmed. Hence, in the mouse, reserves of NPSH are drastically depleted in the liver (50-70%) and lung (70-90%) and heart (25-40%). The rat by contrast shows only some decline and maintains this detoxification pathway throughout BD exposure.

The difference between mice and rats of NPSH depletion by BD in liver or lung tissue can be explained by differences in the rate of formation and detoxification of epoxide intermediates in the organs of these species. In contrast, cardiac tissue does not significantly contribute to xenobiotic metabolism. NPSH depletion in heart tissue of the mouse can be regarded as an indicator for systemically available epoxide intermediates reaching the heart with the efferent blood flow from lung and liver. Although NPSH depletion in heart tissue is only observed from upwards of 500 ppm butadiene, it is expected that endothelial cells of heart tissues will be influenced at lower exposure concentrations, when reactive BD metabolites are provided via the blood circulation. The fact that the heart is passed by the efferent blood flows of two BD metabolizing organs may explain its special susceptibility as a

target organ in BD carcinogenesis.

The above indication that the 1,2-epoxy 3-butene and the diepoxybutene remain longer in the blood of the mice, and the findings of Gervasi, et al. (1985) that the biological half-lives (determined in vitro in Tris buffer; pH 7.4) of the mono- and diepoxide are 14 and 100 hours respectively, indicate that circulating levels of these epoxides should be able to infiltrate target tissues and react with target macromolecules. Thus, it is these epoxide metabolites rather than BD itself which are critical in the biological response. The DNA and nucleoprotein binding of reactive BD metabolites (see cytogenicity) supports this contention.

Studies have been conducted to investigate whether repeated exposure to BD could induce BD metabolism. Using exposures of 7600 or 750 ppm, six hours/day for five days, no induction of BD metabolism was found in the liver or lung of mice or rats (Bond et al. 1988).

In summary, it is clear that there are species differences in the uptake of BD and in the formation and elimination of its toxic epoxide metabolites. Such differences result in blood monoepoxide levels at least 2 times higher in the mouse than the rat, and 590 times higher than in the primate, following comparable exposures to BD (~10 ppm).

Thus, although the metabolism of BD may be qualitatively similar across species, at occupational exposures or even at exposures 10 fold greater, quantitative differences exist which can account for the greater sensitivity of the mouse to BD

carcinogenic activity. In addition, differences in cytogenetic and bone marrow response to BD between species, and the contribution of the MULV retrovirus in the $B_6C_3F_1$ mouse, discussed below are also likely contributing factors to the very different carcinogenic response seen between mouse and rat and man.

C. Cytogenetic Activity/Bone Marrow Response

Butadiene is mutagenic in *Salmonella typhimurium* bacterial strain TA 1530 but only in the presence of a metabolic activating system (De Meester et al., 1980, Poncelet et al., 1980). Hence, BD *per se* is not a direct acting mutagen. Thus, cross-species differences in metabolism will have an important bearing not only on BD's carcinogenic activity but also its mutagenic potential.

Both the 1,2-epoxy 3-butene and the diepoxybutane metabolites are bacterial mutagens and do not require the presence of enzymes in the metabolic activation system for this response. Epoxybutene is mutagenic in Escherichia coli (Hemminki 1980, Voogd, et al. 1981) and the diepoxide produces point mutations in Salmonella strains as well as eukaryotic organisms. Exposure of mice to the epoxybutene by intraperitoneal injection produced a dose-related increase in sister chromatid exchanges in bone marrow cells and in the percentage of cells with chromosomal aberrations (Sharief, et al. 1986).

The diepoxide also causes chromosome changes (Dean and Hodson-Walker 1979) in rat liver cells and induces sister chromatid exchanges in hamster ovary cells (Perry and Evans 1975). Both epoxides *per se* have been shown to covalently interact with

DNA (Brookes and Lawley 1961, Lawley and Brookes 1967, Hemminki, et al., 1980, Kreiling 1986). The formation in DNA of 7-(2-hydroxy-3-buten-1-yl) guanine and of 7-(1-hydroxy-3-buten-2-yl) guanine has been demonstrated after chemical reaction of epoxybutene with DNA in vitro (Citti et al., 1984). Further, studies by Jelitto, et al. (1989) showed that exposure of male rats and mice to BD in a closed system resulted in the formation of these same DNA adducts in mouse but not rat liver. Exposure of rats and mice for 2 hours to 0, 500, or 1000 ppm BD resulted in the occurrence of protein-DNA and DNA-DNA cross-links from mouse, but not rat liver. Such studies indicate that 1,2-epoxy 3-butene and the diepoxide (all of its isomeric forms) are active genotoxic metabolites of BD. The species difference in DNA and protein binding is probably a consequence of quantitative differences in metabolism across species. There is also evidence for carcinogenicity of at least the diepoxide in mice since the D, L, and meso isomeric forms of the diepoxide caused benign and malignant skin tumors in mice following dermal exposure (Van Duuren, et al., 1963 and 1965).

There is also in vivo evidence derived from BD exposure. Tice, et al. (1987) exposed male mice to 6.25, 62.5, and 625 ppm BD for ten exposure days. Five endpoints were evaluated: average generation time (AGT), mitotic index (MI), chromosomal aberrations (CA), sister chromosome exchange (SCE), and micronuclei (MN). BD exposure induced a dose dependent response in all these endpoints. In the 6.25 ppm group, the frequency of sister chromatid exchanges in bone marrow cells was significantly elevated and in the 62.5

ppm group the frequency of MN-polychromatic erythrocytes in the peripheral blood was significantly increased. Cytotoxicity was shown by the extended AGT in the 62.5 ppm group, and by the reduced MI in the 625 ppm group. This dose related response in these multiple cytogenetic endpoints indicates a strong genotoxic and cytotoxic action in the mouse.

Cunningham, et al. (1986) evaluated these responses across species by conducting a two day, six hour/day BD exposure of mice and rats and examining the bone marrow cells for the induction of micronuclei and sister chromatid exchanges. Significant dose dependent increases in micronuclei induction and frequency of SCE were observed in mice at exposures of 100 ppm and greater. The highest tested no observed effect level for both endpoints was 50 ppm BD in air. In contrast, rat bone marrow cells did not show any significant increases in micronucleated polychromatic erythrocytes nor in the frequency of SCE. This lack of response from this two day exposure to BD is consistent with the lack of changes in the bone marrow of rats exposed in the two year cancer bioassay which involved BD exposure as high as 8,000 ppm.

Exxon has conducted micronucleus assays on simultaneous exposures of mice and hamsters to 0 or 1000 ppm BD for 6 hours/day for 2 consecutive days (Exxon 1990). BD produced an 11.2-fold increase in micronuclei formation in the $B_6C_3F_1$ mouse and only a 1.4-fold increase in the number of micronuclei in the Syrian hamster exposed under the same conditions. In primate studies by Sun, et al. (1988), monkeys were exposed to 10, 300, and 8000 ppm BD in air for two hours. At no exposure level was micronuclei

induction found to occur nor was there an increase in frequency of SCE. Thus, the mouse is clearly sensitive to BD induced bone marrow toxicity in contrast to the lack of response of other species.

The above cytogenetic changes in the mouse are in agreement with the findings (Liederman et al., 1986, Irons et al., 1986) that BD affects bone marrow stem cell development and induces macrocytic megaloblastic anemia in the mouse. Irons et al. (1986) exposed the $B_6C_3F_1$ mouse and the NIH Swiss mouse to 1250 ppm BD for six weeks. This resulted in a decrease in circulating erythrocytes, in total hemoglobin and hemocrit, and an increase in mean corpuscular volume and an increase in circulating micronuclei in both strains of mice. No such hematologic changes were seen in the primate (two hour exposure) and in the rat (2 year exposure). This suggests that the primate and rat do not share the genotoxic response of the mouse to BD.

The above studies indicate that the bone marrow is a target organ of BD exposure for the mouse. In addition, the incidence of malignant lymphomas of thymic origin in the $B_6C_3F_1$ mouse cancer bioassay of BD provides additional evidence of bone marrow involvement. The bone marrow is known to have an essential role in radiation induced murine T cell leukemia/lymphomas and also accompanies many instances of chemically induced T cell lymphoma.

Butadiene interferes with bone marrow differentiation and/or DNA synthesis in replicating mouse bone marrow cells. Leiderman et al. (1986) studied the effect of BD on the proliferation and differentiation of hematopoietic stem cells and found that while

BD exposure (1250 ppm BD for six weeks) resulted in no alterations of the frequency of spleen colony forming units (CFU-S) the colonies derived from treated animals were smaller. That this may be due to an alteration in the proportion of immature to mature pluripotent stem cells available following BD exposure was confirmed from long term bone marrow culture studies in which a shift in the course of differentiation of the granulocyte/macrophage precursor cell was found from changes in the number of the granulocytic/macrophage colony forming units (CFU-GM) in BD exposed animals compared to controls. Longer term exposure (30-31 weeks) caused a decrease in CFU-S and CFU-GM. The above observations indicate that BD causes alteration in stem cell development in the mouse. Since depletion of the bone marrow stem cell population is often a precursor of radiation or chemical induced thymic neoplasms (Kaplan 1967, 1977; Seidel et al., 1983) the BD induced stem cell depletion may play an essential role in BD induced lymphoma in the mouse.

Data by Irons (1986a) show a significant increase in the proportion of bone marrow cells in the S cell cycle phase in BD exposed mice, although there was no significant change in the total number of cells. The lengthening of the average generation time and depression of mitotic index, described in previous reference to the studies by Tice (1987), indicate that the effect of BD is to extend the S phase. Tice (1937) notes that the induction of SCE and the chromosome changes (chromatid type breaks and exchanges) are consistent with the induction of DNA damage by an S phase dependent clastogen. Hence, the mechanistic data support the observed cytogenetic response in the mouse and in turn

may have bearing on the lymphoma/leukemia response in the mouse; no such cytogenetic or bone marrow changes have been observed in the rat or primate and are only marginally present in the hamster.

D. The Role of the MULV Retrovirus in Butadiene Induced Leukemogenesis

The initial and subsequent understanding of the extensive influence of a retrovirus in the BD induced murine lymphoma is due to the studies of Irons, et al. under the auspices of the Chemical Industry Institute of Toxicology. Most murine lymphomas/leukemias are now known to result from the transformation of a single cell by a mechanism involving a recombinant retrovirus. In general, the best characterized viral leukemia model has been radiation leukemogenesis in certain strains of mouse including $C_{57}BL$ and C_3H, the parental strains of the $B_6C_3F_1$ mouse. Irons, et al. (1987) found that chronic exposure to BD (1250 ppm) six hour/day, five days/week for three to 21 weeks markedly increased the quantity of the ecotropic (capable of infecting mouse cells) MULV retrovirus recoverable from the bone marrow, thymus and spleen of the $B_6C_3F_1$ mouse. Expression of other endogenous retroviruses was not enhanced. Age-matched controls not exposed to BD either yielded no ecotropic virus or very small numbers of virus-producing cells.

Irons, et al. (1987) also examined the viral trophism to gain a mechanistic understanding. Isolates from exposed and control mice showed no change in FV-1 restriction. This gene governs infection and spread of murine leukemia viruses (Tennant, et al., 1974, 1976). Irons, et al. (1987) also determined that the viral

isolates were N-tropic and were not altered to be outside of the FV-1 gene range of control. Because the changes in FV-1 tropism of the replicating viruses or changes in FV-1 restriction are not found, their conclusion was that the mechanism of this increase in ecotrophic retrovirus in the $B_6C_3F_1$ mouse is due to de novo activation in greater numbers of cells.

Irons,, et al. (1989) subsequently conducted a comparative study in the $B_6C_3F_1$ hybrid mouse and the NIH Swiss mouse. The latter strain only rarely expresses any type of endogenous retrovirus (Jenkins, et al., 1982; Chattapadhyay 1980). $B_6C_3F_1$ mice and NIH Swiss mice were chronically exposed to 1250 ppm BD six hours/day, five days/week for one year. The incidence of thymic lymphomas in the $B_6C_3F_1$ mouse was 57% at the end of one year. These lymphomas were all of T cell origin and exhibited elevated expression of the endogenous ecotropic retrovirus (eMULV). Leukemogenesis was preceded by anemia, bone marrow cytogenetic abnormalities and an increase in the amount of eMULV recoverable from the bone marrow, thymus and spleen. In contrast, NIH Swiss mice similarly exposed to 1250 ppm BD resulted in a 14% incidence of thymic lymphoma, with no increase in eMULV. However, the same hematologic and cytogenetic abnormalities observed with the $B_6C_3F_1$ mouse also occurred with the NIH Swiss mouse indicating that it is the eMULV background that influences the susceptibility to BD induced leukemogenesis in the mouse.

The above comparative data also indicate that the presence of the ecotropic retrovirus is not an absolute requirement for thymic lymphoma development following BD exposure, since some increased

incidence of TL was also observed in the NIH Swiss mouse. However, the retrovirus could well account for the marked difference in the thymic lymphoma incidence between these strains and possibly between species. In addition, the bone marrow damage also appears to be a requisite for the enhanced murine leukemia.

III. Implications for Human Risk Assessment

In using animal data for assessing human cancer risk from chemical exposure, only relevant information should be utilized. This information may include pharmacokinetic, metabolism and genotoxicity studies. Species differences should also be taken into account, as well as the carcinogenic mechanism of action, i.e. genotoxic or nongenotoxic action. BD is a representative of a genotoxic carcinogen. Metabolic activation is required to convert BD to electrophilic metabolites, the mono- and di-epoxides, which then react with nucleophilic target sites such as DNA. This reaction can initiate genetic damage which appears to play a role in the carcinogenic process. It is important to take all this information, i.e. effective dose, species differences, into account in order to provide a proper assessment of human cancer risk.

As have been documented in Section II, there are several differences between mouse, rat, man, and primate in the metabolism and pharmacokinetics of BD which would result in marked differences in genotoxicity and cancer. These differences are:

1. Mice retain a larger dose of inhaled BD when adjusted for body weight and exposure time than rats or primate (at 10 ppm this

difference is 7 fold for rats and 6 fold for monkeys).

2. Mice metabolize BD to the epoxide at about twice the rate as rats.
3. Elimination of the epoxide 1,2-epoxy-3-butene is saturable in mice but not in rats.
4. Mice attain a higher concentration of epoxide metabolites in the blood than rats or primates when exposed at similar concentrations. Given comparable exposure concentrations of BD at 10 ppm, the epoxide level in the blood of primate is 590 fold lower than in the mouse.
5. The reactive epoxides which are formed are direct acting mutagens and have carcinogenic activity. BD itself, unmetabolized, does not have mutagenic activity.
6. Cytogenetic and bone marrow changes have been found in the mouse exposed to BD and possibly a marginal response in the hamster but at very high BD exposures. No such effects have been seen in the rat or primate.
7. The presence of an endogenous retrovirus in the mouse is a critical part of a multifactorial process of BD induced leukemia in this species. It is not possible to determine the contribution of the retrovirus in BD induced leukemia in the $B_6C_3F_1$ mouse, but it is a significant and confounding factor.

The above findings clearly demonstrate that the mouse will attain a significantly higher amount of the epoxides over a longer period of time than the rat (greater than 40 fold) or primate (590 fold) when exposed to butadiene. Results of both <u>in vivo</u> and <u>in vitro</u> studies have implicated 1,2-epoxy-3-butene and di-epoxybutene, the epoxide

metabolites of BD, as causing the genotoxic and carcinogenic effects of BD exposure. The internal doses of the toxic epoxides are related to the rate of formation and the rate of disappearance by elimination pathways as well as the amount of uptake of BD, but it is the concentrations of these reactive metabolites which will largely dictate the biological responses seen in the species tested and which can account in large part for the demonstrated sensitivity of the BD exposed mouse in terms of tumor formation and bone marrow toxicity.

Based on the foregoing, there are a number of significant implications for the human risk assessment of BD.

1. There are major quantitative differences in the uptake and metabolism of BD in the mouse, rat, primate, and man. These differences help explain the greater sensitivity of the mouse to BD carcinogenic activity.
2. In any quantitative assessment of the human cancer risk, one should attempt to account for species differences in uptake and metabolism by using the internal dose of epoxybutene as the measure of dose. One should not assume humans receive equal internal doses of BD or epoxybutene from equal concentrations of BD in air. In fact, primates have lower internal concentrations of BD (by a factor of about 6) and epoxybutene (by a factor of 590) than mice from the same administered concentrations of BD (~10 ppm), due in part to differences in the rates of enzyme mediated metabolic processes. Results from studies of BD metabolism in the lung and liver microsomes have shown that the man is more similar to the primate with respect to 1,2-epoxy-3-butene formation than the rat or mouse.

3. If the available data on pharmacokinetics and metabolism are not used in the quantitative risk assessment, then the best estimate of human cancer risk should be based on the rat cancer bioassay data, not the mouse cancer bioassay. Based on metabolism studies in different species, the rat is a poor model for man, but is closer to man (and primate) than the mouse. The available evidence suggest the mouse may be uniquely sensitive to BD because of greater uptake, faster metabolism, and saturation of detoxification mechanism. This sensitivity is also seen with the cytogenetic and bone marrow responses seen in the mouse but not in other species.

4. The presence of the endogenous retrovirus in the $B_6C_3F_1$ mouse confounds the quantitative evaluation of the lymphoma/leukemia response in this strain to BD exposure. If the mouse cancer bioassay data are used for quantitative risk assessment, then the incidence of lymphomas should be excluded. The presence of the virus and the unique sensitivity of the mouse bone marrow cast doubt on the relevance of this tumor response for human risk assessment. The absence of an increased incidence of lymphoma at the lower exposure concentration in the NTP 2nd cancer bioassay also cast doubt on the relevance of the mouse lymphomas for low dose risk assessments.

In view of the above, the mouse may be uniquely sensitive to BD as compared to other animal species (rodent and nonrodent) because of pharmacokinetic and metabolic differences (which provide an accumulation of significantly higher levels of the epoxide

intermediates) and the presence of the murine retrovirus. Thus any carcinogenic risk assessment based on mouse data will overstate the human cancer risk. If the mouse is to be used for quantitative risk assessment, then this species difference in uptake and metabolism, with respect to the epoxide metabolites, should be taken into account. Further, due to the confounding factor of the retrovirus, the lymphoma incidence should be excluded from human risk assessment. Regardless of the species used for quantitative risk assessment, the analysis should be based on the internal epoxide concentrations, since it is these metabolites that are implicated in causing the cytogenetic and carcinogenic effects of BD in the rodent.

IV. Additional Comments on OSHA's Discussion of Bone Marrow Toxicity, Metabolism, Structure Activity and Genotoxicity

This Section is intended to provide a complete crique of OSHA's material on these aspects and as such includes discussion of some important material already covered in previous sections.

A. Bone Marrow Toxicity

OSHA's discussion of the animal data is incomplete, in that rat, hamster, and primate data are omitted. OSHA's discussion of the human data is seriously flawed; the study by Checkoway and Williams (1982) does not demonstrate an effect of BD on bone marrow in the 8 workers examined. There is no basis for concluding that the bone marrow is a target of BD toxicity in humans. The available data on bone marrow toxicity, considered as a whole, provide further support for our belief that the mouse is uniquely sensitive and not a good model for human risk assessment.

1. Animal Data

The OSHA data are incomplete. The following studies are pertinent:

a. Rodent

Cunningham, et al. (1986) exposed male $B_6C_3F_1$ mice and Sprague Dawley rats by nose-only inhalation from 0-10,000 ppm 6 hours/day for 2 days, and evaluated their bone marrow cells for induction of micronuclei and sister chromatid exchanges. Starting at 100 ppm, a significant increase in the frequency of micronucleated polychromatic erythrocytes and of sister chromatid exchanges was found in mouse bone marrow cells with a no observed effect level of 50 ppm in each case. In contrast, no significant increases in micronucleated polychromatic erythrocytes or sister chromatid exchanges were found in the bone marrow cells of the rat.

Arce, et al. (1990) treated human whole blood lymphocytes with BD in the absence and presence of mouse, rat, and human S9 metabolic systems. No induction of sister chromatid exchanges were observed with either system at 25-100% BD in nitrogen.

Shelby (1990) extended the observations of cytotoxicity and cytogenetic effects of BD in bone marrow cells of $B_6C_3F_1$ mice exposed to 6.25-625 ppm BD 6 hours/day after 10 exposure days (Tice, et al. 1987) by evaluating these activities in the peripheral blood of mice exposed to the same concentrations of BD 6

hours/day, 5 days/week for 13 weeks. In the 10 day study, BD induced statistically significant increases in sister chromatid exchanges at 6.25 ppm, micronucleated polychromatic erythrocytes at 625 ppm, and the percentage of cells with chromosomal aberrations and micronucleated normochromatic erythrocytes at 625 ppm. In the second study using blood from BD exposed mice, the previous finding's were confirmed except that the frequency of micronucleated normochromatic erythrocytes was increased at 62.5 ppm and higher concentrations; this was attributed to the increased statistical power of the peripheral blood micronucleus assessment where 10,000 erythrocytes were scored per animal rather than 1,000 cells in the case of the bone marrow samples.

A comparative study of the bone marrow response to BD exposed mice and hamsters has been completed (Exxon 1990). Mice and hamsters were simultaneously exposed to a concentration of 1,000 ppm BD in air for six hours on each of two consecutive days; this concentration is known to produce hematotoxicity and micronuclei in mice but the genotoxicity of BD in the hamster had not been previously evaluated. As expected, BD produced substantial hematotoxicity and an 11.2 fold increase in micronuclei formation ($p < 0.01$) in the $B_6C_3F_1$ mouse. By comparison, no statistically significant hematotoxicity (only a small downward trend) and only a marginal increase (1.4 fold) in micronuclei ($p < 0.05$) were seen in the Syrian hamster

compared to the negative control (air) for each species.

b. Primate

Sun, et al. (1989) exposed three male Cynomolgus monkeys (Macaca fascicularis) by nose-only inhalation to 10 or 300 or 8,000 ppm [^{14}C] BD for 2 hours progressing from low to high exposure levels and with a minimum of 3 months separating the re-exposure. Prior to exposure, the monkeys were fitted with a venous catheter that was used to administer anesthetic (pentobarbital) and to withdraw blood during exposures. No cytogenetic changes were detectable after these acute exposures at concentrations as high as 8,000 ppm (National Toxicology Program 1989).

Interpretation

The foregoing studies clearly demonstrate that the mouse is singular in its bone marrow response to BD exposure as only a marginal response was seen in the hamster under identical experimental exposure to a high concentration (1000 ppm) of BD. No such responses have been observed in the rat or primate from subacute exposures to 10,000 ppm BD (rat) or 8,000 ppm BD (monkey) nor have these effects been seen in the rat following 2 year exposures to 8,000 ppm (Owen, et al. 1987; Owen and Glaister 1990).

2. Human Data

Checkoway and Williams (1982) evaluated personal air

samples and blood specimens from 157 production workers at a styrene-butadiene plant. The objectives were to quantify exposure levels for styrene, butadiene, benzene, and toluene and to relate these levels to variation in hematological parameters. The highest mean styrene (13.7 ppm) and butadiene (20.0 ppm) concentrations were found in the Tank Farm area. Comparison of mean hematologic parameters adjusted for age and medical history status indicated that eight Tank Farm workers had slightly lower levels of circulating erythrocytes, hemoglobin, platelets and neutrophils, and slightly higher mean corpuscular volumes and neutrophil band forms than 145 workers in other areas. There were no indications of bone marrow toxicity. With respect to the changes observed in Tank Farm workers, the authors could not ascribe a cause directly as exposure and clinical outcomes were measured concurrently and at only one point in time. Overall, among the plant workforce, they concluded that there was no striking indication of bone marrow toxicity related to exposure (Checkoway and Williams 1982, p. 168)

Interpretation

The analysis only involved eight Tank Farm workers, a number too small for reliable statistical tests for significance. Any possible differences between the two groups are, therefore, difficult to interpret. Because of the small numbers of Tank Farm workers (N = 8), the authors stated that this precluded reliable statistical tests of significance. Instead, the patterns of the differences in direction and

magnitudes can be examined ... (Checkoway and Williams 1982, p. 167).

The same data was also analyzed differently by adjusting for age and medical status using regression and correlation analysis. In this analysis, the association studied was between hematological changes and chemical exposure in all job categories. The results showed an association between workers exposed to butadiene and higher total leukocyte count and higher mean corpuscular hemoglobin concentration. The increase in total leukocyte count is opposite to what would have been expected if butadiene exposure produced bone marrow depression.

There are other data and statements by the authors that do not support the butadiene-bone marrow depression and cellular immunity hypothesis. For example, the authors state the following about 5 cases of hematologic malignancy identified among the styrene-butadiene rubber workers: "There is a diversity of clinical disease represented in this case group. While it is possible that styrene-butadiene rubber exposures may have been the common causative factor, there is a lack of specificity of the exposure-disease association" (Checkoway and Williams 1982, p. 167).

Finally, cross-sectional studies are inadequate for inferring causation because it is often difficult to determine the temporal relationships between exposure and disease. The authors' reason for the study was "to explore for possible associations rather than to test specific a priori hypotheses

regarding exposure effects." Typically, results from hypothesis-generating studies are not given much credence until they have been replicated in different populations. To date, there have been no attempts to replicate the findings of the Checkoway and Williams study, but likewise, no such associations have become apparent through routine medical surveillance of the SBR workers studied in the epidemiologic cohorts.

The authors (Checkoway and Williams 1982, p. 164) state that: "Overall, in this population there was no pronounced evidence of hematologic abnormality, as determined from examination of peripheral blood." The results do not suggest significant effects of butadiene exposure on hematologic parameters for the following reasons:

1. The analysis of the Tank Farm workers versus the other production workers is based on too small a number of workers to perform statistical analysis to indicate an association.
2. The results from the correlation and regression analysis, adjusted for age and medical status, suggest no apparent effect of BD exposure. If anything, the results are opposite to what would be expected if BD exposure caused bone marrow depression.
3. Cross-sectional studies are inadequate for inferring causation.
4. Evidence from mice studies by Irons, et al. (1989) do not support a link between hematological parameters and

leukemia. Using two different strains of mice, the $B_6C_3F_1$ and the NIH Swiss, Irons, et al. found almost identical hematological changes and bone marrow toxicity after BD exposure; yet marked differences were observed with respect to the incidence of lymphomas. This difference was attributed to the presence of an endogenous MuLV in one strain, but not present in the other.

Confounding factors including race, smoking habits and ethanol intake (all of which have been shown to effect the CBC parameters measured) were not controlled for in this study and may well have influenced the results seen, especially in as small a population as the eight Tank Farm employees. In addition, although the differences seen between the Tank Farm workers and the other workers studied were statistically significant, there is nothing in this paper to suggest that these differences were clinically significant, or that any medical surveillance program would have identified Tank Farm employees as being in need of additional medical follow-up.

B. **Metabolism**

Overview

OSHA's description of the metabolic pathways for BD is largely correct except that the detoxification pathway for the diepoxybutane has been omitted as has reference to the detoxification of the epoxide metabolites with glutathione (as evidenced by the depletion of non protein sulhydryls) and the

species differences that exist in this elimination process. While OSHA acknowledges that mouse/rat differences in uptake and metabolism of BD help explain why dramatically different responses have been seen in carcinogenicity studies, OSHA needs to include in its quantitative risk assessment the species differences in elimination of toxic metabolites of butadiene. OSHA cites much of the original data on species differences in uptake and metabolism of BD but fails to include more recent and complete data which significantly impact previous assumptions concerning the percent absorption at lower concentrations. OSHA needs to revise downward its conservative assumption of 100% BD absorption to 20% absorption in mice at 1 ppm based on these recent data.

Metabolism

The metabolic pathways depicted in OSHA's Figure 1 are those described by Malvoisin and Roberfroid (1982). Since then, studies by Bolt (1983) and Deutschmann and Laib (1989), provide evidence that at least the 1,2 epoxy 3-butene forms a conjugate with glutathione. Bolt (1983) showed that addition of glutathione, especially glutathione plus rat liver cytosol, to an incubation mixture containing rat liver microsomes and butadiene resulted in a decrease in the amount of 1,2 epoxy 3-butene measured. Deutschmann and Laib (1989) showed an extensive depletion of nonprotein sulfhydryls in the liver, lung, and heart of mice exposed to BD, but a depletion of nonprotein sulfhydryls observed in the rat was not nearly as pronounced. These reductions in nonprotein sulfhydryls and the difference between species can be explained by differences in the amount of

circulating epoxide and in the species' differences in glutathione S-transferase.

Another metabolic pathway, though less well substantiated than the above, is the formation of crotonaldehyde (Duescher, et al. 1990). This has presently only been identified in vitro. With the exception of this minor metabolic pathway, all metabolism of BD involves conversion to the biologically active epoxybutene before detoxification or further metabolism to other forms such as the diepoxybutane. Thus, the internal concentration of epoxybutane is likely to be a significantly better predictor of cancer risk than the internal dose of BD itself.

Uptake

OSHA uses early experimental data of Bond, et al. (1986) to extrapolate a relationship between exposure concentration and percent of inhaled BD retained at 7 or 80 or 1040 ppm for mice and at 70 or 950 or 6100 ppm for rats. Based on regression equations developed with these data, OSHA estimated that 100% absorption of BD was achieved by mice at just over 2 ppm. Recent data by Bond, et al. (1986) show that this is incorrect and that the absorption at 0.8 ppm (1.4 µg/l) BD in mice should be 20%. In the complete study, Bond et al. (1986) extended the exposure concentrations for both rats and mice to 0.08, 0.8, 7.8, 78, 1040 ppm, and (for rats only) 7100 ppm for a 6 hour period. The internal doses (retained concentrations) of butadiene at 6 hours are considerably lower. Correcting an error in the paper by Bond (1986), these new absorption data for the mouse are 16% (0.08 ppm), 20% (0.8 ppm), 13% (not 20%) at 7.8 ppm, 8% at 78 ppm, and 4% at 1040 ppm

compared with the earlier obsolete data for the mouse of 53% at 7.8 ppm, 9% at 78 ppm and 4% at 1040 ppm. Thus, at approximately 1 ppm, actual absorption data show a 20% retention in contrast to OSHA's extrapolated value of 100%. These new data should be included in the OSHA assessment.

Dahl, et al. (1990) have also published data allowing the estimation of retained BD doses in rodents and primates. In this study, three male primates (Macaca fascicularis) were exposed by nose-only inhalation for 2 hours to concentrations ranging from 0.08-1000 ppm. When normalized to body weight, the data from Bond, et al. (1986) show that the rate of uptake of butadiene at approxim tely 10 ppm [^{14}C] BD in mice was substantially greater than for rats or monkeys. When normalized to body weight for each species and expressed on a μ mole per hour per 10 ppm basis, the measured retention rates were determined to be 3.30, 0.46, and 0.52 μ mole/Kg BD for the mouse, rat, and monkey respectively. Thus, for equivalent BD exposures, the rat and primate respectively receive 7.2 and 6.3 fold lower doses of BD than the mouse. The level (77 p mol/mL/ppm) of the biologically active 1,2 epoxy-3-butene metabolite in the blood of mice inhaling 7.8 ppm BD were 590 times higher than in the blood of the monkeys exposed to 10 ppm BD (0.13 p mol/mL/ppm). After exposures to greater than 130 ppm [^{14}C] BD, mice and monkeys had roughly comparable levels. Similarly for rats, Dahl et al. (1990) show that blood levels of 1,2-epoxy 3-butene are some 40-fold lower in the blood of rats exposed to 78 ppm BD than in the blood of mice similarly exposed.

OSHA comments that while there are species differences in the amount of BD at the target site both the rat and mouse metabolized BD to the same reactive metabolites suspected of being the ultimate carcinogens. The data by Dahl, et al. (1990) clearly show that levels of circulating active metabolites are very different between species; there are pharmacokinetic and metabolic data and rationale to support these observations. While qualitatively, the metabolism across species is similar, it is the amount of reactive metabolite at the target sites which is critical. This will be very different between species, and hence the biological response would be expected to be different between species.

It should be noted that the rat exposed to high BD concentrations had no lymphomas and no lung, liver, or heart tumors in contrast to the tumors at these sites occurring in the mouse. As has been described in Section II, there is a rationale for this in terms of the species differences in the enzymes present for the epoxide formation and detoxification in at least the lung and liver. Therefore, the different amount of reactive metabolites at the target site is more critical than qualitative similarities in metabolism between species.

Metabolism studies cited by OSHA only indicate that there are significant differences between the rat and mouse in the metabolism of BD and that these differences correlate with dramatically different tumor responses. When the missing data are added, including the primate and limited human data, it becomes clear that the mouse is a poor model for man, and species

differences in uptake and metabolism must be addressed in any qualitative or quantitative assessment of the human cancer risk.

Instead of presuming that BD absorption and metabolism is the same in humans and rodents, OSHA should examine BD metabolism in humans (using primate data) and compare to rodents before drawing any firm conclusions about the cancer risk to humans. Available data suggest humans are likely to be less sensitive than rat or mouse. At occupational exposure levels, which are two orders of magnitudes below the low dose in the rat study, BD may not present a human cancer risk.

C. Structure Activity

A review of the toxicity of other compounds with similar structures to butadiene or which are metabolities of butadiene shows that the metabolism and detoxification of these compounds is a determinant in the different interspecies responses observed. In the case of butadiene, the epoxide metabolites have definite biological activity in the rodent and it is these reactive metabolites rather than the parent butadiene that should be used as a measure of internal dose.

OSHA recognizes the carcinogenic activity of the diepoxide metabolite of butadiene and its IARC classification as a category 2B animal carcinogen. OSHA should note that intraperitoneal injection of the diepoxide metabolite into mice has been found to cause a dose related increase in the number of tumors per mouse and in the incidence of lung tumors (IARC 1976). Intraperitoneal injection of diepoxybutane dissolved in water or tricaprylin were given 3 times per week for 4 weeks to groups of 15 male and 15

female mice. Total doses in water were 1.7, 6.7, 27, 108, and 192 mg/Kg body weight, resulted in incidences of lung tumors of 21, 40, 55, 64, and 78% respectively. Total doses of tricaprylin were 3, 12, 48, and 192 mg/Kg body weight, and the resulting incidences of lung tumors were 40, 43, 46, and 50% respectively. The vehicle (water or tricaprylin) control incidence of lung tumors was 35% (Shimkin 1986). The increases in numbers of lung tumors were significant ($p < 0.01$) at the 3 highest dose levels of diepoxybutane administered in water (IARC 1976). This may have bearing on the increased incidence of lung tumors seen in the recent NTP carcinogenicity study of BD.

OSHA also examines 4-vinyl cyclohexene (VCH), butadiene dimer, for evidence of the formation of biologically active metabolites and cites the NTP carcinogenicity study on VCH. In this study, rats and mice were administered by gavage 5 days/week for two years to 0, 200 or 400 mg/kg body weight VCH in corn oil. Survival was poor in dosed rats and male mice, making these studies inadequate for determining the presence or absence of carcinogenicity. Nevertheless, in VCH-treated female mice, there was clear evidence of carcinogenicity due to the development of rare ovarian neoplasms.

In another study, Van Duuren, et al. (1963) showed that chronic application of VCH diepoxide (in benzene) induced squamous cell carcinomas in male mice. In a two year carcinogenicity study by NTP, mice were exposed by dermal application to 0, 2.5, 5 and 10 mg/day and rats 0, 15 and 30 mg/day of VCH diepoxide (in acetone). VCH diepoxide induced squamous cell carcinomas at the

site of application in both sexes and species. In addition, a species difference was observed in the induction of ovarian tumors, with ovarian tumors induced in the ovaries of mice but not of rats.

As with butadiene, VCH is a carcinogen in which the greater sensitivity of the mouse versus the rat to ovarian tumors can be explained by species differences in metabolism.

VCH is metabolized by rat and mouse microsomes forming VCH-1,2-epoxide and to a lesser extent, VCH-7,8-epoxide and VCH diepoxide (Watabe, et al. 1981; Gervasi, et al. 1981). These epoxides can be hydrolysed to their corresponding diols by epoxide hydrolase.

VCH itself was not mutagenic to S. typhimurium when incubated with or without Aroclor 1254-induced rat or hamster S9 microsomal fraction. However, the epoxide metabolites and genotoxic VCH-1,2-epoxide was mutagenic to S. typhimurium (Simmon and Baden 1980) and caused chromosomal aberrations in chinese hamster V79 cells (Turchi, et al. 1981). VCH diepoxide was mutagenic in both bacterial and mammalian cells (Turchi, et al. 1981).

Smith et al. (1990a) administered [^{14}C] VCH by gavage (400 mg/Kg). Animals were killed 1, 4, 8, 24 and 48 hours after dosing and tissue burdens of [^{14}C] were determined. Major depots of [^{14}C] VCH were adipose muscle and skin, but ovaries contained about the same concentration as the liver. In another study (Smith et al. 1990b), doses of VCH, VCH-1,2- epoxide and VCH-7,8- epoxide (in mice only) were given in corn oil, ip, to mice and rats at doses

ranging from 0.07-7.4 m mol/kg body weight/day for 30 days. All compounds destroyed the oocytes in the ovaries of the mice, the diepoxide was the most potent and the VCH-^{1,2}- monoepoxide and VCH-^{7,8}- were more potent than VCH. In rats, VCH itself had no effect on the oocytes. This finding was correlated with the results of a further in vivo metabolism study in which mice and rats were given 800 μg/Kg VCH by intraperitoneal injection. VCH epoxide was found in the blood of mice at a peak level of 40.7 μ mol/ml, 2 hours after dosing. The diepoxide was not detected. Neither the epoxide or the diepoxide was detected in the rat.

To determine if metabolic differences could explain this difference in the blood concentration of VCH-1,2-epoxide, the rate of metabolism of VCH to VCH-1,2-epoxide, as well as the rate of degradation of VCH-1,2-epoxide to VCH-1,2-dihydrodiol, was measured in vitro using liver microsomes. VCH-1,2-epoxide was formed at a faster rate in microsomes from mice than from rat. On the other hand, VCH-1,2-epoxide degradation was 2-fold higher in microsomes from the rat as compared to the mouse. It is evident from these studies that the mouse has a greater capacity to convert VCH to reactive intermediates than the rat. A large part of the susceptibility of the mouse to ovarian toxicity is likely due to the metabolism of VCH to the epoxides.

There would seem to be close analogy between VCH and BD metabolism. Structure activity comparison support the contention that the metabolites rather than the parent compounds are toxic and carcinogenic and that there are major species differences in the formation and biological consequences of these metabolites.

OSHA should, therefore, recognize the quantitative interspecies data for the metabolites of BD in its assessment of human risk.

D. Genotoxicity

OSHA's description is largely correct and includes rat data (Cunningham 1990) that should have been included elsewhere. However, once again OSHA emphasized qualitative similarities instead of quantitative differences. The genotoxicity data demonstrate interspecies differences in the formation and elimination of the reactive metabolites. Really all of the data on metabolism, bone marrow toxicity, structure activity, and genotoxicity lead to this conclusion. OSHA should expressly recognize in these sections the quantitative interspecies differences rather than the qualitative aspect, and then implement this conclusion in the quantitative risk assessment of butadiene.

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November 7, 1990
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EDUCATION:

B.Sc. Joint Honors Chemistry/Zoology, Microbiology subsidiary, Nottingham University, U.K. (1970)

M.Sc. Toxicology, Surrey University, U.K. (1974)

Ph.D. Biochemistry, Surrey University, U.K. (1985)

HONORS/POSITION:

Awarded Zoological Society Prince Philip prize (1967).

President of the University Biological Society (1970) and University Canoe Club (1969).

DISSERTATION RESEARCH:

Early biochemical and morphological changes in the skin with particular reference to skin tumor promotion.

EXPERIENCE:

1985-Present: Toxicology Associate, Special Products Unit, Exxon Biomedical Sciences, Inc., East Millstone, N.J.

Company focus for health research and regulatory activity on 1,3 butadiene and benzene.

Representative on the International Institute of Synthetic Rubber Producers and the Chemical Manufacturers Association health committees to develop toxicity research programs and to provide input to regulatory agencies on risk assessment. Liaison link with the National Toxicology Program.

Co-organizer of NIEHS/Industry 1988 symposium on 1,3-butadiene.

Chairman of the International Institute of Synthetic Rubber Producers/National Toxicology Program working group on isoprene.

Participant in the American Petroleum Institute Task Forces on benzene metabolism and pharmacokinetic research. Toxicology focus for interaction with the Chemical Industry Institute of Technology.

Involved in emergency exposure and community exposure guidelines, in health aspects of refining clean-up operations and in molecular modeling.

Responsible for comparative systems for compliance with EEC labeling legislation, and the OSHA Communication Standard, and WHMIS regulations.

Consultant to Exxon Basic Chemicals Product group worldwide including PMN and other regulatory submissions, eg. SARA Title 302 and 313 delisting petitions.

1982-1985: Chemical Toxicology Unit Head/Senior Staff Toxicologist Exxon Research & Environmental Health Div., E. Millstone, NJ.

- Responsibility for the planning, budget (>3 million dollars) and progress of all Exxon Chemical toxicology programs which included supervisory responsibilities for 6 Ph.D staff toxicologists.
- Coordination contact for chemical affiliate toxicology programs.
- Supervision of unit's FDA advisory service to Chemical and Petroleum operations.
- Study monitor for 2 year cancer bioassay (feeding study) of a plasticizer at Exxon Environmental Health Science Laboratory.
- Developed systems for provision of toxicology information for compliance with EEC Labeling of Dangerous Substances and OSHA Hazard Communication Act (this has included development of computerized matrix algorithm programs).

- Collaborative research degree studies with Professor B. D. Goldstein and Dr. G. Witz, CMDNJ Rutgers Medical School, N.J. in conjunction with Professor D. V. Parke, Surrey University, U.K.; practical work included mouse skin explant and in vivo enzymatic studies to determine early changes in the skin following application of petrochemical and petroleum derived materials.

1978-1981: ECEU Toxicologist, Essochem Europe Brussels, Belgium

Provision of toxicology advice to all aspects of the European regional chemicals operations and to Esso Europe petroleum activities including product & process relevant test programs, customer questions, in-house and external (University) training programs/ presentations on all aspects of toxicology. Designed and initiated contracted joint industry toxicity and mutagenicity studies on isobutylene to answer concerns of Belgian and EEC authorities. Carried out round robin toxicity test comparison of European contract laboratories and a comparison of skin sensitization techniques in guinea pig.

Participated in UK Institute of Petroleum (IP) used/ unused oil cancer bioassay; European Additives Technical Committee (ATC) mutagenicity studies of zinc dialkyldithiophosphates (lubricant additives).

Chairman of toxicity subcommittee of Health Advisory Group for CONCAWE (the oil companies' international study group for Conservation of Clean Air and Water Europe).

Chairman MEK-JACC group of ECETOC (European Chemical Industry Ecology and Toxicology Centre).

Committee member; IP Advisory Health Committee Toxicology subgroup; UK Chemical Industry Health and Safety Executive Committee; ATC Toxicology Subgroup.

1977-1978: Assigned to Exxon Corp. Medical Department's Research & Environmental Health Division (REHD), Linden, NJ, USA.

- Design and monitoring of contracted 90 day oral feeding studies in rat and beagle dog. Interpretive reports prepared and data presented in person to the German BGA (and also subsequently to UK authorities) for indirect food additive clearance.

- Identified association between previous human respiratory cancer cases (IPA/Ethanol processes) and dialkyl sulfates; participated in Exxon TSCA 8e submission.

- Responsible for toxicology programs in Solvent and additive programs including animal inhalation studies.

1976: ECEU Toxicologist Essochem Europe Brussels, Belgium (2 month orientation and plan development)

1975-1976: Toxicologist, BP Group.

- Design and arrangement of animal testing with contract laboratories and monitoring of studies conducted. Provision of interpretive reports and recommendations.

- Liaison with British Industrial Biological Research Association over sponsored studies.

- BP toxicology representative in Joint Industry Group studies e.g., whole body inhalation carcinogenicity studies of chloroprene.

Initiation within BP of short term mutagenicity studies and responsibility for the incorporation into the overall toxicity assessment.

- Provision of toxicology consultancy to BP Group.

- Member of Chemical Industry Safety and Health Executive Committee (CISHEC).

1973-1974: Sponsored by British Petroleum for new M.Sc. Course in Toxicology at Surrey University. Awarded M.Sc. based on examination and research project - Liver enlargement and associated phenomena on oral administration of the antioxidant butylated hydroxy toluene (BHT) to mice. (Supervisor Professor P. Grasso).

1971: Transferred to BP Group's Occupational Health Unit - Assisted with toxicological aspects of Unit's work including skin and respiratory sensitization studies of protein derived from petroleum hydrocarbons, and skin absorption of phenol in pigs.

1970: Technologist, British Petroleum Co., Sunbury, Middx, UK - Member of project team on synthetic lubricants for supersonic aircraft in particular Concorde, this involved organic chemical synthesis, TLC and quantitative densitometric determinations.

SOCIETIES:

Chemical Society, European Society of Toxicology, European Environmental Mutagen Society, Society for Drug Research, Laboratory Animal Science Association, Mid-Atlantic Society of Toxicology, British Toxicology Society, British Occupational Hygiene Society.

PERSONAL:

Birthplace: Walton-on-Thames, Surrey, UK
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Summit, NJ USA (October 1, 1954)

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OUTSIDE INTERESTS:

Squash, Canoeing, Inland Waterways, and Industrial Archeology.

FOREIGN LANGUAGES:

French and German - write, read, (speak)

PUBLICATIONS:

Lynch, J., Hanis, N.M., Bird, M.G., Murray, K.J., and Walsh, J.P. An Association of Upper Respiratory Cancer with exposure to dialkyl sulfate. J. Occup Medicine 21: 333-341

Reed, K.G., Bird, M.G., (1979) Forg och Lack The Use of Dearomatized Aliphatic Solvents in Household Paints and Wood Preservative Solutions

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Bird, M.G. Environmental Health Perspectives (in press) Future Directions - Toxicology Studies of Butadiene and Isoprene.

Clark, D.G., Butterworth, S.T.G., Martin, J., Roderick, H.R., and Bird, M.G. Toxicology and Industrial Health (in press) Inhalation Toxicity of High Flash Aromatic Naphtha.

Acquavella, J.F., Owen, G.V., Bird, M.G., Yarborough, C.M., and Lynch, J. Amer. J. Epidermiol (Libienfield prize paper) submitted for publication. An adenamatous polyp case control study to assess occupational risk factors for a workplace colorectal cancer cluster.

Owen, C., Acquavella, J.F., Lynch, J. and Bird, M.G. Amer. Ind. Hyg. Assn. Journal (submitted for publication) An Exposure Estimating Methodology in Support of a Retrospective Morbidity Case-Control Study.

ABSTRACTS/POSTER PRESENTATIONS:

Bird, M.G. (1979)

American Industrial Hygiene Assoc. Meeting, Minneapolis, USA
A comparative study of liver enlargement and associated phenomena on oral administration of butylated hydroxytoluene (BHT) to mice.

Bird, M.G., Witz, G. and Goldstein, B.D. (1983)

Mid-Atlantic Society of Toxicology Meeting, Wilmington, DE
Hydrogen Peroxide Formation in Mouse Skin following topical application of phorbol esters; its possible association with macrophage infiltration.

Bird, M.G., Traul, K.A., Witz, G. and Goldstein, B.D. (1984)
Toxicologist 4 (1) #155

The effect of tumor promotor phorbol-12-myristate-13-acetate (PMA) on cultured mouse skin explants.

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Docket No. H-041

INFORMAL PUBLIC HEARING ON THE PROPOSED OSHA STANDARD ON OCCUPATIONAL EXPOSURE TO 1,3-BUTADIENE

TESTIMONY REGARDING THE TOXICOLOGY OF 1,3-BUTADIENE

Testimony by

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Testimony Regarding the Toxicology of 1,3-Butadiene

I. Introduction

1. I am Professor of Toxicology and Occupational Medicine at the University of Dortmund (since 1982) and head of the Department of Toxicology and Occupational Medicine.

   I have been Professor of Toxicology at the Universities of Tübingen (1978-1979) and Mainz (1979-1982). My CV is attached.

   Current memberships of boards and committees:
   - EUROTOX (Executive Board member)
   - German Society of Occupational Medicine (Executive Board member)

- "Specialized Expert" in the Field of Carcinogenicity/Mutagenicity/Teratogenicity (European Communities, DG XI)
- European MAC-Commission (European Communities, DG V)
- Commission for the Investigation of Health Hazards of Chemical Compound in the Work Area, F.R.G.
- Committee of Dangerous Substances, F.R.G.
- Scientific Advisory Board of the Institute of Drugs, Federal Health Office, and of the GSF (research institution), München (F.R.G.)
- Editor in Chief of the journal "Archives of Toxicology", Editor of several other scientific journals

2. I have been active in 1,3-butadiene research since 1980 and have authored and co-authored many scientific articles on this topic. The most important articles of our group are included in the references list (see "Appendix a" to this testimony).

I have been involved in the work of the 1,3-Butadiene Working Group of the F.R.G. Committee of Dangerous Substances which has set the workplace limit ("Technical Guiding Concentration", TRK) for 1,3-butadiene. The present TRK in Germany is for processing after polymerisation, loading: 15 ppm (34 mg/m^3), and for all other workplace areas 5 ppm (11 mg/m^3). Basis of this regulation was the current state of industrial processing methods and no conflicting experience from the fields of occupational medicine or toxicology.

3. The purpose of the statement is to summarize our studies on butadiene conducted in Germany and to discuss the implications of the results for human risk assessment.

4. The available data demonstrate that long-term studies on butadiene in mice do not adequately reflect the human situation because of species differences in the activating/deactivating enzymes.

   Hence, direct quantitative extrapolations of risk from the mouse studies on 1,3-butadiene to man seem not to be justified.

II. Description and Discussion of the Studies

1. Aim of the studies

1,3-Butadiene, a major component in synthetic rubber and adiponitrile manufacture, has produced cancer at multiple sites in two species of experimental animals. In an inhalation bioassay with Sprague-Dawley rats (exposure to 1000 and 8000 ppm for 110 weeks) butadiene affected exclusively organs with endocrine functions and was considered a weak onkogen (Hazleton Laboratories Europe, 1981). A recent long-term study with $B6C3F_1$-mice (exposure to 625 and 1200 ppm for 60 weeks) revealed a considerably higher carcinogenic activity (Huff et al., 1985). Target organs were lymphatic tissue, heart, lung, mammary gland and

possibly liver. This study was corroborated by a second mouse study (Melnick et al., Environmental Health Perspect. 86: 27, 1990) at lower exposure concentrations ranging from 6.25 to 625 ppm 1,3-butadiene.

The purpose of our studies was

(1) to investigate the routes and mechanisms of activation and deactivation of butadiene and its reactive metabolite(s),

(2) to investigate species differences in metabolism to explain the apparent differences in carcinogenic activity of 1,3-butadiene between rats and mice,

(3) to set a basis of an understanding as to whether butadiene action in man should be similar to that in one of both rodent species.

The studies were performed, under my supervision, at:

- Institute of Pharmacology and Toxicology, University of Mainz (1980-1982), and
- Institute of Occupational Health (Institut für Arbeitsphysiologie), University of Dortmund (1983-1989).

2. Knowledge on butadiene metabolism at the time when our research program started

In microsomal incubates butadiene was metabolized by cytochrome P-450 enzymes to 1,2-epoxybutene-3 (I). Further metabolic transformation of epoxybutene by epoxide hydrolase and/or monooxygenase led to 3,4-epoxy-1,2-butanediol (III, via 3-butene-1,2-diol) and to diepoxybutane (II, see Fig. 1). Epoxybutene was conjugated with glutathione and metabolized by glutathione-S-transferase (Malvoisin et al., 1979; Malvoisin et al., 1982; Malvoisin and Roberfroid, 1982; Bolt et al, 1983). 1/

3. Pharmacokinetics and metabolism of butadiene and epoxybutene in rats and mice

To investigate the species differences in butadiene induced carcinogenesis comparative studies on inhalation pharmacokinetics, metabolism and DNA-binding of inhaled butadiene were conducted in rats and mice.

The pharmacokinetics of distribution and metabolism of butadiene and epoxybutene in mice were investigated on inhalation (Kreiling et al., 1986; Kreiling et al., 1987) at the Universities of Mainz and Dortmund (see above). Kinetic parameters were determined based on a pharmacokinetic model developed by Filser and Bolt (1981). A comparison of these results with the pharmacokinetics parameters of both compounds in rats was performed (Bolt et al., 1984; Filser and Bolt, 1984). The following results were obtained.

3.1 Metabolism of butadiene

To compare the metabolic elimination rates of butadiene in rats and mice, we placed mice and rats in separate closed chambers

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1/ For convenience, details of study methodologies and results are presented in single-spaced text. This is customary in Germany.

with fixed concentrations of butadiene in the air, and then measured the decline in butadiene concentration over time. The decline in concentration indicates that there has been uptake and metabolism of butadiene by the test animal.

Calculated metabolic elimination rates of butadiene for rats and mice depending on the atmospheric concentration of the compound are shown in Fig. 2. Up to ambient concentrations of about 1000 ppm butadiene metabolic elimination of butadiene is proportional to the exposure concentration in mice and rats. Above 1000 ppm saturation kinetics of butadiene metabolism become apparent in both species.

Fig 2 shows that the metabolic elimination rate of butadiene in mice is about twice that in rats, both under conditions of low and high exposure concentrations.

This means that mice metabolize 1,3-butadiene to the reactive metabolite epoxybutene at about twice the rate seen in the rat.

3.2 Metabolism of epoxybutene, the reactive intermediate

We also examined epoxybutene metabolism by measuring the exhalation of epoxybutene in a closed chamber.

Fig. 3 shows the (calculated) metabolic elimination rates of epoxybutene for rats and mice depending on the atmospheric concentration of the compound in this experimental system. A comparison of both species reveals that at lower exposure concentrations mice show a higher metabolic rate for epoxybutene than rats. Epoxybutene metabolism in rats is linearly dependent on the atmospheric concentration of the compound up to exposure concentrations of about 3000 ppm (Kreiling et al., 1987). In mice saturation of epoxybutene metabolism can be observed at about 500 ppm.

Thus, with increasing exposure concentration the metabolic capacity for epoxybutene becomes rate limiting in mice but not in rats.

This means that the mouse, which forms epoxybutene at twice the rate of the rat, is less capable of eliminating this reactive metabolite, particularly at butadiene exposure levels above 500 ppm. One would expect, therefore, to see higher levels of epoxybutene in the blood and tissue of the mouse compared to the rat under identical exposure conditions.

3.3 Exhalation of epoxybutene (reactive metabolite) during exposure to butadiene

Exhalation of epoxybutene into the atmosphere of a closed exposure system can be measured when mice or rats are exposed continuously to high butadiene concentrations (above 2000 ppm; Filser and Bolt, 1984; Kreiling et al., 1987). Fig. 4 shows that exhalation of epoxybutene by mice leeds to an increase of epoxybutene in the atmosphere of the system with exposure time, until a peak concentration of about 10 ppm is reached after 10 h. Epoxybutene exhaled by rats reaches a plateau concentration of about 4 ppm after 2 h of butadiene exposure. The subsequent decline in epoxybutene concentration in the experiment with mice is the result of a decreased butadiene metabolism. From about 12 h onwards mice showed signs of acute toxicity and liver non-protein sulfhydryl content of the animals was practically depleted. In rats using the same protocol the hepatic non-protein sulfhydryl content showed no major depletion and no toxicity was observed (Kreiling et al., 1988).

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These observations can be explained by pharmacokinetic differences between the rat and the mouse. Since the metabolic elimination of 1,2-epoxy-3-butene in mice is a saturable process, the concentration of 1,2-epoxy-3-butene metabolized from 1,3-butadiene gradually increases and is exhaled. This results in an increase in the atmospheric concentration of 1,2-epoxy-3-butene in the closed-chamber system. As long as the atmospheric butadiene concentration remains high enough to saturate epoxybutene metabolism, there is a gradual increase in the internal concentration of 1,2-epoxy-3-butene. In rats, this increase in the internal concentration of 1,2-epoxy-3-butene does not occur since its metabolism does seem to saturate even at external butadiene concentrations of 5000 ppm.

The difference in epoxybutene exhalation between both species indicates that <u>in mice the elimination process of epoxybutene proceeds under saturation conditions even when the compound is metabolically generated during exposure to high butadiene concentrations.</u>

4. Butadiene-induced depletion of non-protein sulfhydryl content in different tissues of rats and mice

The effects of different exposure concentrations of butadiene on the cellular non-protein sulfhydryl (NPSH) content of liver-, lung- and heart tissue were investigated in mice and rats (Deutschmann and Laib, 1989). NPSH depletion is an indication of the amount of systemically available epoxide intermediates. This is because reactive epoxides, such as epoxybutene, react with

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intracellular glutathione which is the major constituent of NPSH compounds. For a comparison of the data with the animal bioassays, the experimental outline of the study was chosen as close as possible to the longterm inhalation studies (i.e., the rat bioassay and the first mouse bioassay, the data of which were available at the time when the studies described here were started). Groups of male animals of both species were exposed for 7 h in an "open" exposure system to different concentrations of butadiene. Tissue NPSH content was determined immediately after exposure according to a modified Ellman procedure.

A comparison of both species is shown in Fig. 5. In liver and lung of rats only moderate effects of butadiene could be measured. A comparison of the dose-dependent depletion of tissue NPSH with the actual exposure concentrations used in the bioassay with rats should be possible under the assumption that butadiene metabolism and NPSH resynthesis remain unchanged during chronic exposure. On this basis a recurrent daily depletion of NPSH content in liver of about 25 or 60 %, and in lung of about 20 or 30 % can be estimated for the low (1000 ppm) and high (8000 ppm) exposure concentration respectively. Drastic effects of butadiene on NPSH content in tissues of mice could be demonstrated. Under conditions of the (first) animal bioassay with mice (and with the assumptions detailed above) the estimated recurrent daily depletions of NPSH content were in liver about 50 and 70 %, in lung about 70 and 90 % and in heart about 25 and 40 %, for the low (625 ppm) and high (1250 ppm) exposure concentration respectively. Cardiac tissue does not significantly contribute to xenobiotic metabolism. NPSH depletion in heart tissue of the mouse can be regarded as an indictor for systemically available epoxide intermediates. That the heart is passed by the efferent blood flows of two butadiene metabolizing organs may explain its special suspectibility as a target organ of butadiene carcinogenesis in the mouse.

5. Alkylation of nulear proteins and DNA by reactive butadiene metabolites

The tumor initiating properties of 1,3-butadiene according to our data and the data of others, are causally connected with the

DNA-alkylating properties of reactive metabolites of 1,3-butadiene (e.g., epoxybutene). Hence, it was important to comparatively study alkylation of nuclear proteins and of DNA after exposure of mice and rats to 1,3-butadiene. Furthermore, the chemical nature of DNA-binding products was investigated (section 5.1).

When mice ($B6C3F_1$) and rats (Wistar) were exposed to (1,4-^{14}C)1,3-butadiene (spec. radioactivity 11,2 mCi/mmol) butadiene derived radioactivity was covalently bound to liver nucleoprotein fractions and to total liver DNA of both species (Kreiling et al., 1986). Covalent binding of reactive butadiene metabolites to liver nucleoproteins of mice was about twice as high as in rats. This shows that, parallel to the about 2-fold higher metabolic rate of butadiene in mice, the formation of reactive protein-binding metabolites is proportionally increased in this species. Comparable amounts of (^{14}C)-butadiene derived radioactivity were associated with total liver DNA of both species.

5.1 Formation of the DNA adducts 7-N-(1-hydroxy-3-buten-2-yl)-guanine and of 7-N-(2,3,4-trihydroxybutyl)guanine

7-N-(2-hydroxy-3-buten-1-yl)guanine (I), 7-N-(1-hydroxy-3-buten-2-yl)guanine (II) as (expected) reaction products of guanine residues with epoxybutene were synthesized according to Citti et el. (1984). 7-N-(2,3,4-trihydroxybutyl)guanine (III) as an expected reaction product of guanine residues with diepoxybutane was synthesized by reaction of diepoxybutane with guanosine and subsequent acid hydrolysis. The compounds (see Fig. 6) were characterized by physico-chemical methods. Male Wistar rats and male $B6C3F_1$ mice were exposed to initial concentrations of 500 ppm (1,4-^{14}C)1,3-butadiene (spec. radioactivity 11,2 mCi/mmol) in a closed exposure system. Liver-DNA was isolated and purified by hydroxylapatite chromatography. DNA with I, II and III added as non-radioactive markers was subjected to acid depurination and the DNA hydrolysates were analysed by columnn chromatography

(Jelitto et al., 1989). Analysis of mouse liver DNA revealed the elution of radioactivity within the first fractions and further 2 peaks of radioactivity associated with the added marker compounds I and III (see Fig. 7). Upon analysis of rat liver DNA only radioactivity associated with the first fractions of the column was eluted.

This shows that 7-N-(1-hydroxy-3-buten-2-yl)guanine and 7-N-(2,3,4-trihydroxybutyl)guanine, reaction products of epoxybutene and diepoxybutane with DNA can be identified in liver DNA of mice, but not of rats after exposure of the animals to 14C-butadiene.

5.2 Formation of DNA-DNA and DNA-protein crosslinks

Male Sprague-Dawley rats and male $B6C3F_1$ mice were exposed for 7 h to 250, 500 and 1000 ppm butadiene. Immediately after exposure cell nuclei of liver and lung tissue were isolated and subjected to alkaline elution according to a modification of the method of Sterzel et al. (1984). Alkaline elution curves obtained from the tissues of mice show the occurrence of protein-DNA and DNA-DNA crosslinks from about 250 ppm butadiene onwards (Fig. 8). No crosslinking activity of butadiene was observed for rats (Jelitto et al., 1989). The crosslinking activity of butadiene in the mouse can be attributed to its reactive and bifunctionally alkylating intermediate diepoxybutane.

III. Implications for human risk assessment

Our investigations on inhalation pharmacokinetics of butadiene and its primary reactive intermediate epoxybutene in mice and rats have reasonably demonstrated that the species differences observed in butadiene carcinogenicity are related to species differences in metabolism of this compound. In addition to the higher metabolic rate of butadiene in mice, limited detoxification and accumulation of its reactive epoxide intermediate epoxybutene must be major determinants for the higher susceptibility of mice.

The detection of alkylation products of epoxybutene and diepoxybutane with guanine residues in liver DNA of mice exposed to butadiene indicates that epoxybutene is further biotransformed to diepoxybutane in this species. This view is supported by the crosslinking activity of butadiene towards DNA and proteins in mice which can be attributed to the bifunctional alkylating diepoxybutane.

The quantitative differences in butadiene metabolism and in biological effectivity of the reactive epoxide intermediates between rats and mice reflect the different enzyme activities involved in butadiene metabolism which are important in view of human risk assessment.

Tab. 1 shows the ratios of specific enzyme activities in subcellular preparations of rodent and human liver and lung, as calculated from published data by Lorenz et al. (1984). The estimated

ratios of specific enzyme activities of hepatic enzymes in mice vs. rats are for monooxygenases 2:1, for epoxide hydrolases 1:4 and for glutathione-S-transferases 5:1. For lung tissue these ratios are about 7:1 (monooxygenases), 1:1 (epoxide hydrolases) and 10:1 (glutathione-S-transferases). Although these specific enzyme activities were not established with butadiene or its epoxides as substrates the ratios demonstrate, that the higher capacity for epoxidation in mice vs. rats can not be adequately counterbalanced by epoxide detoxification through epoxide hydrolase in this species. Thus the major part of epoxide detoxification in mice should proceed via glutathione-S-transferase mediated pathways with the result of glutathione depletion and a subsequently increased toxicity and covalent binding of reactive butadiene intermediates. The drastic depletion of tissue NPSH observed in mice but not in rats after acute exposure of the animals to butadiene is supportive of this view.

All the available data demonstrate that from a quantitative point of view the long-term studies with mice do not adequately reflect the human situation. Table 1 shows that, with respect to the activating and de-activating enzymes involved in the biotransformation of 1,3-butadiene in liver and lung, the mouse is only a very poor model for the situation occurring in man. Closer to the human situation is the rat of which the tumor response to 1,3-butadiene is only very weak.

These conclusions from our own studies are supported by studies of others which have included monkeys. Schmidt and Löser (Arch. Toxicol. 57, 222, 1985) have incubated 1,3-butadiene with

post-mitochondrial fractions from $B6C3F_1$-mice, Sprague-Dawley rats, rhesus monkeys and man (lung and liver). Metabolism was faster with preparations from mice, followed by rats, then followed by the primates (man, monkey).

Dahl et al. (Environmental Health Perspect. 86, 65, 1990) have reported about metabolism studies on 1,3-butadiene in several species including monkeys. Blood levels of toxic metabolites of 1,3-butadiene were lower in monkey than in rodents.

These studies are in line with ours and demonstrate that the quantitative metabolism of 1,3-butadiene is very much different between mice and primates.

IV. Summary and Conclusions

1. Summary

Comparative investigations of inhalation pharmcokinetics of 1,3-butadiene and of its reactive metabolic intermediate, epoxybutene, revealed major differences in metabolism of these compounds in rats (Sprague-Dawley) and mice ($B6C3F_1$).

In both species metabolism of butadiene to epoxybutene follows saturation kinetics, but mice metabolize butadiene at about twice the rate of rats. Epoxybutene metabolism is saturable in mice at low atmospheric concentrations of the compound, whereas in rats no indication of saturation of epoxybutene metabolism can be observed. Under conditions of saturation of butadiene metabolism, considerably higher amounts of epoxybutene are exhaled by mice when compared to rats. When mice or rats are exposed for 7 h to different concentrations of butadiene, a dose-dependent depletion of cellular nonprotein sulfhydryl (NPSH) content can be observed in mice for all tissues (liver, lung, heart) examined. Depletion of NPSH content starts from about 250 ppm butadiene onwards. In rat tissue NPSH content is significantly reduced at high exposure concentrations and in liver only. After exposure of rats and mice to ^{14}C-butadiene, radioactive 7-N-(1-hydroxy-3-buten-2-yl)guanine and 7-N-(2,3,4-trihydroxybutyl)guanine, reaction products of epoxybutene and possibly diepoxybutane with guanine can be detected in liver DNA of mice but not of rats. In addition DNA elution curves obtained from liver and lung tissue of mice show the occurrence of protein-DNA and DNA-DNA cross-links from about 250 ppm butadiene onwards. No such crosslinking activity of butadiene was observed for rats. These data indicate that epoxybutene and diepoxybutane are ultimate genotoxic principles in butadiene metabolism and responsible for the carcinogenic properties of this compound.

The quantitative differences, in biological effectivity of the reactive epoxide intermediates between rats, mice and possibly man reflect the different ratios of specific enzyme activities involved in butadiene metabolism in these species.

2. Conclusions

1,3-Butadiene shows a carcinogenic response in Sprague-Dawley rats and $B6C3F_1$ mice. However, both species behave much different: 1,3-butadiene is a relatively strong carcinogen in the mouse, but only a very weak one in the rat.

The species differences in carcinogenic response to butadiene can reasonably be explained by species differences in metabolism and are due to different activities of activating and deactivating enzymes. According to literature data on the activities of these enzymes in mice, rats and humans, it must be concluded that, quantitatively, the mouse is a poor model for the carcinogenicity of 1,3-butadiene. The rat, in which 1,3-butadiene exhibits a very weak carcinogenic response only, is much closer to the human situation, as far as the activities of the butadiene-transforming enzymes are concerned.

Hence, quantitative human risk estimates for 1,3-butadiene based on mouse data can be expected to extensively overestimate the actual risk for man.

Dortmund, October 9, 1990

Hermann Bolt

(Prof. Dr. Dr. Hermann M. Bolt)

Appendix: (a) References

(b) Figures and table, including legends

(c) Curriculum vitae

Appendix: (a) References

(b) Figures and table, including legends

(c) Curriculum vitae

(a) References (Authors of publications from our own research program underlined).

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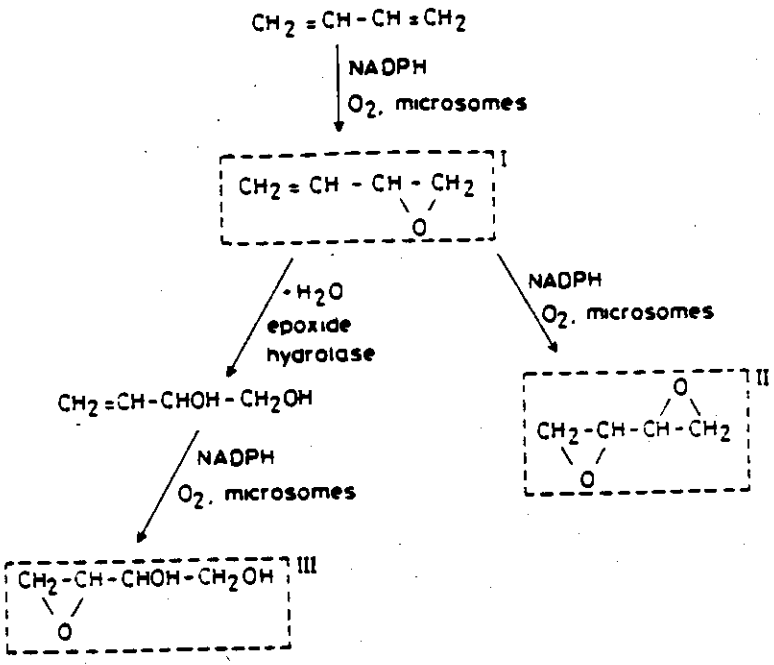


Fig. 1: Metabolism of butadiene according to Malvoisin and Roberfroid (1982). The potential reactive intermediates are framed.

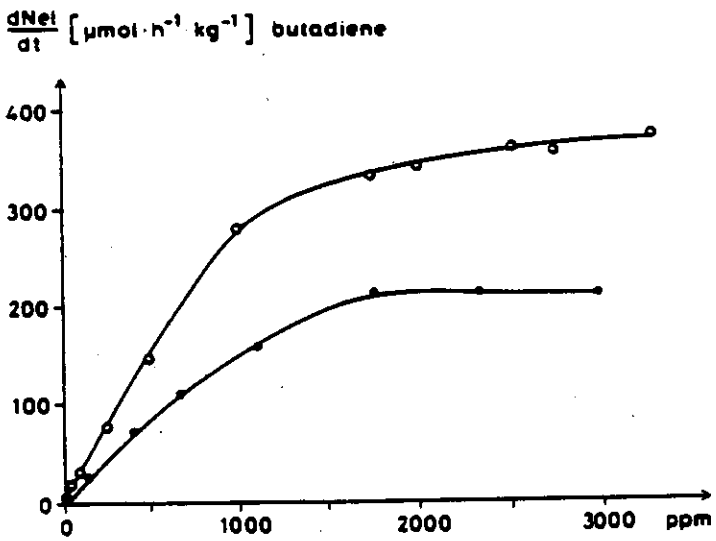


Fig. 2: Metabolic elimination rate of butadiene in mice (o) ($B6C3F_1$), and rats (•) (Sprague-Dawley) depending on the atmospheric concentration of the compound.

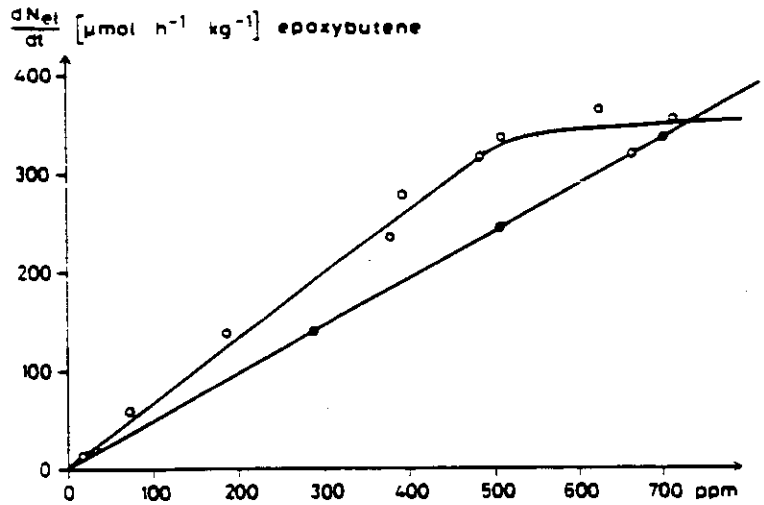


Fig. 3: Metabolic elimination rate of epoxybutene in mice (o) ($B6C3F_1$) and rats (•) (Sprague-Dawley) depending on the atmospheric concentration of the compound. Metabolism of epoxybutene in rats is linearly dependent on the concentration of the compound up to 5000 ppm epoxybutene.

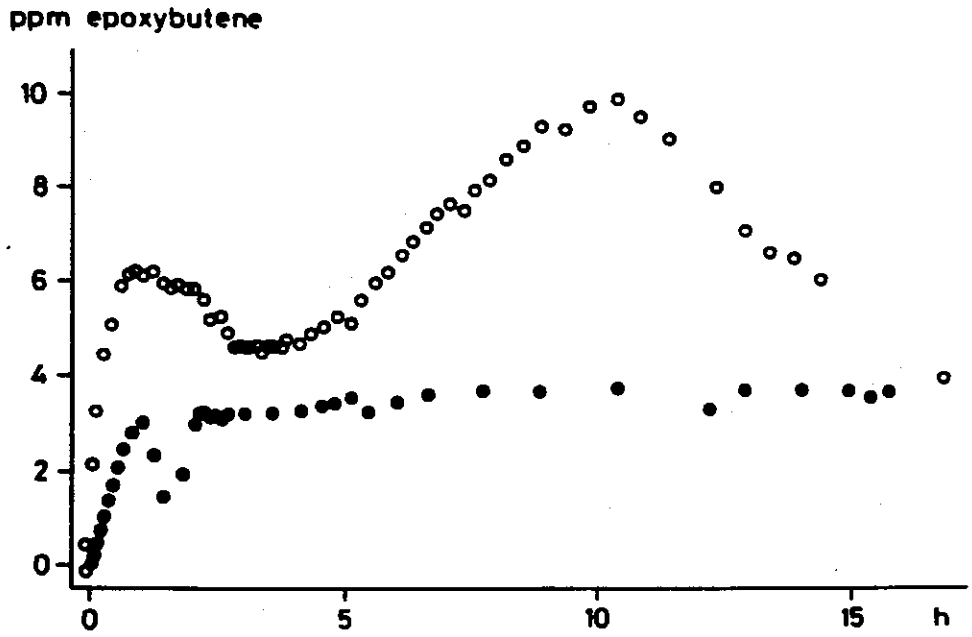


Fig. 4: Time courses of epoxybutene concentrations exhaled into the atmosphere of a closed exposure system (6,4 l) by two Sprague-Dawley rats • or six $B6C3F_1$ mice ○ during continuous exposure to high butadiene concentrations

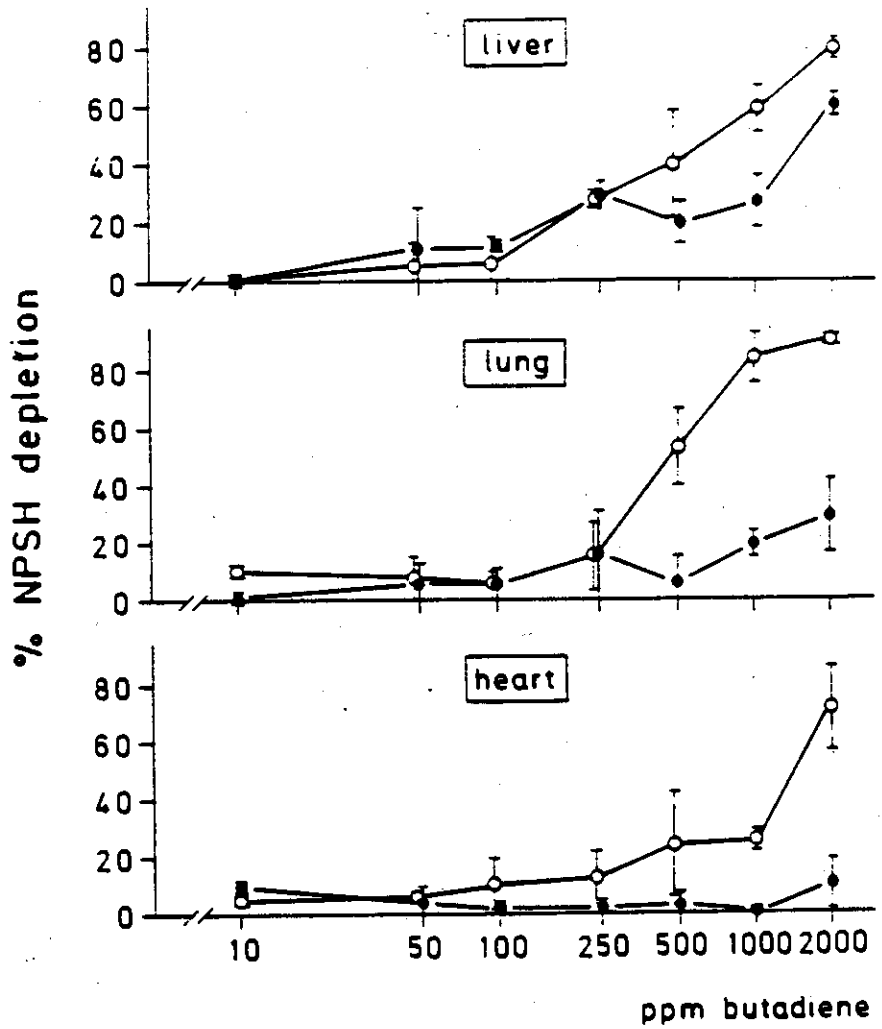
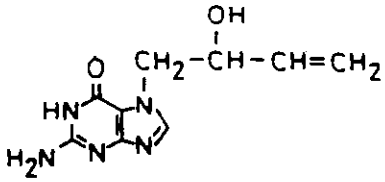
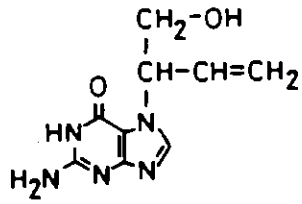


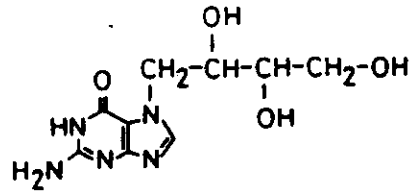
Fig. 5: Depletion of NPSH content in liver-, lung-, and heart tissue of Sprague-Dawley rats and $B6C3F_1$ mice after a 7 h exposure to butadiene. Depletion of NPSH content is expressed in % of the corresponding control value (± SD) and plotted as a function of the logarithm of the expo- exposure concentration.



I 7-N-(2-hydroxy-3-buten-1-yl)guanine



II 7-N-(1-hydroxy-3-buten-2-yl)guanine



III 7-N-(2,3,4-trihydroxybutyl)guanine

Fig. 6: Reaction products of metabolic intermediates of butadiene with guanine residues.

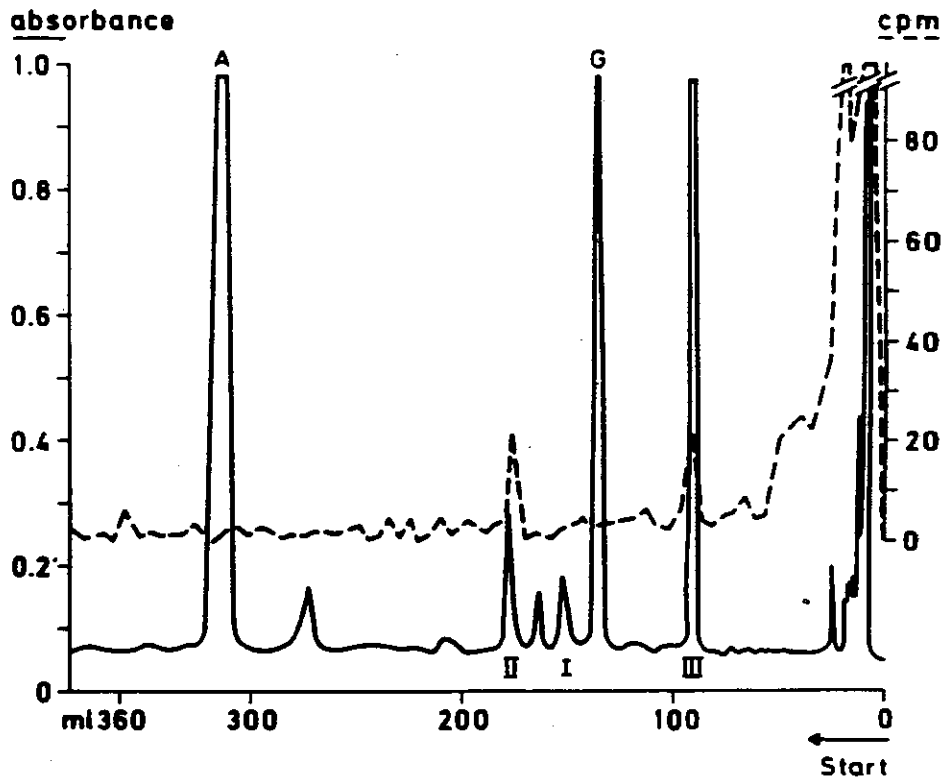


Fig. 7: Analysis on Aminex-A6 of a hydrolysate of liver DNA from mice ($B6C3F_1$) exposed to ^{14}C-butadiene. The non-radioactive markers I, II and III had been added prior to hydrolysis. Elution of radiactivity (top) and of UV-absorbing material (bottom)

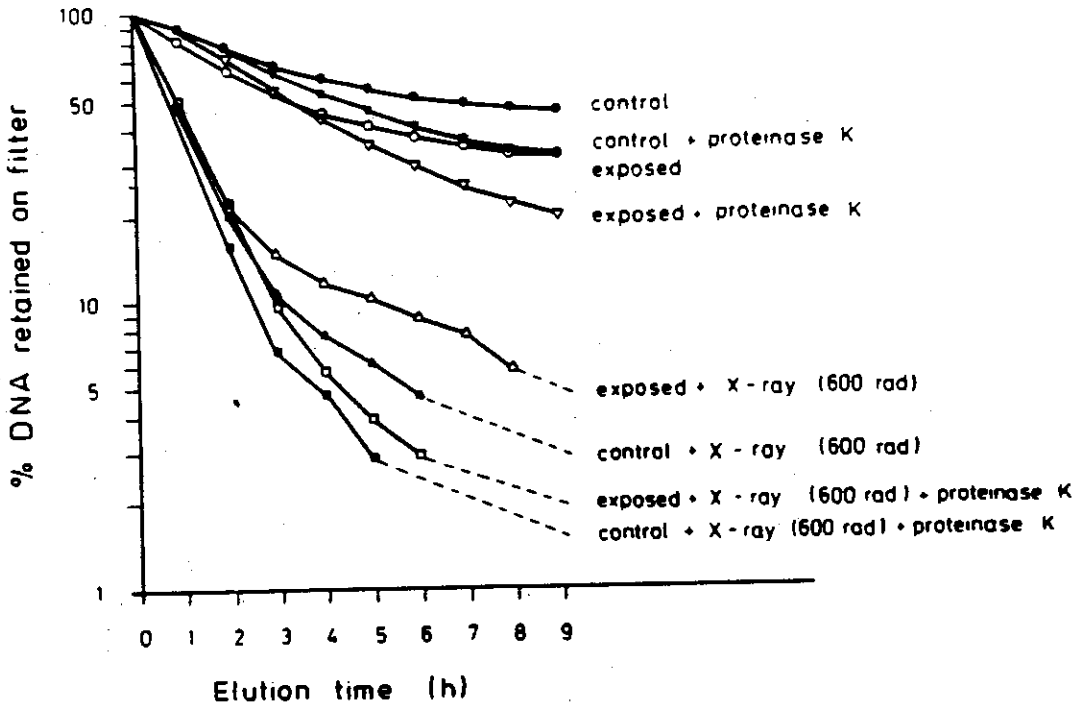


Fig. 8: Alkaline elution curves of liver DNA from $B6C3F_1$ mice exposed to 500 ppm butadiene for 7 h. Controls were exposed to air only.

Table 1: Ratios of specific enzyme activities in subcellular preparations of rodent and human liver and lung (calculated from Lorenz et al., 1984)

	Monooxygenase		Epoxide-hydrolase		Glutathione-S transferase	
	liver	lung	liver	lung	liver	lung
NMRI mouse	4	1220	0.25	0.7	4	10
Sprague-Dawley rat	2	180	1	0.7	0.8	1
Man	1	1	1	1	1	1

Short Curriculum Vitae

Hermann M. Bolt,
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Born Jan. 13, 1943 in Kirchen/Sieg (Germany)

Education in Cologne, then
- studies of medicine, University of Cologne, 1962-1967;
- studies of biochemistry, University of Tübingen, 1968-1971.

Doctoral degrees: Dr. med., 1968; Dr. rer. nat., 1973.

1971-1979: At the Institute of Toxicology, University of Tübingen (Head: Prof. Dr. H. Remmer), as scientific assistant and lecturer.

1974: Habilitation in Biochemical Pharmacology.

1979-1982: Head of Section of Toxicology at the Institute of Pharmacology, University of Mainz.

Since 1982: Director at the Institute of Occupational Health (Institut für Arbeitsphysiologie), University of Dortmund, and Chairman of the Department of Toxicology and Occupational Medicine.

Commission and committee memberships (as of 1990)

- Commission for the investigation of health hazards of chemical compounds in the work area, Deutsche Forschungsgemeinschaft,
- Committee on dangerous substances, Federal Minister of Labor and Social Affairs, F.R.G.
- Specialized experts in the field of carcinogenicity, mutagenicity and teratogenicity, Commission of the European Communities, Bussels,
- Medicines commission of the German physicians,
- Cosmetics commission, Federal Heelth Office, F.R.G.

Executive board (committee) memberships

- Federation of the European Societies of Toxicology (FEST)/EUROTOX
- German Society of Occupational Medicine
- German Society of Pharmacology and Toxicology
  (Chairman of the Section of Toxicology)

Editorial board memberships

- Archives of Toxicology (Editor-in-Chief)
- International Archives of Occupational and Environmental Health (Cooperating Editor)
- Journal of Biochemical Toxicology (Associate Editor)
- CRC Critical Reviews in Toxicology (Editorial Advisory Board)

A list of publications is available on request.

Feb. 8, 1990

Hermann Bolt

(Hermann M. Bolt)

TESTIMONY OF DR. THOMAS B. STARR

My name is Thomas B. Starr. I am currently Principal, Health Sciences Division, ENVIRON Corporation, Arlington, Virginia, a consulting firm providing counsel in health and environmental sciences, and Adjunct Assistant Professor of Biostatistics, University of North Carolina School of Public Health, Chapel Hill, North Carolina. I received by Ph.D. in Physics from the University of Wisconsin at Madison in 1971. Following a National Science Foundation Postdoctoral Fellowship in the Institute for Environmental studies at Wisconsin, I served on that Institute's academic staff and faculty from 1972 to 1981. I subsequently joined the Chemical Industry Institute of Toxicology (CIIT), Research Triangle Park, North Carolina, serving first as Scientist in the Department of Epidemiology from 1981 to 1987, and then from 1987 to 1989 as Director of the CIIT Program on Risk Assessment.

Since 1984 I have served on the Halogenated Organics Subcommittee of the United States Environmental Protection Agency's Science Advisory Board Environmental Health Committee. I have testified before the United States Occupational Safety and Health Administration (OSHA) regarding human health risks posed exposures to formaldehyde and cadmium. I have served on the Air Toxics Panel of the North Carolina Academy of Sciences, the North Carolina Environmental Management Commission's Ad Hoc Committee for Air Toxics and Air Quality Committee, and I currently serve on the Secretary's Scientific Advisory Board on Toxic Air Pollutants for the North Carolina Department of Environmental Health and Natural Resources.

I am an active member of the American Statistical Association, Society for Epidemiological Research, Society for Occupational and Environmental Health, American Association for the Advancement of Science, Sigma Xi, New York Academy of Sciences, Society for Risk Analysis, and the Society of Toxicology. In 1988-89 I served as first President of the newly formed Society of Toxicology Specialty Section on Risk Assessment, and I currently serve as President of the Research Triangle Chapter of the Society for Risk Analysis. Since 1987 I have served on the editorial board of *Fundamental and Applied Toxicology*.

Over the past twenty years, I have published more than 80 scientific papers, abstracts, and book chapters. My scientific research has focused primarily on developing effective means for incorporating information regarding the mechanisms by which chemicals cause toxicity into quantitative risk assessments, and also on improving

epidemiologic surveillance methods for assessing effects of chemical exposure on worker health.

Recently, the Chemical Manufacturer's Association (CMA) requested that ENVIRON critically review certain aspects of OSHA's preliminary risk assessment for 1,3-butadiene (BD), commenting specifically on the extent to which assumptions and procedures used by OSHA to quantify internal doses and potential cancer risks in relation to airborne BD concentration were consistent with currently available scientific information regarding BD toxicity and carcinogenicity in laboratory animals and humans. CMA further asked that ENVIRON recommend improved methods for quantifying the potential cancer risks resulting from BD exposure if aspects of the approach taken by OSHA appeared deficient in light of current knowledge regarding BD's mechanisms of toxic action. Our observations and conclusions to date are set forth in the attached report, "*Comments on the Occupational Safety and Health Administration Quantitative Risk Assessment for 1,3-Butadiene*," which is offered for consideration by OSHA.

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TESTIMONY OF DR. JOSEPH V. RODRICKS

My name is Joseph V. Rodricks. I am one of the founding Principals of ENVIRON Corporation, with considerable background and experience in assessing the risks to human health of exposure to toxic substances. I received my B.S. from M.I.T. in 1960, and my Ph.D. in biochemistry from the University of Maryland in 1968. In 1969-70, I was a postdoctoral scholar at the University of California, Berkeley. I am Certified as a Diplomate of the American Board of Toxicology. Since becoming a consultant, I have conducted and directed numerous risk assessments for private clients, trade associations, and government agencies. I have provided such analyses for a variety of pesticides, occupational carcinogens, environmental pollutants, food and color additives, and drugs. In addition, I have been involved in the development of risk assessment methods to serve a variety of purposes.

Before working as a consultant, I spent nearly fifteen years at the Food and Drug Administration. In my final two years, I was Chief Science Advisor to the Commissioner of FDA, with special responsibility for risk and safety on chemical and toxicological evaluation of food contaminants and additives. I was a member of the National Academy of Sciences Board on Toxicology and Environmental Health Hazards, and have also served on and chaired other Academy Committees and Subcommittees. I have written more than seventy scientific publications on food safety and risk assessment and have lectured widely on these subjects. I have also provided expert testimony before U.S. Congress, in administrative proceedings and in court.

In 1986, the Chemical Manufacturers Association (CMA), requested that ENVIRON Corporation review the available scientific evidence regarding the toxicity of 1,3-butadiene (BD) and conduct a quantitative assessment of the potential risks to workers from exposure to BD. I participated in and supervised that review and assessment, the results of which are contained in a 1986 ENVIRON report, "*Assessment of the Potential Risks to Workers from Exposure to 1,3-Butadiene*". While that report is already a part of the OSHA docket for BD, an updated summary of that work has recently been published in *Environmental Health Perspectives* and is offered for consideration by OSHA.

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COMMENTS ON THE OCCUPATIONAL SAFETY AND HEALTH ADMINISTRATION QUANTITATIVE RISK ASSESSMENT FOR 1,3-BUTADIENE

Prepared for

The Chemical Manufacturers Association
Washington, DC

Prepared by

ENVIRON® Corporation
Arlington, Virginia

November 1990

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I. INTRODUCTION

A. Purpose

In 1986, at the request of the Chemical Manufacturers Association (CMA), ENVIRON Corporation, a consulting firm specializing in the health and environmental sciences, conducted a detailed assessment of the potential risks to workers from 1,3-butadiene (BD) exposure. The final report of that effort (ENVIRON 1986) is now part of the U.S. Occupational Safety and Health Administration (OSHA) docket for BD. When OSHA recently announced that public hearings would be held regarding its new proposed rule for BD (OSHA 1990), CMA again approached ENVIRON for assistance with the risk assessment issues raised by worker exposures to BD. CMA requested that ENVIRON critically review certain aspects of OSHA's preliminary risk assessment for BD, commenting specifically on the extent to which assumptions and procedures used by OSHA to quantify internal doses and potential cancer risks in relation to airborne BD concentration were consistent with currently available scientific information regarding BD toxicity and carcinogenicity in laboratory animals and humans. CMA further asked that ENVIRON recommend improved methods for quantifying the potential cancer risks resulting from BD exposure if aspects of the approach taken by OSHA appeared deficient in light of current knowledge regarding BD's mechanisms of toxic action. This document describes our observations and conclusions to date.

B. Summary of Principal Conclusions

Our principal finding is that a strong, scientific basis exists for concluding that all estimates of cancer risk to which OSHA has referred, including its own "best" estimate, actually overpredict the human cancer risks expected from BD exposure by a substantial margin. This basis is comprised of several distinct elements.

First, data now published in the peer-reviewed literature indicate that BD retention by rodents has been inaccurately represented by both the U.S. Environmental Protection Agency (EPA) and OSHA. Specifically, these data demonstrate that at airborne concentrations of BD on the order of 2 ppm, OSHA has overestimated the dose of BD retained by B6C3F1 mice by an approximately 5-fold factor. Second, similar data obtained from exposed monkeys show that primates retain approximately 6-fold less BD than mice at airborne concentrations of 10 ppm or less. Thus, based solely on the new comparative data regarding BD retention, one can reasonably conclude that humans would experience about 30-fold lower cancer risks than would identically exposed mice.

Third, additional data from the same studies indicate that blood levels of a highly DNA-reactive and mutagenic metabolite of BD, namely, 1,2-epoxybutene-3, are 40-fold lower in monkeys than in rats, and nearly 600-fold lower in monkeys than in mice at airborne BD concentrations of 10 ppm or less. Thus, significant interspecies differences exist in the metabolism of retained BD to toxic intermediates. At the present time, the concentrations of 1,2-epoxybutene-3 in the blood of BD exposed monkeys and rodents provide the best available measures of internal or "delivered" dose upon which extrapolations of cancer risk from laboratory animals to humans can be made. Based upon these measures, one can reasonably conclude that humans would experience anywhere from 40-fold to nearly 600-fold lower cancer risks than rodents when exposed to BD concentrations in the range of concern to OSHA. Fourth, results from epidemiologic studies of exposed workers are consistent with such small predicted risks and, in addition, confirm the improbability of OSHA's having underestimated the human cancer risks posed by BD exposure.

It is our principal recommendation that OSHA base its risk estimates on the measurements of 1,2-epoxybutene-3 levels in rodent and primate blood, since these data provide the best presently available measures of "delivered" dose for BD. If instead OSHA continues to base its risk estimates solely on the amounts of BD retained by mice or rats, then it must replace the inaccurate estimates of these quantities with the correct values.

Because great uncertainty remains regarding the role of the murine leukemia virus in modulating the amplitude of the tumor responses seen in the B6C3F1 mouse, we further recommend that OSHA continue to exclude the B6C3F1 mouse lymphomas from its analyses, and give at least equal weight to the risk estimates that can be derived from tumor responses seen in the Hazleton Laboratories Europe Ltd. (HLE) rat study of BD. Moreover, we recommend that OSHA exclude the mammary fibroadenomas from its analysis of pooled female rat tumors from the HLE study because the risk estimates obtained by including these benign lesions do not accurately portray the much lower risks of true malignancy. Pooling of the benign fibroadenomas with malignant tumors seen in female rats yields risk estimates that are virtually identical to those that would arise from a separate analysis of the fibroadenomas alone.

II. THE OSHA APPROACH

A. Selection of Carcinogenic Response

As noted in its proposed rule (OSHA 1990), OSHA's "best" estimate of human cancer risk from occupational exposure to BD was derived from a two-stage model fit to female B6C3F1 mouse heart hemangiosarcoma incidence as observed in the National Toxicology Program BD inhalation bioassay (NTP 1984). OSHA employed "crudely adjusted" estimates of tumor incidence, which are obtained by eliminating from consideration those animals in a study that die prior to the time of first appearance of the specific tumors of interest. Recognizing that the retention of BD following exposure is both dose- and species-dependent, OSHA appropriately employed estimates of retained BD dose specific to the B6C3F1 mouse strain, in contrast to corresponding administered concentrations of BD in air, as the independent variable in its "best" estimate analysis. Then, assuming that humans would retain BD to the same extent as mice and would thus experience the same cancer risk as mice at any given airborne BD concentration, OSHA estimated that 51 excess cancer deaths would occur among every 10,000 workers exposed to 2 ppm of BD for a working lifetime (45 years).

In addition to this "best" estimate, OSHA generated three other risk estimates using different data sets in an effort to portray the range of potential risks that might be posed by BD exposure. Specifically, OSHA also analyzed the incidence of pooled tumors (excluding lymphoma) in the same female B6C3F1 mice, and pooled tumors (both with and without the inclusion of nonmalignant mammary fibroadenomas) in female Charles River CD rats as observed in the Hazleton Laboratories bioassay (HLE 1981) sponsored by the International Institute of Synthetic Rubber Producers. The crudely adjusted incidences of these endpoints were fit with one- and two-stage versions of the multistage model, and both maximum likelihood point estimates of risk and upper 95% confidence limits on estimated risk were calculated. Estimates of the retained BD doses specific to either the mouse or rat species were also employed in these alternative analyses, and the conversion to human cancer risks was again predicated on the assumption that humans would experience equal BD retention, and thus cancer risk, from equivalent exposure conditions.

We support OSHA in the exclusion of lymphomas from its risk calculations based upon the mouse data, principally because of the questionable relevance of this endpoint to potential human cancer risk. Given the strong evidence that MuLV expression is critically involved in the production of bone marrow toxicity and leukemogenesis by BD in the B6C3F1 mouse, inclusion of the lymphomas in OSHA's analyses would serve only to encumber additionally

its risk estimates with the enormous uncertainty regarding the relevance of this endpoint to humans.

For several reasons, we also recommend that the mammary fibroadenomas be excluded from OSHA's analysis of pooled female rat tumors taken from the Hazleton Laboratories study (HLE 1981). First, as previous commenters and OSHA have noted, mammary fibroadenomas are not known to progress to malignancy. The relevance of this lesion to human cancer risk is thus highly questionable. Second, combination of these benign lesions with malignant tumors was not necessary to establish qualitatively the carcinogenicity of BD in female rats. Thyroid follicular tumors were significantly elevated in the high dose group, and significant dose-related trends were noted for this lesion and Zymbal gland carcinomas as well. Third, the mammary fibroadenomas were very common among unexposed female rats (40% incidence), and their incidence was even higher in the exposed groups (approximately 75% at 1000 ppm and 67% at 8000 ppm). In contrast, the malignant rat tumors considered by OSHA were quite rare in control animals, and in combination achieved only about 5% incidence in the 1000 ppm group and 18% incidence in the 8000 ppm group. As a consequence, the risk estimates obtained from pooled tumors including the mammary fibroadenomas are virtually identical to estimates that would be obtained from a separate analysis of these benign lesions alone. Therefore, the risk estimates obtained from the combination of significant malignant tumors and benign mammary fibroadenomas do not accurately reflect the far greater importance that the malignant lesions should play in the determination of potential human cancer risks from BD exposure.

B. Choice of Study

While OSHA made use of data from the rat inhalation bioassay (HLE 1981) in defining a range of potential human cancer risks, the Agency argued that numerous asserted limitations and deficiencies in this study produced "greater uncertainty in risk estimates derived from the rat data." We disagree, and believe that the concerns OSHA has expressed regarding the HLE study are largely unfounded. A number of these concerns have been addressed in detail by previous commenters. We agree with their principal conclusion that the HLE study was well-conducted: the randomization process by which individual animals were assigned to treatment groups was not flawed; the histopathology was more than adequate; and the quality control audits were sufficient to assure the validity of the study and the reliability of its results. Furthermore, we are convinced that any discrepancies in tumor counts among

different analyses of the study can be readily resolved by undertaking a detailed examination of the individual animal data contained in the HLE study final report. Thus, we believe that OSHA has overstated the degree of uncertainty that can justifiably be associated with the Hazleton Laboratories rat study (HLE 1981).

At the same time, OSHA dismissed several important criticisms of the NTP study, stating that it "does not believe that the deviations from the study protocol which occurred in the first NTP bioassay invalidate the conclusions of the study." However, the single most important question regarding the suitability of the NTP mouse study for quantitative risk assessment remains unanswered, namely, what role did the endogenous murine type C leukemia virus (MuLV) play in modulating the amplitude of the carcinogenic responses seen in the B6C3F1 mice? Irons et al. (1987) demonstrated an approximately 4-fold difference in lymphoma incidence between B6C3F1 mice (60%), which express MuLV, and NIH Swiss mice (14%), which do not, following one year of exposure to 1250 ppm BD. Clearly, viral expression modulates the incidence of this malignant lesion.

The presence of the endogenous MuLV virus and its activation by BD exposure could also impact quantitatively on the incidence of other tumor types in exposed B6C3F1 mice. Consequently, even though OSHA has excluded the lymphoma endpoint from its analyses and based its "best" estimates of risk on the incidence of heart hemangiosarcomas among female mice, there is still great uncertainty regarding the relevance of these estimates, especially in quantitative terms, to the potential human cancer risks from BD exposure. We therefore believe that highly credible risk estimates can be derived from the 1981 Hazleton Laboratories rat study. Indeed, such estimates are likely to be much less uncertain than any that could be obtained from the 1984 NTP mouse study, even if the known high to low dose and interspecies differences in BD pharmacokinetics are taken appropriately into account.

C. Other Risk Estimates Considered by OSHA

OSHA also considered previous risk assessments that had been prepared by the EPA Office of Toxic Substances (OTS), the EPA Carcinogenicity Assessment Group (CAG), ICF/Clement, and ENVIRON. These assessments employed the same two carcinogenicity studies as OSHA, but they utilized incidence rates of somewhat different tumor types in both females and males of each rodent species. In addition, some of these assessments employed dose-response models other than the conventional multistage model, and some employed the airborne BD concentration directly as the exposure measure.

As OSHA noted, its "best" estimate of human cancer risk at 10 ppm BD is lower than almost all of the other estimates derived from the NTP mouse bioassay. (The only exception is the maximum likelihood estimate from OSHA's own 2-stage analysis of pooled female

mouse tumors, for which the coefficient of the linear term (q_1) was driven to zero in the fitting process.) OSHA has expressed concern that by relying upon the female mouse heart hemangiosarcoma data for its "best" estimate of risk, it may be underestimating BD's carcinogenic potential. However, as is amply demonstrated in the following sections, there is a strong, scientific basis for concluding that all of the existing BD cancer risk estimates, including OSHA's "best" estimate, overpredict human cancer risk from BD exposures by a substantial margin.

III. NEW BD RETENTION DATA NOT USED BY OSHA

A. High to Low Dose Retention Differences

The 1984 NTP BD inhalation bioassay exposed mice to BD concentrations of 625 and 1250 ppm, while the 1981 HLE study exposed rats to BD concentrations of 1,000 and 8,000 ppm. It was thus necessary to extrapolate risks from these high exposure conditions to the much lower BD levels of concern to OSHA. In performing these extrapolations, OSHA made use of information regarding the retention of inhaled BD in rodents.

OSHA's calculations of internal BD dose in rodents are based on preliminary results from the Lovelace Research Institute (NTP 1985) study of BD retention in B6C3F1 mice and Sprague-Dawley rats. These data indicated that 52.8% of the inhaled BD had been retained by mice at the end of 6 hour exposures to 7 ppm BD, with progressively smaller percentages (9.3 and 4.4%) retained at higher airborne concentrations (80 and 1040 ppm). Similarly, rats exposed for 6 hours to 70 ppm were reported to retain 6.9% of the inhaled BD, with progressively smaller percentages (2.1 and 1.4)%) retained at higher airborne concentrations (950 and 6100 ppm).

OSHA employed power law regression equations developed with these data to estimate retention percentages at other airborne BD concentrations. As OSHA has noted, their equation for mice predicts that 100% retention is achieved at just over 2 ppm, and the Agency assumed that 100% retention would also occur at all lower concentrations. In comparison, OSHA's equation for rats indicates that 100% retention would be achieved only at concentrations below 1 ppm.

A final report of the Lovelace BD retention study has now been published in the peer-reviewed literature (Bond et al. 1986), and the measured BD retention percentages reported therein differ significantly from the earlier preliminary estimates employed by EPA, OSHA, and others. A very large difference occurred at 7 ppm where the earlier estimate (52.8%) for mice was reduced to 13% (0.4/3 x 100). OSHA should note that an error exists in the 1986 Bond et al. paper which results in this value being reported as 20% (James A. Bond, personal communication). Furthermore, at just under 1 ppm (1.4 μg/l), the BD retention percentage for mice was determined to be just 20%, in contrast to the 100% that OSHA's methodology has predicted.

Thus, on the basis of this new experimental data regarding retained BD dose in mice, it can be seen that OSHA's "best" estimate has overstated internal dose of BD and the attendant

cancer risks at airborne BD concentrations near 2 ppm by an approximately 5-fold factor. Smaller but still significant errors in the percentage of BD retained at higher airborne concentrations imply similar overestimates of risk there as well.

It is important to note that EPA has recognized the recent change in estimated BD retention percentages and has revised the CAG upper bound estimate of BD potency accordingly (Cote and Bayard 1990). Clearly, it is also appropriate for OSHA to take proper account of the new information provided by Bond et al. (1986) in developing its maximum likelihood point and upper bound estimates of risk based on both the mouse and rat data. To accomplish this, OSHA must recalculate the coefficients of its power law regression equations for mouse and rat retained doses with the correct BD retention data reported by Bond et al. (1986).

B. Interspecies Retention Differences

In another very recent report, Dahl et al. (1990) have published additional findings that are highly relevant to the comparative doses of BD retained by rodents and primates. Specifically, while OSHA has assumed that humans would retain BD doses equal to those retained by either identically exposed mice or rats, the Dahl et al. (1990) findings indicate that this would not likely be the case.

B6C3F1 mice and Sprague-Dawley rats were identically exposed for 6 hours to 7.8 ppm BD. Male monkeys (Macaca Fascicularis) were exposed for 2 hours to 10 ppm BD. When Dahl et al. normalized the amount of retained BD to the different species body masses and expressed retained BD on a per 10 ppm-hour basis, the measured BD retention rates were determined to be 3.30, 0.46, and 0.52 μmol/kg in the mouse, rat, and monkey respectively. Thus, if these species were identically exposed to the same airborne BD concentration, they would <u>not</u> retain the same BD dose per unit body mass. Rather, the mouse would retain a BD dose that is approximately 7.2-fold higher than that retained by the rat, and 6.3-fold higher than that received by the monkey.

These findings indicate that rats and monkeys would be at significantly less risk of developing cancer than mice if all were identically exposed to the same airborne BD concentration because they would retain significantly less BD per kilogram of body mass than do mice. Because the human species is far more closely related to the monkey than the mouse, both anatomically and physiologically, it is reasonable to expect that humans would also retain significantly less BD than would identically exposed mice. Indeed, were this the only relevant information available regarding BD disposition in different species, it would argue that the rat provides a better animal model than the mouse for evaluating human cancer risk from BD exposure. If OSHA continues to rely on the mouse data for its "best" estimates

of risk, it should therefore adjust those estimates downward by an approximately 6-fold factor so as to properly account for the smaller retention of BD by primates relative to the mouse.

C. Combining High to Low Dose and Interspecies Retention Differences

As noted in section III.A, OSHA has overestimated the amount of BD retained by mice at low concentrations by an approximately 5-fold factor. To fully correct this error, OSHA must recalculate the coefficients of its power law regression equation for retained dose in mice with the corrected BD retention data reported by Bond et al. (1986). In addition, since OSHA is concerned with human cancer risk, it is necessary to extrapolate low dose risk estimates from mice or rats to humans, and in doing so, OSHA has assumed that humans would experience the same retained BD dose as mice or rats if both were identically exposed to BD. However, as noted in the previous section, the Dahl et al. (1990) study indicates that monkeys retain about 6.3-fold less BD than mice if both are identically exposed to BD. It follows that OSHA must also adjust its low dose estimates of human cancer risk based on the mouse data by this 6.3-fold factor.

Thus, based on the best data presently available regarding BD retention, the adjustments for high to low dose and interspecies differences in retained BD must be multiplied together, yielding estimated human cancer risks from BD exposure that are approximately 30-fold lower than OSHA's current estimates. Moreover, as described in the next section, new data regarding interspecies differences in the metabolism of retained BD show that even further reductions in OSHA's current estimates are scientifically appropriate.

IV. NEW BD METABOLISM DATA NOT USED BY OSHA

The metabolism and pharmacokinetics of BD have been described in considerable detail by previous commenters as well as in ENVIRON's earlier risk assessment of BD (ENVIRON 1986). Here we shall only summarize certain critical facts relevant to the quantitation of estimated human cancer risk from BD exposure.

Many important aspects of the metabolism of BD are well-established. The first step, namely, oxidation of BD to the monoepoxide 1,2-epoxybutene-3 was first demonstrated with hepatic microsomes over ten years ago by Malvoisin et al. (1979). Subsequently, these investigators demonstrated further oxidation in vitro of 1-2-epoxybutene-3 to 1,2:3,4-diepoxybutane, as well as the epoxide hydrolase-mediated reduction of 1,2-epoxybutene-3 to 3-butene-1,2-diol, followed by second oxidation step yielding 3,4-epoxy-1,2-butane-diol. The 1,2-epoxybutene-3 intermediate is a monofunctional alkylating agent, while 1,2:3,4-diepoxybutane is bifunctional and is known to form DNA-DNA crosslinks. Both of these metabolites of BD are DNA-reactive intermediates that also show mutagenic activity in bacterial and other test systems, while BD per se does not. Thus, both may play critical roles in the carcinogenicity of BD. It is therefore especially important to establish accurately the quantitative relationships between airborne BD concentrations and corresponding internal "delivered" doses of these BD metabolites for the relevant species.

An extensive series of studies by Bolt and colleagues (c.f., Laib et al. 1990) has confirmed the production of 1,2-epoxybutene-3 following in vivo BD exposure, and further established the saturable character, at least at very high airborne BD concentrations (> 1000 ppm) of the initial conversion of BD to this monoepoxide in both rats and mice. These investigators also determined that at lower airborne BD concentrations, where linear pharmacokinetics prevail, mice metabolize BD to 1,2-epoxybutene-3 at about twice the rate as do rats. In addition, they determined that the subsequent metabolism of 1,2-epoxybutene-3 is saturable in mice at a far smaller rate (350 μmol/kg/hr) than in rats (> 2600 μmol/kg/hr). Taken together, these findings imply that internal concentrations of 1,2-epoxybutene-3 would reach much higher levels in mice than in rats when both are identically exposed to airborne BD. In fact, Laib et al. (1990) have concluded that the limited 1,2-epoxybutene-3 detoxication capacity of mice relative to that of rats is a primary determinant of the higher susceptibility of mice to BD-induced carcinogenesis. Knowledge of internal concentrations of 1,2-epoxybutene-3 is thus critical to the development of accurate estimates of the cancer risks posed by BD exposure.

The study of Bond et al. (1986) and the more recent study by Dahl et al. (1990) have both developed significant new information regarding quantitative differences among species in internal concentrations of several BD metabolites. The Dahl et al. (1990) study is of particular interest, since it reports comparative measurements of blood levels of the mutagenic 1,2-epoxybutene-3 obtained not only in mice and rats, but also in monkeys, following inhalation exposures to BD for 2 hours.

Specifically, B6C3F1 mice were exposed to 7.8 and 78 ppm BD; Sprague-Dawley rats were exposed to 78 ppm BD; and monkeys were exposed to 10 ppm BD. Dahl et al. determined that monoepoxide levels in the blood of mice and rats identically exposed to 78 ppm BD were 26 and 5.2 pmol/ml/ppm respectively (blood epoxide levels were normalized by airborne BD concentrations, i.e., expressed per ppm BD, so as to permit direct comparisons even when the different species were not identically exposed). Thus, at 78 ppm BD, mice exhibited approximately 5-fold higher 1,2-epoxybutene-3 levels in their blood than did rats. Even more importantly, in the concentration range of interest to OSHA, mice exposed to 7.8 ppm BD exhibited 77 pmol/ml/ppm of 1,2-epoxybutene-3 in their blood, while monkeys exposed to 10 ppm exhibited only 0.13 pmol/ml/ppm. In other words, mice developed more than 590-fold higher blood concentrations of 1,2-epoxybutene-3 than did monkeys. Thus, making the very reasonable assumption that humans metabolize BD in the same manner as monkeys, the Dahl et al. (1990) results imply that humans should be approximately 590-fold less sensitive to BD's carcinogenicity than are mice.

A similar conclusion can be drawn from a comparison of the rat and monkey blood epoxide levels observed by Dahl et al. Specifically, rats exposed to 78 ppm exhibited 5.2 pmol/ml/ppm blood epoxide, while monkeys similarly exposed to 10 ppm exhibited only 0.13 pmol/ml/ppm blood epoxide, as noted above. Again, provided that humans metabolize BD in the same manner as monkeys, these data show that humans should be approximately 40-fold less sensitive to BD's carcinogenicity than are rats.

When OSHA conducted its risk assessment for BD, the best data available regarding internal doses of BD or its metabolites were the preliminary estimates of retained BD percentages obtained from the Lovelace Institute study (NTP 1985). As was noted previously, OSHA appropriately utilized this preliminary information in constructing all of its estimates of cancer risk. The new data of Dahl et al. (1990) regarding internal 1,2-epoxybutene-3 concentrations now provide a much superior alternative measure of "delivered" dose even relative to the correct BD retention data. This highly reactive and mutagenic epoxide metabolite is far more likely to be responsible for the carcinogenicity of BD than is BD per se. It is therefore essential that OSHA employ these new pharmacokinetic data in extrapolating its risk estimates from mice and rats to humans.

A summary of the approximate impacts on risk estimates of using the different measures of "delivered" BD dose discussed in this and preceding sections is provided in Table 1. At 2 ppm, we have utilized a 5-fold reduction (from 100% to 20%) in retained BD dose for mice, but no change in retained BD dose for rats. Thus, the lower risks predicted from the HLE rat study with the new BD retention data arise solely from rat-monkey differences in BD retention. Note also that the exact risk estimates arising from use of the correct BD retention data will depend in part on the specific regression equations utilized by OSHA in predicting retained BD dose versus airborne BD concentration.

TABLE 1
Approximate Impact of Different Measures of "Delivered" Dose on Maximum Likelihood (Upper Bound) Estimates of Cancer Deaths Per 10,000 Workers Exposed for a Working Lifetime to 2 ppm BD

Data Base	Current OSHA Estimates	Using Correct Retention Data	Using New Metabolism Data
Pooled Female Mouse Tumors[a]	183. (230.)	5.9 (7.4)	0.31 (0.39)
Female Mouse Heart Tumors[b]	51. (71.)	1.7 (2.3)	0.09 (0.12)
Pooled Female Rat Tumors with Fibroadenomas[b]	29. (33.)	40.[c] (45.)[c]	0.73 (1.00)
Pooled Female Rat Tumors without Febroadenomas[b]	5. (5.7)	8.[c] (9.)[c]	0.13 (0.20)

[a] Assuming a one-hit model analysis.
[b] Assuming a two-stage model analysis.
[c] Assuming no change in retained BD dose for rats.

V. COMPARISON OF PREDICTED AND OBSERVED HUMAN RISKS

As noted previously, OSHA has expressed concern that by relying upon the female mouse heart hemangiosarcoma data for its "best" estimate of risk, it may be underestimating BD's carcinogenic potential. Indeed, it called attention to the fact that its "best" risk estimate of 128/10,000 at 10 ppm BD is lower than almost all of the other estimates derived from the NTP mouse bioassay. The alternative estimates larger than OSHA's are 1) the ICF one-hit analysis of pooled male mouse tumors, which predicted more than 10,000 deaths per 10,000 exposed workers, 2) the ICF estimate of 3,435/10,000 based on pooled female mouse tumors, 3) the OTS estimate of 821/10,000, again based on pooled female mouse tumors, 4) the CAG estimate of 482/10,000, based on pooled male and female mouse tumors, 5) the ENVIRON estimate of 456/10,000, based on pooled male mouse tumors, and 6) the OTS estimate of 392/10,000, based on male mouse hemangiosarcomas. It is instructive to examine whether the observed cancer mortality data for BD exposed workers are consistent with these larger predicted risks.

CAG (EPA 1985) described a consistency check for its "point" estimate of risk (defined below), namely, 0.025/ppm lifetime continuous exposure using the Meinhardt et al. (1982) and Matanoski et al. (1982) studies of worker mortality in the styrene-butadiene rubber industry. Results from similar exercises have also been reported by ENVIRON (1986) and Acquavella (1990).

CAG combined its "point" estimate of BD potency with estimated exposure levels and the sample sizes of different worker groups to generate predicted numbers of extra deaths attributable to BD exposure under the hypothesis that the CAG "point" estimate of BD potency was accurate. Fewer deaths were observed than were predicted in all but one of the six groups that CAG analyzed (USEPA 1985). For example, in the Matanoski et al. (1982) study, for the worker group whose jobs last held were in the production category, there were significantly fewer observed deaths from lymphopoietic cancer than CAG's "point" estimate of BD potency predicted (11 deaths observed versus 20.6 predicted). Nevertheless, CAG concluded that its "point" estimate was still not inconsistent with the observations if the observed deficits in cancer deaths could be attributed instead to an underascertainment of deaths in the Matanoski et al. study.

It must be noted here that CAG's "point" estimate was identified as a geometric mean of the sex-specific point estimates that resulted from its analysis of the NTP mouse data. However, the point estimate (denoted by q_1) that CAG obtained from its analysis of the female mouse data was actually zero (see p. 6-49 in EPA (1985)). The "point" estimate

employed by CAG for its consistency check is actually the geometric mean of the q_1 for male mice and q_2, the coefficient of the quadratic term in the multistage model, for female mice. However, the coefficient q_2 does not even have the correct dimensional units to be a cancer potency factor. Thus, CAG's "point" estimate of cancer potency, i.e., the geometric mean of q_1 and q_2, is a meaningless quantity.

Nevertheless, the approach CAG took in evaluating the consistency of its risk estimates with epidemiologic observations is useful in objectively assessing OSHA's concern that it may have underestimated human cancer risk from BD exposure. If we assume, for example, that the 3,269 workers in the production category of the Matanoski et al. (1982) study were occupationally exposed to 10 ppm BD for 10 of 50 working years and followed for 18 of 50 remaining years of life, then the probability of observing 11 or fewer deaths from lymphopoietic cancer in this group can be readily determined for each of the specific alternative estimates of risk noted above, as well as for OSHA's estimate of 128/10,000. These probabilities are summarized in Table 2, and results from a similar calculation for all cancer deaths combined are presented in Table 3.

Tables 2 and 3 clearly demonstrate that all of the alternative estimates of cancer risk larger than OSHA's are statistically inconsistent with the observed numbers of lymphopoietic cancer deaths (11) and all cancer deaths (94) among workers in the production group of the Matanoski et al. (1982) study. Even OSHA's risk estimate of 128/10,000 is large enough to be improbable: there is less than 1 chance in 4 ($p \sim .24$) that 11 or fewer lymphopoietic cancer deaths would have arisen in this group if the risk associated with their BD exposure were in fact 128/10,000. Similarly, there is less than 1 chance in 12 ($p \sim .08$) that 94 or fewer cancer deaths of any type would have arisen in this group under the same assumptions.

In summary, epidemiologic observations indicate that OSHA has not significantly underestimated human cancer risk from BD exposure. All of the higher alternative estimates noted by OSHA are statistically inconsistent with actual observations. Furthermore, while the epidemiologic data are not sufficiently powerful to reject predicted risks as small as OSHA's "best" estimates, they are also consistent with the far smaller risks that would be predicted by the BD retention and metabolism differences that are now known to exist between high and low doses and between rodents and primates.

TABLE 2
Consistency Check of Alternative Estimates of BD Cancer Risk Based on the 11 Lymphopoietic Cancer Deaths Observed Among 3,269 Production Workers (Matanoski et al. 1982)

Risk Assessment	Predicted Extra Deaths Per 10,000	Predicted Total Deaths Per 3,269	Probability of 11 or Fewer Deaths
OSHA	128	13.6	.2388
OTS	392	19.8	.0238
ENVIRON	456	21.3	.0129
CAG	482	21.9	.0099
Clement	3,435	91.4	<< .0001

TABLE 3
Consistency Check of Alternative Estimates of BD Cancer Risk Based on the 94 Total Cancer Deaths Observed Among 3,269 Production Workers (Matanoski et al. 1982)

Risk Assessment	Predicted Extra Deaths Per 10,000	Predicted Total Deaths Per 3,269	Probability of 94 or Fewer Deaths
OSHA	128	108.6	.0808
OTS	392	114.8	.0262
ENVIRON	456	116.3	.0192
CAG	482	116.9	.0170
Clement	3,435	186.4	<< .0001

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Docket No. H-041

REPRODUCTION AND DEVELOPMENTAL TOXICITY OF 1,3-BUTADIENE

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REPRODUCTION AND DEVELOPMENTAL TOXICITY OF 1,3-BUTADIENE

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I. EXECUTIVE SUMMARY

1,3-Butadiene (BD) has been tested in long-term inhalation studies in Sprague-Dawley rats and B6C3F1 mice. In comparison with the rat study, significantly reduced survival occurred in the mouse study at lower concentrations of BD, and the malignant tumor incidence, considered the cause of most deaths, was increased. There were also significant increases in non-neoplastic lesions in tissues that rapidly grow or proliferate. Because the results of these two studies strongly suggested that B6C3F1 mice were more susceptible than Sprague-Dawley rats to the effects of BD, lower concentrations were tested. Preliminary, unaudited results of the second B6C3F1 mouse carcinogenicity study indicate that the lowest BD concentration tested, 6.25 ppm, caused ovarian atrophy and other effects. In view of the health requirements of the mouse for ovarian maintenance, and the age of the mice when these observations were present, the relevance of this observation in sick animals is questionable, at best. It is concluded that the pharmacokinetic differences in the uptake, metabolism and excretion of BD in man and mouse, in combination with the functional differences in the ovary of these two species, indicate that the ovarian atrophy observations in the mouse are not relevant to assessment of human reproductive risk.

Other studies in mice that were also considered by OSHA to be indicative of reproductive hazard for humans identified developmental toxicity at 40 ppm and reduced testicular weights, testicular atrophy, increased incidences of abnormal sperm heads, and a reversible increase in resorption (dominant lethal study) at 200 ppm. It is concluded that these studies in mice are also inappropriate for use in identifying NOELS for BD, because of the metabolic and functional differences between humans and mice. In addition, the results of some of these studies indicate that higher NOELS should be identified (the developmental toxicity NOEL should be 40 ppm; the sperm head morphology and dominant lethal studies should have reproductive effects NOELS of 5000 ppm). OSHA's identification of a safety factor of 100 for these mouse data is considered to be inappropriate, because of the special sensitivity of the mouse to BD.

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It is my conclusion that BD is not a reproductive or developmental hazard to humans at permitted exposure levels of an 8-hour TWA of 2 ppm. This conclusion is supported when information available from other studies of BD in rats, guinea pigs, rabbits and dogs is considered. It is my opinion that OSHA's preliminary document describing a proposed rule by the U.S. Department of Labor, Occupational Safety and Health Administration (1990) did not give appropriate weight to these other studies.

Review of the rat and mouse developmental (embryo-fetal toxicity/ teratogenicity) toxicity studies demonstrates that the mouse was more susceptible to effects of BD than the rat. The maternal and developmental NOELS for the rat were identified as 200 and 1000 ppm BD, respectively, and the maternal and developmental NOELS for BD in the mouse were 40 ppm. In dominant lethal and sperm head morphology studies in male mice, transient, minimal increases in resorption that did not affect live litter sizes were reported at 1000 and 5000 ppm BD, and these concentrations of BD were also reported to affect the more mature stages of spermatogenesis in male mice. It should be noted that the interpretation of these data is questionable, and that concentrations of BD as high as 5000 ppm did not affect mating or fertility, even in this sensitive species.

In a series of carcinogenicity studies of BD, concentrations of 200 and 625 ppm reduced absolute and relative testicular weights in B6C3F1 mice, after 40 and 65 weeks of exposure, and increased testicular atrophy at 200, 625 and 1250 ppm in this species and strain. In Sprague-Dawley rats, neither ovarian nor testicular atrophy was produced by exposure to concentrations of BD as high as 8000 ppm for 105 (females) or 111 (males) weeks. Exposure of cohabited rats, guinea pigs and rabbits to BD at concentrations as high as 6700 ppm for a period of eight months did not impair fertility or cause testicular or ovarian atrophy in these three species, and female dogs similarly exposed to concentrations of BD as high as 6700 ppm did not demonstrate ovarian atrophy.

II. QUALIFICATIONS

I am Mildred S. Christian, Ph.D., ATS. My doctoral degree was obtained from Thomas Jefferson University in the field of anatomy (developmental toxicology/teratology), and I am a Diplomate of the Academy of Toxicological Sciences. Since 1967, I have worked in the field of developmental/reproductive toxicology, designing, conducting and evaluating the results of safety evaluations in laboratory animals. These tests were performed in order to identify the potential of drugs and other chemicals to cause adverse effects on the development of embryos or the reproductive performance of adult animals. I have actively contributed to the scientific development of these specialty areas of research. I have served on committees of the U.S. Food and Drug Administration, the U.S. Environmental Protection Agency and the Organization for Economic Cooperation and Development, whose purpose was to develop appropriate methods for: 1) conducting and analyzing these types of animal tests; and 2) identifying how the results of animal studies should be used in human risk assessment. I am currently the President and Executive Director of Research at Argus Research Laboratories, Inc., the largest international, independent consulting laboratory dedicated to performing and evaluating these types of animal tests. In this capacity, I currently annually supervise and evaluate approximately 250 of these studies. I have served as the President of the Teratology Society, and am currently the President of the International Federation of Teratology Societies Scientific Program and the President-Elect of the American College of Toxicology. I am a charter member of the Society for Risk Assessment. I have multiple publications and have written over 600 "teratology" and reproductive toxicology studies for private industry or regulatory bodies of the United States government. I am an editor of a major textbook used for risk assessment in developmental and reproductive toxicology, and since 1982, I have been the Editor-in-Chief of the "Journal of the American College of Toxicology". I have participated or chaired multiple courses in reproductive/developmental toxicology conducted at universities, private corporations, and meetings of professional societies and United States and international regulatory groups.

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III. REPRODUCTIVE EFFECTS - My interpretation of the reports contrasted with OSHA's

A. Ovarian and Testicular Atrophy - rat vs. mouse

1. Lifetime Studies

Three lifetime studies, one in rats and two in mice, have been conducted to evaluate the carcinogenic potential of BD. Rats exposed to BD at concentrations as high as 8000 ppm did not have increases in mortality or ovarian or testicular atrophy after exposure for 105 or 110 weeks. Mice exposed for 60 to 61 weeks to 625 ppm or 1250 ppm concentrations of BD had significantly reduced lifespan, as the result of tumors at multiple sites, ovarian and testicular atrophy, and lesions in other rapidly proliferating tissues. In a subsequent chronic study in mice, ovarian atrophy was preliminarily reported at levels as low as 6.25 ppm. These studies are described below. It is concluded that the evidence of ovarian atrophy in mice is not relevant to human risk assessment and does not provide evidence of a human reproductive hazard at the proposed standard of 2 ppm.

a. Hazleton, Europe, 1981a - rat

A carcinogenicity study in which ovarian and testicular atrophy was assessed was conducted in rats at Hazleton, Europe (1981a). Groups of 110 male and 110 female Sprague-Dawley rats were exposed to 0, 1000 or 8000 ppm BD, 6 hours per day, 5 days per week for 105 weeks (female) or 111 weeks (male). Ovarian atrophy in rats occurred as follows: 0-4.3% and 8000 ppm-4.2%; testicular atrophy occurred in rats as follows: 0-31.0% and 8000 ppm-19.3% These results are consistent with the results of the study by Carpenter et al. (1944), described in section IV. In that study, rats were exposed to BD levels up to 6700 ppm over a period of eight months (7.5 hours per day, 6 days per week, for a maximum of 202 exposures). No testicular or ovarian pathology was seen in the sections examined histologically.

The rat studies do not provide any indication that BD presents a reproductive hazard for humans at any exposure level tested.

b. National Toxicology Program (NTP), 1982, 1984, Battelle - mouse

The first NTP study at Battelle (1982, 1984) exposed 50 male and 50 female B6C3F1 mice, 6 hours per day, 5 days per week to 0, 625 and 1250 ppm BD. The study was originally designed to run for 104 weeks but was terminated at week 60 for the males and week 61 for the females because survival in exposed groups was reduced (male: 0-98%, 625 ppm-22%, 1250 ppm-14%; female: 0-92%, 625 ppm-30%; 1250 ppm-60%). In this mouse study the incidences of ovarian atrophy were 0-4.1%, 625 ppm-88.9% and 1250 ppm-83.3%, and those for testicular atrophy were 0-0%, 625 ppm-40.4% and 1250 ppm-22.9%.

c. National Toxicology Program (NTP), 1989a, Battelle, - mouse (preliminary data)

A second carcinogenicity study in mice in which ovarian and testicular atrophy was assessed was conducted at Battelle (1989) exposing 50 male and 50 female B6C3F1 mice, 6 hours per day, 5 days per week to 0, 6.25, 20, 62.5, 200 and 625 ppm BD. Preliminary, unaudited results of the mouse study have been reported. The study was scheduled for 103 weeks with 5 mice per sex per group killed at 40 and 65 weeks, in order to follow the progressions of the lesions.

An independent review of the pathology data (Keller, D.A., 1990) identified that ovarian atrophy was interrelated with the age of the mice at sacrifice. Ovarian atrophy did not occur for any of the ten mice in each group that was sacrificed after 40 weeks (nine months) of exposure to BD at concentrations as high as 62.5 ppm. After 65 weeks of exposure to BD, none of the ten mice sacrificed in the control or 6.25 ppm groups had ovarian atrophy identified. In the 20 and 62.5 ppm groups, 1 of 10 and 9 of 10 mice, respectively, were noted to have ovarian atrophy at the 65-week sacrifice; this observation also occurred for 4 of 6 mice in the 62.5 ppm group that were found dead between the 40- and 65-week sacrifice periods. This pattern of effect is expected, based on the normal time reproductive senescence occurs in mice (12 to 15 months of age), with cycle changes occurring as early as eight months of age (Nelson and Felicio, 1990). The majority of the observations of ovarian atrophy occurred after the mice had been exposed to BD for more than

15 months; all incidences of ovarian atrophy that occurred in the control and 6.25 ppm groups, and all except one event in the 20 ppm group occurred after this prolonged exposure period and the expected end of reproductive life in the mouse. In the 0, 6.25, 20 and 62.5 ppm groups, ovarian atrophy was identified for totals of 6 of 55, 19 of 55, 32 of 48 and 33 of 45 mice that died or were sacrificed in extremis after 15 months of exposure to BD or were sacrificed after 24 months of exposure to BD.

Significant reductions in absolute and relative testicular weights occurred at the 200 and 625 ppm concentrations of BD. A reduction in absolute testis weight that occurred for the 62.5 ppm group at 40 weeks is considered unrelated to BD because: 1) the absolute testis weight was not reduced for this group following exposure for 65 weeks; 2) the values at 40 weeks were not dose-dependent; and 3) the testis weight relative to body weight values were unaffected for this group at both 40 and 65 weeks. Histopathologically, testicular atrophy in mice occurs at levels ranging from 625 ppm to 1250 ppm.

2. Considerations Relevant to Ovarian Atrophy in the Mouse

a. Preliminary reports of Unaudited Data

OSHA should exercise caution in relying on preliminary reports of ovarian atrophy at 6.25 ppm in the B6C3F1 mouse. These preliminary reports are based on unaudited data. The nature or severity of the effects have not been described in the preliminary reports. Although this change was reportedly evaluated using a grading system of minimal, slight, moderate and marked, no criteria were provided to define these subjective terms. It should also be noted that marked atrophy was never reported for mice exposed for 15 or more months to concentrations of BD as high as 62.5 ppm.

As described above, examination of the raw data reveals that atrophy was not seen at 6.25 or 20 ppm until after completion of the reproductive life of the animals. This important fact was not included in preliminary reports. These findings indicate that the ovary was not a uniquely sensitive tissue during the expected reproductive life of the exposed mice, and that concentrations of BD that affect rapidly growing tissues did not cause ovarian atrophy at the 6.25 and 20 ppm concentrations until after the expected,

age-related atrophy of the ovary was initiated. Although the incidence of ovarian atrophy was increased at the 62.5 ppm concentration after 15 months of exposure, a time some level of ovarian atresia is expected, the onset of normal atrophy was not abnormally accelerated, based on the absence of this finding at this exposure level in mice exposed for nine months, even in this then proliferating tissue in this uniquely sensitive species. OSHA should review the final report of the audited data before reaching any conclusions concerning the significance or relevance of these data. Additional comments, beyond what is provided below, could be provided once a final report is available for review, and in response to OSHA's analysis of the complete, final data set.

b. Relevance of Ovarian Atrophy Seen After the End of the Reproductive Life of the Mouse

As described, ovarian atrophy was not seen at 40 or 65 weeks for mice in the 6.25 and 20 ppm groups; this change occurred in these groups only in mice that were found dead or sacrificed after 65 weeks of exposure or at the terminal sacrifice at 24 months (although 1 of 10 mice in the 20 ppm group had ovarian atrophy noted at 65 weeks, this incidence should not be considered an effect level because it is within the expected range for mice of this age). The reproductive life of the mouse normally ends at approximately 12 to 15 months (Nelson and Felicio, 1990).

As occurs in humans, ovulation in the mouse is directly correlated with age, although it can also be suppressed by many other factors including malnutrition, acute illnesses, and diseases affecting the hypothalamus, the pituitary and the thyroid. However, the major cause of cessation of reproductive activity is the depletion of the oocytes in the ovaries, a process which begins at birth in all mammals. Mitosis is completed *in utero*, and even within the same inbred strain, mice show extensive individual differences in initial oocyte stocks and in the numbers of oocytes remaining at the approach to acyclicity (Gosden, *et al*., 1983). Age-related ovarian depletion has been demonstrated to occur in rodents (Nelson, *et al*., 1982).

OSHA should not regard the finding of ovarian atrophy at the 6.25 and 20 ppm concentrations of BD only after the end of the mouse's reproductive

life as evidence of a "reproductive" effect. The 20 ppm concentration should be considered a NOEL for ovarian atrophy. In addition, for reasons discussed below, the unique sensitivity of the B6C3F1 mouse to BD toxicity makes the evidence of ovarian atrophy in the B6C3F1 mouse irrelevant to human risk assessment.

c. Severity of Ovarian Atrophy

OSHA expresses concern that ovarian atrophy, if sufficiently extensive, would cause a failure of implantation or early death of the fetus. 55 Fed. Reg. 32772. In humans, clinical problems reflecting ovarian atresia typically arise only when major functional changes are noted, as might be induced with antineoplastic agents, exposure to synthetic hormones or hereditary disfunction. As described previously, ovarian atrophy after completion of the expected reproductive life would not result in a failure to conceive or death of the fetus. Therefore, BD concentrations of 6.25 and 20 ppm should not be considered to be reproductive hazards. In addition, the preliminary data do not indicate marked atrophy at concentrations as high as 62.5 ppm (only minimal to moderate levels of ovarian atrophy were reported for exposures of 15 to 24 months), although in the absence of defined criteria, the actual severity of these changes and their biological significance is unknown.

d. Health Status of Animals

The presence of ovarian atrophy in dying animals or animals with tumors should not be identified as an unusual observation. Non-neoplastic changes identified in female mice at the 6.25 ppm concentration and higher included bone marrow hyperplasia, extramedullary hematopoiesis in the liver, etc., thymic necrosis (atrophy), cardiac endothelial hyperplasia, alveolar epithelial hyperplasia, forestomach epithelial hyperpolasia, ovarian atrophy, and ovarian granulosa cell hyperplasia. No additional non-neoplastic lesions were associated with the 20 ppm concentration of BD. At higher concentrations, additional non-neoplastic lesions included forestomach ulcers and ovarian angiectasis (62.5 ppm and higher), uterine atrophy (200 and 625 ppm)

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and myocardial mineralization (625 ppm). As previously described, the ovary is sensitive to the general health and nutrition of the animals, and just as other rapidly proliferating tissues were affected by BD-exposure in the mouse, the rapidly proliferating ovarian and testicular tissues were, and should be expected to be, sensitive to the toxic effects of BD. However, the relevance of the sensitivity of the mouse ovary to human risk becomes questionable, because estrous cycling occurs at four- to five-day intervals in a rodent, in comparison with the approximately 28-day ovarian cycle that occurs for human females, and the mouse is uniquely sensitive to BD.

It also cannot be forgotten that these mice were not healthy, and that although these effects of BD on rapidly proliferating tissues, as well as production of tumors, did not necessarily occur in the same animals, ovarian atrophy was only one of many effects of BD in the mouse. As the onset and duration of reproductive life are closely correlated with the lifespan of most mammals, the dose-dependent incidences of effects on proliferating tissues and reduced lifespan of mice exposed to BD would be expected to result in ovarian atresia in response to altered health status, in addition to the age-related ovarian depletion (Nelson, et al., 1982) in the numbers of oocytes that occurs in rodents.

An additional factor contributing to the overall health status of the mouse is the interaction of the toxic effects of BD and the nutritional status and stress of the study procedures. In the NTP chronic studies, the mice were feed deprived during exposure, but not water deprived. The mouse is uniquely sensitive to water and diet deprivation, and it has long been recognized that physiological changes and an increased response to toxicants are caused in mice by feed and water deprivation for five or more hours. Even when compensatory feed and water consumption occurs, the effects of transient pertubations of the physiological state of the mouse are unknown, and it is also unknown whether the preexisting status is regained. These factors have historically made the mouse model of questionable relevance in prolonged inhalation studies because the mice must experience the stress of daily feed and water deprivation for many months or be exposed orally to the test substance as the result of ingestion of contaminated water and diet. It is well documented that water or feed deprivation can increase the incidences of

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congenital malformations in many mouse strains, although the dam apparently recovers, and that the mouse ovary has been shown to be particularly sensitive to nutritional deprivation. For example, the onset of puberty in the mouse can be delayed by lowering caloric intake; when caloric intake is restored, previously deprived females are reproductively competent and can reproduce when their littermate controls are infertile or dead (Visscher, et al., 1952).

Based on studies by Mattison (1979), it appears that inbred mouse strains are more sensitive than rats to the effects of polycyclic aromatic hydrocarbons on oocyte and follicle destruction (Christian, et al., 1983). The C57BL/6J mouse is the strain most commonly used to study the relative contributions of age-associated changes in neuroendocrine and ovarian function as well as chronic exposure to ovarian hormones and their effect on changes and ultimately, cessation of ovarian cyclicity. As identified by Nelson and Felicio (1990), in this strain, cyclicity changes from a four-day to a five-day cycle length at approximately eight months of age, with the cycle then becoming progressively longer, and cycling generally stopping when the mice are 12 to 15 months of age. These ages do not remarkably differ from the 12-month period during which exposure to BD occurred in the first lifetime study in mice, indicating related ovarian atrophy should be expected, as previously described.

e. Unique Sensitivity of the B6C3F1 Mouse to BD

Mice are more sensitive to some toxicants than are other species, and there are many strain differences in susceptibility. This attribute of the mouse has frequently provided a tool for studying biochemically-mediated effects. For example, genetic differences in metabolism of some drugs can be studied using inbred mouse strains, with highly inducible levels of a drug-metabolizing enzyme activity (aryl hydrocarbon hydroxylase) reflecting a Mendelian dominant trait over low inducible levels. Fraser (1965) performed elegant studies demonstrating multiple genetically-mediated differences in the response of the various strains of mice to toxicants. Although most of this research was performed in order to demonstrate differences in the response of genetically different embryos, it also demonstrated the great variability in the genetically-mediated strain-specific response of the adult mouse.

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Based on the previously described chronic studies, it is clear that there are remarkable differences between the Sprague-Dawley rat and the B6C3F1 mouse strains in their response to BD. As described in the discussion of developmental effects (Section IV), significant species differences in response to BD also have been demonstrated between the Swiss CD-1 mouse and Sprague-Dawley rat in inhalation developmental toxicology studies. In the Sprague-Dawley rat, the maternal NOEL for BD is 200 ppm, and the developmental NOEL is 1000 ppm. In contrast, in the CD-1 mouse, the maternal and developmental NOELS for BD are 40 ppm. In comparison to the rat strain tested, the increased sensitivity of these mouse strains to the effects of BD, as demonstrated in developmental toxicity testing and assessment of ovarian and testicular atrophy, appears to be related to quantitative and qualitative differences in uptake, metabolism and excretion of BD.

Pharmacokinetic studies have demonstrated that remarkable differences exist in these measures among man, primate, rat and mouse. These data are presented in statements by Drs. Bird and Bolt. BD is metabolized to the epoxide, 1,2-epoxy-3-butene (cytochrome P-450 in microsomal preparations in the presence of a NADPH-regenerating system). The epoxide intermediate can be further metabolized either by epoxide hydrolase and/or monoxygenase to 3,4-epoxy-1,2-butanediol (via 3-butene-1,2-diol) and to diepoxybutane, or by conjugation with glutathione by glutathione-S-transferases. As occurs for many chemicals, activation to the epoxide appears to be necessary for carcinogenicity, and the more rapid uptake and slower excretion of the epoxide in the mouse, in comparison with other species, is believed to be the mechanism by which BD is tumorogenic. Species differences in the rate of formation of 1,3-epoxy-3-butene from BD in liver microsomes exist, with the mouse > rat > man > monkey, and the ratio between mouse and monkey approximately 7:1 (Schmidt and Loeser, 1985).

Data also exist in other systems demonstrating that the epoxides are genotoxic and cytotoxic in mouse but not in rat (de Meester, 1988). As described by Arce, et al., (1990), sister chromatid exchange (SCE) and micronucleus induction results indicated that BD was genotoxic in the bone marrow of the mouse but not the rat, paralleling the results of the chronic

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bioassays. BD did not induce unscheduled DNA synthesis (UDS) in mouse or rat hepatocytes following in vivo exposure nor in vitro in rat and mouse hepatocytes induced by EB or DEB. Gene mutation (Ames) tests of BD in rat, mouse and human liver S9 metabolic systems indicate that BD was genotoxic in vivo but only weakly, if at all, genotoxic in vitro.

As previously described, the epoxides of BD are the toxicants identified as responsible for the effects of BD on rapidly proliferating cells, including the ovarian atresia (oocyte destruction), the reduced proliferation of other tissues, and the tumors that occur in multiple tissues. The mouse is uniquely sensitive to BD because of the pharmacokinetic profile of this chemical in this species, in comparison with that identified in other species, including man. As described in the following section, two chemicals that are structurally-related to BD also demonstrate species-specific responses that reflect their pharmacokinetic profiles and formation of epoxides.

f. Effects of Structurally-Related Chemicals

Although pharmacokinetic data for BD in Fischer-344 rats are unavailable, it appears that this rat strain has a reduced response to two structurally-related chemicals, in comparison with the B6C3F1 mouse, and that for both of these chemicals oocyte destruction by epoxides of the chemical is the cause of the ovarian carcinogenicity and atrophy in the mouse, with the mouse producing remarkably higher levels of the epoxides than the rat.

1) 4-Vinylcyclohexene

In a study of 4-vinylcyclohexene (VCH), VCH induced ovarian tumors in B6C3F1 mice but not in Fischer-344 rats (Sipes, et al., 1989). The production of the ovarian tumors appears related to destruction of oocytes by the epoxides of VCH. VCH-1,2-epoxide is formed in vivo in mice and is the apparent cause of the ovotoxicity and ovarian carcinogenicity.

2) 4-Vinyl-1-Cyclohexene Diepoxide

VCH is a structural analog and starting material for production of 4-vinyl-1-cyclohexene diepoxide (VCHD). In a study of VCHD in Fischer-344

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rats and B6C3F1 mice (Chhabra, et al., 1990 and National Toxicology Program, 1989b), the mouse was again remarkably more sensitive to the carcinogenic effects of this chemical, and ovarian follicular atrophy and tubular hyperplasia occurred in mice but not in rats, as also did benign or malignant granulosa cell tumors and benign mixed tumors in the ovary. Again, differences in the levels of epoxides present in the various tissues were the apparent cause of the differences in the responses of the two species.

g. Appropriate Safety Factors for Mouse Data

For the reasons presented in the preceding pages, the evidence of ovarian atrophy in the mouse is not relevant for human risk assessment. The data that support this conclusion also demonstrate that if any mouse data are used for an assessment of developmental or reproductive hazards, a safety factor of 100 would not be appropriate.

OSHA's safety factor of 100 includes a factor of 10 based on the assumption that the mouse metabolism causes the critical organs in the mouse to receive a smaller dose of the toxicant, compared to larger animals, including humans. For BD, the available data, presented in other testimony, indicates the opposite is true -- the mouse receives a larger internal dose of BD epoxides, by more than a factor of 10 (based on a comparison with primates). This indicates that the safety factor of 10 based on metabolism is not necessary. Based on the mouse's demonstrated unique sensitivity to BD, there does not appear to be a need for any safety factor.

B. National Toxicology Program Sperm Studies

These studies of potential effects of BD on reproduction emphasize evaluation of sperm in mice, through dominant lethal and sperm-head morphology studies. OSHA identifies 200 ppm as a NOEL for morphologically abnormal sperm heads and a LOEL for dominant lethal effects. These values are based on effects that should be disregarded either because they are not dose-related or because they are not biologically important. The correct NOEL for each study is 5000 ppm.

1. Sperm-head Morphology, Battelle, 1988a - mouse

In the Sperm-head Morphology Study, (Battelle, 1988a) B6C3F1 mice, 20 per group, were exposed to BD for 6 hours per day for 5 consecutive days at concentrations of 0, 200, 1000 or 5000 ppm. During the fifth postexposure week the males were killed, examined for gross lesions of the reproductive tract, and their sperm examined. Increased incidences of altered sperm-head morphology were reported for the 1000 and 5000 ppm groups. The relevancy of these observations is questionable because the statistical methods used to identify changes appear to be mathematically biased as the result of the relative sizes of the means and procedures (500 sperm heads from each mouse were categorized as normal or abnormal). The mean percentage of normal sperm heads per total examined was 98.40, 98.08, 97.23* and 96.34* for the 0, 200, 1000 and 5000 ppm groups, respectively. Changes of less than 10% in animal parameters are generally considered not biologically important. These values were 99.7, 98.8 and 97.9 percent of the control group value for the 200, 1000 and 5000 ppm groups, respectively, and these 1 to 2 percent reductions in normal sperm heads are probably biologically meaningless. As noted by the authors, there were no significant differences among the classes of sperm-head abnormalities (mean number of type of abnormal sperm head per total abnormal sperm heads).

Thus, these small changes in sperm-head morphology associated with changes in late spermatogonia or early primary spermatocytes are considered to be of minimal biological importance. It should also be noted that these morphological abnormalities do not necessarily correlate with developmental abnormalities or reduced fertility and are reversible in nature.

2. Dominant Lethal, Battelle, 1988b - mouse

The dominant lethal study was conducted by Battelle (1988b) in CD-1 male mice under the same conditions as those described above for the sperm-head

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* Significantly different from the control group value ($P \leq 0.05$).

morphology study (i.e., 20 males per group were exposed to 0, 200, 1000 and 5000 ppm concentrations of BD for 6 hours per day on 5 consecutive days). Following exposure, the mice were mated for 8 consecutive weeks (two female mice were mated with each male mouse per week). The female mice were sacrificed 12 days after the last day of cohabitation, and the uterine contents examined.

Although the authors report slight effects during the first two weeks postexposure, evaluation of the data demonstrates that these statistically significant observations were neither dose-dependent nor biologically important (live litter sizes were unaffected). In week 1 postexposure, there were 37, 40, 38 and 37 pregnant females in the 0, 200, 1000 and 5000 ppm groups, respectively. As compared with control group values, dead implantations per total implantations per litter averaged 6.87, 10.31, 12.27* and 7.81 percent in these same respective groups; the percentage of females with more than one resorption was 56.8, 67.5, 73.7 and 54.1, and with more than two resorptions, 13.5, 38.5*, 42.1* and 37.1*. Implantations per litter averaged 11.49, 11.45, 11.45 and 12.08, of which 10.70, 10.25, 10.03 and 11.14 were live.

During week 2 postexposure, there were 35, 39, 36 and 38 pregnant females in the four respective groups. The average number of implantations was significantly reduced for the 5000 ppm group, as compared to the 1000 ppm group value. This significant difference was unrelated to BD because the value (11.45 implants per litter) is well within the expected range for this strain (there were averages of 10.79 to 12.74 implants per control group litter during the eight weeks of mating), and the values were not dose-dependent (in the 0, 200, 1000 and 5000 ppm groups, implantations in week 2 postexposure averaged 11.89, 11.87, 12.78 and 11.45, respectively). Differences in resorptions were also not dose-dependent (there were averages of 0.80, 1.36*, 1.36* and 0.90 resorptions per litter in these same groups). At week 4 postexposure, the percentage of dead implantations in the control group was significantly higher than that in the 5000 ppm group, an observation that is not uncommon in a study of this size, but one that is not generally

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* Significantly different from the control group value ($P \leq 0.05$).

interpreted as a protective effect of an agent. In the four respective groups, the percentage of dead implantations averaged 8.18, 5.09, 5.73 and 4.04* per litter.

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* Significantly different from the control group value ($P \leq 0.05$).

IV. DEVELOPMENTAL TOXICITY - My interpretation of the reports contrasted with OSHA's

A. Carpenter, 1944 - rat

Four developmental toxicity studies have been performed with 1,3-Butadiene (BD). The first of these was reported by Carpenter, et al. (1944) in a study in which rats (12 per sex per group), guinea pigs (6 per sex per group), rabbits (2 per sex per group) and dogs (1 female per group) were exposed to 0, 600, 2300 or 6700 ppm BD in air for a maximum of 202 exposures over a period of eight months (7-1/2 hours per day, 6 days per week). It is unclear from the data provided when mating occurred; male and female rats, guinea pigs and rabbits may have been continuously cohabitated throughout the study.

Although the information provided is limited, and these studies were conducted prior to either the development of, or requirement for, many regulatory guidelines, they were conducted in conformance with expected requirements for the time. These studies are of value because they demonstrate that prolonged exposure to BD at 600, 2300 or 6700 ppm concentrations did not affect the reproductive performance of rats, guinea pigs or rabbits, the three species mated. Toxic levels of BD were given, based on reduced body weight gains of rats and guinea pigs (there were insufficient numbers of rabbits and dogs to evaluate effects on body weight). At the end of the exposures, rats were reported to have weights that were 90.5, 86.3 and 81.2 percent of the control group value at 600, 2300 and 6700 ppm BD, respectively. Data were not provided for the other species. Livers of the 6700 ppm group of rats had light cloudy swelling. No other effects of BD were reported (liver and kidney weights and hematology, blood chemistry and urinalysis parameters were reported as unaffected).

The authors reported that the litter frequency (fecundity) in the rat was reduced (there were 3.3, 2.7, 2.5 and 2.6 litters produced by the 0, 600, 2300 and 6700 ppm groups, respectively). However, these numbers do not indicate an effect of BD because: 1) the values do not decrease in a

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dose-dependent manner, despite a wide range of exposure levels; and 2) average litter size, another measure of fertility, is not affected. The litter sizes reported were 8.4, 7.9 and 7.8 pups per litter for the 600, 2300 and 6700 ppm groups, with the 600 ppm group reported to be larger than the control group, and the mid and high exposure groups equivalent to the control group. All litters were larger than the expected historical value (6.0 pups per litter). These results stand in sharp contrast to OSHA's conclusion that the 2300 and 6700 ppm concentrations produced "maternal toxicity in rats consisting of decreased litter size", 55 Fed Reg. 32753. It is also noteworthy that no testicular or ovarian pathology was seen in the sections examined histologically.

B. Hazleton, Europe (IISRP), 1981b - rat

The second developmental toxicity study was conducted by Hazleton Laboratories, Europe (1981b), sponsored by IISRP. Sprague-Dawley rats were exposed to 0, 200, 1000 or 8000 ppm BD in air for 6 hours a day on days 6 to 15 of gestation (24 rats per group). Negative (40 rats) and positive (26 rats) controls were used appropriately. The methods used followed present guidelines and are sufficient to assess the developmental toxicity of BD. Although this study was not used by OSHA for risk assessment considerations, a reanalysis of this study is provided in order to clarify results that may have been considered to indicate that BD affected the development of rat conceptuses at the 200 ppm concentration or that it was teratogenic at the 8000 ppm concentration.

1. Maternal Body Weights

Maternal body weight gain during the exposure period was significantly reduced by 200 to 8000 ppm concentrations of BD. The reduction in weight gain was most severe during the first three days of exposure, with weight loss evident for the 8000 ppm group. Weight gain for the 200 and 1000 ppm groups was equivalent to control values on days 9-12 and 12-15 of gestation. Significantly reduced weight gain occurred for the 8000 ppm group on days 9-12 of gestation; this group had weight gain equivalent to the control group on days 12-15 of gestation. During the postexposure period, increased weight gains, a compensatory effect that commonly occurs in these types of studies,

were evident for each group that had been exposed to BD. From days 0 to 20 of gestation, the percent maternal body weight gain was 51.5, 50.0, 47.9 and 45.2** at exposure levels of 0, 200, 1000 and 8000 ppm BD. Adjusted mean body weight gains (maternal body weight minus the weight of the gravid uterus) for the entire gestation period (days 0-20) were significantly reduced for the 1000 and 8000 ppm groups. OSHA incorrectly reported weight loss for all groups in the first few days, rather than for only the 8000 ppm group.

2. Caesarean-Sectioning Observations, Including Postimplantation Loss

Exposure to BD at concentrations as high as 8000 ppm did not affect pregnancy incidences, corpora lutea, implantations, percent preimplantation loss, early and late intrauterine deaths, percent postimplantation loss, live litter sizes or the percent fetuses per implantations.

Both the authors and OSHA noted that postimplantation loss was slightly higher in all BD-exposed groups, as stated by OSHA. I do not agree that these increases were related to BD, for the following reasons: 1) the effect was biologically important and statistically significant for only the positive control group; 2) the values for groups exposed to BD did not significantly differ from those for the control group; 3) the slightly increased values for the groups exposed to BD were not dose-dependent; and 4) the small differences in postimplantation loss did not cause differences in live litter sizes (the percent postimplantation loss was 3.6, 6.0, 4.9, 7.3 and 43.7** in the filtered air control group, 200, 1000 and 8000 ppm BD groups, and the positive control group, respectively; mean litter sizes were 12.5, 12.0, 13.4 12.8 and 7.9 fetuses in these same respective groups).

** Significantly different from the control group value ($P \leq 0.01$).

3. Fetal Weights and Crown-Rump Lengths

Fetal weights and crown-rump lengths were significantly reduced for the 8000 ppm group. I disagree that there were effects for all dosage groups, as reported by OSHA. Fetal body weights averaged 3.3, 3.2, 3.2 and 3.1* g, and crown-rump lengths averaged 37.8, 37.2, 37.2 and 35.9** mm in the groups exposed to 0, 200, 1000 and 8000 ppm BD, respectively. As compared with the control group values, the values for fetal body weights and crown-rump lengths for the 200 and 1000 ppm groups did not have differences that were biologically important or statistically significant. In both cases, the values for the 200 and 1000 ppm groups were identical, observations that would not be expected in consideration of the five-fold difference in magnitude between the two exposure levels. The statistical significance of the reduced fetal body weight for the 8000 ppm group is marginal and may be inappropriate for the following reasons: 1) it appears that a one-way analysis of variance (ANOVA) was followed by multiple comparisons using one-tailed t-tests; these methods are generally considered inappropriate because body weights can increase or decrease (making a two-tailed t-test more appropriate), and the use of multiple t-tests increases type 1 error (i.e., increases the probability that a value will be statistically significant; Muller, et al., 1984; Neter, et al., 1985; and SAS Institute Inc., 1988); 2) it appears that the positive control group value was included in the analysis, (values for a positive control group should be excluded from the comparisons of the negative or vehicle control group with the groups exposed to the test substance). When these fetal body weight data were reanalyzed at my laboratory followed by two-tailed t-tests excluding the values for the positive control group, there were no statistically significant differences among the four groups. This finding indicates that the cause of the statistically significant reduction in the fetal body weight for all fetuses in the 8000 ppm group (and not for male and female fetuses, when calculated separately by sex) may be the use of inappropriate statistical analyses.

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* Significantly different from the control group value ($P \leq 0.05$).
** Significantly different from the control group value ($P \leq 0.01$).

Regardless of questions regarding statistical significance of fetal body weights in this study, it is agreed that the 8000 ppm group had reduced fetal body weights and crown-rump lengths in this study, and that these are interrelated observations indicating delays in development. It should be noted that such growth retardations are usually considered to be reversible and commonly occur when maternal body weight gain is retarded. Maternal body weight gain was reduced during the dosage period for groups provided each concentration of BD, with the 8000 ppm group having actual weight loss during the first three days of exposure.

4. Fetal Anatomical Defects

a. Classification System Used

The testing laboratory classified fetal abnormalities as: major (rare and/or possibly lethal), minor (commonly occurring, nonlethal defects) or variants (variations in skeletal ossification). In addition to this procedure, the laboratory graded abnormalities of the rib cage, citing slight and moderate waviness as a minor defect and marked and severe waviness as a major defect. Because the fetuses in all groups in this study were considerably smaller than those observed in previous studies performed at the laboratory, they noted that skeletal ossification was less advanced than normal, and that some aspects of retardation of ossification which they would normally regard as minor defects were classified as variants in this study.

It should be noted that the use of these arbitrary categories and tabulation of fetal incidences, and not both fetal and litter incidences, make it very difficult to independently evaluate the fetal observations or to make interlaboratory comparisons. This may be the reason that the conclusions of the authors were generally accepted and repeated in the OSHA document. It appears that OSHA did not attempt to further analyze these observations.

b. Delays in Ossification

The authors concluded that a higher incidence of minor fetal defects and variants was observed in the BD-exposed groups than in the control group, and

that this was associated with the smaller fetal size in the BD-exposed groups. I agree with this conclusion for the 8000 ppm group. As noted by the authors and OSHA, the significant increase in the number of litters with fetuses with skeletal variants that occurred for the 8000 ppm group was interrelated with the reduced body weights and smaller crown-rump lengths that occurred for this group. These variants consisted of delays in ossification of the thoracic centra (bipartite thoracic centra) and incomplete ossification of the sternum, delays in ossification (Hannah and Moore, 1971) that are generally believed to reverse. I would also note that the 8000 ppm group had statistically significant increases in the fetal and/or litter incidences of delays in ossification of the ribs (wavy ribs), an alteration that has been shown to be reversible and often associated with small fetal body weights and sizes (Nishimura, et al., 1982; Sterz, et al., 1985).

c. Absence of Major Fetal Defects

The authors also concluded that there was an indication of teratogenicity due to the presence of major fetal defects at the 8000 ppm concentration of BD. OSHA repeated this conclusion, although I disagree for the reasons provided below.

The report states that the fetal incidences of major skeletal defects were increased in all BD-exposed groups, with significant increases in litter incidences for the 1000 and 8000 ppm groups. These "major" skeletal defects principally consisted of wavy ribs, which are known to be reversible delays in ossification (Nishimura, et al., 1982; Sterz, et al., 1985), and should not have been categorized as major defects. Other "major" skeletal defects reported included abnormalities of the skull, spine, sternum, long bones and ribs.

Several of these "major" skeletal defects appear to be delays in ossification, rather than major defects (frontals and nasal foreshortened, 2 fetuses in the 8000 ppm group; short ileum, 2 fetuses in the 8000 ppm group; humerus, radius and ulna short, 1 fetus in the 8000 ppm group; femur, fibula and tibia short, 1 fetus in the 8000 ppm group). All of these "major" alterations occurred at incidences that were not statistically significant and

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are within the historical ranges for this rat strain (Lang, 1988, and my laboratory). "Major" alterations generally classified as malformations included curvature of spine due to an additional vertebral arch, 1 fetus in the 8000 ppm group; fused ribs, 1 fetus in the 1000 ppm group and 1 fetus in the 8000 ppm group; malformed rib, 1 fetus in the 8000 ppm group. Major fusion of sternebrae occurred for 2 fetuses in the 8000 ppm group, but this is an observation I usually consider to represent accelerated development, not a "major" alteration, because the sternum is fused in the adult. Although some alterations were increased for the 8000 ppm group, or occurred only for this group, as described above, all incidences are within the ranges observed historically for this rat strain (Lang, 1988, and my laboratory), and single, high dosage group events that are not statistically significant are generally considered to be spontaneous and unrelated to the test substance.

d. Other Changes Unrelated to BD

1) Minor Skeletal Defects

The number of litters with fetuses with minor skeletal defects was significantly higher for the 200 ppm group, an observation that should be interpreted as unrelated to the test substance because the values were not dose-dependent.

2) Minor External/Visceral Defects

As reported by the author of the report and OSHA, the number of minor external/visceral defects was higher in the BD-exposed groups than in the filtered air control group (16.9, 23.8, 24.4* and 25.5 percent of the fetuses per litter had this observation in the four respective groups), and the litter incidence for this observation was significantly increased for the 1000 ppm group (13, 18, 20* and 19 litters had fetuses with minor external and visceral defects). Neither of these observations should be attributed to BD because the values were not dose-dependent.

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* Significantly different from the control group value ($P \leq 0.05$).

3) Subcutaneous Hemorrhagic Areas

Statistical analysis revealed significant increases in the fetal incidences of subcutaneous hemorrhagic areas (hematoma) in the 200 and 1000 ppm groups. There were 6, 8*, 14* and 7 fetuses in the control, 200, 1000 and 8000 ppm groups with this alteration, respectively; the litter incidence was not significant (5, 8, 9 and 6 litters in these respective groups had fetuses with this alteration). It should be noted that subcutaneous hemorrhage is generally secondary to trauma during removal of the fetus from the uterus, and the absence of dose-dependency for this observation tends to indicate that trauma was the cause of these observations, and is also in agreement with the author's conclusions regarding the small fetal sizes that occurred in this study.

4) Lens Opacities

It was also reported that the number of fetuses with lens opacities was significantly increased in the 8000 ppm group (lens opacities were reported as unilateral or bilateral); there were totals of 37, 35, 38 and 44* fetuses in which one or both eyes had a lens opacity in the respective groups; although there appears to be a dose-dependency for this minor alteration, this observation probably is an artifact interrelated with the examination procedure and/or fixation. The numbers of fetuses with only unilateral changes or only bilateral changes were not dose-dependent in the respective four groups, supporting the conclusion that this finding is an artifact. Unilateral lens opacities occurred for 19, 11, 8 and 13 fetuses, and bilateral lens opacities occurred for 18, 24, 30 and 31 fetuses. The litter incidences for unilateral and bilateral lens opacities combined were 14, 10, 12 and 13 in these same respective groups.

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* Significantly different from the control group value ($P \leq 0.05$).

5) Heart Malformations

Although it is true that two fetuses in the 8000 ppm group had heart malformations, it is doubtful that these malformations were effects of the test substance. Even in combination, the incidences were not statistically significant, and the malformations were different. One of these fetuses had multiple external/soft tissue malformations [abnormal facial shape, subcutaneous oedema and sunken eyes (interrelated observations reflecting oedema), persisting truncus arteriosus, and undescended testes], and the other fetus had an interrupted aortic arch as its only external/visceral defect. The types of alterations that occurred in the fetus with multiple malformations occur spontaneously in this strain (Lang, 1988, and my laboratory).

6) Minor Skeletal Defects

It remains clear that the numbers of fetuses with significant increases were not dose-dependent for minor skeletal defects. It should be noted that when analyzed in terms of the litter incidences, 10, 21, 18 and 15 litters had fetuses with minor skeletal defects, incidences that are also clearly not dose-dependent.

C. Battelle, 1987a - rat

My interpretation of the data from the initial developmental toxicity study is supported by the results of a second rat developmental toxicity study, conducted by Battelle (1987a). In this study, Sprague-Dawley rats (24 to 28 per group) were exposed to BD for 6 hours per day at concentrations of 0, 40, 200 or 1000 ppm on days 6 through 15 of gestation. The dams were Caesarean-sectioned on day 20 of gestation, and the fetuses subsequently examined. Acceptable procedures were used to identify maternal and developmental effects.

The 40 and 200 ppm concentrations of BD were not toxic to the dams. Body weight gain was significantly reduced for the 1000 ppm group on days 6 to 11 of exposure. This effect did not persist thereafter. The corrected average maternal body weight gain during gestation minus the weight of the gravid uterus was significantly reduced for the 1000 ppm group. Thus, the maternal NOEL was clearly defined as 200 ppm, and maternal toxicity was evident as significant reduction in body weight gain at 1000 ppm. All other parameters including percent pregnant, and averages for implantations, resorptions, live litter sizes, placental weights, fetal sex ratios and fetal body weights were unaffected by maternal exposure to BD at concentrations as high as 1000 ppm. There were no significant differences among the groups in the incidences of fetal malformations and variations. All fetal alterations that occurred were within the ranges observed historically (Lang, 1988, and my laboratory). Based on these data, the developmental NOEL for BD in the rat is greater than 1000 ppm, the highest concentration tested.

D. Battelle, 1987b - mouse

The fourth developmental toxicity study was conducted by Battelle (1987b) in Swiss CD-1 mice (18 to 22 per group) exposed to BD for 6 hours per day at concentrations of 0, 40, 200 or 1000 ppm on days 6 through 15 of gestation. Although this study had an extremely poor pregnancy rate, as sometimes occurs in mouse studies, there were adequate numbers of pregnant mice in the groups for analysis (pregnancy rates of 56, 57, 68 and 67 percent in the 0, 40, 200 and 1000 ppm dosage groups, respectively, resulted in 18, 19, 21 and 20 pregnant dams with litters available for examination). The mice were sacrificed on day 18 of gestation, and standard Caesarean-sectioning and fetal examinations were made.

The 200 and 1000 ppm concentrations were toxic to the dams. Maternal body weight gains for the 200 and 1000 ppm groups were significantly reduced on days 11 to 16 of gestation. Gravid uterine weights for these groups were also significantly reduced, as were the corrected maternal body weight gains

during gestation (maternal body weight gain on days 0 to 18 of gestation minus the weight of the gravid uterus). The 1000 ppm group had significantly reduced maternal body weights on day 18 of gestation. The 40 ppm group was unaffected.

There were no dose-dependent, significant differences in the averages for implantations, resorptions, live fetuses or sex ratios per litter. The only significant value was a reduction in early resorption for the 200 ppm group, as compared with the control group value, an observation that was not dose-dependent and does not indicate developmental toxicity.

The authors and OSHA report significant reductions in male fetal body weights for the 40, 200 and 1000 ppm groups; combined male and female fetal body weights and female fetal body weights were significantly reduced only at 200 and 1000 ppm. The significant decrease for the 40 ppm group male fetal body weight was an effect of litter size and not an effect of BD for the following reasons:

1) the significant reduction was caused by the increased numbers of fetuses in this group. When fetal body weights were analyzed at my laboratory using an analysis of covariance and litter size as the covariate, there were no significant differences in the 40 ppm group fetal weights;

2) the method of analysis used by the testing laboratory (multiple comparison using a t-test) is known to increase the probability of a type 1 error (i.e., increase the probability that a value will be statistically significant; Muller, et al., 1984; Neter, et al., 1985; and SAS Institute Inc., 1988). This criticism that the statistical significance for the value resulted from use of an inappropriate statistical analysis is further substantiated because: a) the testing facility no longer uses this method (Staples, R.E., personal communication with an author, T.J. Mast, 1990); and b) in the companion developmental toxicity study of BD in rats, an alternate method, one generally considered to be appropriate, Duncan's multiple-range test, was used for further statistical analyses if the results of an ANOVA were significant.

Placental weights were significantly reduced for the 200 and 1000 ppm groups (combined sexes), for male but not female fetuses in the 200 ppm group, and for both sexes in the 1000 ppm group.

No significant differences occurred among the groups in the incidences of malformation, although extra ribs, a common variation in mice, was significantly increased in the 200 and 1000 ppm groups, and delayed ossification of the sternum, a variation that is reversible and commonly occurs in fetuses with small body weights, was significantly increased for the 1000 ppm group.

Based on these data in CD-1 mice, the maternal and developmental NOELS for BD are 40 ppm. Maternal body weight gains were significantly reduced by 200 and 1000 ppm concentrations of BD. These concentrations resulted in reduced fetal and placental weights and supernumerary ribs; the 1000 ppm concentration also caused retarded sternal ossification. No concentration of BD tested caused embryo-fetal deaths or malformations. These findings are in agreement with the conclusions of the authors regarding the absence of teratogenicity at any concentration tested, and their conclusions regarding retarded growth, placental weights and increases in supernumerary ribs at the 200 and 1000 ppm concentrations of BD, and the retardation of sternal ossification and general ossification at 1000 ppm concentrations of BD.

Thus, the expected greater toxicity of BD in mice, as compared with rats, was evident (the maternal NOEL in mice was 40 ppm, as compared with the maternal NOEL of 200 ppm in rats), and the increase in interrelated effects on embryo-fetal development followed the same pattern (the developmental NOEL in mice was 40 ppm, as compared with the developmental NOEL of 1000 ppm in rats). Adverse effects on the conceptuses of both species were limited to reversible growth retardations and variations in ossification that occurred at concentrations that were toxic to the dams (caused significant reductions in maternal body weight gains). These conclusions are also in agreement with OSHA's conclusion that the 40 ppm concentration of BD was a NOAEL (no adverse effect level, page 32772).

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These conclusions contrast with those of the authors of the reports that were repeated in the OSHA document, "that the fetus [may] be more susceptible than the dam". Rather, they demonstrate that the method of statistical analysis and the larger litter sizes of the 40 ppm group were the causes of the apparent significant decrease in male fetal weight for this group, and that all significant effects occurred only at concentrations of 200 ppm and higher in mice and concentrations of 1000 ppm and higher in rats. The developmental NOELS in mouse and rat were 40 and 1000 ppm, respectively.

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V. DISCUSSION AND CONCLUSIONS

An examination of the data collected from the reproduction, developmental toxicity and carcinogenesis studies of BD in rats and mice leaves little doubt that the mouse is extremely sensitive to BD and/or its metabolites. Studies in rats identify a developmental NOEL of 1000 ppm and a maternal NOEL of slightly less than or 200 ppm. Data from the mouse, on the other hand, identify maternal and developmental NOELS of 40 ppm. Lifetime studies suggest an even greater sensitivity in adult mice exposed over many weeks because: 1) ovarian atrophy occurs in B6C3F1 mice at 62.5 ppm at 65 weeks of exposure, and after completion of the reproductive life of this species at 6.25 and 20 ppm (65 or more weeks of exposure); 2) testicular atrophy occurs in both B6C3F1 and CD-1 mice at 625 ppm BD, with reduced relative testicular weights at 200 and 625 ppm concentrations of BD; and 3) adult rats exposed over a longer period to concentrations of BD as high as 8000 ppm did not demonstrate increases in ovarian or testicular atrophy.

Pharmacokinetic data strongly suggest that the metabolism of BD to its epoxide, 1,2-epoxy-3-butene, is responsible for these differences between the two species. The more rapid uptake and slower excretion of the epoxide in the mouse, in comparison to the rat (and the primate), is believed to be the mechanism by which BD is tumorogenic, produces ovarian and testicular atrophy, affects other rapidly proliferating tissues, and causes a lower NOEL for the developmental toxicity of BD in the mouse.

It is my opinion that OSHA currently has applied too much weight to the mouse studies, a species that is not relevant for use in calculating appropriate safety factors for humans because of the pharmacokinetic differences between these two species. OSHA appears to overlook the large margin of safety provided by the more appropriate species, the rat. OSHA's conclusion that humans are at increased risk of reproductive injury at 2 ppm

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is incorrect and based on studies in mice (developmental toxicity study, dominant lethality study, sperm-head morphology study, carcinogenicity study), an inappropriate species for use in identifying NOELS for use in identifying the human risk.

OSHA has identified developmental and reproductive effects as follows:

Study/Species	OSHA's NOEL	Appropriate NOEL
Sperm-head Morphology/ B6C3F1 Mice (Battelle, 1988a)	200 ppm	5000 ppm
Dominant Lethal Study/ CD-1 Mice (Battelle, 1988b)	200 ppm (LOEL)	5000 ppm
Testicular Atrophy/ Lifetime Study/ B6C3F1 Mice (NTP, 1982, 1984)	200 ppm	200 ppm
Ovarian Atrophy/ Lifetime Study B6C3F1 Mice (NTP, 1989a, preliminary data)	6.25 ppm	20 ppm
Developmental Toxicity/ CD-1 Mice (Battelle, 1987b)	40 ppm (NOAEL)	40 ppm (developmental) 40 ppm (maternal)

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Each of these studies was conducting using mice, a species of questionable relevance to humans primarily because of the unique sensitivity of the mouse to BD. OSHA did not consider the following data base of negative data in other species:

Study/Species	NOEL	
Developmental and Reproductive Studies, Rats, Guinea Pigs, Rabbits, Dogs (Carpenter, et al., 1944)	6700 ppm	Exposure occurred for a period of eight months; rats, guinea pigs and rabbits were bred and their fertility was not impaired; no ovarian or testicular pathology was identified (only female dogs were tested). Although few animals were tested, no remarkable hazard was identified following prolonged exposure to high concentrations (600, 2300 or 6700 ppm).
Testicular Atrophy/ Lifetime Study/ Sprague-Dawley Rats (Hazleton, Europe, 1981a)	8000 ppm (111 weeks)	
Ovarian Atrophy/ Lifetime Study/ Sprague-Dawley Rats (Hazleton, Europe, 1981a)	8000 ppm (105 weeks)	
Developmental Toxicity Sprague-Dawley Rats (Hazleton, Europe, 1981b)	1000 ppm (developmental) 8000 ppm retarded growth ≤200 ppm (maternal)	
Developmental Toxicity Sprague-Dawley Rats (Battelle, 1987a)	1000 ppm (developmental) 200 ppm (maternal)	

Over emphasis of the preliminary findings of atresia of the mouse ovary after completion of the reproductive life of this species and following prolonged exposure at 6.25 and 20 ppm is particularly inappropriate, in consideration of the sensitivity of the species and the anatomical and physiological differences between human and mouse ovaries. As noted above, there is also disagreement with OSHA's interpretation of many of the studies regarding reproduction and development.

Use of a safety factor of 100, based on all other reproductive and developmental NOELS for BD, other than those for the mouse ovary and mouse developmental toxicity study, observations that are considered inappropriate for use in reproductive risk assessment because the mouse is a uniquely sensitive species, does not indicate an increased risk to humans at 2 ppm. If considerations are restricted to observations in a more appropriate species, the rat, the lowest reproductive/developmental NOEL is 1000 ppm, with only retarded fetal growth identified at 8000 ppm. Neither testicular nor ovarian atrophy was produced by exposure of rats to 8000 ppm for 105 or 111 weeks or exposure of rats, guinea pigs, rabbits and dogs (female) to 6700 ppm for eight months. Application of a safety factor of 100 to these data indicates no increased risk of reproductive or developmental toxicity to humans at 10 ppm.

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COMMENTS ON AND ALTERNATIVE RISK ASSESSMENT FOR OSHA-PROPOSED BUTADIENE STANDARD

NOVEMBER 9, 1990

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5.0 SUMMARY

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1.0 SUMMARY

The information cited by OSHA was reviewed and an independent alternative risk assessment was conducted. The risk assessment utilized new data on pulmonary retention and species differences in pharmacokinetics of butadiene, allowing for more accurate quantitative dose-response evaluations.

The first portion of the attached comments correct the OSHA raw potencies resulting in risk estimates of less than 1 in 1000 for both the mouse and rat for the 2 ppm occupational exposure scenario (45 years of a 74 year lifetime, 250 days per year, 8 hours per day). The corrections are based on the species differences in retention and metabolism of butadiene.

Next, the risk assessment develops independent alternative raw cancer potency estimates based on the second NTP mouse bioassay and the IISRP rat bioassay. The raw cancer potencies are then adjusted based on dose level effects and species differences in the retention and metabolism of butadiene.

It is demonstrated that the range of risk estimates spans 16 orders of magnitude and the median risk estimate is in the 1 in 10,000 range considering 56 different cases based on both the rat and mouse. The highest risk estimates result from the female mouse and the lowest risk estimates result from the male rat with risk estimates of near 1 in 1,000,000.

It is concluded that the mouse is unique in its ability to produce higher levels of butadiene epoxides and has less capacity for detoxifying the biologically active epoxides. Thus the mouse is at significantly higher risk than is the rat, primate, or man.

OSHA's concern that ovarian atrophy seen in female mice exposed to low levels of butadiene might indicate potential risk to humans is not warranted. As detailed below, the mouse is extraordinary and unique in the ovarian response following butadiene exposure because of the high levels of butadiene epoxide formed by the mouse. The ovarian atrophy seen in the mouse but not in the rat, is also observed in the mouse with epoxides of related compounds. The CMA Butadiene Panel through its consultant Dr. M. Christian has provided a careful, detailed analysis of the animal toxicity data. It is concluded that mouse ovarian atrophy is not a hazard for work place exposure to 2 ppm and that butadiene does not represent a reproductive hazard at low ppm exposures.

In summary, the conclusion is reached that the risk resulting from work place exposure to 2 ppm, even including some "worst case" assumptions, is less than 1 in 1000. In reality, residual risk is likely to be substantially smaller than 1 in 1000 based on the weight of the evidence.

It is concluded that a 2 ppm PEL is protective for all known toxicological endpoints. The setting of the 10 ppm STEL is optional.

2.0 INTRODUCTION

Comments on a number of issues pertaining to OSHA's estimation of risk resulting from exposure to butadiene are offered in the following discussion.

Butadiene presents a challenge to the risk assessor. If one only had the epidemiology studies to review, the conclusion would be reached that there is no apparent cancer problem resulting from present occupational exposures. It is recognized that within the total epidemiology data set, there are isolated anomalies that either represent very unusual circumstances that run counter to conventional dose-response principles or are statistical artifacts. On the other hand if one only had the mouse oncogenicity studies, great concern could be raised over statistically significant increases in lung tumors at exposure levels as low as 6.25 ppm. The reality for the risk assessor and ultimately the risk manager is that not only are there results of epidemiology studies and 2 mouse oncogenicity studies, but there is also a high dose lifetime rat oncogenicity study and fairly extensive mutagenicity and metabolism data on butadiene and related compounds that aid in characterizing the risk.

The present "state of the art" of risk assessment does not allow a precise determination of the risk. What the risk assessment can do is to incorporate all the relevant information and present the resulting range of risks alone with the assumptions and uncertainties. From such an analysis a weight of the evidence based conclusion can be derived. It is clear based on more recent data and other considerations, that the OSHA risk estimate is overstated. We are encouraged that OSHA went beyond the simple but less meaningful conservative worst case analysis and utilized information beyond applying the linearized multistage model to the animal tumor counts in its risk assessment. We note particularly OSHA's acknowledgement of the confounding role of the murine retrovirus, and the concept that absorbed dose is a better indicator of exposure than butadiene concentration in chamber air.

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The major differences in the risk assessment presented in the following comments and that presented by OSHA include incorporating new pulmonary uptake data, greater utilization of the species specific butadiene metabolism data, an approximate time-to-tumor analysis of the second NTP mouse oncogenicity study (NTP-II), and an increased emphasis on the rat study as being more directly relevant to man than is the mouse. The resulting range of risk estimates are presented. The weight of the evidence clearly demonstrates that the best informed estimates of risk are smaller than OSHA's criteria for significant risk.

3.0 ALTERNATIVE CANCER RISK ESTIMATIONS

The approach used in the following sections are presented from 2 perspectives. Firstly, the OSHA risk assessment is reviewed (Section 3.1) in light of new information on pulmonary retention and species differences. Secondly, an independent alternative risk assessment is presented in Section 3.2 based on the NTP-II mouse study and the IISRP rat study. This review and further analysis clearly demonstrate that OSHA has overestimated the risk based on mouse hemangiosarcomas and pooled tumors from NTP-I.

3.1 QUANTITATIVE ALTERNATIVE ANALYSIS OF OSHA'S RISK ASSESSMENT

First, the OSHA estimates of potency based on the NTP-I mouse study will be corrected by incorporating current information on retention and metabolism discussed in Appendix 1. The parameters and values used in modifying the OSHA estimates are given in Table 1 and the data OSHA used are presented in Table 2. In Section 3.2 (ALTERNATIVE RISK ASSESSMENT BASED ON NTP-II AND IISRP STUDIES) the results of a preliminary analysis of the NTP-II mouse study are presented and the results examined for consistency with NTP-I.

Next, OSHA's potency estimate based on the IISRP rat study will be adjusted to reflect current knowledge of retention efficiency and pharmacokinetics of butadiene.

3.1.1 OSHA Estimates of Risk Based on the NTP-I Mouse Study

It is agreed that the retrovirus played an important role in the incidence of malignant lymphoma and that risk estimates should be developed based on both including and excluding the malignant lymphomas in order to explore the full range of risk and uncertainty. The confounding role of the retrovirus in the magnitude of the malignant lymphoma and possibly

the hemangiosarcoma response is discussed in more detail in Appendix 2.

OSHA's "best" estimate of risk from occupational exposure was based on the female mouse hemangiosarcoma response in NTP-I (Table 3, line 2) and is shown in the tabulation at the end of this section. OSHA's highest estimate of risk (183/10,000) was based on female mouse pooled tumors for which the high dose group was dropped and a one-hit model fitted (Table 3, line 9). Risks were calculated for maximally exposed persons over a period of 45 years (45 years of a 74 year lifetime, 250 days per year, 8 hours per day). The best estimate of risk was reported (OSHA Table 21) as 51 per 10,000 based on the multistage model incorporating 2 stages applied to hemangiosarcoma. These risks are maximum likelihood estimates.

For reasons discussed below, these estimates should not be viewed as estimates of risk per se, but rather as initial "raw" potency estimates requiring incorporation of additional biologically relevant information.

OSHA RAW POTENCY RISK ESTIMATES: NTP-I MOUSE

Same Risk Expressed in 3 Notations

OSHA Best Estimate-Mouse Hemangiosarcoma, Multistage Model

Raw Potency	51/10,000	1 in 200	5E-3

OSHA Mouse Pooled Tumors-2 stage model

Raw Potency	2/10,000	1 in 5000	2E-4

OSHA Mouse Pooled Tumors, One-Hit Model

Raw Potency	183/10,000	1 in 55	1.8E-2

3.1.1.1 Effect of Overestimation of Human Retention Efficiency

An immediate adjustment of OSHA's raw potency can be made for the fact that data initially reported by CAG (EPA,1985) were used in determining the retention of butadiene at levels of occupational interest. This data has since been revised in a later report by Bond et al (Bond et al,1986) summarized in Table 4 and Figure 1. For example, at 2 ppm Figure 1 shows retention in the mouse and

rat to be 17% and 5% respectively. The earlier data used by OSHA on the other hand suggested 100% and 28% respectively (OSHA Notice of Hearing: Table 20). OSHA then ascribed these retention efficiencies to humans in extrapolating from the animal studies.

There is additional pharmacokinetic modeling support that human retention is lower than assumed by both EPA and OSHA. A study by Hattis and Wasson (1987), referenced by OSHA, has also examined the question of human respiratory retention. Using a physiologically based pharmacokinetic (PBPK) model with experimental metabolic data from rats and mice, these authors employed the amount of butadiene metabolized as their dose measure and reached a number of conclusions of direct relevance to the OSHA calculations. Among these was the fact that their model indicated that both human absorption and metabolite formation at low doses is likely to be much lower than assumed by EPA. As OSHA assumed even higher absorption than EPA, this conclusion applies in particular to the OSHA calculations. The PBPK model indicated humans would likely exhibit a 10% retention efficiency at 2 ppm rather than the 100% assumed by OSHA for their mouse to man extrapolation, and the 28% assumed by OSHA in their rat to man extrapolation. As 10% is also intermediate between the interpolated values in figure 1 for rats and mice, 10% was adopted for use in correcting OSHA's risk assessment as the best informed and most reasonable estimate for human retention at 2 ppm. At the end of this section is a tabulation displaying the correction of the various raw potencies.

The effect of using 10% rather than 100% retention on OSHA's best estimate of risk based on hemangiosarcoma is to reduce the apparent risk by a factor of 10, from 51 per 10,000 to approximately 5 per 10,000 or, 0.5 in 1,000 (Table 3, line 4) or less than 1 in 1000.

The risk based on pooled mouse tumors using the same 2-stage model would be reduced 100 fold from 2 in 10,000 to **2 in 1 million**(Table 3, line 12) (the 100 rather than 10 fold reduction follows from the quadratic nature of the fitted model for pooled tumors). Even the one-hit model considered by OSHA to be less plausible would imply a 10 fold reduction in risk to 18 per 10,000 (Table 3, line

11) rather than 183 per 10,000 as originally estimated by OSHA.

REVISED RISK ESTIMATE AFTER ADJUSTING FOR RETENTION-MOUSE

Same Risk Expressed in 3 Notations

OSHA Best Estimate-Mouse Hemangiosarcoma

Raw Potency	51/10,000	1 in 200	5E-3
Retention	5/10,000	1 in 2000	5E-4

OSHA Mouse Pooled Tumors-2 stage model

Raw Potency	2/10,000	1 in 5000	2E-4
Retention	0.02/10,000	1 in 500,000	2E-6

OSHA Mouse Pooled Tumors, One-Hit Model

Raw Potency	183/10,000	1 in 55	1.8E-2
Retention	18/10,000	1 in 550	1.8E-3

Using the pulmonary retention correction factor of 10% rather than 28%, the OSHA rat based risk estimate considering mammary tumors would be reduced from 115/10,000 to 42/10,000 for the one-hit model and from 29/10,000 to 10/10,000 for the two-stage model.

REVISED RISK ESTIMATE AFTER ADJUSTING FOR RETENTION-RAT

Same Risk Expressed in 3 Notations

OSHA Rat One-Hit, With Mammary Tumors

Raw Potency	115/10,000	1 in 87	1.1E-2
Retention	42/10,000	1 in 238	4.2E-3

OSHA Rat, Two Stage model, with Mammary Tumors

Raw Potency	29/10,000	1 in 344	2.9E-3
Retention	10/10,000	1 in 1000	1E-3

OSHA Rat One-Hit, Without Mammary Tumors

Raw Potency	115/10,000	1 in 87	1.1E-2
Retention	42/10,000	1 in 238	4.2E-3

OSHA Rat, Two Stage model, without Mammary Tumors

Raw Potency	29/10,000	1 in 344	2.9E-3
Retention	10/10,000	1 in 1000	1E-3

3.1.1.2 Effect of Underestimation of Animal Dose

The OSHA measure of dose was taken to be the amount of butadiene retained on termination of the experiment at 6 hours, and ignored both metabolism and excretion during the exposure, as well as, the potential for further metabolism after the 6 hour period. The Hattis and Wasson study concluded that this led to an underestimation of the actual dose received by mice and rats by factors of about 2 and 4.5 respectively.

The effect of a 2 fold underestimation of actual dose on OSHA'S best estimate of risk based on hemangiosarcoma is to further reduce the apparent risk by 50% from 5 per 10,000 (from section 3.1.1.1) to 2.6 per 10,000 (Table 3, line 6).

The risk based on pooled mouse tumors using the same 2-stage model would be reduced more than 2 fold from 2 in 1 million to 1 in 1.6 million (Table 3, line 14), the non-linear reduction follows from the quadratic nature of the fitted model for pooled tumors. Even the one-hit model considered by OSHA to be less plausible would imply a 2 fold reduction in risk to 9 per 10,000 or less than 1 in 1000 (Table 3, line 13).

The inclusion of the two-fold animal dose underestimation with the ten-fold overestimation of human retained dose results in **all** the OSHA mouse based risk estimates, including those based on pooled tumors, being reduced to a risk of less than 1 in 1,000.

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REVISED RISK ESTIMATE AFTER ADJUSTING FOR UNDERESTIMATION OF ANIMAL DOSE -MOUSE

Same Risk Expressed in 3 Notations

OSHA Best Estimate-Mouse Hemangiosarcoma

Raw Potency	51/10,000	1 in 200	5E-3
Retention	5/10,000	1 in 2000	5E-4
Underest.	2.6/10,000	1 in 3800	2.6E-4

OSHA Mouse Pooled Tumors-2 stage model

Raw Potency	2/10,000	1 in 5000	2E-4
Retention	0.02/10,000	1 in 500,000	2E-6
Underest.	0.006/10,000	1 in 1,600,00	6.2E-7

OSHA Mouse Pooled Tumors, One-Hit Model

Raw Potency	183/10,000	1 in 55	1.8E-2
Retention	18/10,000	1 in 550	1.8E-3
Underest.	9/10,000	1 in 1100	9.2E-4

Applying the 4.5 fold risk reduction correction for underestimating dose to the rat based potency which considered mammary tumors to be relevant for estimating risk, results in risk estimates of 1 per 1100 (Table 5, line 3) or 9 per 10,000 for the one-hit model and to 1 in 4500 (Table 5, line 4) or 2.2 per 10,000 for the two-stage model. The following tabulation shows the effect of correcting the raw potency estimates for underestimation of animal dose.

The corresponding OSHA rat based risks without mammary tumors, that is rat mammary tumors considered to be irrelevant to human risk, would decline to less than 1 in 1,000 for both one-hit (risk of 1 in 23,000, Table 5, line 9) and two-stage models (risk of 1 in 26,000, Table 5, line 10). The corrected rat based risk estimates are summarized below.

REVISED RISK ESTIMATE AFTER ADJUSTING FOR UNDERESTIMATION-RAT

Same Risk Expressed in 3 Notations

OSHA Rat One-Hit, With Mammary Tumors

Raw Potency	115/10,000	1 in 87	1.1E-2
Retention	42/10,000	1 in 238	4.2E-3
Underest.	9/10,000	1 in 1100	2.9E-3

OSHA Rat, Two Stage model, with Mammary Tumors

Raw Potency	29/10,000	1 IN 344	2.9E-3
Retention	10/10,000	1 in 1000	1E-3
Underest.	2.2/10,000	1 in 4500	2.2E-4

OSHA Rat One-Hit, Without Mammary Tumors

Raw Potency	6/10,000	1 in 1700	6.0E-4
Underest.	0.4/10,000	1 in 23000	4.4E-5

OSHA Rat, Two Stage model, without Mammary Tumors

Raw Potency	5/10,000	1 in 2000	5.0E-4
Underest.	5/10,000	1 in 26000	3.9E-5

3.1.1.3 Effect of Using Biologically Effective Dose on OSHA's Risk Estimates

A more significant adjustment is made to OSHA's estimates based on current knowledge regarding butadiene metabolism in rodents and monkeys (Dahl et al 1990), and relative enzyme activity between rodents, primates and humans. (Appendix 1) The latter data suggest human metabolism likely is more similar to the primate than the mouse.

The mutagenic epoxide metabolites of butadiene are the primary suspected carcinogens. (Appendix 2) As such, the appropriate dose measure for interspecies extrapolation is likely to be related to the epoxide concentrations in the organisms. For a given exposure to butadiene, the epoxide level in the mouse is considerably greater than in the monkey, and rat. According to the data of Dahl et al (1990), this ratio could be between 2 and 3 orders of magnitude, with a mouse to primate ratio of 590/1 at the exposure concentration

corresponding most closely to 2 ppm. Although the appropriate factor for humans has not been determined with certainty, factors in this range would be supported from relative enzyme activity across species (Appendix 1). The factor of 590 was therefore used to delineate likely lower bounds on the mouse based risks from the models. A factor of 40 was indicated as the likely rat to primate factor to use in delineating the lower bound on rat based risks. The procedure for making this adjustment is detailed in Table 6. The reductions in risk estimates using blood epoxide levels as the biological effective dose (BED) are substantial and are summarized below.

The corresponding adjustments to the OSHA mouse based risks show for hemangiosarcoma, a risk of 9 in **1 million**(Table 3, lines 7,8) and for pooled tumors, risks of 32 in **1 million** (Table 3, line 15) and 7 in **10 billion** (Table 3, line 16) for the one and two stage models respectively.

REVISED RISK ESTIMATE AFTER ADJUSTING FOR BIOLOGICAL EFFECTIVE DOSE-MOUSE

Same Risk Expressed in 3 Notations

OSHA Best Estimate-Mouse Hemangiosarcoma

Raw Potency	51/10,000	1 in 200	5E-3
Retention	5/10,000	1 in 2000	5E-4
Underest.	2.6/10,000	1 in 3800	2.6E-4
BED	0.09/10,000	1 in 110,000	9E-6

OSHA Mouse Pooled Tumors-2 stage model

Raw Potency	2/10,000	1 in 5000	2E-4
Retention	0.02/10,000	1 in 500,000	2E-6
Underest.	0.006/10,000	1 in 1,600,00	6.2E-7
BED	7E-6/10,000	1 in 1.4E+9	7E-10

OSHA Mouse Pooled Tumors, One-Hit Model

Raw Potency	183/10,000	1 in 55	1.8E-2
Retention	18/10,000	1 in 550	1.8E-3
Underest.	9/10,000	1 in 1100	9.2E-4
BED	0.32/10,000	1 in 31,000	3.2E-5

For pooled rat tumors including mammary tumors, OSHA risk estimates would be reduced to about 1 in 6,600 (Table 5, line 5) and 1 in 28,000 (Table 5,

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line 6) for their one and two stage models, respectively.

REVISED RISK ESTIMATE AFTER ADJUSTING FOR UNDERESTIMATION-RAT

Same Risk Expressed in 3 Notations

OSHA Rat One-Hit, With Mammary Tumors

Raw Potency	115/10,000	1 in 87	1.1E-2
Retention	42/10,000	1 in 238	4.2E-3
Underest.	9/10,000	1 in 1100	2.9E-3
BED	1.5/10,000	1 in 6600	1.5E-4

OSHA Rat, Two Stage model, with Mammary Tumors

Raw Potency	29/10,000	1 IN 344	2.9E-3
Retention	10/10,000	1 in 1000	1E-3
Underest.	2.2/10,000	1 in 4500	2.2E-4
BED	0.36/10,000	1 in 28,000	3.6E-5

Utilization of all the information and using a weight of the evidence approach, both the rat and the mouse based risk estimates indicate risks less than 1 in 1000 for the occupational scenario of 2 ppm. In fact the risk may be quite small given the uncertainty about the relevance of the mouse to man and the low risks predicted by the male rat response.

3.2 INDEPENDENT ALTERNATIVE RISK ASSESSMENT BASED ON NTP-II AND IISRP STUDIES

In this section, independently developed potency estimates for butadiene are calculated based on unaudited NTP-II mouse data, and rat data from the IISRP study. Use is made of published data relating to retention data in rats and mice (Bond et al,1986), pharmacokinetic behavior of butadiene in mice, rats and monkeys (Dahl et al 1990), and relative enzyme activity across species (Appendix 1). The calculated risks are compared with those derived by OSHA (1990).

As will be discussed later in greater detail, the risk assessment based on NTP-II is a preliminary screening analysis using summary data.(Appendix 3) The dose-response data used for modeling is summarized in Tables 7 through 11.

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3.2.1 MODELS AND ASSUMPTIONS

OSHA restricted its choice of models to the Multistage and One-hit Models in its analysis of the NTP-I and IISRP studies. To the best of our knowledge, no modeling results have been reported to date for the more recent NTP-II mouse study.

The summary data in the NTP-II report presented the results either by individual tumor type or total malignant tumors. While an analysis based on total malignant tumors without malignant lymphomas would be needed to examine the impact of the retrovirus related malignant lymphomas (see Appendix 4) on the risk estimates, it was not possible from the available summary information. It is recognized that additional dose-response modelling using individual animal data is needed to more fully explore the risk estimates from NTP-II.

Interpretation of the tumor response in the mouse presents a challenge. It is likely that more than one tumorigenic mechanism is at work in the variety of tumors observed. The malignant lymphomas are likely to be linked to a large extent with a threshold-like mechanism related to activation of an endogenous retrovirus. The short term STOP-exposure experiments (Table A3-2, Appendix 3) demonstrated that high dose level over a short time was more potent in inducing lymphomas than the same cumulative dose over a longer period. The hemangiosarcomas may also be linked in some way to the retrovirus (Appendix 4), and the dose-response in female mice suggests a threshold or non-linear response between 200 and 63 ppm.

The implications of having both threshold-like and non-threshold-like mechanisms is that all the models used herein for dose-response extrapolation assume non-threshold behavior. Therefore, including the total spectrum of tumor types most likely overestimates the risk especially at low exposure levels.

3.2.1.1 QUANTAL MODELS

Quantal models are so called because they deal only in proportions. They do not allow use of time information in latency and tumor progression. They are the most common type of model applied and include the Multistage, Linearized Multistage (LMS) and One-hit models used by OSHA.

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The LMS in fact is not a model but rather a bounding procedure for the Multistage model in which the linear term is forced to its maximum positive value consistent with the data at the 95% confidence level. It therefore provides an upper 95% confidence level for the true risk at low doses assuming the model is the true model. This procedure is notably insensitive to the form of the data being modeled and tends to give high estimates for the potency compared to other models. The LMS procedure increasingly is becoming viewed as unrealistic and unreasonably conservative. A similar procedure can be used to estimate the lower 95% bound on risk. The distance between these bounds is a measure of the tightness of the risk bounding procedure. A negative value indicates that, even if the model were correct, the data is also consistent with the compound having no low dose carcinogenic activity. Thus, the Most Likely Estimate (MLE), together with the lower and upper bounds provides a measure of the possible range of potencies consistent with the experimental data; again, assuming the multistage is the appropriate model.

The multistage and one-hit models were applied in this independent alternative analysis, but it should be noted that they are all low dose linear and, therefore, more conservative than other possible quantal models like the Weibull, Probit and Logit. The latter models may well be more appropriate for tumors exhibiting threshold behavior. The use of alternative models provides a range of most likely estimates based on differing approaches conventionally used to model carcinogenic risk. The resulting range provides a measure of the possible range of risks, and addresses the uncertainty introduced by lack of knowledge concerning the true model.

From the possible quantal models which could be applied, this analysis has restricted itself to those models used by OSHA. The multistage model was applied to hemangiosarcoma and pooled malignant tumors in mice and pooled tumors in rats.

3.2.1.2 TIME TO TUMOR MODELS USED TO MODEL NTP-II

Time to tumor models more fully utilize available information and characterize the latency and onset

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of tumors as a function of the dosing pattern. Such models are also capable of distinguishing the differences between early and late tumor onset which provides another criterion for severity of effect.

Ideally, to fully utilize such models, individual animal records must be available; however, in this analysis crude time to tumor data consisting of early deaths to 40 weeks, 40 week interim sacrifice, deaths to 65 weeks, 65 week interim sacrifice, deaths to 104 weeks and terminal sacrifice were used in-lieu of individual animal data.

The incidence of pooled malignant tumors in the female NTP-II mice was modeled using the Multistage-Weibull time to tumor model. The resulting hypothetical risk to a person exposed to 2 ppm for the occupational exposure scenarios was then calculated.

3.2.2 PHARMACOKINETIC PARAMETERS USED IN ALTERNATIVE RISK ASSESSMENT.

A range of risks were calculated based on the various measures of dose with and without the pharmacokinetic adjustments described earlier in Sections 3.1.1.1, 3.1.1.2 and 3.1.1.3. The human retention efficiency for butadiene was assumed to be 10%.

Based on the ratios of epoxide levels across species, risks were based on "corrected butadiene retained", assuming mouse and rat epoxide levels of 590 and 40 times respectively, than that of a human exposed at 2 ppm. These are the same factors applied earlier to adjust the OSHA estimates of risk based on NTP-I. This procedure involved calculation of the risk to a "human size" mouse exposed to 2 ppm for 45 years using butadiene retained as the dose measure (Table 4). The relative difference in blood epoxide ratios were then applied to correct for the relative human epoxide dose. However, the use of NTP-II data rather than NTP-I also allows the <u>direct</u> use of epoxide as the dose measure in the modeling. This is because the Dahl et al, (1990) data were developed in the exposure range covered in NTP-II, therefore little extrapolation is required beyond the conditions of the Dahl experiments. The relationship between exposure concentration, butadiene retained and epoxide in blood is summarized in Table 12 and Figure 2. Details of how epoxide in blood was calculated from the Dahl et al data are given in Table

13. Potential risks are then estimated assuming human blood levels would be similar to that in the monkey.

All results are presented as risks for the maximally exposed individual assumed by OSHA. This person is exposed for 250 days per year, 8 hours per day for 45 years out of an assumed 74 year life. Results are presented based on the NTP-II mouse study (Table 14) followed by the IISRP rat study (Table 15).

3.2.3 RISK ESTIMATES FROM NTP-II MOUSE STUDY

In the following 2 sections the risk estimates based on pooled malignant tumors and hemangiosarcoma from the NTP-II bioassay are presented. The pooled malignant tumors predicted a risk of 1 in 4000 using the time to tumor model, the highest risk of any combination of tumors examined in a screening analysis and represents a conservative case. The hemangiosarcoma case was selected to compare with OSHA's analysis.

3.2.3.1 Risk Estimates Based on Pooled Tumors:

When butadiene retained is utilized as the dose measure, modeling of pooled tumors in NTP-II (Table 14) results in estimates similar to those of OSHA based on NTP-1 (Table 3). The Multistage-Weibull time-to-tumor model and using butadiene retained (Table 14, line 18) indicates a raw potency risk almost identical to that of OSHA (approximately 180 per 10,000) for 45 years exposure at 2 ppm. However, this estimate does not take into account the pharmacokinetic behavior of butadiene and considerably lower risks are indicated when the epoxide metabolites are used as the surrogate for the biologically effective dose. As discussed earlier, this was accomplished in two ways for NTP-II data. Based on pooled tumors the Multistage-Weibull time-to-tumor model calculated risk falls to 3 in 10,000 using butadiene retained with the epoxide correction (Table 14, line 19). Using epoxide directly in the modeling resulted in a similar risk of 2 in 10,000 (Table 14, line 20). The quantal Multistage model indicates somewhat higher risks whether 1 or 2 stages are fitted with risks ranging from 10 in 10,000 to 5 in 10,000, as indicated in Table 14, lines 15 and 17.

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SUMMARY OF RISK ESTIMATES FROM NTP-II, FEMALE, POOLED TUMORS, TIME TO TUMOR MODEL

	Same Risk Expressed in 3 Notations		
Raw Potency	180/10,000	1 in 56	1.8E-2
Corrected for:			
Butad. retain. and			
Epoxide	3.3/10,000	1 in 3000	3.3E-4
Epoxide direct	2.4/10,000	1 in 4200	2.4E-4

SUMMARY OF RISK ESTIMATES FROM NTP-II, FEMALE, POOLED TUMORS, MULTISTAGE, 2 STAGE

	Same Risk Expressed in 3 Notations		
Corrected for:			
Butad. retain. and			
Epoxide	3.9/10,000	1 in 2500	3.9E-4
Epoxide direct	2.9/10,000	1 in 3400	2.9E-4

SUMMARY OF RISK ESTIMATES FROM NTP-II, FEMALE, POOLED TUMORS, MULTISTAGE, 1 STAGE

	Same Risk Expressed in 3 Notations		
Corrected for:			
Butad. retain. and			
Epoxide	9/10,000	1 in 1100	9.1E-4
Epoxide direct	4.8/10,000	1 in 2100	4.8E-4

3.2.3.2 Risk Estimates Based on Hemangiosarcoma

OSHA's best estimate of risk (51/10,000) was based on a two-stage fit to the NTP-I data (Table 3, line 2). Modeling of NTP-II indicates that this is a considerable overestimate of the risk. There was no hemangiosarcoma response in the NTP-II study at either 6.25 ppm or 20 ppm and only a single response at 62.5 ppm (Table 9). As a result risk estimates were less then 1 in 145 million (Table 14, lines 1 and 2). Survival at 20 ppm and above was statistically different from controls, which raises the possibility of animals dying before expressing the tumor. We can examine the effect of this by assuming a massive and unlikely response at 20 ppm of 90%. The result, using butadiene retained as the dose measure, was a risk of only 5 in 10,000 (Table 14, line 7). Using butadiene retained with the epoxide correction or using epoxide directly as the dose measure results in a disproportionate lowering of the risk to considerably less than 1 in a million (estimated 1 in 16 million) due to the quadratic response (Table 14, line 8). A less than quadratic response is unlikely as mortality was not affected at 6.25 ppm.

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The range of risks from the various models (Multistage and Multistage-Weibull), dose assumptions and tumor end-points selected from NTP-I and NTP-II are presented in Tables 3 and 14, respectively. The estimates span 16 orders of magnitude. A cumulative distribution of the calculated risks based on mice is shown in Figure 3. Approximately 80% of the estimates are below the OSHA best estimate. Only 2 of the cases predict more risk than OSHA's best estimate, while 34 of the cases predict less risk with 10 cases predicting risk 2 or more orders of magnitude smaller. Based on the unique response of the mouse it is concluded that the real risk is less than 1 in 1000 and may be as low as 1 in a 1,000,000 or lower.

3.2.4 RISK ESTIMATES BASED ON IISRP RAT STUDY

The industry sponsored rat study is discussed in Appendix 5. Results are summarized in Table 15 and Figure 4.

The results for rats are based on the tumor counts summarized by Environ (1986). There is considerable debate as to the relevance of the rat mammary fibroadenomas to human risk. Such non-malignant tumors were considered in this analysis as providing possible upper bounds on the risk. Risks were therefore calculated using female rat responses both including and excluding the mammary tumors. It may be that the enhanced level of naturally occurring mammary gland tumors represents a promotional effect due to the 1000 and 8000 ppm high doses used in the rat study; a response unlikely to be seen at low exposures such as 2 PPM. Pooled tumors in males were also used to generate possible risk estimates.

Using butadiene retained, the highest risk obtained was 8 in 10,000 for rat female tumors including mammary fibroadenomas (Table 15, line 1). This resulted from dropping the high dose and fitting a one-hit model (multistage with one stage). The high dose was dropped because both the 1000 ppm and 8000 ppm group had similar high incidence of mammary gland tumors. The corresponding OSHA risk was 115 in 10,000 (Table 5, line 1). The difference was primarily due to the use in the alternative analysis of current retention data and the observations of Hattis and Wasson (1987) regarding underestimation of rat doses. Fitting a two stage model lowered this analysis' and OSHA risks to 2 (Table 15, line 2) and 29 (Table 5, line 2) per 10,000, respectively. Adjusting OSHA risks to reflect current understanding of animal and human retention of

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butadiene gave a risk of 2 per 10,000 (Table 5, line 4), similar to the results in the alternative analysis.

Using epoxide as the measure of biologically effective dose suggested risks are probably much lower. Calculations using epoxide as the dose measure yield risk estimates smaller than 3 in 100,000 (Table 15, line 4).

SUMMARY OF RISK ESTIMATES FROM IISRP-RAT, FEMALE, POOLED TUMORS WITH MAMMARY TUMORS, ONE-HIT MODEL

	Same Risk Expressed in 3 Notations		
Corrected for:			
Retention and underestimat.	7.8/10,000	1 in 1300	7.8E-4
Blood epoxide	1.3/10,000	1 in 7600	1.31E-4

If mammary tumors are not relevant, then risks are calculated to be at least an order of magnitude lower (Table 15, line 5 and 6). Use of male data indicates risks of the order of 1 in a million (Table 15, line 7 and 8).

SUMMARY OF RISK ESTIMATES FROM IISRP-RAT, FEMALE, POOLED TUMORS WITHOUT MAMMARY TUMORS, ONE-HIT MODEL

	Same Risk Expressed in 3 Notations		
Corrected for:			
Retention and underestimat.	0.5/10,000	1 in 19,000	5.3E-5
Blood epoxide	0.086/10,000	1 in 110,000	8.6E-6

Application of alternative models would be expected to widen the range of possible risks indicated, but generally in the direction of lower risk. Higher risks were often predicted as a result of dropping higher dose levels and using one-hit models.

The range of risks based on the different dose assumptions, tumor types and model stages for rats are presented in Table 15. The estimates span over 4 orders of magnitude. A cumulative distribution of the calculated risks based on rats is shown in Figure 4. Approximately 90% of the estimates are smaller (less risk) than the OSHA favored estimate. The weight of the evidence from the rat based risk estimates predicts the risk to man is less than 1 in 1000 and may be as small as 1 in 1,000,000. Of the 2 rodent species, the metabolism data indicates that the rat is the more

relevant to man and should be given greater weight in a weight of evidence conclusion.

4.0 REPRODUCTIVE TOXICITY

The ovarian atrophy observed in the mouse is considered to be extraordinary and unique to the female mouse ovary because of the high levels of butadiene epoxide formed by the mouse. (Appendix 1 and 6) Epoxides of related chemicals also affect the mouse ovary but not the rat ovary. This effect is not seen in the rat or any other species. There is no evidence of functional abnormalities in butadiene exposed animals in reproduction studies. The available evidence suggests that this mouse ovarian effect is not a hazard for exposures to 2 ppm.

5.0 SUMMARY

The range of risks based on both rats and mice based on different dose assumptions, tumor types and models are summarized graphically in Figure 5. The estimates span 16 orders of magnitude. A cumulative distribution of the calculated risks is shown in Figure 5. Approximately 90% of the estimates are smaller (less risk) than the OSHA "best" estimate. The mouse based risk estimates tend to be higher, and because the rat is more relevant to man the rat should be given greater weight.

As a result of this analysis, there is a high confidence that occupational risk from exposure to butadiene at 2 ppm for 45 years is below 1 in 1,000, and is likely to be considerably lower and may be insignificant.

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TABLE 1

ASSUMPTIONS USED IN CORRECTING OSHA RISK ASSESSMENT

MOUSE WEIGHT	.03 KG
INHALATION RATE	0.052 M^3/DAY
RAT WEIGHT	.35 KG
INHALATION RATE	0.29 M^3/DAY
HUMAN WEIGHT	70 KG
INHALATION RATE	9.6 M^3/8 HOUR WORKING DAY
EXPOSURE	45/74 YEARS, 250/365 DAYS PER YEAR 8 HOURS PER DAY

MOUSE RETENTION EFFICIENCY:

RANGE 0.8 - 1,000 PPM:

LOG(% RETAINED) = 1.30 - 0.226LOG(CONC PPM) R^2 = 0.995

(CALCULATED FROM DATA OF BOND (1986)

RAT RETENTION EFFICIENCY:

2 PPM: 5% (FROM BOND DATA)

625-1250 PPM AS EPA/ENVIRON

HUMAN RETENTION EFFICIENCY: 10% (HATTIS AND WASSON/BOND ET AL)

WORKER DOSE AT 2 PPM FOR 45 YEARS: 0.025 MG/KG/DAY LIFETIME AVERAGE

MOUSE DOSE AT 2 PPM OCCUPATIONAL CONDITIONS: 0.26 MG/KG/DAY LIFETIME AVERAGE (NORMALIZED BY 9.6M3/20M3 FACTOR)
(THIS DOSE IS USED TO CORRECT FOR EPOXIDE RATIO OF 590 : EQUIVALENT DOSE = 0.26/540 = 4.4E-4 mg/kg/d)

RAT DOSE AT 2 PPM OCCUPATIONAL CONDITIONS : 0.036 MG/KG/D LIFETIME AVERAGE (NORMALIZED BY 9.6M3/20M3 FACTOR)
(THIS DOSE IS USED TO CORRECT FOR EPOXIDE RATIO OF 40 : EQUIVALENT DOSE = 0.036/40 = 9.1E-4 mg/kg/d)

TABLE 2

DOSE-RESPONSE DATA FROM NTP-I USED BY OSHA* FOR FEMALE MICE

Dose (ppm)	0	625	1250
Dose (mg/kg/d lifetime)	0	16.16	23.81
Pooled Tumors	3/47	23/38	41/45
Heart Hemangiosarcomas	0/47	11/38	18/45

Parameters of fitted multistage model:

Extra Risk = 1 - exp-($q1*d + q2*d^2$)
where d = lifetime average retained dose (mg/kg/day)

q1 $(mg/kg/d)^{-1}$
q2 $(mg/kg/d)^{-2}$ = 0 by definition for One-hit model.

Results:

Pooled Tumors:

One-hit : q1 = 0.074;
Two stage : q1 = 0; q2 = 0.0038

Hemangiosarcoma:

One-hit : q1 = 0.021
Two stage : q1 = 0.021; q2 = 0.0004

Female Rats Pooled Tumors:

Dose (ppm)	0	1,000	8,000
Dose (mg/kg/d lifetime)	0	6.4	24.13
With Mammary Fibroadenomas	40/99	77/97	72/96
Without Mammary Fibroadenomas	0/90	4/85	15/82

Parameters of fitted multistage model:

With mammary fibroadenomas:

One-hit : q1 = 0.166 High Dose Dropped;
Two stage : q1 = 0.04; q2 = 0;

Without mammary fibroadenomas:

One-hit : q1 = 0.008
Two stage : q1 = 0.007; q2 = 0.00005

* OSHA (1990)

TABLE 3
COMPARISON OF OSHA AND CORRECTED OSHA RISKS
FROM THE NTP-I MOUSE STUDY
BASED ON 45 YEARS OCCUPATIONAL EXPOSURE AT 2 PPM

LINE #	TUMOR	SPEC/SEX	STUDY	MODEL	DOSES	STAGES	SOURCE	RISK	1 IN X	
1	HEMANGIOSARCOMA	MOUSE F	NTP-I	M-STAGE	2	1	OSHA	5.3E-03	1.9E+02	OSHA (FR AUG 10, 1990)
2	HEMANGIOSARCOMA	MOUSE F	NTP-I	M-STAGE	3	2	OSHA	5.1E-03	2.0E+02	OSHA (FR AUG 10, 1990)
3	HEMANGIOSARCOMA	MOUSE F	NTP-I	M-STAGE	2	1	AIRA	5.0E-04	2.0E+03	OSHA ADJUSTED FOR RETENTION
4	HEMANGIOSARCOMA	MOUSE F	NTP-I	M-STAGE	3	2	AIRA	5.0E-04	2.0E+03	OSHA ADJUSTED FOR RETENTION
5	HEMANGIOSARCOMA	MOUSE F	NTP-I	M-STAGE	2	1	AIRA	2.6E-04	3.8E+03	OSHA ADJUSTED FOR RETENTION AND UNDERESTIMATION OF MOUSE DOSE
6	HEMANGIOSARCOMA	MOUSE F	NTP-I	M-STAGE	3	2	AIRA	2.6E-04	3.8E+03	OSHA ADJUSTED FOR RETENTION AND UNDERESTIMATION OF MOUSE DOSE
7	HEMANGIOSARCOMA	MOUSE F	NTP-I	M-STAGE	2	1	AIRA	9.0E-06	1.1E+05	OSHA ADJUSTED FOR ANIMAL RETENTION AND EPOXIDE RATIO
8	HEMANGIOSARCOMA	MOUSE F	NTP-I	M-STAGE	3	2	AIRA	9.0E-06	1.1E+05	OSHA ADJUSTED FOR ANIMAL RETENTION AND EPOXIDE RATIO
9	POOLED TUMORS	MOUSE F	NTP-I	M-STAGE	2	1	OSHA	1.8E-02	5.5E+01	OSHA (FR AUG 10, 1990)
10	POOLED TUMORS	MOUSE F	NTP-I	M-STAGE	3	2	OSHA	2.0E-04	5.0E+03	OSHA (FR AUG 10, 1990)
11	POOLED TUMORS	MOUSE F	NTP-I	M-STAGE	2	1	AIRA	1.8E-03	5.6E+02	OSHA ADJUSTED FOR RETENTION
12	POOLED TUMORS	MOUSE F	NTP-I	M-STAGE	3	2	AIRA	2.0E-06	5.0E+05	OSHA ADJUSTED FOR RETENTION
13	POOLED TUMORS	MOUSE F	NTP-I	M-STAGE	2	1	AIRA	9.2E-04	1.1E+03	OSHA ADJUSTED FOR RETENTION AND UNDERESTIMATION OF MOUSE DOSE
14	POOLED TUMORS	MOUSE F	NTP-I	M-STAGE	3	2	AIRA	6.2E-07	1.6E+06	OSHA ADJUSTED FOR RETENTION AND UNDERESTIMATION OF MOUSE DOSE
15	POOLED TUMORS	MOUSE F	NTP-I	M-STAGE	2	1	AIRA	3.2E-05	3.1E+04	OSHA ADJUSTED FOR ANIMAL RETENTION AND EPOXIDE RATIO
16	POOLED TUMORS	MOUSE F	NTP-I	M-STAGE	2	2	AIRA	7.0E-10	1.4E+09	OSHA ADJUSTED FOR ANIMAL RETENTION AND EPOXIDE RATIO

TABLE 4

RESPIRATORY RETENTION OF BD IN RATS AND MICE*

EXPOSURE CONCENTRATION		PERCENTAGE RETAINED AT 6 HOURS	
(ug/l)	ppm	RATS	MICE
0.14	0.08	17	16
1.4	.8	6	20
13	7	4	13.3
130	70	8	8
1800	1,000	2.5	4
13,000	7,100	1.5	N.A.

* Taken from table 2 of Bond et al (1986). The mouse retention for 7 ppm was erroneously listed as 20% in the paper.

N.A. Not Available

TABLE 5

COMPARISON OF OSHA AND CORRECTED OSHA RISKS
FROM THE IISRP RAT STUDY
BASED ON 45 YEARS OCCUPATIONAL EXPOSURE AT 2 PPM

LINE #	TUMOR	SPEC/SEX		STUDY	MODEL	DOSES	STAGES	SOURCE	RISK	1 IN X	
1	POOLED/MAMMARY	RAT	F	IISRP	M-STAGE	2	1	OSHA	1.1E-02	8.7E+01	OSHA (FR AUG 10, 1990)
2	POOLED/MAMMARY	RAT	F	IISRP	M-STAGE	3	2	OSHA	2.9E-03	3.4E+02	OSHA (FR AUG 10, 1990)
3	POOLED/MAMMARY	RAT	F	IISRP	M-STAGE	2	1	AIRA	9.2E-04	1.1E+03	OSHA ADJUSTED FOR RETENTION + UNDERESTIMATION OF RAT DOSE
4	POOLED/MAMMARY	RAT	F	IISRP	M-STAGE	3	2	AIRA	2.2E-04	4.5E+03	OSHA ADJUSTED FOR RETENTION + UNDERESTIMATION OF RAT DOSE
5	POOLED/MAMMARY	RAT	F	IISRP	M-STAGE	2	1	AIRA	1.5E-04	6.7E+03	OSHA ADJUSTED FOR EPOXIDE DOSE+RETENTION
6	POOLED/MAMMARY	RAT	F	IISRP	M-STAGE	3	2	AIRA	3.6E-05	2.8E+04	OSHA ADJUSTED FOR EPOXIDE DOSE+RETENTION
7	POOLED/NO MAMMARY	RAT	F	IISRP	M-STAGE	3	1	OSHA	6.0E-04	1.7E+03	OSHA (FR AUG 10, 1990)
8	POOLED/NO MAMMARY	RAT	F	IISRP	M-STAGE	3	2	OSHA	5.0E-04	2.0E+03	OSHA (FR AUG 10, 1990)
9	POOLED/NO MAMMARY	RAT	F	IISRP	M-STAGE	3	1	AIRA	4.4E-05	2.3E+04	OSHA ADJUSTED FOR RETENTION + UNDERESTIMATION OF RAT DOSE
10	POOLED/NO MAMMARY	RAT	F	IISRP	M-STAGE	3	2	AIRA	3.9E-05	2.6E+04	OSHA ADJUSTED FOR RETENTION + UNDERESTIMATION OF RAT DOSE
11	POOLED/NO MAMMARY	RAT	F	IISRP	M-STAGE	3	1	AIRA	7.3E-06	1.4E+05	OSHA ADJUSTED FOR EPOXIDE DOSE+ANIMAL RETENTION
12	POOLED/NO MAMMARY	RAT	F	IISRP	M-STAGE	3	2	AIRA	6.4E-06	1.6E+05	OSHA ADJUSTED FOR EPOXIDE DOSE+ANIMAL RETENTION

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TABLE 6

ADJUSTMENT OF OSHA RISKS

A. Adjustment for Overestimation of Human Retention

At 2 ppm, OSHA assumed the mouse retention efficiency was 100% and the rat retention efficiency was 28%. This was based on their regression equation based on older Bond data. The new data indicates retention efficiencies of 17% and 5% at 2 ppm for mouse and rat, respectively. The data of Hattis and Wasson suggests 10% may be apprpriate for humans. This figure is intermediat beween the rat and mouse data and we take 10% as representing human retention efficiency.

The Equivalent human dose for input to the OSHA model is listed in Table 20 of the OSHA Notice. These are based on rat and mice retention efficiency and therefore require adjusting based on our assumption of 10% human retention. For example, based on mice at 2 ppm, OSHA estimated the human dose at 0.25 mg/kg/d, assuming 100% absorption based on their erroneous regression equation. This should be adjusted to 0.025mg/kg/d based on 10% in humans. Similarly, based on rats, a human dose of 0.07 mg/kg/d is calculated assuming humans are like rats and absorb what OSHA mistakenly calculates rats absorb (28%). Updated Bond data indicate only 5% for rats at 2 ppm. We apply the more appropriate factor of 10% instead to give a dose of 10/28*0.07 = .025 mg/kg/day.

The modified doses are simply re-entered into OSHA's multistage equations.

B. Adjustment for Underestimate of Animal Dose

According to Hattis and Wasson (1987), the doses to mice and rats in NTP-I were underestimated by a factor of 2-4.5. The OSHA multistage parameters are corrected to reflect this:

The OSHA multistage equation parameters used to calculate extra risk are q1 and q2. If the factor by which dose is underestimated is x, then q1 is modified to q1/x and q2 is modified to $q2/x^2$.

C. Adjustment for Epoxide as Relevant Dose Measure

Where a risk based on BD retained is modified based on epoxide, the following procedure was followed. The dose of BD retained to a "worker mouse" exposed for 45 out of 74 "mouse years" was calculated. Thus, at 2 ppm, mouse BD retained is simply:

2ppm x 2.2 mg/m3/ppm x 1.73 m3/kg/d x 9.6m3/20m3 x 250d/365d x 45y/74y X 0.17 retention = 0.26 mg/kg/d

This level of BD retained is equivalent to a certain blood level in the mouse. If the corresponding level in man is 590 times less, as Dahl's data indicate, then the effective dose of bd retained for the human is equal to 0.26 mg/kg/d/590 = 4.4×10^{-4} mg/kg/d. This human dose was then input to the original OSHA models in Table 21 of the Notice.

The corresponding adjustment based on the rat is as follows:

For the "worker " rat, the lifetime average BD retained is given by:

2ppm x 2.2 mg/m3/ppm x 0.83 m3/kg/d x 9.6m3/20m3 x 250d/365d x 45y/74y x 0.05 retention= 0.0364 mg/kg/d

This level of BD retained is equivalent to a certain blood level in the rat. If the corresponding level in man is 40 times less as looks probable from the data of Dahl et al, then the effective dose of BD retained for the human is equal to 0.0364mg/kg/d/40 = 9.1×10^{-4} mg/kg/d. This human dose was then input to the original OSHA models in Table 21 of the Notice.

TABLE 7

QUANTAL DOSE RESPONSE DATA FROM NTP-II
POOLED MALIGNANT TUMORS

Dose (ppm)	0	6.25	20	62.5	200	625
Dose (mg/kg/d lifetime)	0	0.56	1.38	3.32	8.17	20.0
Pooled Tumors[1]	17/59	38/49	41/57	44/60	56/70	79/90

Parameters of fitted multistage model:

Extra Risk = $1 - \exp-(q1*d + q2*d^2)$
where d = lifetime average retained dose (mg/kg/day) or epoxide in blood (pmol/ml)

Results:

Pooled Tumors:

mg/kg/d basis:

One-hit : $q1 = 2.06\ (mg/kg/d)^{-1}$; 2 dose levels
Two stage : $q1 = 0.881\ (mg/kg/d)^{-1}$; $q2 = 0$; 3 dose levels

epoxide in blood basis:

One-hit : $q1 = 0.0134\ (pmol/ml)^{-1}$; 2 dose levels
Two stage : $q1 = 0.0079\ (pmol/ml)^{-1}$; $q2 = 0$; 3 dose levels

1 Corrected for time of first tumor. See Table 6

TABLE 8

DOSE RESPONSE DATA FROM NTP-II USED FOR TIME TO TUMOR MODELING

POOLED FEMALE MALIGNANT TUMORS*

	Exposure Concentration(ppm)					
	Controls	6.25	20	62.5	200	625
Deaths to 40 weeks	0/0	0/1	0/3	0/0	3/3	19/21
40 week sacrifice	0/10	0/10	0/10	0/10	0/10	1/8
Deaths to 65 weeks	0/1	0/0	1/1	4/6	15/16	57/60
65 week sacrifice	1/10	0/10	0/10	0/10	8/10	2/2
Deaths to 104 weeks	16/49	38/49	40/46	40/44	30/31	-

Multistage-Weibull Model Applied: P = 1-exp-(F)

$$F = [\, q0 + q1*d + q2*d^2 + q3*d^3 + q4*d^4 + q5*d^5 \,] \times [t - tau]^n$$

d = dose (lifetime average mg/kg/d)
t = time of exposure (weeks) = 104 weeks (We assume 104 weeks in mouse = 74 years in man)
tau = latency (weeks) = 18
n = 2.9 (dimensionless)
q0 = 1.3E-6; q1 = 1.7E-6; q2 = 1.7E-19; q3 = 9.2E-23; q4 = 3.1E-20; q5 = 7.8E-11

For worker exposure at 2 ppm for 45 years using BD retained as the dose measure, the dose is 0.025 mg/kg/day for life based on a 10% retention factor.

* Compiled from pathology summary tables in Battelle Report (1990).

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TABLE 9

QUANTAL DOSE RESPONSE DATA FROM NTP-II
FEMALE HEART HEMANGIOSARCOMAS

	Exposure Concentration(ppm)				
	Controls	6.25	20	62.5	200
Heart Hemangiosarcomas	0/60	0/59	0/57	1/60	20/57
Mortality Adjustment (1)	0/60	0/59	29/57	54/60	
Mortality Adjustment (2)	0/60	0/59	51/57		

Extra Risk = $1 - \exp-(q1*d + q2*d^2 + q3*d^3 + \ldots)$
where d = lifetime average retained dose (mg/kg/day)

q0 (dimensionless)
q1 $(mg/kg/d)^{-1}$
q2 $(mg/kg/d)^{-2}$ = 0 by definition for One-hit model.
q3 $(mg/kg/d)^{-3}$

Original Data Set:

Three stage 4 dose levels : q0 = 0; q1 = 0; q2 = 0; q3 = 4.3×10^{-4}

Pseudo-mortality correction (1): 3rd dose response = 50%; 4th dose response = 90%;

Three stage 4 dose levels : q0 = 0; q1 = 0; q2 = .24; q3 = 0

Pseudo-mortality correction (2): Three dose levels; 3rd dose response = 90%;

Two stage 3 dose levels : q0 = 5.7×10^{-4}; q1 = 0; q2 = .76;

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TABLE 10

DOSE RESPONSE DATA FROM NTP-II FOR TIME TO TUMOR MODELING

HEMANGIOSARCOMAS

	Exposure Concentration(ppm)					
	Controls	6.25	20	62.5	200	625
Deaths to 40 weeks	0/0	0/1	0/3	0/0	0/3	0/21
40 week sacrifice	0/10	0/10	0/10	0/10	0/10	1*/8
Deaths 40 to 65 weeks	0/1	0/0	0/1	0/6	3/16	24/59
65 week sacrifice	0/10	0/10	0/10	0/10	1/10	2/2
Deaths 65 to 104 weeks (Including Terminal Sacrifice)	0/49	0/49	0/46	1/44	16/31	-

Multistage-Weibull Model Applied: P = 1-exp-(F)

625 ppm dose level dropped (5 dose levels fitted)

$$F = [\, q0 + q1*d + q2*d^2 + q3*d^3 + q4*d^4 + q5*d^5 \,] \times (t-tau)^n$$

d = dose (lifetime average mg/kg/d)
t = time of exposure (weeks) = 104 weeks (We assume 104 weeks in mouse = 74 years in man)
tau = latency (weeks) = 40 (upper bound input to model)
n = 1.6 (dimensionless)
q0 = 1.1E-13; q1 = 2.0E-24; q2 = 3.0E-19; q3 = 8.5E-13; q4 = 2.2E-07; q5 = 1.3E-12

For worker exposure at 2 ppm for 45 years using BD retained as the dose measure, the worker dose is 0.025 mg/kg/day for life based on a 10% retention factor.

* Compiled from pathology summary tables in Battelle Report (1990).

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TABLE 11

DOSE RESPONSE DATA FOR RATS USED IN CORRECTING OSHA DATA*

Dose (ppm)	0	1,000	8,000
Dose (mg/kg/d lifetime average)	0	7.4	27.5
Female rats:			
Pooled tumors including mammary	41/99	77/97	72/96
Pooled tumors without mammary	1/94	8/95	21/92
Male rats:			
Pooled tumors	2/96	4/96	20/87

Parameters of fitted multistage model:

Extra Risk = $1 - \exp-(q1*d + q2*d^2)$
where d = lifetime average retained dose (mg/kg/day)

q0 (dimensionless)
q1 $(mg/kg/d)^{-1}$
q2 $(mg/kg/d)^{-2}$ = 0 by definition for One-hit model.

Results:

Female rats: Pooled Tumors:
with mammary:

One-hit : q0 = 0.534; q1 = 0.14; Top dose dropped
Two stage : q0 = 0.72; q1 = 0.034; q2 = 0; 3 dose levels

Without mammary tumors:

Two stage : q0 = 1.2E-2; q1 = 9.3E-3; q2 = 0; 3 dose levels

Male rats: Pooled tumors:

Two stage : q0 = 2.1E-2; q1 = 7.6E-4; q2 = 2.9E-4

* According to Environ Corporation (1986)

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TABLE 12

RELATION BETWEEN EXPOSURE DOSE AND ABSORBED DOSE

MOUSE:

DOSES IN NTP-II	LIFETIME AVERAGE	
CONCENTRATION PPM	BD RETAINED[1] (MG/KG/D)	EPOXIDE IN BLOOD[2] (PMOLE/ML)
0	0	0
6.25	0.56	86
20	1.38	152
62.5	3.32	306
200	8.17	805

RATS :

EXPOSURE CONCENTRATION PPM	EQUIVALENT LIFETIME AVERAGE ABSORBED DOSE MG/KG/DAY[3]
0	0
1,000	7.4
8,000	27.5

1 Calculated from a regression equation fitted to the Bond et al (1986) data. The equation is (base 10): log(percent bd retained) = 1.30 - 0.226log(bd concentration in ppm) Respiratory parameters assumed were 0.052 m^3/day and a body weight of 0.03kg.

2 Calculated from data of Dahl et al (1990) by linear interpolation and extrapolation. See Table 11.

3 From Environ (1986)

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TABLE 13

CALCULATED MOUSE EPOXIDE BLOOD LEVELS FOR GIVEN EXPOSURE[1]

BD EXPOSURE CONC PPM	CALCULATED EPOXIDE BLOOD LEVEL (PMOL/ML)	EQUIVALENT LIFETIME AVERAGE LEVEL[2]	METHOD OF CALCULATION
0	0	0	
2	154	28	LINEAR EXTRAPOLATION: 2/7.8 X 600.6
6.25	481	86	LINEAR EXTRAPOLATION: 6.25/7.8 X 600.6
7.8	600.6	107	DAHL DATA POINT (77 pmol/ml/ppm x 7.8 = 600.6 pmol/ml)
20	849	152	LINEAR INTERPOLATION: EPOXIDE = 600.6 + 20.33 X (20-7.8)
62.5	1713	306	LINEAR INTERPOLATION: EPOXIDE = 600.6 + 20.33 X (62.5-7.8)
78	2028	362	DAHL DATA POINT (26 pmo;/ml/ppm x 78 ppm = 2,028 pmol/ml)
200	4508	805	LINEAR EXTRAPOLATION: EPOXIDE = 600.6 + 20.33 X (200-7.8)
625	13148	2348	LINEAR EXTRAPOLATION: EPOXIDE = 600.6 + 20.33 X (625-7.8)

1 Calculated from Dahl et al (1990) Table 4: This Table indicated normalized blood levels of epoxide in mice of 77 pmol/ml/ppm at 7.8 ppm and 26 pmol/ml/ppm at 78 ppm. These levels were used in the table above to estimate epoxide levels at other exposure concentrations. The concentrations apply to a 2 hour exposure.

2 Epoxide level during eposure X 6h/24h X 5days/7days

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TABLE 14
COMPARISON OF ALTERNATIVE INDEPENDENT RISK ASSESSMENT (AIRA) AND OSHA RISKS
FROM THE NTP-II MOUSE STUDY
BASED ON 45 YEARS OCCUPATIONAL EXPOSURE AT 2 PPM

LINE #	TUMOR	SPEC/SEX	STUDY	MODEL	DOSES	STAGES	SOURCE	RISK	1 IN X	
1	HEMANGIOSARCOMA	MOUSE F	NTP-II	M-STAGE	4	3	AIRA	6.7E-09	1.5E+08	USING BD RETAINED
2	HEMANGIOSARCOMA	MOUSE F	NTP-II	M-STAGE	4	3	AIRA	3.7E-14	2.7E+13	USING BD RETAINED AND EPOXIDE RATIO OF 590
3	HEMANGIOSARCOMA	MOUSE F	NTP-II	M-STAGE	4	3	AIRA	1.5E-04	6.7E+03	USING BDRETAINED WITH PSEUDOMORTALITY CORRECTION (I)
4	HEMANGIOSARCOMA	MOUSE F	NTP-II	M-STAGE	4	3	AIRA	4.7E-08	2.1E+07	USING BD RETAINED+EPOXIDE RATIO+MORTALITY CORRECTION (I)
5	HEMANGIOSARCOMA	MOUSE F	NTP-II	M-STAGE	4	3	AIRA	2.4E-14	4.2E+13	USING BLOOD EPOXIDE MEASUREMENTS OF DAHL ET AL
6	HEMANGIOSARCOMA	MOUSE F	NTP-II	M-STAGE	4	3	AIRA	8.8E-09	1.1E+08	BLOOD EPOXIDE + PSEUDOMORTALITY CORRECTION (I)
7	HEMANGIOSARCOMA	MOUSE F	NTP-II	M-STAGE	3	2	AIRA	4.7E-04	2.1E+03	USING BD RETAINED WITH PSEUDOMORTALITY CORRECTION (II)
8	HEMANGIOSARCOMA	MOUSE F	NTP-II	M-STAGE	3	2	AIRA	1.5E-07	6.7E+06	USING BD RETAINED+EPOXIDE RATIO+MORTALITY CORRECTION (II)
9	HEMANGIOSARCOMA	MOUSE F	NTP-II	M-STAGE	3	2	AIRA	6.3E-08	1.6E+07	BLOOD EPOXIDE + PSEUDOMORTALITY CORRECTION (II)
10	HEMANGIOSARCOMA	MOUSE F	NTP-II	MS-WEIBULL	5	5	AIRA	6.4E-11	1.6E+10	USING BD RETAINED
11	HEMANGIOSARCOMA	MOUSE F	NTP-II	MS-WEIBULL	5	5	AIRA	6.2E-18	1.6E+17	USING BD RETAINED AND EPOXIDE RATIO OF 590
12	HEMANGIOSARCOMA	MOUSE F	NTP-II	MS-WEIBULL	5	5	AIRA	1.3E-14	7.7E+13	USING BLOOD EPOXIDE MEASUREMENTS OF DAHL ET AL
13	POOLED TUMORS	MOUSE F	NTP-II	M-STAGE	3	2	AIRA	3.9E-04	2.6E+03	USING BD RETAINED AND EPOXIDE RATIO OF 590
14	POOLED TUMORS	MOUSE F	NTP-II	M-STAGE	2	1	AIRA	5.0E-02	2.0E+01	USING BD RETAINED
15	POOLED TUMORS	MOUSE F	NTP-II	M-STAGE	2	1	AIRA	9.1E-04	1.1E+03	USING BD RETAINED AND EPOXIDE RATIO OF 590
16	POOLED TUMORS	MOUSE F	NTP-II	M-STAGE	3	2	AIRA	2.9E-04	3.4E+03	USING BLOOD EPOXIDE MEASUREMENTS OF DAHL ET AL
17	POOLED TUMORS	MOUSE F	NTP-II	M-STAGE	2	1	AIRA	4.8E-04	2.1E+03	USING BLOOD EPOXIDE MEASUREMENTS OF DAHL ET AL
18	POOLED TUMORS	MOUSE F	NTP-II	MS-WEIBULL	5	5	AIRA	1.8E-02	5.6E+01	USING BD RETAINED
19	POOLED TUMORS	MOUSE F	NTP-II	MS-WEIBULL	5	5	AIRA	3.3E-04	3.0E+03	USING BD RETAINED AND EPOXIDE RATIO OF 590
20	POOLED TUMORS	MOUSE F	NTP-II	MS-WEIBULL	5	5	AIRA	2.4E-04	4.2E+03	USING BLOOD EPOXIDE MEASUREMENTS OF DAHL ET AL

Pseudomortality corrections involved artificially increasing the response in Table 7 to simulate the possible effect of animals dying before they had time to express the tumor. The first pseudomortality correction (I) assumed a response of 50% at 20 ppm and a 90% response at 62.5 ppm. The second adjustment (II) assumed a 90% response at 20 ppm and dropped higher dose levels in the modeling.

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TABLE 15

COMPARISON OF ALTERNATIVE INDEPENDENT RISK ASSESSMENT (AIRA) AND OSHA RISKS
FROM THE IISRP RAT STUDY
BASED ON 45 YEARS OCCUPATIONAL EXPOSURE AT 2 PPM

LINE #	TUMOR	SPEC/SEX		STUDY	MODEL	DOSES	STAGES	SOURCE	RISK	1 IN X	
1	POOLED/MAMMARY	RAT	F	IISRP	M-STAGE	2	1	AIRA	7.8E-04	1.3E+03	USING BD RETAINED/ 4.5 UNDERESTIMATION OF RAT DOSE
2	POOLED/MAMMARY	RAT	F	IISRP	M-STAGE	3	2	AIRA	1.9E-04	5.3E+03	USING BD RETAINED/ 4.5 UNDERESTIMATION OF RAT DOSE
3	POOLED/MAMMARY	RAT	F	IISRP	M-STAGE	2	1	AIRA	1.3E-04	7.7E+03	USING BD RETAINED/ EPOXIDE RATIO OF 40
4	POOLED/MAMMARY	RAT	F	IISRP	M-STAGE	3	2	AIRA	3.1E-05	3.2E+04	USING BD RETAINED/ EPOXIDE RATIO OF 40
5	POOLED/NO MAMMARY	RAT	F	IISRP	M-STAGE	3	1	AIRA	5.3E-05	1.9E+04	USING BD RETAINED/ 4.5 UNDERESTIMATION OF RAT DOSE
6	POOLED/NO MAMMARY	RAT	F	IISRP	M-STAGE	3	1	AIRA	8.6E-06	1.2E+05	USING BD RETAINED/ EPOXIDE RATIO OF 40
7	POOLED	RAT	M	IISRP	M-STAGE	3	2	AIRA	4.0E-06	2.5E+05	USING BD RETAINED/ 4.5 UNDERESTIMATION OF RAT DOSE
8	POOLED	RAT	M	IISRP	M-STAGE	3	2	AIRA	7.0E-07	1.4E+06	USING BD RETAINED/ EPOXIDE RATIO OF 40

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LIST OF FIGURES

FIGURE 1 - BD RETENTION DATA FROM BOND 1986 FOR RATS AND MICE

FIGURE 2 - EPOXIDE CONCENTRATION IN MOUSE BLOOD
Calculated from DAHL et al 1990

FIGURE 3 - RANGE OF MOUSE BASED RISKS
36 Model/Tumor/Dose Combinations

FIGURE 4 - RANGE OF RISKS FROM RATS
20 Model/Tumor/Dose Combinations

FIGURE 5 - RANGE OF RAT AND MOUSE BASED RISKS
56 Model/Tumor/Dose Combinations

FIG 1: BD RETENTION DATA FROM BOND 1986 FOR RATS AND MICE

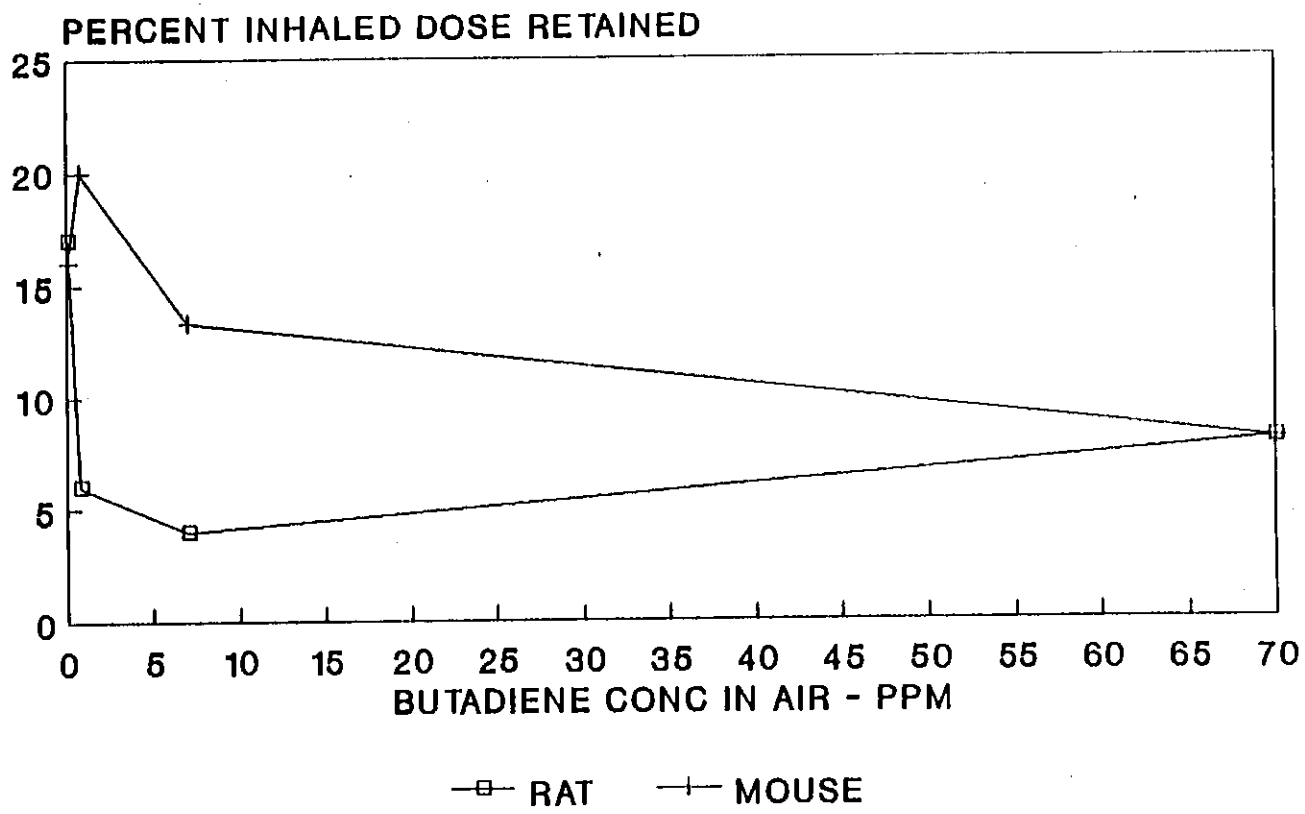

Figure 1

BD Retained as Function of Exposure Concentration

in Rats and Mice

This figure summarizes the data of Bond et al (1986). Rats and mice were exposed nose only for 6 hours to the indicated air concentrations of radiolabeled BD and then sacrificed. The amount of label in the carcass was determined and the retention defined as the amount retained divided by the amount inhaled. The amount inhaled was calculated from the measured air concentration and the measured respiratory rates of the animals.

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FIG 2: EPOXIDE CONC IN MOUSE BLOOD

CALCULATED FROM DAHL ET AL 1990

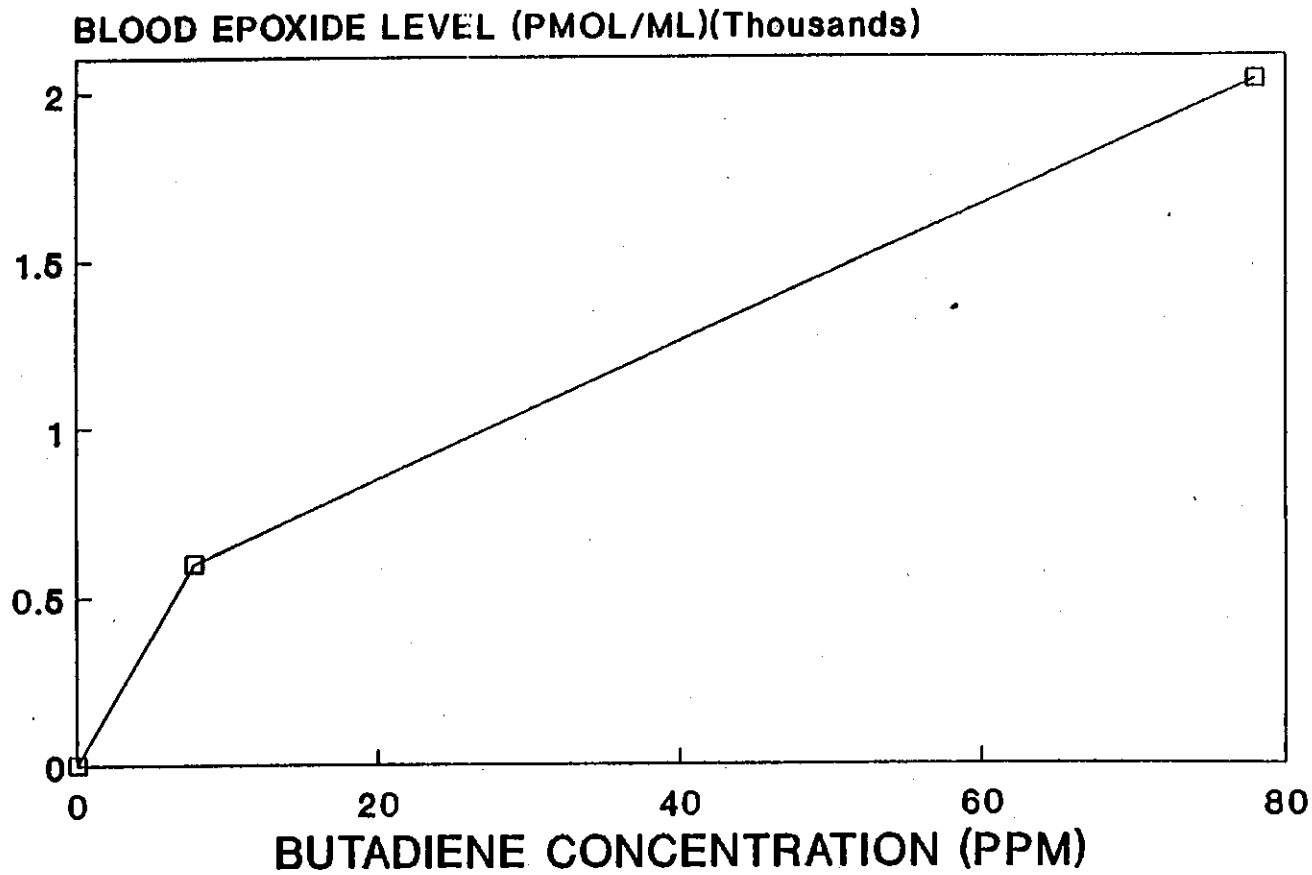

Figure 2

Blood levels of Epoxide in Mice Versus Exposure Concentration

This figure was constructed from the data in Table 4 of Dahl et al (1990). This Table indicated normalized blood levels of epoxide in mice of 77 pmol/ml/ppm at 7.8 ppm and 26 pmol/ml/ppm at 78 ppm. These levels were used to estimate epoxide levels at other exposure concentrations by interpolation or extrapolation (see Table 11 of this assessment). The concentrations apply to a 2 hour exposure.

662000

FIG 3: RANGE OF MOUSE BASED RISKS
36 MODEL/TUMOR/DOSE COMBINATIONS

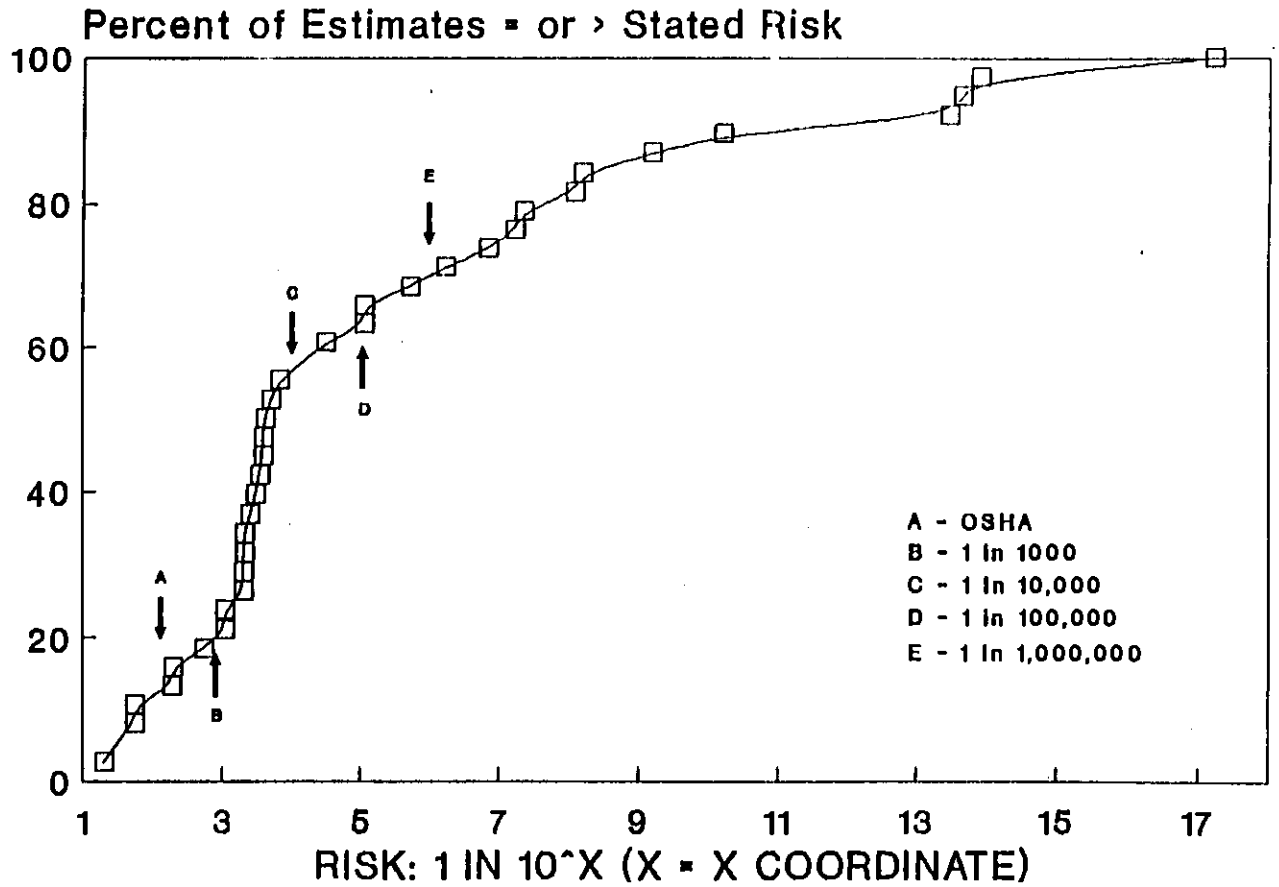

Figure 3

Range of Possible Mouse Based Risks
Based on Occupational Exposure for 45 years at 2 ppm

This figure displays the range of worker risks determined from the various models and assumptions applied to mice. The x axis indicates the exponent of the risk. For example 5 on the x axis indicates a risk of 1 in 10^5, or 1 in 100,000. The y axis is the cumulative percentile of the risk estimates derived having a greater risk than indicated by the x co-ordinate. Thus, figure 3 indicates that approximately 50% of the risk estimates were greater than 1 in 10^4, or 1 in 10,000. The OSHA best estimate is also indicated in the figure (arrow A). Arrows B - E indicate log intervals of decreasing risk from 1 in 1,000 to 1 in 1,000,000. The majority of the cases predict risks to man of less than 1 in 1,000.

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FIG 4: RANGE OF RISKS FROM RATS

20 MODEL/TUMOR/DOSE COMBINATIONS

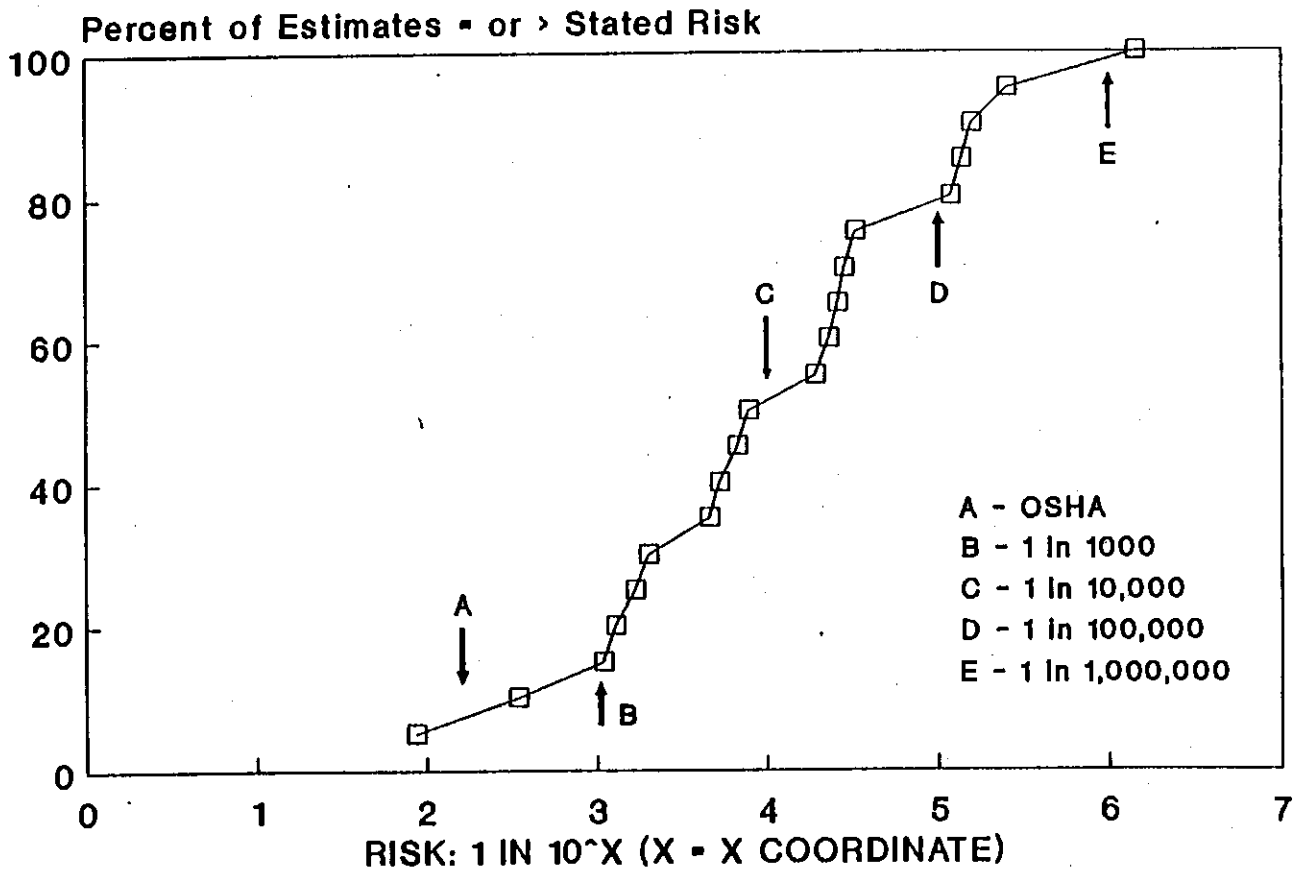

Figure 4

Range of Possible Rat Based Risks
Based on Occupational Exposure for 45 years at 2 ppm

This figure displays the range of worker risks determined from the various models and assumptions applied to rats. The x axis indicates the exponent of the risk. For example 5 on the x axis indicates a risk of 1 in 10^5, or 1 in 100,000. The y axis is the cumulative percentile of the risk estimates derived having a greater risk than indicated by the x co-ordinate. Thus, figure 4 indicates that approximately 50% of the risk estimates were greater than 1 in 10^4, or 1 in 10,000. The OSHA best estimate is also indicated in the figure (arrow A). Arrows B - E indicate log intervals of decreasing risk from 1 in 1,000 to 1 in 1,000,000.

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FIG 5: RANGE OF RAT & MOUSE BASED RISKS

56 MODEL/TUMOR/DOSE COMBINATIONS

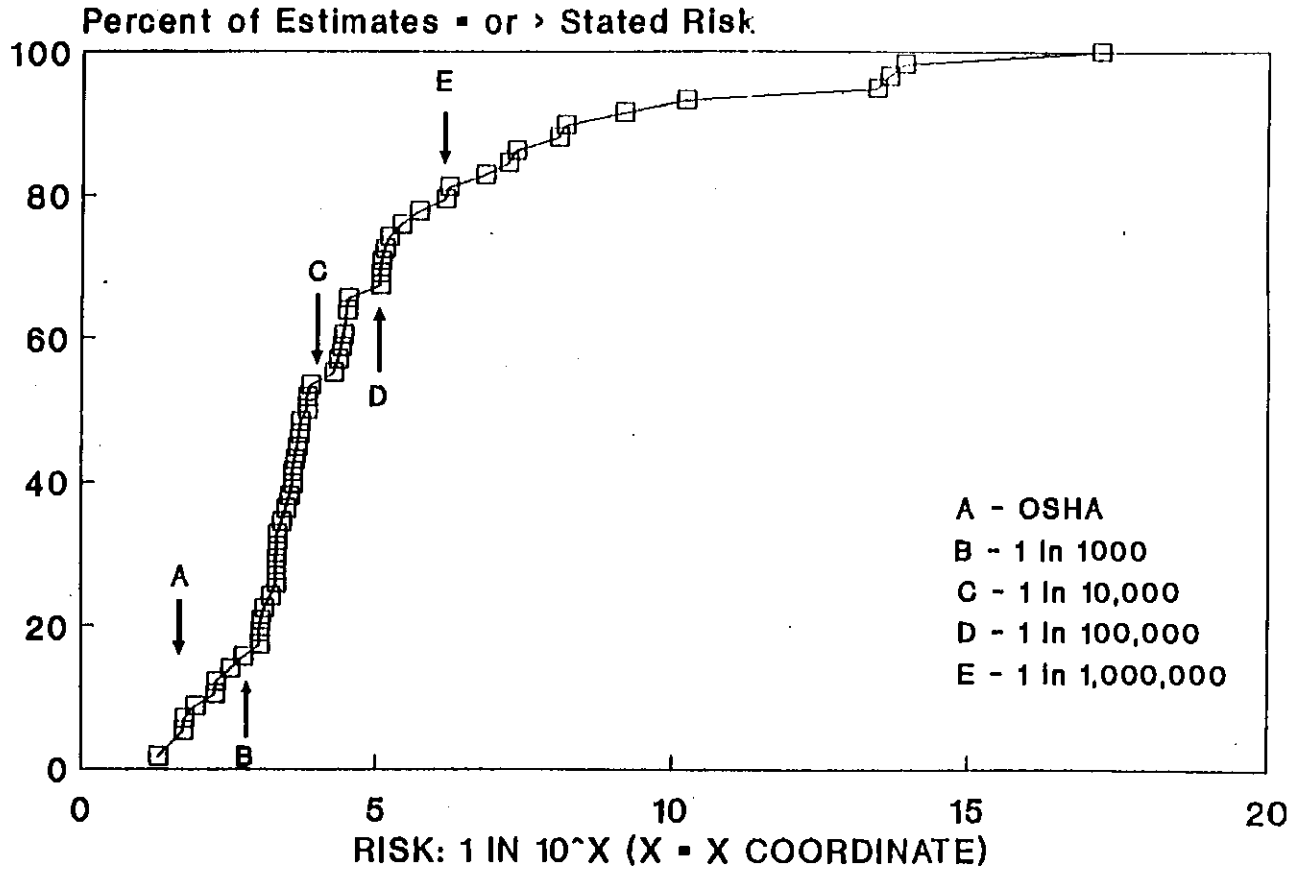

Figure 5

Range of Possible Rat and Mouse Based Risks
Based on Occupational Exposure for 45 years at 2 ppm

This figure displays the range of worker risks determined from the various models and assumptions applied to both rats and mice. The x axis indicates the exponent of the risk. For example 5 on the x axis indicates a risk of 1 in 10^5, or 1 in 100,000. The y axis is the cumulative percentile of the risk estimates derived having a greater risk than indicated by the x co-ordinate. Thus, figure 5 indicates that approximately 50% of the risk estimates were greater than 1 in 10^4, or 1 in 10,000. The OSHA best estimate is also indicated in the figure (arrow A). Arrows B - E indicate log intervals of decreasing risk from 1 in 1,000 to 1 in 1,000,000. Most of the estimates are substantially lower than 1 in 1,000 and range down to significant risks many orders of magnitude smaller.

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LIST OF APPENDICES

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APPENDIX 1: METABOLISM AND PHARMACOKINETICS

The generally accepted premise that epoxide metabolites are the agents responsible for BD induced mutagenicity and carcinogenicity carries with it the opportunity to express the dose response curve in terms of the biologically effective internal dose. Such calculations are now possible and should be performed in order to get a more accurate and informed description of the dose/response relationship in each species.

OSHA used BD retained in the carcass after 6 hours exposure as their measure of dose to allow interspecies extrapolation. This data was obtained in independent experiments. In the absence of other information, this is a reasonable approach. The primary drawbacks are:

1) It is unlikely that BD is the biologically active agent.

2) The BD dose measure was BD retained ("retention following 6 hours"). This was an underestimate of the actual BD dose received.

3) The older data on which OSHA depended to estimate the retention efficiency is flawed and has been recently updated by the original authors.

These factors and their implications and use in risk assessment based on NTP-I, NTP-II and the IISRP rat study are fundamental to the analysis.

It is unlikely that BD is the biologically active agent. There is general consensus that metabolic epoxide products are responsible for the carcinogenic activity. A better measure of dose would be the amount of this biologically active material.

There are sufficient data to support use of the epoxide as a dose surrogate. There have also been sufficient experimental data on respiratory uptake, distribution, metabolism and enzyme activity across species to allow the use of epoxide levels in estimating possible risks to workers (Bond et al, 1986; Dahl et al, 1990; Schmidt and Loeser, 1985; Lorenz, et. al., 1984).

The recent studies of Dahl et al (1990) in monkeys, rats and mice have shown strikingly different blood levels of epoxide metabolites across these species. Mice and rats were exposed for 6 hours to 8 ppm or 80 ppm BD and monkeys were exposed for 2 hours to 10 ppm BD. The resulting epoxide levels in the blood, normalized to account for differing

exposure conditions, indicated mouse levels from 200 to over 590 times that of the monkey and 5 to 40 times that of the rat. Interspecies extrapolation can be performed based on these relative epoxide levels corresponding to the amount of BD retained at exposure levels of occupational interest. The OSHA dose measure of BD retained (calculated from the updated 1986 Bond paper) was used to calculate the risk for a mouse or rat exposed at 2 ppm under occupational conditions. The human risk was then estimated from the ratio of epoxide expected in a monkey to that in a mouse or rat at 2 ppm. Although the appropriate epoxide ratio for humans is unknown, it is likely that from the in vitro relative enzyme activity data that the human will respond more like a monkey. Accordingly, factors of 40 and 590 based on the rat and mouse respectively were used to delineate possible lower bounds on the risks from the models.

Metabolism and excretion in the mouse and rat studies continued **after** each 6 hour exposure raising the possibility that use of BD retained at this time as the dose measure may have overestimated the amount of BD processed. On the other hand, there was undoubtedly continued metabolism and excretion occurring **during** the exposure leading to a possible underestimate of the amount of BD processed. Hattis and Wasson (1987) addressed this problem with the aid of a pharmacokinetic model. They concluded that in the NTP-I study the BD retained at 6 hours was an underestimate of the actual dose by a factor of 2 in the mouse and 4.5 in the rat. This knowledge was used to modify the OSHA estimates based on the NTP-I mouse and IISRP rat accordingly. However, such factors have not been utilized in this analysis of the NTP-II mouse as Hattis and Wasson did not model the low dose situation pertinent to NTP-II.

Hattis and Wasson also concluded that their models indicated that both human absorption and metabolite formation at low doses is likely to be lower than assumed by EPA. As OSHA assumed even higher absorption than EPA, this conclusion applies in particular to the OSHA calculations. The PBPK model indicated humans would likely exhibit a 10% retention efficiency at 2 ppm rather than the 100% assumed by OSHA.

The latter factor is supported by the animal data of Bond et al (1986) shown in Figure 1 and Table 1. The Bond data was used in determining the animal BD retained dose in NTP-II. The older data was apparently sufficiently accurate to determine animal BD retained for the high exposures of NTP-I but should not be extrapolated to low doses.

OSHA risks were adjusted to reflect this lower retention in humans assuming the 10% factor suggested by the pharmacokinetic analysis and the animal retention data.

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Finally, Shell agrees with OSHA that dose-response relationships are likely to scale according to body weight **assuming the appropriate active species is identified**.

The appropriate dose scale to use is determined by the biologically effective quantity of material at the target organ of interest and may not bare a simple relation to amount of **parent** material inhaled and bodyweight. Shell believes the latter statement is demonstrably true for BD in mice and rats. Shell believes that pharmacokinetic data and the observed systemic distribution of tumors implies that concentration **of the biologically active species** in body fluids is the appropriate dose measure. This in turn supports the selection of bodyweight for scaling purposes. Bodyweight scaling is also consistent with analyses of correlations between observed potencies in animals with epidemiology data (Crouch and Wilson, 1979; Allen et al, 1988).

OSHA risks have been further adjusted to reflect this information on the biologically active species.

To summarize, there is now sufficient interspecies pharmacokinetics information available to include three factors in a most informed risk assessment for BD:

(1) The use of updated animal retention data for BD after inhalation exposure (Bond et al,1986) and pharmacokinetic data predicting human retention (Hattis and Wasson,1987).

(2) Adjustment of retention values in rats and mice because of metabolism occurring during the 6 hour exposure period (Hattis and Wasson, 1987).

(3) Adjustment for internal dose of BD epoxides rather than the relatively nonmutagenic BD parent compound based on the work of Dahl et al (1990).

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APPENDIX 2: EVIDENCE FOR ADDUCT FORMATION AND MUTAGENICITY OF BUTADIENE EPOXIDES

It is important to acknowledge that all present evidence implicates butadiene epoxides, as the putative toxic agents responsible for the mutagenic and carcinogenic action of butadiene in rodents.

In in-vivo experiments, repeated exposure of B6C3F1 or NIH Swiss mice to 1250 ppm of butadiene for 3 - 24 weeks results in a macrocytic anemia, with a large increase in the frequency of micronuclei in circulating peripheral red blood cells and in precursor cells in bone marrow (Irons et al. 1986a,b). Irons et al. (1987) treated B6C3F1 and NIH Swiss mice to a single 6-hour exposure of 1250 ppm butadiene, and evaluated bone marrow cell preparations for clastogenic effects at 24, 48, 72, and 96 hours after cessation of treatment. A comparable high frequency of chromatid breaks, as well as increases in chromatid and isochromatid gaps in both strains were observed. Moderate increases in fragments and chromatid exchanges were noted; however, chromosome-type abnormalities were not observed. The maximum frequency of abnormalities in both strains was observed at 24 hours, and diminished with time.

Other investigators (Cunningham et al. 1986) used a nose-only inhalation apparatus to expose male B6C3F1 mice and Sprague-Dawley rats to 100 or 10,000 ppm of butadiene vapor, 6 hours per day, for 2 days. They observed a significant increase in the frequency of micronuclei and sister chromatid exchange (SCE) in the bone cells of mice at both doses, but no apparent effect on these measures in rats.

Tice et al. (1987) found that exposing male B6C3F1 mice to 6.25 ppm and higher of butadiene vapor (6 hours per day, for 10 exposure days during a 15-day period) induced a significant increase in the frequency of SCE in bone marrow cells. A 62.5 ppm exposure produced a significant increase in micronuclei in circulating polychromatic erythrocytes and a significant lengthening of the average generation time in the bone marrow. Exposure at 625 ppm induced a significant increase in chromosomal aberrations, in the relative level of polychromatic erythrocytes in the circulation, as well as in the frequency of micronuclei in normochromic erythrocytes. A trend toward a decreased mitotic index in the bone marrow was observed in exposed mice. Likewise, the induction of micronuclei in polychromatic erythrocytes and of SCE were well correlated with dose.

Evaluating the frequency of micronuclei in circulating polychromatic and normochromic erythrocytes, Jauhar et al.

(1988) observed an increase in both sexes of B6C3F1 mice exposed to 6.25, 62.5, or 625 ppm of butadiene vapor, 6 hours per day, 5 days per week, for 13 weeks.

An atmospheric concentration of approximately 70% butadiene vapor was shown to be mutagenic in Salmonella strains TA1530 and TA1535 in the presence of a metabolic activation system. No significant mutagenic activity was seen in strains TA1537, TA1538, TA98, or TA100 (DeMeester et al. 1978). Early suggestions that butadiene may be "directly" mutagenic in the Salmonella assays was later questioned by the same research group (DeMeester et al. 1980; Poncelet et al. 1980) who suggested that butadiene was metabolized to one or more volatile, mutagenic derivatives which contaminated the atmosphere in the incubation chamber and acted on the bacteria present in the neighboring plates. The metabolites of 1,3-butadiene responsible for mutagenic activity are butadiene epoxides and diepoxide (DeMeester et al. 1978; Gervasi et al. 1985).

To investigate the activity of the putative mutagenic metabolites, Sharief et al. (1986) injected C57BL/6 mice with butadiene monoepoxide intraperitoneally (i.p.) at doses of 10, 25, 50, 100, or 150 mg/kg body weight. Three of four mice injected with 150 mg/kg of the monoepoxide died. The authors found a dose-related increase in the frequency of SCE in bone marrow cells, but no effect on replication index of the marrow except a decrease in the one survivor in the 150 mg/kg group. Butadiene monoepoxide at i.p. doses of 25, 50, 100, or 150 mg/kg also produced a dose-related increase in several measures of clastogenicity in the bone marrow (i.e., number of chromatid and chromosome gaps, total chromosomal aberrations other than gaps, percentage of cells with aberrations, and the number of aberrations per cell).

Several metabolites of butadiene (including butadiene epoxides) are chemically reactive, and have been shown to form covalent adducts with macromolecules (Citti et al. 1984; Gervasi et al. 1985; Kreiling 1986; Laib et al. 1990; Vangala et al 1987).

<u>Butadiene monoepoxide</u> is a monofunctional alkylating agent,and has been detected in exhaled air of rats exposed to butadiene (Bolt et al 1983; Filser et al. 1984). It has been shown to form covalent adducts with deoxyguanosine and DNA in vitro (Citti et al. 1984; Gervasi et al. 1985), to be mutagenic to Salmonella typhimurium (De Meester et al. 1978), and tumorigenic in animal bioassays (Van Duuren et al. 1963, 1966). Gervasi et al. (1985) have reported that the half-life of butadiene monoepoxide in Tris buffer (pH 7.4) is 14 hours.

Butadiene diepoxide is a bifunctional alkylating agent (Jelitto et al. 1989). It has also been shown to be mutagenic in bacterial assays (Gervasi et al. 1985), and to be tumorigenic in experimental animals (McCammon et al. 1957; Weil et al. 1963; Shimkin et al. 1966; Van Duuren et al. 1963, 1965, 1966; IARC 1976). The half-life of butadiene diepoxide in Tris buffer (pH 7.4) is reportedly 100 hours (Gervasi et al. 1985).

Butadiene diol-epoxide is a monofunctional alkylating agent, and is assumed to be capable of reacting with cellular macromolecules. However, no specific studies of this metabolite have been identified in the published literature.

These results all confirm that metabolites of BD (epoxides and diexpoxides) are mutagenic and clastogenic in experimental systems. The actions of these metabolites have been most clearly seen in mice.

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APPENDIX 3: DISCUSSION OF TUMORS OBSERVED IN NTP-II MOUSE STUDY

Because of the magnitude and rapidity of tumor responses seen in the first mouse bioassay, NTP decided to conduct a multidose evaluation of the carcinogenic potential of 1,3-butadiene. In this study, groups of 50 male and 50 female B6C3F1 mice were exposed to 0, 6.25, 20, 62.5, 200, or 625 ppm of butadiene vapor, 6 hours per day, 5 days per week for 103 weeks. Groups of 10 animals of each sex were similarly exposed for sacrifice at 40 and 65 weeks.

While the in-life and analytical portions of NTP-II are complete, the study has not been reviewed by the NTP Pathology Work Group and therefore full details of this study have not been fully released by NTP. A final report is anticipated in 4Q90. However, preliminary indications of the findings of NTP-II are available from Melnick, et. al. (1990a,b).

The preliminary data from NTP-II indicate an increased mortality among mice exposed to butadiene vapor at concentrations of 20 ppm and higher. It is not clear at this time whether the mortality reported at these lower levels of butadiene vapor is directly tumor-related, or is a reflection of some other toxicity of butadiene in the B6C3F1 mouse.

PPM	0	6	20	63	200	625
MALES						
Survival at 2 yr (%)	70	78	48*	44*	8*	0*
Malignant tumors (%)	33	55	62*	68*	73*	86*
FEMALES						
Survival at 2 yr (%)	74	66	48*	22*	0*	0*
Malignant tumors (%)	24	63*	68*	63*	80*	88*

In general, the tumor observations of NTP-II confirm those of NTP-I, an increased incidence of a wide variety of tumors in the B6C3F1 mouse is associated with chronic exposures to 1,3-butadiene (including thymic lymphoma and hemangiosarcoma of the heart). These results are summarized in Table A3-1, but several comments are warranted.

The incidence of lymphocytic lymphomas (assumed to be primarily thymic lymphoma) in NTP-II is reported to be 69% in male and 40% in female B6C3F1 mice chronically exposed to 625 ppm of butadiene vapor, that is, male mice appear to be more susceptible to butadiene-associated lymphomas than are female mice, consistent with the results of NTP-I. The incidence of lymphocytic lymphoma in male mice treated at 200 ppm and less was essentially

baseline, providing a clear demonstration of the dose threshold for butadiene-associated lymphomas at some level between 200 and 625 ppm in the mouse. A similar pattern is seen in female mice, with a marginally significant increase in lymphoma incidence seen at 200 ppm and a clear response seen at 625 ppm. These observations are consistent with the hypothesis that the development of thymic lymphoma in butadiene-exposed mice is dependent on viral activation - a toxicological process which requires that some minimum dose threshold of butadiene be exceeded.

Other Tumors: Statistically increased incidence of a wide variety of common and uncommon tumors in a total of 9 tissues were seen in B6C3F1 mice exposed chronically to butadiene vapor levels as low as 6 ppm (lung neoplasms in female mice). Similar to findings in NTP-I, the high mortality among butadiene-treated mice due to lymphoma (males), and the combination of lymphoma and cardiac hemangiosarcoma (females), clearly suppresses the observed incidence of these other tumors at 625 ppm. This censoring of tumor data is less apparent at butadiene concentrations of 200 ppm and below.

Dose is often expressed in terms of the amount of time (T) exposed to a specified concentration (C) of the test agent (e.g., expressed as ppm-weeks of exposure). Melnick, et al (1990a,b) also included preliminary results from an as-yet-unaudited NTP-sponsored study in which male B6C3F1 mice were exposed to various regimens of butadiene exposure, resulting in equivalent CxT exposures (expressed in ppm-weeks). These data, summarized in Table A3-2, are discussed in greater detail later with individual tumor types, but they strongly suggest that CxT is a poor measure of dose in the quantitative risk analysis of some of the butadiene tumor responses in mice. The implications of these findings for the quantitative risk modeling of epidemiological findings in butadiene-exposed workers should be noted.

For lymphocytic lymphoma, for example, the concentration (C) of butadiene vapor in the chamber atmosphere seems to be the more critical factor. As can be seen in the last two columns of Table A3-2, highly different lymphoma responses are seen in two groups with equivalent CxT exposures (e.g., 16,250 ppm-weeks). Animals exposed to 625 ppm for 26 weeks express 10 times more lymphocytic lymphomas than those exposed to half that dose for twice as long. Such pattern is consistent with the hypothesis that a specific threshold dose must be exceeded to effect viral activation and lymphomagenesis.

For certain non-lymphoma tumor endpoints, a cursory examination of the tumor data would suggest that the

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duration of exposure (T) is the more important factor in the CxT relationship. That is to say, doubling the duration appears to result in a higher tumor yield in butadiene-treated animals, in spite of the fact that they are being exposed to only half the concentration of butadiene vapor (e.g., hemangiosarcoma in male mice exposed to 625 ppm x 26 wk compared to 312 ppm x 52 wk). This interpretation, however, may be confounded by a relatively high intercurrent mortality due to thymic lymphoma in all groups exposed to 625 ppm, and we await further details from NTP on this study.

SPECIFIC COMMENTS ON INDIVIDUAL TUMOR TYPES IN NTP-II

The following comments are pertinent to risk analysis of the various observed tumor endpoints of NTP-II and are prepared in anticipation of doing a complete time to tumor analysis at a later date using individual animal data.

Lymphocytic Lymphoma: The incidence of lymphocytic lymphoma in male B6C3F1 mice exposed to 1,3-butadiene in NTP-II lymphoma was as follows:

	0	6	20	63	200	625 ppm
Male	2/70 (3%)	1/60 (2%)	2/60 (3%)	4/69 (6%)	2/70 (3%)	62/90* (69%)
Female	2/70 (3%)	4/60 (7%)	6/60 (10%)	3/70 (4%)	11/70* (16%)	36/90* (40%)

In male B6C3F1 mice, the occurrence of lymphocytic lymphoma is substantially increased in the 625 ppm group, while the incidence in the 6, 20, 63 and 200 ppm groups are not different from that in the controls. A similar pattern is seen in butadiene-treated female B6C3F1 mice, with a small but statistically significant increased incidence occurring in the 200 ppm group, and a more profound increase at 625 ppm. Data from the stop-exposure study (Table 4) suggest that the lymphoma incidence is related to magnitude of the peak exposure. As discussed in the previous section, this tumor response is assumed to represent primarily T-cell lymphomas of the thymus, a tumor which is etiologically associated with a mouse-specific virus (MuLV) which is apparently activated by butadiene.

Because of the interpretative complication of MuLV, and because of the threshold pattern apparent in the dose-response relationship, this high-dose-only mouse response is not appropriate for low dose extrapolation.

All Malignant Lymphomas: As discussed in previous sections, the category "all malignant lymphomas" probably includes the T- and B-cell lymphocytic lymphomas of the previous category, as well as reticulum cell sarcoma. The incidence of all malignant lymphomas in NTP-II was as follows:

	0	6	20	63	200	625 ppm
Male	4/70 (6%)	3/60 (5%)	8/60 (13%)	11/69* (16%)	9/70* (13%)	69/90* (77%)
Female	10/70 (14%)	14/60 (23%)	18/60* (30%)	10/70 (14%)	19/70* (27%)	43/90* (48%)

It is apparent from these data that the contribution of lymphocytic (especially, T-cell) lymphomas to this category is substantial. In both the male and female mice of NTP-II, the lymphoma incidence at 625 ppm are high. Responses in the 20, 63 and 200 ppm groups, in spite of statistical significance, are equivocal.

The increased tumor incidence of this category are to a large extent due to the high incidence of T-cell lymphoma of the thymus. Therefore, for the same reasons as discussed above, the data for "all malignant lymphomas" are not appropriate for low dose risk extrapolation.

Heart, Hemangiosarcoma: Hemangiosarcoma in the heart is a rare tumor in the mouse, but was seen at high frequency in the butadiene treated mice of NTP-II.

	0	6	20	63	200	625 ppm
Male	0/70 (0%)	0/60 (0%)	1/60 (2%)	5/58* (9%)	20/70* (29%)	6/90* (7%)
Female	0/70 (0%)	0/60 (0%)	0/60 (0%)	1/59 (2%)	20/70* (29%)	26/90* (29%)

This tumor response appears to follow a conventional dose-response. The high dose group needs to be eliminated for modelling purposes since the high lymphoma response was a competing risk that does not give an accurate picture of the risk of hemangiosarcoma in the 625 ppm dose group.

The hemangiosarcoma response may be relevant to man, although the potential role of the virus in the full expression of hemangiosarcomas should be kept in mind. The tumor responses seen in the 6, 20, 63 and 200 ppm groups are adequate for low-dose extrapolation. The models used should be capable of fitting the observed data.

Lung, Alveolar-Bronchiolar Neoplasm:

	0	6	20	63	200	625 ppm
Male	22/70 (31%)	23/60 (38%)	20/60 (33%)	33/69* (48%)	42/70* (60%)	12/88* (13%)
Female	4/70 (6%)	15/60* (25%)	19/60* (32%)	27/70* (39%)	32/70* (46%)	25/88* (28%)

Many strains of mice are prone to develop spontaneous tumors of the lung, ranging from 70-90% incidence in 12-18 month Strain A mice, to 5-15% incidence reported in C3H, to approximately 1% in C57BL mice (Shimkin 1955). In NTP-II, a 31% incidence is seen in untreated B6C3F1 males and 6% is seen in untreated B6C3F1 females.

In butadiene-treated male and female mice, the lung tumor response shows a dose-response pattern which is appropriate for quantitative risk analysis. However, the 625 ppm dose group in males, and the 200 and 625 ppm groups in females need to be eliminated for modelling purposes since early mortality due to the high incidence of thymic lymphoma was a competing risk which obscured the true risks of lung tumors in these dose groups.

Combining the lung tumor observations in male B6C3F1 mice from the classical bioassay with those from the stop-exposure study results in the following tabulation:

Cumulative Dose			
C x T	Dose(ppm)	Weeks	Response
65,000	625	104	14%
20,800	200	104	60%
16,250	625	26	36%
16,692	321	52	64%
8,125	625	13	54%
8,000	200	40	70%
6,552	63	104	48%
2,080	20	104	33%
624	6	104	38%
0	0	104	31%

Several comments are warranted. First, comparison of the lung tumor incidence seen in those groups exposed to 625 ppm of butadiene vapor for varying periods of time shows an inverse incidence with duration of exposure. This is most likely due to the increasing incidence of lymphocytic lymphoma and resulting mortality seen in the male mice exposed to 625 ppm with increasing duration (34% incidence as a result of 13 weeks of exposure, 60% with 26 weeks, 69% with 104 weeks). Second, a cursory analysis of the stop-exposure data (i.e., treatment groups exposed to equivalent CxT doses of butadiene vapor) shows, for example, that the incidence of tumors observed at 625 ppm for 26 weeks (36%) is only about half that seen at 321 ppm for 52 weeks (64%). Such data suggest that length of exposure is more important than is concentration in determining the lung tumor response in butadiene-treated mice. On closer examination, however, it should be appreciated that the mortality due to lymphoma seen in all groups treated at 625 ppm of butadiene vapor obscures the interpretation of the stop-exposure study for all endpoints other than lymphoma. Finally, comparing data for groups treated at 200 ppm of butadiene, a dose that was not associated with lymphoma expression, provides strong evidence that the assumption that equivalent CxT doses produce equal responses does not hold for butadiene-associated mouse lung tumors. As seen in the above table, 200 ppm of butadiene vapor for 104 weeks resulted in about the same incidence of lung tumors (60%) as did the same butadiene concentration for only 40 weeks (70%). Two interpretations for this are 1) the CxT assumption does not hold, or 2) that competing risks and death rates in the 104-week group have resulted in an underestimate of the true incidence of lung tumors.

In spite of the fact that mice are especially prone to develop lung tumors, this response might be relevant to man, and appropriate for quantitative risk analysis. Confounding by lymphoma-associated mortality in the 625 ppm group in males, and in the 200 and 625 ppm groups in females, suggests that these groups should be eliminated from the risk modeling (until individual animal data become available to allow for appropriate statistical corrections for early mortality).

<u>Forestomach, Squamous Cell Neoplasm</u>: Forestomach tumors are not uncommon in rodents exposed to chemicals. What is unusual in this situation is that the exposure is via inhalation and not oral. How and why the tumor response occurred in the forestomach is not explainable at this time.

Man does not have the forestomach equivalent to the rodent, although some suggest the esophagus may be relevant.

However, the chemical environment and the duration of exposure for tissues in the stomach versus the esophagus are quite different.

The incidence of squamous cell neoplasms of the forestomach of butadiene-treated mice is summarized below.

	0	6	20	63	200	625 ppm
Male	1/70 (1%)	0/60 (0%)	1/60 (2%)	5/60 (8%)	12/70* (17%)	13/89* (15%)
Female	2/70 (3%)	2/60 (3%)	3/57 (5%)	4/68 (6%)	7/70* (10%)	28/89* (31%)

The tumor responses above shows a dose-response pattern in male mice chronically exposed to butadiene vapor. Competing mortality due to lymphoma is obvious in the high-dose males. For this reason, the 625 ppm group in males should be eliminated for the purposes of risk modeling. By the same logic, lymphoma-associated mortality may be suppressing the expression of forestomach tumors in the female mice. If that be the case, the true incidence of forestomach tumors in the 200 and 625 ppm groups is likely to be higher than that listed in the table above, and the true dose-response pattern in female mice would be suggestive of a threshold. It should be noted that eliminating the female 200 and 625 ppm groups from the risk analysis (because of competing lymphoma response) would remove all of the treatment groups showing a significant tumor response. For this reason, all dose groups of female mice should be included for risk modeling with the caveat that the available data from NTP-II do not presently allow for statistical correction of early competing mortality.

The table below summarizes tumor responses in butadiene-treated male B6C3F1 mice according to decreasing cumulative dose, and suggests that for the relatively low-incidence of mouse forestomach tumors, the CxT assumption appears to hold.

Cumulative Dose			
C x T	Dose(ppm)	Weeks	Response
65,000	625	104	15%
20,800	200	104	17%
16,250	625	26	22%
16,692	321	52	26%
8,125	625	13	16%
8,000	200	40	12%
6,552	63	104	8%
2,080	20	104	2%
624	6	104	0%
0	0	104	1%

There are significant biological differences between the forestomach of the mouse and any of the structures of the human digestive tract. For this reason, the forestomach tumors cannot be totally ignored, but should be given less weight in risk assessment. Quantitative risk modeling should exclude data from the 625 ppm group in males because of confounding lymphoma mortality. All dose groups should be included in the risk analysis of female mouse data, with the caveat that available information on NTP-II do not presently allow appropriate corrections to be made for early competing mortality. In the analysis of both male and female data, a threshold model should be considered.

Harderian Gland, Neoplasm:

	0	6	20	63	200	625 ppm
Male	6/70 (9%)	7/60 (12%)	11/60 (18%)	24/69* (35%)	33/70* (47%)	7/90* (8%)
Female	9/70 (13%)	10/60 (17%)	7/60 (12%)	16/69* (23%)	22/70* (31%)	7/90 (3%)

The Harderian gland is a secretory gland found in the "third eyelid" of a variety of animals, which secretes an oily substance that lubricates the eyeball. It may also have an endocrine function. The Harderian gland is not present in man, but the type of glandular structure is similar to other glands in man and animals. Tumors in this gland occur naturally, but are increased as a result of exposure to radiation and a number of chemicals. Naturally occurring tumors occur late in life.

The tumor responses in butadiene-treated male and female mice show a dose response pattern. The high-dose group needs to be eliminated for modeling purposes since the lymphoma response was a competing risk that suppressed the true risk of Harderian gland tumors in the 625 ppm dose groups.

000322

The tumor response in male B6C3F1 mice is tabulated by cumulative dose (ppm-weeks) versus percent of animals bearing tumors in the following:

Cumulative Dose			
C x T	Dose(ppm)	Weeks	Response
65,000	625	104	8%
20,800	200	104	47%
16,250	625	26	22%
16,692	321	52	56%
8,125	625	13	46%
8,000	200	40	54%
6,552	63	104	35%
2,080	20	104	18%
624	6	104	12%
0	0	104	8%

There are several apparent inconsistencies in the tumor responses displayed above. The tumor incidence in these male mice is about the same (or slightly higher) following exposures to 200 ppm for 40 weeks (57%) than for the same dose given for 104 weeks (47%). One possible explanation for this is that there may be competing non-lymphoma risks in the 200 ppm x 104 week group, which artificially suppress the true tumor incidence at this dose. As an alternative explanation, it may be that once some threshold dose is exceeded, the maximum tumor response (say 50 - 60%) occurs.

Man does not have Harderian glands. For male mice, quantitative risk modeling should utilize the tumor observations in the 0, 6, 20, 63, and 200 ppm groups, with the 625 ppm data eliminated because of intercurrent mortality. For female mice, a similar analysis should be conducted on the data set (i.e., excluding the 625 ppm group from the analysis). A second risk analysis of the female data is suggested which also excludes data from the 200 ppm group.

000323

Liver, Hepatocellular Neoplasm: As can be seen in the table below the B6C3F1 mouse is prone to develop hepatocellular tumors naturally. Chronic exposure to butadiene vapor, however, may increase slightly the incidence of these lesions.

	0	6	20	63	200	625 ppm
Male	31/70 (44%)	27/60 (45%)	35/59 (59%)	32/59 (54%)	39/70* (56%)	12/89 (13%)
Female	17/69 (25%)	20/60 (33%)	23/60* (38%)	24/60* (40%)	20/60* (33%)	3/90 (3%)

In the male mice, the incidence of liver tumors is only slightly above that in the concurrent control group, although statistical significance is achieved at 200 ppm. The suppressive effect of high intercurrent mortality is clearly visible in the 625 ppm group. In the female mice, the incidence of liver tumors in treated animals is statistically increased at 20, 63, and 200 ppm. The tumor incidence in the 200 and 625 ppm groups of female mice suggest suppression by high intercurrent mortality.

The data suggest that 1,3-butadiene has only a weak tumorigenic effect in the livers of male and female B6C3F1 mice. Liver tumors are well known to occur commonly in untreated mice. The potential relevance to human health is debatable. For male mice, quantitative risk modeling should exclude the 625 ppm group; for female mice, the 200 and 625 ppm groups should be excluded from the analysis.

Preputial Gland, Neoplasm: Preputial glands in the mouse are a pair of prominent specialized sebaceous glands at the distal end (prepuce) of the penis. The preputial glands in man are a series of much smaller glandular structures which are widely distributed over the mucosal aspect of the prepuce. The female equivalent are termed clitoral glands, and were apparently not affected by butadiene treatment.

	0	6	20	63	200	625 ppm
Male	0/70 (0%)	0/60 (0%)	0/60 (0%)	0/69 (0%)	5/70* (7%)	0/90 (0%)

As can be seen, the incidence of this tumor is extremely low. In spite of this, approximately 7% of male mice in the 200 ppm group were found to carry preputial gland neoplasms. The lack of response in the mice exposed to 625 ppm of

butadiene vapor suggests suppression by high intercurrent mortality.

Analysis of the tumor response according to cumulative dose (CxT) to the animal is tabulated below.

Cumulative Dose			
C x T	Dose(ppm)	Weeks	Response
65,000	625	104	0%
20,800	200	104	7%
16,250	625	26	6%
16,692	321	52	8%
8,125	625	13	10%
8,000	200	40	2%
6,552	63	104	0%
2,080	20	104	0%
624	6	104	0%
0	0	104	0%

In spite of certain biological differences between the preputial glands of mice and man, this response may be relevant to human health. Because of high lymphoma-associated mortality, data from the 625 ppm group should be excluded from quantitative risk analysis.

Mammary Gland, Adenocarcinoma: The incidence of adenocarcinoma of the mammary gland in butadiene-treated female mice is summarized below.

	0	6	20	63	200	625 ppm
Female	0/70 (0%)	2/60 (3%)	2/60 (3%)	6/70* (9%)	13/70* (19%)	13/90* (14%)

The incidence of mammary gland tumors appears to increase in a dose-related manner, and are statistically increased at butadiene vapor concentrations of 63 ppm and higher. Competing intercurrent mortality appears to be suppressing the true tumor incidence at 200 and 625 ppm.

000325

The incidence of mammary adenocarcinoma in NTP-II may have potential relevance to human health. Quantitative risk analysis of these data should exclude data from the 200 and 625 ppm groups because of reported competing mortality; a separate analysis should include data from the 200 ppm group in spite of the likelihood that the incidence of mammary gland tumors in that group is artificially low.

Ovary, Granulosa Cell Neoplasm: Spontaneous granulosa cell neoplasms in the mouse are uncommon (Frith et al. 1981) as confirmed by the zero incidence in control animals of NTP-II.

	0	6	20	63	200	625 ppm
Female	0/69 (0%)	0/59 (0%)	0/59 (0%)	9/70* (13%)	11/70* (16%)	6/89 (7%)

Histologically diverse, both granulosa cells and thecal cells may be involved in what is termed a granulosa cell tumor of the ovary. Of interest, granulosa cell tumors are readily induced in mice by treatments as diverse as irradiation, treatment with the carcinogen 7,12-dimethylbenz(a)anthracene (DMBA), neonatal removal of the thymus, as well as following parabiotic linkage of normal and oophorectomized animals. They also develop within tubular adenomas in strain C57BL/6J x C3H/He mice with abnormal genes of the W series which result in a premature depletion of oocytes in this hybrid. Such animals would appropriately be referred to as B6C3F1, although it is not clear that they are genetically identical to the specific hybrid strain used by NTP.

It is interesting to note that ovarian atrophy (i.e., deletion of oocytes) was reported in the butadiene-treated mice of NTP-I. Alison & Morgan comment that there appears to be a common sequence of events between experimental induction of granulosa cell tumors and their occurrence in animals with genetic deletion of oocytes. This sequence consists of oocyte destruction, degeneration of follicular granulosa cells and ovarian atrophy, compensatory elevation of circulating pituitary gonadotropins, and subsequent proliferation and eventual neoplasia of granulosa cells. Such a process would suggest that granulosa cell tumors in the mouse are the result of non-genotoxic mechanisms.

The development of granulosa cell tumors in the mouse is probably a non-genotoxic process and the selection of an appropriate quantitative risk model must take this into consideration. Granulosa cell tumors also occur in women,

and therefore the findings in NTP-II may have possible human health relevance. Because of high competing intercurrent mortality, the risk analysis should most appropriately exclude data from both the 200 and the 625 ppm groups. A separate risk analysis should add back the 200 ppm group in spite of the likelihood that the incidence in that group is artificially low.

TABLE A3-1

1,3-BUTADIENE

TUMOR OBSERVATIONS FROM NTP-II

PRELIMINARY TUMOR OBSERVATIONS IN MALE B6C3F1 MICE (NTP-II)

(ppm)	0	6	20	63	200	625
Lymphocytic lymphomas	3%	2%	3%	6%	3%	69%
All malig. lymphomas	6%	5%	13%	16%	13%	77%
Hemangiosarcoma (heart)	0%	0%	2%	9%	29%	7%
Lung neoplasms	31%	38%	33%	48%	60%	14%
Forestomach neoplasms	1%	0%	2%	8%	17%	15%
Harderian gland neoplasm	9%	12%	18%	35%	47%	8%
Hepatocellular neoplasm	44%	45%	59%	54%	56%	13%
Preputial gland neoplasm	0%	0%	0%	0%	[illegible]	0%

PRELIMINARY TUMOR OBSERVATIONS IN FEMALE B6C3F1 MICE (NTP-II)

(ppm)	0	6	20	63	200	625
Lymphocytic lymphomas	3%	7%	10%	4%	16%	40%
All malig. lymphomas	14%	23%	30%	14%	27%	48%
Hemangiosarcoma (heart)	0%	0%	0%	2%	29%	29%
Lung neoplasms	6%	25%	32%	39%	46%	28%
Forestomach neoplasms	3%	3%	5%	6%	10%	31%
Harderian gland neoplasm	13%	17%	12%	23%	31%	8%
Hepatocellular neoplasm	25%	33%	38%	40%	33%	3%
Mammary gland adenocarcinoma	0%	3%	3%	9%	19%	14%
Ovarian neoplasm	0%	0%	0%	13%	16%	7%

TABLE A3-2

1,3-BUTADIENE
STOP-EXPOSURE STUDY IN MALE MICE (FROM NTP-II)

	0 ppm-wks	8000 ppm-wks		16,250 ppm-wks	
	Control	40w-200	13w-625	52w-312	26w-625
Lymphocytic lymphomas	3%	12%	34%	6%	60%
All malig. lymphomas	6%	24%	48%	30%	74%
Hemangiosarcoma (heart)	0%	30%	14%	66%	26%
Lung neoplasms	31%	70%	54%	64%	36%
Forestomach neoplasms	1%	12%	16%	26%	22%
Harderian gland neoplasm	9%	54%	46%	56%	22%
Preputial gland neoplasm	0%	2%	10%	8%	6%

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APPENDIX 4: CONFOUNDING EFFECT OF THE MURINE RETROVIRUS

In mice from both NTP-I and NTP-II studies, the malignant lymphoma and hemangiosarcoma tumor types stand out with respect to their potential causal relationship with a retrovirus.

Malignant T-cell Lymphoma: Almost 60% of the high-dose male and 20% of the high-dose female B6C3F1 mice developed T-cell lymphoma by week 61 of the study. The B6C3F1 is NTP's preferred test animal because it is considered to be a low-leukemia mouse strain (i.e., the incidence of spontaneous leukemia/lymphoma in the B6C3F1 is low, and untreated animals are expected to survive the two-year bioassay period). From NTP's point-of-view, therefore, it was biologically significant to find such a lymphoma response in this experiment.

T-cell lymphoma is seen in mice treated with ionizing radiation, and has been associated with a mouse-specific RNA virus (i.e., a retrovirus) called Murine Leukemia Virus (MuLV; see Irons et al. 1989). Following radiation, MuLV is "activated" -- that is, the MuLV genome is rapidly replicated in the form of RNA and then packaged as complete virus particles which are found in various tissues and in the circulation. It is apparently during this replication phase that genetic recombination of viral and cellular genes may occur, resulting occasionally in an oncogenic variant of MuLV. Activation of MuLV can also occur spontaneously during normal aging in the B6C3F1 strain, and a low incidence of spontaneous thymic lymphoma is seen in groups of aging B6C3F1 mice. The relationship between MuLV and thymic lymphoma has suggested that activation of the MuLV genome would likely be a threshold phenomenon, and not appropriately modeled by non-threshold quantitative risk approaches.

Shortly after publication of NTP-I, CIIT initiated studies examining the possible etiological involvement of MuLV in the expression of thymic lymphoma in butadiene-treated mice. Results from these studies at CIIT indicate that exposing mice to high concentrations of butadiene appears to enhance viral activation substantially, and that this phenomenon seems to correlate with subsequent development of thymic lymphoma in these animals (Irons et al. 1987; Irons et al. 1989).

Hemangiosarcoma of the Heart: Primary hemangiosarcoma of the heart is an extremely rare neoplasm in the B6C3F1 strain. In NTP-I vascular lesions were seen with an overall

incidence of 30% in males and 43% in females (Solleveld et al. 1988). Based on histological criteria, NTP pathologists classified these cardiac lesions as either endothelial hyperplasia, or hemangiosarcoma. The lesions in the heart were often multifocal and non-uniform in histological appearance suggesting a multicentric origin to NTP pathologists, as opposed to secondary metastatic disease (which would be expected to present a more uniform histological pattern). The portions of the heart most often affected were the left ventricular wall and the interventricular septum. Vascular lesions in the liver, lung, and kidney were found in a significant number of the animals with primary cardiac hemangiosarcoma; these lesions were assumed by NTP pathologists to represent metastasis from the heart, although one high-dose female was noted to have a liver lesion but no heart lesion.

At the present time, the role of MuLV in the total incidence of hemangiosarcoma of the heart is circumstantial. MuLV is endogenous in the mouse, that is the viral genome is present in every cell of the mouse's body, including the endothelial cells of the heart. The multicentric localization of the cardiac lesions observed in NTP-I is certainly consistent with a viral mechanism. In addition, Irons et al. (1989) found a lower, although non-zero, incidence of cardiac hemangiosarcoma in butadiene-treated NIH Swiss mice, which lack a competent MuLV proviral genome, than in similarly treated B6C3F1 mice. This observation may also be suggestive of a possible contributory role for MuLV in this tumor, although Iron's finding may alternatively be attributable to differences in metabolic activation or deactivation of butadiene in the two strains of mice.

The mouse tumor response might have been significantly influenced by the presence of the MuLV. In this sense, certain tumor types would not be relevant as a human hazard. Although OSHA discounts only the mouse lymphoma response, other tumors, such as hemangiosarcomas, might also be significantly influenced by the presence of MuLV and, therefore, this would also be a mouse-only response.

000331

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APPENDIX 5: DISCUSSION OF IISRP CHRONIC RAT STUDY

Sprague-Dawley CD rats (110 males and 110 females per group) were exposed to butadiene vapor concentrations of 0 (control), 1000, or 8000 ppm, 6 hours per day, 5 days per week until 20% survival was reached (111 weeks for males and 105 weeks for females). Chamber air was monitored for butadiene dimer (4-vinyl-1-cyclohexene) and for inhibitor (tertiary butyl catechol) in order to minimize contributions of these components to the results of this study.

Observations were made of the general appearance and behavior of the animals, survival, body weight, hematology, clinical chemistry, urine analysis, necropsy findings and histopathology. Appropriate statistical analysis was made of the findings.

On the first exposure day the majority of animals, including controls, showed a slight red-colored secretion about the nose. The incidence of this finding decreased with time and by week five was seen in only a few animals. In the second month, and continuing until the fifth month, some rats in the high dose group, especially females, showed wet and ruffled fur together with slight limb weakness or incoordination (ataxia) during the first three hours of the first exposure day of the week. Other minor abnormalities were noted from time to time but could not unequivocally be attributed to 1,3-butadiene exposure.

During the course of the study a dose-related increase in mortality was seen. However, analysis of the individual treatment groups indicated that only the 8000-ppm group was statistically different from controls.

	Number of Dead Rats	
	Males	Females
Controls	55	54
1000 ppm	50	68
8000 ppm	68	76

Increased mortality in the female rats was associated with humane sacrifice of animals with large mammary tumors. Increased mortality in the male rat was believed to be due to nephropathy (i.e., kidney dysfunction). It should be emphasized here that nephropathy is, in fact, the most common natural cause of death in aging male rats. However, the incidence of kidney lesions in the IISRP study was significantly increased among the butadiene-treated animals compared to controls. It is also worth noting here that

according to a pathological evaluation of male rat kidney sections from the IISRP study the histological appearance of the lesion in butadiene-treated male rats is not consistent with the so-called "hydrocarbon nephropathy" reported in isoalkane-treated male rats.

The body weight gains of the treated animals were generally depressed during the first 12 weeks of the study but were compensated later by an increased gain, so that by the end of the first year the control and treated groups were similar. Reduced weight gains were not observed in an earlier IISRP 3-month study using exposures of up to 8000 ppm (Crouch et al. 1979).

Hematology and clinical chemistry did not reveal any definite effect that could be associated with 1,3-butadiene exposure.

Organ weight data suggested an effect from 1,3-butadiene exposure on both liver and kidneys. The liver weight and the ratio to body weight were elevated for both sexes and both doses with the exception of the low dose male liver weight. There was no associated pathological change in the liver even at the ultrastructural level. Kidney weight and the ratio to body weight were increased in male rats at the 8000 ppm level. This was associated with a tendency for a more severe level of nephrosis compared with control males and some slight, early evidence of functional change reflected in the amount of protein in the urine and urine volume. Higher heart weight and ratio to body weight may have been associated with the kidney changes.

Necropsy and histopathological evaluation of all major organs and tissues revealed very little treatment-related, non-tumor effect. There was a higher incidence of focal epithelialization (metaplasia) in the lungs of males only at 8000 ppm and this was associated with an increase in lung weight and the ratio to body weight.

A number of tumors were present in larger numbers in the treated animals than in the controls and these differences were statistically valid (Table A5-1). The tumors identified as having increased incidence were:

<u>Zymbal gland carcinoma</u> - predominantly in the high dose females. According to Hazleton's experience with the Sprague-Dawley rat, the number of Zymbal gland tumors also was close to that expected as part of the background. Most of the tumors of this type were clustered in animals killed in a short period (76-90 weeks) but none were found at the end of the study despite an intensive search.

Thyroid, follicular-cell adenoma - particularly in the high dose females. It should be noted that the historical background incidence of thyroid tumors ranges from 0 - 6% for this strain, in this laboratory.

Testis, Leydig-cell adenoma - in both dose groups. Interstitial (Leydig) cell tumors are the most common testicular tumor of the rat (Mostofi & Bresler 1976). Their incidence increases with age and they can be bilateral. The higher numbers of testicular tumors observed in the high-dose male rats was close to the historical control range (0-6%) in the same strain and laboratory.

Uterus, sarcoma - in both dose groups in similar numbers. The numbers of uterine sarcomas were close to those expected from the background in untreated rats of the strain used and do not suggest, in isolation, a treatment-related effect.

Pancreas, exocrine adenoma - only in high dose males. It should be noted here that the diagnosis of neoplasia is questionable for the pancreatic adenomas. These were classified as tumors by convention but, according to the study pathologist, the small lesions equally could be classified as hyperplasia, and excluded from any analysis of tumor incidence.

Mammary gland, adenoma and carcinoma - in both female dose groups. There was evidence that the mammary gland tumors appeared earlier in the study in the treated females, suggesting a relation to treatment. There is, however, some question concerning the interpretation of the numbers of mammary tumors since they occur with a high and variable frequency in untreated rats of the strain used.

On the basis of these findings and of the statistical analysis, the study pathologist concluded that: "The weight of evidence suggests that the test article (1,3-butadiene) is a weak oncogen to the rat under these conditions of exposure". In this study using whole body exposure of rats to concentrations of 1000 or 8000 ppm 1,3-butadiene, there was no "no effect" level.

In summary, the general (non-tumor) effects in rats associated with 1,3-butadiene exposure for two year include a transient increase in nasal secretion, slight ataxia, early mortality (high dose), and early and transient reduced weight gain, increased severity of spontaneous nephropathy (high dose males), increased liver weight and ratio to body weight and an increased metaplasia of the lung (high dose males). The overall conclusion from the evaluation of the tumor incidence is that although there was an increase in the numbers at six sites, in only three of these could the

pattern of increases be clearly related to treatment. This pattern of small increases in a range of tumors or increases of a tumor with a high background incidence suggest that, although treatment may have influenced the number, any effect is to be considered as weak.

TABLE A5-1

1,3-BUTADIENE

TUMOR OBSERVATIONS FROM HAZLETON BIOASSAY (IISRP)

(AS SUMMARIZED BY ENVIRON 1986)

NUMBER OF TUMOR-BEARING MALE SPRAGUE-DAWLEY RATS (IISRP)

	0 ppm	1000 ppm	8000 ppm
Leydig cell tumors	0	3	8
Pancreatic exocrine tumors	2	1	11
Zymbal gland carcinoma	0	1	2
Tumor-bearing rats	2/96	4/96	20/87

NUMBER OF TUMOR-BEARING FEMALE SPRAGUE-DAWLEY RATS (IISRP)

	0 ppm	1000 ppm	8000 ppm
Mammary carcinoma	18	15	26
Mammary fibroadenoma	40	75	67
Thyroid follicular tumors	0	4	11
Zymbal gland carcinoma	0	0	4
Uterine/cervical sarcoma	1	5	7
Tumor-bearing rats w/o mammary	1/94	8/95	21/92
Tumor-bearing rats incl mammary	41/99	77/97	72/96

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Owen PE, Glaister JR, Gaunt IF & Pullinger DH (1987). Inhalation toxicity studies with 1,3-butadiene. 3. Two year toxicity/carcinogenicity study in rats. Amer. Indust. Hyg. Assoc. J. 48: 407-413.

APPENDIX 6: REPRODUCTIVE TOXICITY

A primary non-cancer concern expressed by OSHA was ovarian atrophy seen in female mice exposed to even low levels of BD (see unaudited results - "NTP II") . This mouse effect should be considered to be extraordinary and unique to the female mouse ovary because of the high levels of butadiene epoxide formed by the mouse. This effect is not seen in the rat or any other species. There is no evidence of functional abnormalities in BD reproduction studies. The available current evidence suggests that this mouse ovarian effect should not be considered to be a hazard for human females exposed in the workplace to 2 ppm.

As iterated in Appendix 4 for carcinogenic effects, the mouse is particularly susceptible to toxic effects of BD because of its unique ability to produce and maintain levels of toxic epoxide metabolites of BD. For this reason the mouse is not a good model for estimating human reproductive risk or hazard associated with BD exposure. This argument is particularly compelling in light of the recent findings of similar toxicity to the mouse ovary, but not the rat ovary, due to 4-vinyl cyclohexene, as well as, 4-vinyl cyclohexene epoxide (NTP, 1986, NTP, 1989). These chemicals are structurally similar to BD (4-vinyl cyclohexene is a dimer of BD) and in chronic testing have been found to be highly toxic to the mouse ovary. Furthermore, recent studies by Sipes, et al (1989; also see Smith, et. al., 1990a,b) have shown this mouse specific effect of 4-vinyl cyclohexene to be due to epoxide metabolites and not the parent compound.

The studies with 4-vinyl cyclohexene demonstrate that rare effects to the mouse ovary are only possible because of unique capabilities of the mouse to produce toxic epoxides. It is likely that the mechanisms responsible for similar (rare) ovarian effects in the mouse as a result of BD exposure are likewise due to BD epoxide metabolites. This reasonable assumption would lead to a conclusion that the mouse is unique it its ability to produce such effects. This being the case, it would not be necessary for an agency to utilize the "standard" 10 X 10 or 100 fold safety factor to produce a safe human dose. The mouse is already more sensitive than the most sensitive human. Therefore a 2 ppm standard would be protective against reproductive effects to female workers.

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DOW

Dow U.S.A.

The Dow Chemical Company
P.O. Box 1398

March 21, 1991

Genevieve Shiroma, Chief
Toxic Air Contaminant Identification Branch
Stationary Source Division
Air Resources Board
Attn: 1,3-Butadiene
P.O. Box 2815
Sacramento, CA 95812

RE: COMMENTS ON PRESENCE OF 1,3-BUTADIENE IN CALIFORNIA

Dear Ms. Shiroma:

The comments contained herewith address the issue of the presence of 1,3-butadiene in California, in particular from Styrene-Butadiene Copolymer Production.

On page A-5 of Part A, Exposure Assessment for Proposed Identification of 1,3-Butadiene as a Toxic Air Contaminant, Table III-1, 1,3-Butadiene Emission Inventory, states that Styrene-Butadiene Copolymer Production accounts for 8 tons/year of butadiene emissions.

The Exposure Assessment document then states on page A-11 that there are two latex production facilities in California and that the emissions reported in Table-III-1 are from the northern California facility. The Exposure Assessment document further states that "According to the US EPA, the Northern California facility emitted an estimated 6.95 megagrams (approximately 8 tons) of 1,3-butadiene in 1984 (US EPA, 1989). Approximately 5.1 megagrams of the 1,3-butadiene emissions came from latex process and tank leaks, while 1,85 megagrams of the 1,3-butadiene emissions came from equipment leaks."

The Northern California latex facility that is referred to in the Exposure Assessment is located at the Dow Chemical Co. in Pittsburg, California. We would like the opportunity to correct the Emission Inventory values reported in the Exposure Assessment for the Pittsburg Latex Plant.

The 1,3-butadiene emission values reported in the CARB Exposure Assessment report for the Pittsburg Latex Plant were 11233.5 lb/yr process emissions (5.1 megagrams) and 4074.9 lb/yr (1.85 megagrams) from equipment leaks. It is stated in the CARB report that approximately 8 tons of 1,3-butadiene was emitted from the Pittsburg in 1984 according to EPA.

Data for 1,3-butadiene emissions was submitted to the EPA in August of 1984 pursuant to a Clean Air Act 114 request. The butadiene emissions reported for process and tank vents were 111,653 kg or 7377.95 lb/yr. These were the emissions for the plant as it was operating at that time, at 65% of capacity. Apparently this number was then extrapolated to 100% capacity to arrive at the value reported in the CARB report for 1984 emissions of 11233.5 lb/yr which does not reflect actual emissions.

Since 1984 many improvements have been made at the Latex Plant to control all emissions, including 1,3-butadiene. Authorities to Construct were secured to modernize the Latex Plant and to further abate the Latex Plant emissions to a thermal oxidation device. The emissions from the Latex Plant are now sent to the thermal oxidation process from the Latex Plant scrubber at least 90% of the time as permitted. The emissions of 1,3-butadiene from the modernized Latex Plant were estimated to be 2555 lb/yr with this configuration. The basis of the emission estimate was computer modeling by ASPEN, Advanced System for Process Engineering, a licensed process simulation program developed by MIT and the Department of Energy.

A source test was conducted once the modernized Latex Plant was on-line that confirmed operation below 2555 lb/yr. The vent from the Latex scrubber was analyzed. This is the stream that goes to atmosphere a maximum of 10% of the operating time, the rest of the time it is sent to the thermal processing unit eliminating the butadiene emission.

The results of the source test showed an average daily emission rate of 60 lb/day of total organics (8.52 lb butadiene) based on running at full capacity. Based on the source test data, the annual emissions of butadiene would be 310 lb.yr.

We therefore request that the values shown in the Part A Exposure Assessment document for the Northern California styrene-butadiene copolymer production plant (ie:Pittsburg Plant) for process emissions (which includes tank vents) be changed in the final document to 2555 lb/yr. The modeled emission estimate of 2555 lb/yr is more conservative than the source test result of 310 lb/yr.

On page A-11 of the Exposure Assessment it states that according to the US EPA, 1.85 megagrams (4075 lb/yr) of 1,3-butadiene emissions came from equipment leaks at the Northern California facility in 1984.

As part of the response to the EPA 114 request in 1984, the Pittsburg Latex Plant provided a listing of the valves, flanges, pumps and compressors in butadiene service. The EPA then apparently applied their Average SOCMI Emission Factors for Fugitive Emissions to arrive at the 4075 lb/yr fugitive emission losses from equipment leaks. We believe this number is greatly over estimated.

As part of the Latex Plant modernization, sections of the plant were repiped thus eliminating a number of the valves and flanges present when the 1984 count was submitted to the EPA. In addition the Latex

Plant has had a very effective Fugitive Monitoring and Leak Detection and Repair Program. The program now involves quarterly monitoring of valves and monthly monitoring of pumps and compressors. The results of this monitoring is that no components have been found to leak above the 2 ppm limit of detection of the monitoring instrument.

Because we have actual monitoring data available, the SOCMI 3 Tier Stratified Method for fugitive emission estimating was used for the recently completed AB-2588 emissions inventory for the Latex Plant. The 1,3-butadiene fugitive emission estimate using this SOCMI method was 533 lb/yr.

To conclude, we request that the 1,3-butadiene emission estimates for the Northern California (Dow Pittsburg) Styrene-Butadiene Copolymer Production Plant reflect the current emission values of 2555 lb/yr for process emissions and 533 lb/yr for fugitive emissions rather than the EPA 1984 values currently in the Draft Part A Exposure Assessment document. The use of these values will present a more realistic estimate of the 1,3-Butadiene Emission Inventory in Table III-1.

If you have any questions regarding this information, please call me at 415 432-5639. I plan on attending the meeting on March 27 and I can answer any questions that day as well.

Yours very truly,

Andree Youngson

Andree Youngson
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Enclosures

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SM-2059
March 22, 1991

Genevieve Shiroma, Chief
Toxic Air Contaminant Identification Branch
Stationary Source Division
Air Resources Board
Attn: 1,3-Butadiene
P.O. Box 2815
Sacramento, CA 95812

Dear Ms. Shiroma:

Please find attached General Motors comments on the California Air Resources Board preliminary draft report titled Proposed Identification of 1,3-Butadiene as a Toxic Air Contaminant, dated February 1991. General Motors comments focus on the following three points:

- The report does not properly inform the reader that the risk from motor vehicle 1,3-butadiene emissions has been reduced dramatically over the past 20 years and will continue to decrease substantially due to CARB's regulatory program for controlling criteria pollutants;

- The report does not provide the proper perspective on the risk from motor vehicles relative to the much larger risks from indoor sources such as environmental tobacco smoke; and

- The report should better portray the limitations and uncertainty associated with the range of unit risk factors developed for 1,3-butadiene.

If you have any questions concerning these comments, please contact either J. M. Heuss or J. J. Vostal of my staff at 313-947-1787 or 313-947-1637, respectively.

Sincerely,

for Gerald F. Stoffler
S. A. Leonard, Director
Automotive Emission Control

Attachment

Let's Get It Together
SAFETY BELTS SAVE LIVES

GENERAL MOTORS COMMENTS ON PROPOSED IDENTIFICATION OF 1,3-BUTADIENE AS A TOXIC AIR CONTAMINANT BY THE STATE OF CALIFORNIA

General Motors (GM) has reviewed the preliminary draft prepared by the Staffs of the Air Resources Board and the Department of Health Services and offers the following comments. While the document indicates that 1,3-butadiene may cause or contribute to an increase in mortality and illness, thereby posing a potential hazard to human health, GM is concerned that the risk from present and future ambient concentrations of 1,3-butadiene is not put into the proper perspective in the preliminary draft. In particular, the preliminary draft fails to inform the reader that the risk from motor vehicle 1,3-butadiene emissions has been reduced dramatically over the past 20 years and will continue to decrease substantially due to CARB's regulatory program for controlling criteria pollutants. In addition, the reader is not provided perspective on the substantial human exposures to 1,3-butadiene from other sources, including environmental tobacco smoke. Finally, the limitations and uncertainty associated with the range of unit risk factors developed for 1,3-butadiene should to be better portrayed to the reader.

Comments on Executive Summary

There are several statements in the Executive Summary that are incorrect. First, in response to the question "Are emissions of 1,3-butadiene expected to increase in the state?," the draft indicates:

> "Yes, for the next two years emissions of 1,3-butadiene are expected to increase in California. Approximately 94 percent of California's airborne 1,3-butadiene comes from an increasing number of gas and diesel-fueled motor vehicles. Several regulations have been adopted by the ARB which are expected to result in emission reductions of 1,3-butadiene from light duty motor vehicles starting in 1993."

This statement is incorrect and severely misleading. The fact that 1,3-butadiene emissions from motor vehicles are already substantially controlled is documented by the emission factors given on page A-1 of Appendix A to the Part A Exposure Assessment. The fact that ambient concentrations of 1,3-butadiene in California have been dramatically reduced over the past 20 years by the California Motor Vehicle Emission Control program is documented in our comments on Part A. Factually, existing regulations together

with the numerous new regulations focused on reducing hydrocarbon emissions will continue to dramatically reduce ambient concentrations of 1,3-butadiene for the foreseeable future. Day by day new vehicles with low emissions of 1,3-butadiene are replacing older, higher emitting vehicles. In the preliminary motor vehicle emission inventory developed in 1987,[1] CARB pointed out that the attrition of non-catalyst vehicles would reduce the 1,3-butadiene inventory 61 percent by the year 2000. New regulations adopted since that calculation was made will reduce emissions even further.

Second, GM has concerns related to the precision with which the estimated inventory of 1,3-butadiene emissions from "quantified" sources in California (Exposure A-7) is expressed. As Appendix A indicates, butadiene emissions continue to be derived from the butadiene/TOG ratio and based on "preliminary" inventories calculated by the CARB[1] in 1987 "as a very rough approximation of the true emission inventory." This preliminary estimate was at that time considered to suffer from a possible "error of as much as factor of two." An additional error could have been introduced by unexplained "adjustments" -- an increase of 20% and 50% for gasoline and diesel fueled vehicles respectively. These adjustments were "based on revisions to the emission factors as communicated by MSD"[2] but are not explained in the document.

Because motor vehicle 1,3-butadiene emissions have been measured directly since 1987, the use of oversimplified correction factors is outdated and could seriously misinform the reader. General Motors feels strongly that the emission factors used in paragraph A and B, Appendix A should be replaced by measured butadiene emissions or the potential range of error should be discussed in the text. Butadiene data from the new Auto/Oil Industry research project, in particular, could provide a better basis for statistically robust inventories.[3]

Third, in response to the question "What about indoor exposure to 1,3-butadiene?," the Executive Summary indicates that indoor air may be the major route of exposure to 1,3-butadiene for those individuals exposed to a heavy smoking environment. Based on the available data it is clear that indoor air <u>is</u> the major route of exposure to 1,3-butadiene for smokers as well as those exposed to a heavy smoking environment and that exposure to environmental tobacco smoke (ETS) <u>is</u> a major route of exposure to 1,3-butadiene for most Californians. An estimate of the average daily intake of 1,3-butadiene from ETS is made in the comments to follow; it is 2.8 times the daily intake from the population-weighted statewide average ambient 1,3-butadiene concentration.

GM believes that a knowledge of the relative contributions of individual sources is important for better estimates of public exposure. In the 1980's, the U.S. EPA introduced a new conceptual model of total human exposure (TEAM) and used it to compare benzene emissions vs. exposures. The analysis in Table 1 shows how significant individual sources of exposure are because the general public spends most of its time indoors.

Table 1. Benzene Emissions Inventories vs. Public Exposures based on Personal Monitoring[4]

	Contributions Based on Emissions Inventories	Contributions Based on Personal Monitoring
Autos	82%	18%
Cigarettes	1%	39%
Environmental Tobacco Smoke	--	5%
Industry	14%	3%
Personal & Home	3%	34%
Total	100%	100%

These results suggest that corresponding corrections should be applied in estimating public exposures to 1,3-butadiene. In fact, because the benzene and butadiene content of cigarette smoke is similar while the ambient exposure to benzene is many times that of butadiene, a corresponding comparison for butadiene will probably show an even smaller contribution from motor vehicles than shown in Table 1. An estimate of the sources of personal exposure to 1,3-butadiene would be more useful than the simple proportioning of the ambient inventories given in the Executive Summary.

Fourth, in response to the question "What is the risk assessment for exposure to 1,3-butadiene?," the Executive Summary indicates that "the DHS staff estimates the number of potential excess cancers due to airborne 1,3-butadiene exposure to range from 1 to 115 additional cancers per million people exposed throughout their lives..." GM suggests that the phrase "upper limit" be added so that the range of potential cancers is put in the correct perspective for decision makers. CARB has approached the potential health risks from toxics with great caution. While this is understandable, GM believes extreme care must be taken to keep the estimates in their proper context -- otherwise they will be misinterpreted and the risk from ambient 1,3-butadiene will be improperly perceived as a major threat to public health. For example, Vostal et al.[5] -- considering the situation when all motor

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vehicles will be equipped with current emission controls -- have predicted minimal effects of butadiene from motor vehicles on the U.S. annual cancer incidence (0 to 0.05 cases per million urban population).

As CARB evaluates the risk from exposure to 1,3-butadiene and other airborne toxics, GM recommends that it consider the findings and recommendations from several important reports and reviews by eminent scientific groups. In particular, EPA's Science Advisory Board (SAB) has recently recommended that the Agency should target its environmental protection efforts on the basis of opportunities for the greatest risk reduction.[6] In order to set priorities, the SAB indicated that the Agency "must weigh the relative risks posed by different environmental problems, determine if there are cost-effective opportunities for reducing those risks, and then identify the most cost-effective risk reduction options." Further, the SAB recommended that EPA improve the data and analytical methodologies that support risk assessment and comparison, indicating that risk rankings should be based on the total human exposure to specific toxic agents.

A recent National Research Council review of human exposure assessment for airborne pollutants came to similar conclusions.[7] The NRC committee wrote that "risk reduction strategies that address only outdoor air are only partially effective. Such strategies need to be modified to better address the importance of indoor exposures." To set priorities for reducing risk to potentially harmful pollutants, the committee indicated that all media and all routes of exposure must be assessed.

In order to compare risks from different agents and routes of exposure, Paustenbach, et al.[8], in a review of the current practice of health risk assessment, recommend that "in each portion of an assessment, the weight of evidence approach should be used to identify the most defensible value. In the risk characterization, the best estimate of the potential risk as well as the highest plausible risk should be presented."

GM suggests that CARB follow these recommendations in the continuing evaluation of the risks associated with 1,3-butadiene and other toxic air contaminants.

Specific GM comments on Parts A and B of the preliminary draft follow.

Comments on Part A Exposure Assessment

1,3-Butadiene Emission Inventory. As discussed above, the inventory summarized in Table III-1 is subject to considerable uncertainty; the document should include a discussion of the major uncertainties. Among the uncertainties are several related to emission factors for both on-road and other mobile sources. While many of the emission factors used are given in Appendix A, the original data used to determine the emission factors for on-road motor vehicles are not identified. In addition, it is noted that an update to the emission factors is expected in the late summer of 1990. Since the preliminary draft was issued in February 1991, the footnote is puzzling. The primary data sources are particularly important because many of the organic analyses in the literature do not resolve 1,3-butadiene from butane and other C4 hydrocarbons. Therefore, the estimated values for 1,3-butadiene emissions may not be accurate.

In the late 1980's several high quality data sources reported 1,3-butadiene emissions from motor vehicles. For example, Marshall[9] measured both engine out and catalyst exhaust 1,3-butadiene emissions from nine late model (1985-1987) catalyst equipped passenger cars. The engine out emissions averaged 34.6 mg/mi, which is in reasonable agreement with the emission factor of 27.9 mg/mi for non-catalyst passenger cars given on page A-1 of Appendix A. Marshall measured 0.87 mg/mi in the exhaust after the catalyst demonstrating that the catalyst reduced 1,3-butadiene emissions by 97 percent. This is somewhat greater than the 90 percent reduction in the CARB estimate given in Table A-1 of Appendix A.

Vostal, Williams, and Lipari[5] reported 1,3-butadiene measurements from seven late model GM and Chrysler passenger cars that averaged 0.46 mg/mi -- with 1,3-butadiene representing 0.11 wt percent of the exhaust HC emissions. An even larger data base that includes 1,3-butadiene exhaust measurements has recently been made available to CARB by the Auto/Oil Air Quality Improvement Research Program. This data should be included in any estimates of current and future year inventories.

Exposure to 1,3-butadiene in California. The preliminary draft report includes the results from 360 individual 24-hour samples collected at 20 toxic monitoring stations in California. Based on these results, an overall population-weighted statewide 1,3-butadiene concentration of 0.37 ppbv was calculated. This concentration is referred to as an overall statewide exposure, but GM is concerned that this is a misnomer. At best it could be characterized as an overall statewide <u>outdoor</u> exposure; but because

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people spend over 80 percent of their time indoors, it cannot be considered an overall statewide exposure.

There are also several questions regarding the methodology used to obtain the samples. No description of the site selection criteria for the 20 monitoring sites is given; to aid the reader in judging the appropriateness of the sites, the criteria should be referenced. In addition, the procedure in Appendix B specifically calls for samples to be taken in stainless steel canisters and analyzed as soon as possible. Although the draft indicates that the samples collected in Tedlar bags are of verifiable quality, the reasons for the difference between the written and actual procedures should be documented.

There is another source of current ambient concentrations of 1,3-butadiene in California -- the individual organic species measured in hundreds of samples during the 1987 Southern California Air Quality Study (SCAQS). This data should be used to get a better idea of the spatial and temporal variation in ambient 1,3-butadiene concentrations so that more refined exposure analyses can be carried out. There is another way that the SCAQS data can be used -- to verify overall estimates of 1,3-butadiene emissions from vehicular sources. The SCAQS data can be used to estimate the fraction of 1,3-butadiene in ambient non-methane organic carbon concentrations. In a similar data base involving several hundred samples collected in 1987 in 32 cities, Lonneman found that 1,3-butadiene represented 0.22 wt percent of the carbon in the samples.[10] The analogous fraction of carbon in the SCAQS samples can be used to check the estimated statewide emissions of 1,3-butadiene.

In order to carry out a risk assessment, the long-term trend in ambient 1,3-butadiene concentrations should be documented. Whereas the Executive Summary indicated that 1,3-butadiene concentrations are expected to increase in California in the next several years, there is a substantial body of information documenting that current ambient concentrations are only a fraction of the ambient concentrations that existed 20 years ago. Altshuller, et al.[11] measured individual hydrocarbons in several hundred samples collected in Los Angeles over several months in the fall of 1967 and reported 1,3-butadiene concentrations averaging 2 ppbv with 10 percent of the values exceeding 5 ppbv. Similarly, the average 1,3-butadiene concentration in 218 samples analyzed by the Los Angeles Air Pollution Control District in 1965 was 2 ppbv.[12] Thus, the current 1,3-butadiene concentrations in Los Angeles are roughly one-quarter of the concentrations measured in the middle-to-late 1960's. This dramatic reduction in ambient concentrations occurred

in spite of increasing population, numbers of vehicles or vehicle miles travelled and is a clear indication of the success of the emission controls on motor vehicles.

For the remaining emissions of 1,3-butadiene, it is instructive to consider the fraction that are emitted by non-catalyst vehicles. From the emission factors and VMT estimates shown in Appendix A for passenger cars and light- and medium-duty trucks, it can be shown that non-catalyst vehicles are estimated to emit 60 percent of the remaining 1,3-butadiene emissions even though they account for only 13 percent of the vehicle miles travelled. Clearly, any additional vehicle-related risk reduction efforts should be focused on these older, high-emitting vehicles.

Estimates of Exposure from Indoor Air. The preliminary draft correctly identifies environmental tobacco smoke (ETS) as a major source of 1,3-butadiene in indoor air. However, the draft indicates that there is not sufficient information to make a quantitative analysis at this time. Nevertheless, the draft includes reference to several data sources that can be used to provide some perspective on the exposures from ETS. Obviously, for the 30 percent of persons over 18 that are current smokers, the exposure to 1,3-butadiene from smoking overwhelms that from any other sources. For the roughly equal portion of the population that are former smokers, their lifetime exposure to 1,3-butadiene has probably also been dominated by their smoking experience. For non-smokers, the exposure to ETS is not insignificant. Survey information referenced in the draft report indicates that Californians are exposed to ETS approximately 2.6 hours each day on the average. Because this is (probably) self-reported exposure, it represents exposure over some threshold. In addition, the draft indicates that about four percent of California residents reported attending bars and nightclubs. Lofroth, et al.[13] have estimated that the inhaled dose in two hours in such conditions is in the range of 18 to 32 ug/exposure. For comparison, the daily inhaled dose from the statewide ambient concentration reported in the draft is 16.4 ug. Lofroth, et al. also report measurements of the airborne yield of 1,3-butadiene from sidestream smoke of 400 ug/cigarette. When one considers that roughly 70 billion cigarettes are smoked in California each year -- for the most part indoors -- the potential for significant exposure of non-smokers to 1,3-butadiene from ETS is apparent.

In order to evaluate the relative risk from both outdoor and indoor sources of 1,3-butadiene, GM recommends that CARB carry out additional studies to quantitate this important exposure. While the preliminary draft indicates that there is <u>not</u> sufficient

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information to make a quantitative analysis at this time, GM submits that there is sufficient information to estimate total daily intake of 1,3-butadiene from ETS -- or passive smoking as it is also called -- when the information in Lofroth, et al. is combined with the extensive body of existing information on the exposure to ETS. Estimates of the typical daily intake of various toxic constituents of cigarette smoke for both active and passive exposure are given in the attached Table C-3 from Appendix C of EPA's May 1990 review of the health effects of passive smoking.[14] While it is recognized that exposure to ETS varies widely due to differences in the rate of smoking, types of cigarettes smoked, room volumes, and ventilation rates in indoor environments, the EPA calculated a "typical" exposure condition using representative values of the composition of both mainstream and sidestream cigarette smoke from the NRC assessment of the health effects of ETS.[15] The typical exposures in Table C-3 are consistent with the average concentrations of several airborne components of ETS measured in real indoor settings.

Using the NRC reported value for the average emission rate of respirable suspended particulate matter (RSP) per cigarette in sidestream smoke, 26 mg, EPA calculated a daily intake for passive exposure of 3 mg. Using the same methodology for 1,3-butadiene, with an emission rate of 400 ug per cigarette, one can calculate the average daily intake of a passive smoker to be 46 ug. This can be compared to an average daily intake of 16.4 ug for an individual exposed to the average ambient concentration of 0.37 ppbv (0.82 ug/m^3). Thus the exposure from typical indoor 1,3-butadiene concentrations far exceeds that of typical outdoor concentrations. In addition, the statement that approximately 94 percent of California's airborne 1,3-butadiene comes from motor vehicles is misleading. While motor vehicles are responsible for the bulk of outdoor emissions of 1,3-butadiene, they are responsible for a much smaller percentage of the actual 1,3-butadiene that Californians are exposed to. The Part A Exposure Assessment and the Executive Summary should make this point clear for the reader.

Comments on Part B Health Assessment

GM scientists compliment the CARB and DHS staffs on a thorough review of the available information on the potential health effects of 1,3-butadiene. GM particularly appreciates the DHS attempts to discuss and include toxicokinetic aspects in the hazard evaluation and risk assesssment process. It is, therefore, disappointing that the document concludes on page 4-33 that the assessment cannot rely at present on the pharmacokinetic estimates of metabolized dose for estimating human health risks. GM feels strongly that

considerations of pharmacokinetic principles in the disposition of 1,3 butadiene in the human body should be an integral part of the hazard identification and would significantly improve the final risk estimates.

In general, General Motors concurs with the statement that "adverse noncarcinogenic effects are not expected at the ambient levels of 1,3-butatadiene" -- currently not exceeding 2 ppbv in California. Concerning the carcinogenicity of 1,3-butadiene, GM scientists and the scientific community at large are not absolutely certain that the present evidence satisfactorily documents that the ambient concentrations of 1,3-butadiene "may cause or contribute to an increase in mortality and illness, thereby posing a potential hazard to human health." In view of the recent public discussions[16] pointing out that a high percentage of all chemicals are expected to be carcinogenic in animal tests at chronic exposures near maximum tolerated doses, the automotive industry feels that clarification of cancer-producing mechanisms is an important step before final decisions are made on the real public health hazard of a chemical.[17] Indeed, the 1990 U.S. Clean Air Act Amendments specifically require that "the EPA Administrator shall enter into appropriate arrangements with the National Academy of Sciences to review.. ...risk assessment methodology used to determine the carcinogenic risk associated with exposure to hazardous air pollutants" (the review will be completed before 1993). This represents an important precedent; State Boards should also consider evaluating and improving their risk assessment methodology.

The document acknowledges that the mechanisms by which extremely high concentrations of 1,3-butadiene produce tumors in animals are still unknown. Unfortunately, no adequate experiments have been conducted on animals at low ambient levels to date. The DHS's conclusion that "no threshold mechanisms have been shown to specifically affect the action of butadiene" and the assumption that "no practical threshold exists" are, therefore, in view of these uncertainties premature. The text on page 4-3 recognizes that threshold mechanisms based on saturation of detoxification enzymes have been proposed but fails to mention this possibility when the non-threshold action of butadiene is assumed in the cancer potency estimates.

Additionally, some butadiene-induced tumors such as thymic lymphoma/leukemia in the B6C3F1 mouse were not replicated in other species of laboratory rodents (rat) and not all scientists would agree that the evidence for chemical (genotoxic) carcinogenicity of butadiene in animals is sufficient. First, the in vitro

genotoxicity of butadiene has not been verified in vivo on laboratory rats. Second, criteria for animal carcinogenicity tests are currently under review by the U.S. National Academy of Sciences (NRC). Third, the multiple site character of tumors as well as wide tolerances to tumors in various species of laboratory rodents suggest a potential non-specific action of high doses of the chemical. The incidence of butadiene leukemia varies widely among species and even among different strains of mice (B6C3F1 vs. NIH Swiss mice[18]) indicating that the tumors might be induced by activation of endogenous leukemogenic retroviruses (ecotropic MULV) rather than by the postulated direct chemical action of butadiene.[19] This represents an important argument for a non-specific, threshold-producing effect of butadiene based on secondary tumor-regulating mechanisms. Since thymic lymphomas represent the most substantial tumor response in mice, this factor could significantly influence the resulting calculations of the cancer potency and unit risk from animal data (page 4-20). At least, this evidence questions the DHS conclusion that the mouse bioassay is "superior to that of the rat data" and that "the mouse provides the best estimate for the upper bound for plausible excess cancer risk to humans."

The chemical carcinogenicity of 1,3-butadiene is further challenged by the fact that the "epidemiological evidence of human carcinogenicity is inadequate" in spite of high occupational exposures and numerous studies. A greater emphasis on the existing uncertainties would be, therefore, more appropriate than the reported DHS "finding" of 1,3-butadiene carcinogenicity in humans (page 1-37).

In the quantitative assessment of the "theoretical" risk associated with continuous lifetime exposure to butadiene, wide ranges of scaling procedures and assumptions have been used in deriving unit risks. For example, Chapter 1.1.3.2 on Extrapolation lists an assumption that only 16-17% of a low inhaled dose is absorbed by animals resulting in an absorbed dose of 18 to 38 mg/kg-day. The same text also states that a person living in an area where the average butadiene concentration is 0.0004 ppm "would receive an inhalation dose of 0.00025 mg/kg-day." Not only is this a dose that is approximately five orders of magnitude lower than that in experimental animals, but a simple calculation suggests that the estimate has been based on the assumption that humans retain 100 percent of the inhaled butadiene. Should the lower absorption factor of 17% have been used in man as in animals, the resulting dose would be approximately six times lower.

In assessing the potential risk for humans, the document's

conclusions recognize that:

(1) Because of the large differences in carcinogenic potency between rats and mice, the question of which of the animal models is more appropriate for humans remains unanswered. DHS staff selected the mouse bioassay data but do not list the evidence supporting the claim that the mouse data is "superior to that of the rat data";

(2) DHS staff also arbitrarily accepts that the exposure in humans results in the same absorption rate and handling of the inhaled 1,3-butadiene concentrations as in animals.

These and other assumptions result in a considerable level of uncertainty in the predicted public health effects of ambient concentrations of butadiene and may produce a substantial error in the risk estimates.

Unfortunately, none of these uncertainties are adequately emphasized in the Conclusions of the document. Indeed the concluding chapter includes a statement on page 5-1 that the average ambient levels of butadiene in California could be associated with up to 1 to 128 additional cancers per million lifetime exposed individuals. It would be more appropriate to state that the exposures may result "in up to 0 to 1 or 0 to 128 additional cancer cases per million exposed depending on the selected animal model." Dividing this estimate by the average lifespan of 70 yrs, the prediction would result in up to 0 to 0.014 or 0 to 4.5 additional cancer cases per million exposed people per year. As noted above, the inherent uncertainties in these estimates are important caveats to the DHS conclusion that "1,3-butadiene is an air pollutant that may contribute to an increase in mortality or serious illness or pose a present hazard to human health."

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TABLE C-3. SUMMARY OF CONCENTRATIONS AND DAILY INTAKES FOR CONSTITUENTS OF CIGARETTE SMOKE, ASSUMING FRESH SS

Constituent*	C_i^{**}	$I_P^{\dagger}$	$I_P^{\dagger\dagger}$
Benzene	1.3 $\mu g/m^3$	21 μg	300 μg
Hydrazine	0.5 ng/m^3	8 ng	300 ng
N-nitrosodimethylamine	30.0 ng/m^3	160 ng	300 ng
N-nitrosodiethylamine	3.0 ng/m^3	48 ng	200 ng
N-nitrosopyrrolidine	2.0 ng/m^3	32 ng	200 ng
RSP[a]	200.0 $\mu g/m^3$	3 mg	240 mg
Nicotine[b]	23.0 $\mu g/m^3$	370 μg	14 mg
2-Naphthylamine[a]	0.3 ng/m^3	5 ng	20 ng
4-Aminobiphenyl[a]	0.9 ng/m^3	14 ng	50 ng
Benz(a)anthracene[a]	0.8 ng/m^3	13 ng	500 ng
Benzo(a)pyrene[a]	0.5 ng/m^3	8 ng	300 ng
λ-Butyrolactone[a]	0.3 $\mu g/m^3$	5 ng	150 μg
N-nitrosonornicotine[a]	17.0 ng/m^3	270 ng	15 μg
N-nitrosodiethanolamine[a]	0.3 ng/m^3	5 ng	500 ng
Nickel[a]	6.0 ng/m^3	96 ng	500 ng
Polonium-210[a]	2.0 nC_i/m^3	32 nC_i	1 pC_i

* Only constituents listed as human carcinogens, suspected human carcinogens or animal carcinogens (NRC, 1986) are listed, with the exception of nicotine (a precursor to carcinogens).

** For passive exposures only.

† Intake for passive exposure.

†† Intake for active exposure.

[a] Chemicals located in the particulate phase for both active and passive smokers.

[b] Nicotine is assumed to be entirely in the particulate phase for active smokers and entirely in the vapor phase for passive smokers.

FROM REFERENCE 14

FACSIMILE COMMUNICATION

NUMBER OF PAGES (Including this cover): 16

TO: Genevieve Shiroma

FROM: Jim Ehlmann

Environmental Activities Staff
General Motors Technical Center
30400 Mound Road
Warren, MI 48090-9015

Telephone: 313-947-1799 8-227-

MESSAGE: You will also be receiving a copy in Monday's mail.

DATE: March 22, 1991

GM

Environmental Activities Staff
General Motors Corporation
General Motors Technical Center
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SM-2059
March 22, 1991

Genevieve Shiroma, Chief
Toxic Air Contaminant Identification Branch
Stationary Source Division
Air Resources Board
Attn: 1,3-Butadiene
P.O. Box 2815
Sacramento, CA 95812

Dear Ms. Shiroma:

Please find attached General Motors comments on the California Air Resources Board preliminary draft report titled Proposed Identification of 1,3-Butadiene as a Toxic Air Contaminant, dated February 1991. General Motors comments focus on the following three points:

- The report does not properly inform the reader that the risk from motor vehicle 1,3-butadiene emissions has been reduced dramatically over the past 20 years and will continue to decrease substantially due to CARB's regulatory program for controlling criteria pollutants;

- The report does not provide the proper perspective on the risk from motor vehicles relative to the much larger risks from indoor sources such as environmental tobacco smoke; and

- The report should better portray the limitations and uncertainty associated with the range of unit risk factors developed for 1,3-butadiene.

If you have any questions concerning these comments, please contact either J. M. Heuss or J. J. Vostal of my staff at 313-947-1787 or 313-947-1637, respectively.

Sincerely,

for Gerald F. Stoffler
S. A. Leonard, Director
Automotive Emission Control

Attachment

Let's Get It Together
SAFETY BELTS SAVE LIVES

GENERAL MOTORS COMMENTS ON PROPOSED IDENTIFICATION OF 1,3-BUTADIENE AS A TOXIC AIR CONTAMINANT BY THE STATE OF CALIFORNIA

General Motors (GM) has reviewed the preliminary draft prepared by the Staffs of the Air Resources Board and the Department of Health Services and offers the following comments. While the document indicates that 1,3-butadiene may cause or contribute to an increase in mortality and illness, thereby posing a potential hazard to human health, GM is concerned that the risk from present and future ambient concentrations of 1,3-butadiene is not put into the proper perspective in the preliminary draft. In particular, the preliminary draft fails to inform the reader that the risk from motor vehicle 1,3-butadiene emissions has been reduced dramatically over the past 20 years and will continue to decrease substantially due to CARB's regulatory program for controlling criteria pollutants. In addition, the reader is not provided perspective on the substantial human exposures to 1,3-butadiene from other sources, including environmental tobacco smoke. Finally, the limitations and uncertainty associated with the range of unit risk factors developed for 1,3-butadiene should to be better portrayed to the reader.

Comments on Executive Summary

There are several statements in the Executive Summary that are incorrect. First, in response to the question "Are emissions of 1,3-butadiene expected to increase in the state?," the draft indicates:

> "Yes, for the next two years emissions of 1,3-butadiene are expected to increase in California. Approximately 94 percent of California's airborne 1,3-butadiene comes from an increasing number of gas and diesel-fueled motor vehicles. Several regulations have been adopted by the ARB which are expected to result in emission reductions of 1,3-butadiene from light duty motor vehicles starting in 1993."

This statement is incorrect and severely misleading. The fact that 1,3-butadiene emissions from motor vehicles are already substantially controlled is documented by the emission factors given on page A-1 of Appendix A to the Part A Exposure Assessment. The fact that ambient concentrations of 1,3-butadiene in California have been dramatically reduced over the past 20 years by the California Motor Vehicle Emission Control program is documented in our comments on Part A. Factually, existing regulations together

with the numerous new regulations focused on reducing hydrocarbon emissions will continue to dramatically reduce ambient concentrations of 1,3-butadiene for the foreseeable future. Day by day new vehicles with low emissions of 1,3-butadiene are replacing older, higher emitting vehicles. In the preliminary motor vehicle emission inventory developed in 1987,[1] CARB pointed out that the attrition of non-catalyst vehicles would reduce the 1,3-butadiene inventory 61 percent by the year 2000. New regulations adopted since that calculation was made will reduce emissions even further.

Second, GM has concerns related to the precision with which the estimated inventory of 1,3-butadiene emissions from "quantified" sources in California (Exposure A-7) is expressed. As Appendix A indicates, butadiene emissions continue to be derived from the butadiene/TOG ratio and based on "preliminary" inventories calculated by the CARB[1] in 1987 "as a very rough approximation of the true emission inventory." This preliminary estimate was at that time considered to suffer from a possible "error of as much as factor of two." An additional error could have been introduced by unexplained "adjustments" -- an increase of 20% and 50% for gasoline and diesel fueled vehicles respectively. These adjustments were "based on revisions to the emission factors as communicated by MSD"[2] but are not explained in the document.

Because motor vehicle 1,3-butadiene emissions have been measured directly since 1987, the use of oversimplified correction factors is outdated and could seriously misinform the reader. General Motors feels strongly that the emission factors used in paragraph A and B, Appendix A should be replaced by measured butadiene emissions or the potential range of error should be discussed in the text. Butadiene data from the new Auto/Oil Industry research project, in particular, could provide a better basis for statistically robust inventories.[3]

Third, in response to the question "What about indoor exposure to 1,3-butadiene?," the Executive Summary indicates that indoor air may be the major route of exposure to 1,3-butadiene for those individuals exposed to a heavy smoking environment. Based on the available data it is clear that indoor air <u>is</u> the major route of exposure to 1,3-butadiene for smokers as well as those exposed to a heavy smoking environment and that exposure to environmental tobacco smoke (ETS) <u>is</u> a major route of exposure to 1,3-butadiene for most Californians. An estimate of the average daily intake of 1,3-butadiene from ETS is made in the comments to follow; it is 2.8 times the daily intake from the population-weighted statewide average ambient 1,3-butadiene concentration.

GM believes that a knowledge of the relative contributions of individual sources is important for better estimates of public exposure. In the 1980's, the U.S. EPA introduced a new conceptual model of total human exposure (TEAM) and used it to compare benzene emissions vs. exposures. The analysis in Table 1 shows how significant individual sources of exposure are because the general public spends most of its time indoors.

Table 1. Benzene Emissions Inventories vs. Public Exposures based on Personal Monitoring[4]

	Contributions Based on Emissions Inventories	Contributions Based on Personal Monitoring
Autos	82%	18%
Cigarettes	1%	39%
Environmental Tobacco Smoke	--	5%
Industry	14%	3%
Personal & Home	3%	34%
Total	100%	100%

These results suggest that corresponding corrections should be applied in estimating public exposures to 1,3-butadiene. In fact, because the benzene and butadiene content of cigarette smoke is similar while the ambient exposure to benzene is many times that of butadiene, a corresponding comparison for butadiene will probably show an even smaller contribution from motor vehicles than shown in Table 1. An estimate of the sources of personal exposure to 1,3-butadiene would be more useful than the simple proportioning of the ambient inventories given in the Executive Summary.

Fourth, in response to the question "What is the risk assessment for exposure to 1,3-butadiene?," the Executive Summary indicates that "the DHS staff estimates the number of potential excess cancers due to airborne 1,3-butadiene exposure to range from 1 to 115 additional cancers per million people exposed throughout their lives..." GM suggests that the phrase "upper limit" be added so that the range of potential cancers is put in the correct perspective for decision makers. CARB has approached the potential health risks from toxics with great caution. While this is understandable, GM believes extreme care must be taken to keep the estimates in their proper context -- otherwise they will be misinterpreted and the risk from ambient 1,3-butadiene will be improperly perceived as a major threat to public health. For example, Vostal et al.[5] -- considering the situation when all motor

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vehicles will be equipped with current emission controls -- have predicted minimal effects of butadiene from motor vehicles on the U.S. annual cancer incidence (0 to 0.05 cases per million urban population).

As CARB evaluates the risk from exposure to 1,3-butadiene and other airborne toxics, GM recommends that it consider the findings and recommendations from several important reports and reviews by eminent scientific groups. In particular, EPA's Science Advisory Board (SAB) has recently recommended that the Agency should target its environmental protection efforts on the basis of opportunities for the greatest risk reduction.[6] In order to set priorities, the SAB indicated that the Agency "must weigh the relative risks posed by different environmental problems, determine if there are cost-effective opportunities for reducing those risks, and then identify the most cost-effective risk reduction options." Further, the SAB recommended that EPA improve the data and analytical methodologies that support risk assessment and comparison, indicating that risk rankings should be based on the total human exposure to specific toxic agents.

A recent National Research Council review of human exposure assessment for airborne pollutants came to similar conclusions.[7] The NRC committee wrote that "risk reduction strategies that address only outdoor air are only partially effective. Such strategies need to be modified to better address the importance of indoor exposures." To set priorities for reducing risk to potentially harmful pollutants, the committee indicated that all media and all routes of exposure must be assessed.

In order to compare risks from different agents and routes of exposure, Paustenbach, et al.[8], in a review of the current practice of health risk assessment, recommend that "in each portion of an assessment, the weight of evidence approach should be used to identify the most defensible value. In the risk characterization, the best estimate of the potential risk as well as the highest plausible risk should be presented."

GM suggests that CARB follow these recommendations in the continuing evaluation of the risks associated with 1,3-butadiene and other toxic air contaminants.

Specific GM comments on Parts A and B of the preliminary draft follow.

Comments on Part A Exposure Assessment

1,3-Butadiene Emission Inventory. As discussed above, the inventory summarized in Table III-1 is subject to considerable uncertainty; the document should include a discussion of the major uncertainties. Among the uncertainties are several related to emission factors for both on-road and other mobile sources. While many of the emission factors used are given in Appendix A, the original data used to determine the emission factors for on-road motor vehicles are not identified. In addition, it is noted that an update to the emission factors is expected in the late summer of 1990. Since the preliminary draft was issued in February 1991, the footnote is puzzling. The primary data sources are particularly important because many of the organic analyses in the literature do not resolve 1,3-butadiene from butane and other C4 hydrocarbons. Therefore, the estimated values for 1,3-butadiene emissions may not be accurate.

In the late 1980's several high quality data sources reported 1,3-butadiene emissions from motor vehicles. For example, Marshall[4] measured both engine out and catalyst exhaust 1,3-butadiene emissions from nine late model (1985-1987) catalyst equipped passenger cars. The engine out emissions averaged 34.6 mg/mi, which is in reasonable agreement with the emission factor of 27.9 mg/mi for non-catalyst passenger cars given on page A-1 of Appendix A. Marshall measured 0.87 mg/mi in the exhaust after the catalyst demonstrating that the catalyst reduced 1,3-butadiene emissions by 97 percent. This is somewhat greater than the 90 percent reduction in the CARB estimate given in Table A-1 of Appendix A.

Vostal, Williams, and Lipari[5] reported 1,3-butadiene measurements from seven late model GM and Chrysler passenger cars that averaged 0.46 mg/mi -- with 1,3-butadiene representing 0.11 wt percent of the exhaust HC emissions. An even larger data base that includes 1,3-butadiene exhaust measurements has recently been made available to CARB by the Auto/Oil Air Quality Improvement Research Program. This data should be included in any estimates of current and future year inventories.

Exposure to 1,3-butadiene in California. The preliminary draft report includes the results from 360 individual 24-hour samples collected at 20 toxic monitoring stations in California. Based on these results, an overall population-weighted statewide 1,3-butadiene concentration of 0.37 ppbv was calculated. This concentration is referred to as an overall statewide exposure, but GM is concerned that this is a misnomer. At best it could be characterized as an overall statewide <u>outdoor</u> exposure; but because

people spend over 80 percent of their time indoors, it cannot be considered an overall statewide exposure.

There are also several questions regarding the methodology used to obtain the samples. No description of the site selection criteria for the 20 monitoring sites is given; to aid the reader in judging the appropriateness of the sites, the criteria should be referenced. In addition, the procedure in Appendix B specifically calls for samples to be taken in stainless steel canisters and analyzed as soon as possible. Although the draft indicates that the samples collected in Tedlar bags are of verifiable quality, the reasons for the difference between the written and actual procedures should be documented.

There is another source of current ambient concentrations of 1,3-butadiene in California -- the individual organic species measured in hundreds of samples during the 1987 Southern California Air Quality Study (SCAQS). This data should be used to get a better idea of the spatial and temporal variation in ambient 1,3-butadiene concentrations so that more refined exposure analyses can be carried out. There is another way that the SCAQS data can be used -- to verify overall estimates of 1,3-butadiene emissions from vehicular sources. The SCAQS data can be used to estimate the fraction of 1,3-butadiene in ambient non-methane organic carbon concentrations. In a similar data base involving several hundred samples collected in 1987 in 32 cities, Lonneman found that 1,3-butadiene represented 0.22 wt percent of the carbon in the samples.[10] The analogous fraction of carbon in the SCAQS samples can be used to check the estimated statewide emissions of 1,3-butadiene.

In order to carry out a risk assessment, the long-term trend in ambient 1,3-butadiene concentrations should be documented. Whereas the Executive Summary indicated that 1,3-butadiene concentrations are expected to increase in California in the next several years, there is a substantial body of information documenting that current ambient concentrations are only a fraction of the ambient concentrations that existed 20 years ago. Altshuller, et al.[11] measured individual hydrocarbons in several hundred samples collected in Los Angeles over several months in the fall of 1967 and reported 1,3-butadiene concentrations averaging 2 ppbv with 10 percent of the values exceeding 5 ppbv. Similarly, the average 1,3-butadiene concentration in 218 samples analyzed by the Los Angeles Air Pollution Control District in 1965 was 2 ppbv.[12] Thus, the current 1,3-butadiene concentrations in Los Angeles are roughly one-quarter of the concentrations measured in the middle-to-late 1960's. This dramatic reduction in ambient concentrations occurred

in spite of increasing population, numbers of vehicles or vehicle miles travelled and is a clear indication of the success of the emission controls on motor vehicles.

For the remaining emissions of 1,3-butadiene, it is instructive to consider the fraction that are emitted by non-catalyst vehicles. From the emission factors and VMT estimates shown in Appendix A for passenger cars and light- and medium-duty trucks, it can be shown that non-catalyst vehicles are estimated to emit 60 percent of the remaining 1,3-butadiene emissions even though they account for only 13 percent of the vehicle miles travelled. Clearly, any additional vehicle-related risk reduction efforts should be focused on these older, high-emitting vehicles.

Estimates of Exposure from Indoor Air. The preliminary draft correctly identifies environmental tobacco smoke (ETS) as a major source of 1,3-butadiene in indoor air. However, the draft indicates that there is not sufficient information to make a quantitative analysis at this time. Nevertheless, the draft includes reference to several data sources that can be used to provide some perspective on the exposures from ETS. Obviously, for the 30 percent of persons over 18 that are current smokers, the exposure to 1,3-butadiene from smoking overwhelms that from any other sources. For the roughly equal portion of the population that are former smokers, their lifetime exposure to 1,3-butadiene has probably also been dominated by their smoking experience. For non-smokers, the exposure to ETS is not insignificant. Survey information referenced in the draft report indicates that Californians are exposed to ETS approximately 2.6 hours each day on the average. Because this is (probably) self-reported exposure, it represents exposure over some threshold. In addition, the draft indicates that about four percent of California residents reported attending bars and nightclubs. Lofroth, et al.[13] have estimated that the inhaled dose in two hours in such conditions is in the range of 18 to 32 ug/exposure. For comparison, the daily inhaled dose from the statewide ambient concentration reported in the draft is 16.4 ug. Lofroth, et al. also report measurements of the airborne yield of 1,3-butadiene from sidestream smoke of 400 ug/cigarette. When one considers that roughly 70 billion cigarettes are smoked in California each year -- for the most part indoors -- the potential for significant exposure of non-smokers to 1,3-butadiene from ETS is apparent.

In order to evaluate the relative risk from both outdoor and indoor sources of 1,3-butadiene, GM recommends that CARB carry out additional studies to quantitate this important exposure. While the preliminary draft indicates that there is <u>not</u> sufficient

information to make a quantitative analysis at this time, GM submits that there is sufficient information to estimate total daily intake of 1,3-butadiene from ETS -- or passive smoking as it is also called -- when the information in Lofroth, et al. is combined with the extensive body of existing information on the exposure to ETS. Estimates of the typical daily intake of various toxic constituents of cigarette smoke for both active and passive exposure are given in the attached Table C-3 from Appendix C of EPA's May 1990 review of the health effects of passive smoking.[14] While it is recognized that exposure to ETS varies widely due to differences in the rate of smoking, types of cigarettes smoked, room volumes, and ventilation rates in indoor environments, the EPA calculated a "typical" exposure condition using representative values of the composition of both mainstream and sidestream cigarette smoke from the NRC assessment of the health effects of ETS.[15] The typical exposures in Table C-3 are consistent with the average concentrations of several airborne components of ETS measured in real indoor settings.

Using the NRC reported value for the average emission rate of respirable suspended particulate matter (RSP) per cigarette in sidestream smoke, 26 mg, EPA calculated a daily intake for passive exposure of 3 mg. Using the same methodology for 1,3-butadiene, with an emission rate of 400 ug per cigarette, one can calculate the average daily intake of a passive smoker to be 46 ug. This can be compared to an average daily intake of 16.4 ug for an individual exposed to the average ambient concentration of 0.37 ppbv (0.82 ug/m^3). Thus the exposure from typical indoor 1,3-butadiene concentrations far exceeds that of typical outdoor concentrations. In addition, the statement that approximately 94 percent of California's airborne 1,3-butadiene comes from motor vehicles is misleading. While motor vehicles are responsible for the bulk of outdoor emissions of 1,3-butadiene, they are responsible for a much smaller percentage of the actual 1,3-butadiene that Californians are exposed to. The Part A Exposure Assessment and the Executive Summary should make this point clear for the reader.

Comments on Part B Health Assessment

GM scientists compliment the CARB and DHS staffs on a thorough review of the available information on the potential health effects of 1,3-butadiene. GM particularly appreciates the DHS attempts to discuss and include toxicokinetic aspects in the hazard evaluation and risk assesssment process. It is, therefore, disappointing that the document concludes on page 4-33 that the assessment cannot rely at present on the pharmacokinetic estimates of metabolized dose for estimating human health risks. GM feels strongly that

considerations of pharmacokinetic principles in the disposition of 1,3 butadiene in the human body should be an integral part of the hazard identification and would significantly improve the final risk estimates.

In general, General Motors concurs with the statement that "adverse noncarcinogenic effects are not expected at the ambient levels of 1,3-butatadiene" -- currently not exceeding 2 ppbv in California. Concerning the carcinogenicity of 1,3-butadiene, GM scientists and the scientific community at large are not absolutely certain that the present evidence satisfactorily documents that the ambient concentrations of 1,3-butadiene "may cause or contribute to an increase in mortality and illness, thereby posing a potential hazard to human health." In view of the recent public discussions[16] pointing out that a high percentage of all chemicals are expected to be carcinogenic in animal tests at chronic exposures near maximum tolerated doses, the automotive industry feels that clarification of cancer-producing mechanisms is an important step before final decisions are made on the real public health hazard of a chemical.[17] Indeed, the 1990 U.S. Clean Air Act Amendments specifically require that "the EPA Administrator shall enter into appropriate arrangements with the National Academy of Sciences to review.. ...risk assessment methodology used to determine the carcinogenic risk associated with exposure to hazardous air pollutants" (the review will be completed before 1993). This represents an important precedent; State Boards should also consider evaluating and improving their risk assessment methodology.

The document acknowledges that the mechanisms by which extremely high concentrations of 1,3-butadiene produce tumors in animals are still unknown. Unfortunately, no adequate experiments have been conducted on animals at low ambient levels to date. The DHS's conclusion that "no threshold mechanisms have been shown to specifically affect the action of butadiene" and the assumption that "no practical threshold exists" are, therefore, in view of these uncertainties premature. The text on page 4-3 recognizes that threshold mechanisms based on saturation of detoxification enzymes have been proposed but fails to mention this possibility when the non-threshold action of butadiene is assumed in the cancer potency estimates.

Additionally, some butadiene-induced tumors such as thymic lymphoma/leukemia in the B6C3F1 mouse were not replicated in other species of laboratory rodents (rat) and not all scientists would agree that the evidence for chemical (genotoxic) carcinogenicity of butadiene in animals is sufficient. First, the in vitro

FROM 31094713 03-22-91 01:59 PM P11

genotoxicity of butadiene has not been verified in vivo on laboratory rats. Second, criteria for animal carcinogenicity tests are currently under review by the U.S. National Academy of Sciences (NRC). Third, the multiple site character of tumors as well as wide tolerances to tumors in various species of laboratory rodents suggest a potential non-specific action of high doses of the chemical. The incidence of butadiene leukemia varies widely among species and even among different strains of mice (B6C3F1 vs. NIH Swiss mice[18]) indicating that the tumors might be induced by activation of endogenous leukemogenic retroviruses (ecotropic MULV) rather than by the postulated direct chemical action of butadiene.[19] This represents an important argument for a non-specific, threshold-producing effect of butadiene based on secondary tumor-regulating mechanisms. Since thymic lymphomas represent the most substantial tumor response in mice, this factor could significantly influence the resulting calculations of the cancer potency and unit risk from animal data (page 4-20). At least, this evidence questions the DHS conclusion that the mouse bioassay is "superior to that of the rat data" and that "the mouse provides the best estimate for the upper bound for plausible excess cancer risk to humans."

The chemical carcinogenicity of 1,3-butadiene is further challenged by the fact that the "epidemiological evidence of human carcinogenicity is inadequate" in spite of high occupational exposures and numerous studies. A greater emphasis on the existing uncertainties would be, therefore, more appropriate than the reported DHS "finding" of 1,3-butadiene carcinogenicity in humans (page 1-37).

In the quantitative assessment of the "theoretical" risk associated with continuous lifetime exposure to butadiene, wide ranges of scaling procedures and assumptions have been used in deriving unit risks. For example, Chapter 1.1.3.2 on Extrapolation lists an assumption that only 16-17% of a low inhaled dose is absorbed by animals resulting in an absorbed dose of 18 to 38 mg/kg-day. The same text also states that a person living in an area where the average butadiene concentration is 0.0004 ppm "would receive an inhalation dose of 0.00025 mg/kg-day." Not only is this a dose that is approximately five orders of magnitude lower than that in experimental animals, but a simple calculation suggests that the estimate has been based on the assumption that humans retain 100 percent of the inhaled butadiene. Should the lower absorption factor of 17% have been used in man as in animals, the resulting dose would be approximately six times lower.

In assessing the potential risk for humans, the document's

conclusions recognize that:

(1) Because of the large differences in carcinogenic potency between rats and mice, the question of which of the animal models is more appropriate for humans remains unanswered. DHS staff selected the mouse bioassay data but do not list the evidence supporting the claim that the mouse data is "superior to that of the rat data";

(2) DHS staff also arbitrarily accepts that the exposure in humans results in the same absorption rate and handling of the inhaled 1,3-butadiene concentrations as in animals.

These and other assumptions result in a considerable level of uncertainty in the predicted public health effects of ambient concentrations of butadiene and may produce a substantial error in the risk estimates.

Unfortunately, none of these uncertainties are adequately emphasized in the Conclusions of the document. Indeed the concluding chapter includes a statement on page 5-1 that the average ambient levels of butadiene in California could be associated with up to 1 to 128 additional cancers per million lifetime exposed individuals. It would be more appropriate to state that the exposures may result "in up to 0 to 1 or 0 to 128 additional cancer cases per million exposed depending on the selected animal model." Dividing this estimate by the average lifespan of 70 yrs, the prediction would result in up to 0 to 0.014 or 0 to 4.5 additional cancer cases per million exposed people per year. As noted above, the inherent uncertainties in these estimates are important caveats to the DHS conclusion that "1,3-butadiene is an air pollutant that may contribute to an increase in mortality or serious illness or pose a present hazard to human health."

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TABLE C-3. SUMMARY OF CONCENTRATIONS AND DAILY INTAKES FOR CONSTITUENTS OF CIGARETTE SMOKE, ASSUMING FRESH SS

Constituent*	C_i**	I_p†	I_p††
Benzene	1.3 μg/m³	21 μg	300 μg
Hydrazine	0.5 ng/m³	8 ng	300 ng
N-nitrosodimethylamine	30.0 ng/m³	160 ng	300 ng
N-nitrosodiethylamine	3.0 ng/m³	48 ng	200 ng
N-nitrosopyrrolidine	2.0 ng/m³	32 ng	200 ng
RSP[a]	200.0 μg/m³	3 mg	240 mg
Nicotine[b]	23.0 μg/m³	370 μg	14 mg
2-Naphthylamine[a]	0.3 ng/m³	5 ng	20 ng
4-Aminobiphenyl[a]	0.9 ng/m³	14 ng	50 ng
Benz(a)anthracene[a]	0.8 ng/m³	13 ng	500 ng
Benzo(a)pyrene[a]	0.5 ng/m³	8 ng	300 ng
λ-Butyrolactone[a]	0.3 μg/m³	5 ng	150 μg
N-nitrosonornicotine[a]	17.0 ng/m³	270 ng	15 μg
N-nitrosodiethanolamine[a]	0.3 ng/m³	5 ng	500 ng
Nickel[a]	6.0 ng/m³	96 ng	500 ng
Polonium-210[a]	2.0 nC_i/m^3	32 nC_i	1 pC_i

* Only constituents listed as human carcinogens, suspected human carcinogens or animal carcinogens (NRC, 1986) are listed, with the exception of nicotine (a precursor to carcinogens).

** For passive exposures only.

† Intake for passive exposure.

†† Intake for active exposure.

[a] Chemicals located in the particulate phase for both active and passive smokers.

[b] Nicotine is assumed to be entirely in the particulate phase for active smokers and entirely in the vapor phase for passive smokers.

FROM REFERENCE 14

INTERNATIONAL
INSTITUTE OF
SYNTHETIC RUBBER
PRODUCERS, INC.

March 21, 1991

VIA FEDERAL EXPRESS

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Toxic Air Contaminant Identification Branch
Stationary Source Division
Air Resources Board
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Post Office Box 2815
Sacramento, CA 95812

RE: Proposed Identification of 1,3-Butadiene as a Toxic Air Contaminant (Preliminary Draft, February 1991)

Dear Ms. Shiroma:

The International Institute of Synthetic Rubber Producers (IISRP), representing more than 50 worldwide producers of synthetic rubber, is pleased to provide these comments on the Preliminary Draft "Proposed Identification of 1,3-Butadiene as a Toxic Air Contaminant." IISRP has been an active participant in assessing the toxicity of butadiene. We have sponsored a major lifetime rat inhalation study of butadiene and the largest epidemiology study conducted in the world of butadiene-exposed workers. IISRP has also been a major participant in the current OSHA rulemaking on butadiene.

Our comments here concentrate on the available human evidence. Detailed comments on the available animal evidence, which we also recommend you consider carefully, are being submitted by the Butadiene Panel of the Chemical Manufacturers Association.

Before discussing the available human evidence, we note that the "Preliminary Draft, Part A, Exposure Assessment, (February 1991)" contains old emissions data for Styrene-Butadiene Copolymer Plants. To our knowledge, the only polymer plants using butadiene in California are the two SB latex plants referenced, one in Northern California and another in Southern California. We have been informed that both plants have provided government authorities more current emissions data than that contained at page A-11 of your Preliminary Draft.

The preliminary draft does not include a number of recent publications. Specifically, we refer to five enclosed articles published as a part of the proceedings of the

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International Symposium on 1,3-Butadiene published in the June 1990 issue of Environmental Health Perspectives. The Board is also referred to the extensive materials submitted to the Occupational Safety and Health Administration (OSHA) docket for the 1,3-butadiene proposed rulemaking. We enclose here the testimony submitted by Drs. John Acquavella and Philip Cole on behalf of the International Institute of Synthetic Rubber Producers.

The testimony of Drs. Acquavella and Cole includes detailed analyses of all the available epidemiology studies. We recommend your close review of these analyses. These analyses show that the large available data base demonstrates that butadiene is not a human cancer risk at current occupational exposure levels, and, therefore, also not at lower environmental levels. We summarize those analyses below. Our specific comments on the CARB draft's discussion of these issues are attached.

Several follow-up studies of styrene-butadiene rubber (SBR) workers and of workers in butadiene manufacture contain useful information on the relation between employment in these industries and mortality. These investigations include 17,448 subjects with an average of 22 years of follow-up. The Standardized Mortality Ratio (SMR) for all causes of death and specific cancers are not elevated. Also, mortality rates are low for butadiene exposed subgroups across studies. On this point, there has been universal agreement, both at the butadiene symposium and the OSHA hearings.

Particular attention has been focused on the occurrence of lymphatic and hematopoietic cancer (LHC). These studies collectively reported a total of 36 observed leukemia deaths, compared with 34.1 expected (SMR of 106, with a 95% confidence interval of 74 - 146). These null data show no association between butadiene industry employment and this disease. Similarly, there is little difference between the observed and expected numbers of deaths from other forms of LHC: 20/17.8 (SMR = 112; 95% CI = 69 - 173) for lymphosarcoma and reticulosarcoma; 12/10.2 (SMR = 118; 95% CI = 61 - 206) for Hodgkin's disease; and 22/21.6 (SMR = 102; 95% CI = 64 - 154) for other forms of LHC.

Two of the studies (Divine 1990; Matanoski 1988), permit an evaluation of LHC patterns by duration of employment and time since hire. For subjects with <10 years and with 10+ years of employment, the aggregate data show SMRs of 111 (35/32) and 102 (45/44), respectively. For subjects with <10 years and with 10+ years since hire, the SMRs are 83 (7 observed/8 expected LHC deaths) and 108 (73/67), respectively. Again, these data

support the absence of a causal relation between butadiene industry employment and LHC.

The IISRP SBR worker research program is by far the largest and most significant for the assessment of human risk from butadiene exposure (Matanoski 1988, Matanoski 1990). This study included 12,110 workers at eight SBR plants followed over a 40-year period during which exposures were higher than current occupational exposures.

Lymphopoietic cancer mortality for this large cohort was lower than or consistent with general population rates. Specific findings were: all lymphopoietic cancers (55 observed (obs), 56.7 expected (exp), standardized mortality ratio (SMR) = 97); lymphosarcoma (7 obs, 11.5 exp, SMR = 61); and leukemia (22 obs, 22.8 exp, SMR = 96). Analyses by job category also showed low mortality rates. Importantly, leukemia mortality was not elevated for the two job category subgroups with highest potential for butadiene exposure -- production workers (7 obs, 6.4 exp, SMR = 111) and mechanical workers (6 obs, 8.6 exp, SMR = 70). The overwhelming evidence from this study does not show an excess of leukemia or any other cause of death associated with butadiene exposure or employment in the SBR industry.

The IISRP program also sponsored further research into the relationship between butadiene and lymphopoietic cancers through a nested case control study (Matanoski 1989). The findings and conclusions for leukemia from the case control study were markedly inconsistent with the low leukemia mortality rates found in the cohort study of the same population. That is, despite the lack of a leukemia excess for the large SBR cohort, the case control study found a significantly elevated odds ratio (OR) of 7.6 for butadiene and leukemia. This discrepancy seems to reflect, in part, a deficit of leukemia mortality among unexposed workers and more normal mortality rates among exposed workers. In addition, reanalysis of the case control data shows that the OR of 7.6 was based on a very selective analysis, reflected by the marked decrease in the butadiene-leukemia OR which results from minor changes in exposure categorization (i.e., from 7.6 to 0.9). Until the discrepancies between the SBR worker cohort and case control results are resolved, the OR estimates from the case control study should not be interpreted at face value. Rather, emphasis should be placed on finding an interpretation for the case control results that is consistent with the lack of a leukemia excess for the SBR worker cohort overall.

In conclusion, the epidemiologic evidence relating to employment in the butadiene industries demonstrates that such

employment does not increase the risk of any cause of death including LHC. The studies show collectively that overall cancer mortality is lower than that of the general population.

To assist your review, we are enclosing specific comments on the CARB draft and a set of some of the references noted in this letter. We appreciate the opportunity to comment on this draft and to supply additional materials for the Board's evaluation of butadiene epidemiology. If we can be of further assistance, please call Dr. John Acquavella, Chairman of IISRP's Epidemiology Committee, at (314) 694-8813.

Sincerely,

William E. Tessmer/ed

William E. Tessmer
Managing Director

Enclosures

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COMMENTS OF THE
INTERNATIONAL INSTITUTE OF
SYNTHETIC RUBBER PRODUCERS
ON THE CALIFORNIA AIR RESOURCES BOARD
PRELIMINARY DRAFT "PROPOSED IDENTIFICATION OF
OF 1,3-BUTADIENE AS A TOXIC AIR CONTAMINANT"

MARCH 21, 1991

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General Comments

As discussed in our cover letter the California Air Resources Board (CARB) review of the butadiene (BD) epidemiology studies does not cover a number of recent publications on the topic, including five articles published as a part of the proceedings of the International Symposium on 1,3-Butadiene in the June 1990 Environmental Health Perspectives (enclosed). CARB should also view the extensive materials submitted to the Occupational Safety and Health Administration (OSHA) docket for the 1,3-Butadiene rulemaking. We enclose testimonies submitted to OSHA by Drs. Acquavella and Cole on behalf of the International Institute of Synthetic Rubber Producers.

Four general issues seemed apparent in reading the CARB review.

First, mention is made of studies of tire manufacturing populations. These studies are essentially irrelevant for the assessment of BD, as BD is not liberated during tire manufacturing and is not a solvent used in those plants. At least one of these studies had a small subcohort of workers employed in the "synthetic plant" where styrene butadiene rubber (SBR) was made at times (McMichael 1976). However, even for these workers, it is unclear whether there was butadiene exposure. There was, however, opportunity for exposure to numerous potential confounding factors (e.g., benzene and other solvent exposure, other elastomeric ingredients). Finally, in the McMichael study, although the original publication reported elevated lymphatic leukemia rates among workers in the synthetic

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plants (McMichael 1976), further studies by this research group attributed these findings to solvent exposure, not butadiene (See Checkoway 1984, enclosed). As Matanoski (1990) stated (referring to Checkoway 1984): "In a subsequent study, these investigators associated the leukemia risk with solvent exposure only and did not mention a relationship to the SBR department."

Second, CARB makes reference to a variety of potential health effects from BD exposure including: respiratory disease, cardiovascular disease, and various non-lymphopoietic cancers. Yet, there is general agreement that the only issue of concern relates to lymphopoietic cancers. Mortality rates for other causes of death are low for BD exposed subgroups in studies of workers in SBR and BD monomer production across studies. On this point, there has been universal agreement, both at the BD symposium and at the OSHA hearings.

Third, the elevated SMRs for lymphopoietic cancers in SBR and BD production involve short-term workers and not longer term workers employed during the same time periods. This has been termed the "Paradox of Butadiene Epidemiology" in an article by Dr. Acquavella (Acquavella 1989, enclosed). To many scientists, this suggests no risk from BD exposure, but rather the role of confounding factors among short-term workers. Other scientists have placed greater emphasis on the findings among short-term workers, with the rationale that short-term workers may have higher exposures than long-term workers. This seems illogical since all long-term workers were, at one time, short-

term workers. Therefore, long term workers have not only the exposures characteristic of short-term workers, but additional exposure attributed to continued employment in the industry. Relevant to this issue is a recent National Cancer Institute study (Stewart 1990) which addressed the types of jobs in the formaldehyde industry held by short-term versus long-term workers and concluded: "The results from the comparison of job titles suggest that workers who worked less than a year in these plants did not, in general, hold different jobs than those who went on to work for longer periods of time and the jobs did not have higher exposure levels." Hence, the highest exposure groups in BD studies are almost certainly the longer term workers, and it is most logical to place greatest emphasis in evaluating the evidence for BD on the lack of elevated mortality rates for long-term workers. Thus, the available relevant data would not support the position that butadiene is a human carcinogen.

Fourth, several of the referenced studies of tire manufacturing or other non-SBR workers are incorrectly characterized as studies of SBR workers. We will note these as a part of our comments on specific issues contained in the draft text.

Specific Comments

P. 3-37: Reference is made to the McMichael (1976) study as a study of workers at a single SBR plant. This is incorrect. As McMichael clearly states in the first paragraph of

this article, this is a study of workers at a large tire manufacturing plant.

P. 3-38: In discussing the McMichael study, confidence intervals (CI) are given as 99.9% CIs. The footnote to Table 5 (p. 183) of McMichael's article points out that the method of CI calculation is atypical and suggests more precision than is warranted for the SMRs. For example, the SMR of 620 is based on four cases and, if calculated in the standard way (see Rothman and Boice 1979), a 95% confidence interval would be 166-1580. The 99% CI would be much broader (99-1939) and likewise the 99.9% CI (79-2096). Hence the CIs given for this study are misleading. It would be clearer, at least for this study, to give the observed numbers so that readers can make their own judgments about the precision of McMichael's estimates.

P. 3-39: Mention is made of the Ott study (Ott 1980) as a study of 2904 workers at Dow Chemical SBR plants. Again, this is an incorrect characterization of the employment of these workers. As the title indicates, this was a styrene-based products study, not a study of SBR workers. Only 391 of the 2,904 workers were employed in styrene butadiene latex, while the remainder were employed in polystyrene and benzene alkylation related units. CARB's review of this study and its implications for BD are misleading because it neglects to mention Ott's finding of no leukemias among the styrene butadiene latex workers. Quoting from page 459:

"No cases of leukemia were identified from the Dow styrene-butadiene (latex) production plants." (Ott 1980)

Hence, the SMR for leukemia was 0 and the four leukemias mentioned by CARB were unrelated to the latex operations.

P. 3.41: The document mentions the hematological effects study by Checkoway and the conclusion that the results were suggestive of possible biological effects of unknown clinical consequence. Several points should be mentioned in CARB's review of this study. First, Checkoway also concluded there was no significant difference between the two groups (i.e., tank farm versus other) in this study and that the values for the tank farm workers were within the normal range. This is important information for evaluating possible hematological effects of BD. It is also noteworthy that several variables which affect hematological parameters were not controlled in this study including: smoking, body size, fasting, alcohol, and exercise (see Rodger, et al. 1987). Differences in any one of these factors could produce the sub-clinical differences in this study. It is also apparent that the tank farm group consisted of only 8 workers versus a comparison group of 145 workers. In the small tank farm group, one person's values could have created a slight difference in the group's mean values.

Evaluation of hematological effects might also be extended to consider the available data on SBR and BD monomer worker mortality from non-malignant diseases of the blood and blood forming organs. For the largest SBR workers study, the

findings for non-malignant diseases of the blood and blood forming organs show fewer deaths observed than expected both for the total cohort (5 obs, 7.2 exp, SMR = 70) and for mechanical and process workers (2 obs, 4.1 exp, SMR = 48) (Matanoski 1988). Thus, the evidence from this study would not suggest an excess of non-malignant diseases of the blood or blood forming organs associated with employment in the SBR industry.

P. 3.44: In discussing the Downs study, CARB concludes that the authors found elevated SMRs for the lymphopoietic system regardless of how the cohort was subdivided. Yet, this conclusion, indirectly attributed to Downs, conflicts with his own conclusion (p. 325), namely:

> The patterns in Table IX for latency and duration of employment are contrary to expectation. If a carcinogen were active in this environment, one would expect the SMRs to show a positive relationship to latency, and one would also expect the same pattern with increasing duration of employment. Neither pattern was present for either all lymphohematopoietic cancer or lymphosarcoma.

P. 3-45: The conclusion of the epidemiology review - that there is strong epidemiologic evidence for a BD-lymphopoietic cancer association -- conflicts markedly with other reviews on the topic (Acquavella 1989, Ott 1990, Cole 1990, Acquavella 1990, all enclosed). This conclusion is inconsistent with the known lack of elevated mortality for long-term workers in butadiene related industries -- a situation unprecedented for an occupational carcinogen.

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Direct Testimony re Butadiene Epidemiology
OSHA hearings on 1,3-Butadiene
Dr John F. Acquavella

On behalf of the International Institute
of Synthetic Rubber Producers

November 5, 1990

My name is John Acquavella. I am currently Senior Epidemiology Consultant for the Monsanto Company in St Louis, Missouri and am testifying today on behalf of the International Institute of Synthetic Rubber Producers (IISRP). Until recently, I was the Epidemiology Group Head for Exxon Biomedical Sciences in New Jersey, where I worked for 6 years. Previously, I worked in epidemiology scientific and management positions for the U.S. Environmental Protection Agency (3 years) and the University of California at the Department of Energy funded Los Alamos National Laboratory (3 years).

I received my masters and doctorate degrees in epidemiology from the State University of New York at Buffalo. During my doctoral studies, I was awarded the Lilienfeld prize by the Society for Epidemiologic Research - an annual award presented for the outstanding paper based on research done toward the completion of a graduate degree in epidemiology. My entire professional career has been devoted to environmental and occupational epidemiologic research in government and industry.

Since 1985, I have served as Epidemiology Committee chairman for the IISRP. In that capacity, I have coordinated the butadiene (BD) epidemiologic research program funded by the IISRP members and the BD monomer producers worldwide. This program has evaluated mortality rates from 1943-1982 for 12,000+ styrene butadiene rubber (SBR) workers at eight plants in the U.S. and

Canada - by far the largest BD cohort studied to date. During my affiliation with this program, I have visited numerous BD facilities - both monomer and polymer plants - to familiarize myself with the various types of industrial processes, job categories, personnel practices, and specific tasks performed by workers. I have visited facilities included in the NIOSH, Texaco, and IISRP-sponsored epidemiology studies - the major studies reviewed by OSHA in the proposed BD Rule.

I have published numerous articles on occupational cohort and case control studies, and several review articles - including two articles on BD epidemiology. My curriculum vitae and the two articles on BD epidemiology are being submitted with my testimony.

Executive Summary

My testimony will concentrate on the research program for which I provided scientific oversight and reanalysis: the international styrene butadiene rubber (SBR) worker cohort mortality study (Matanoski 1988, Matanoski 1990) sponsored by the International Institute of Synthetic Rubber Producers (IISRP) and the related lymphopoietic cancer nested case control study (Matanoski 1989). In addition, I will point out several factual errors in OSHA's epidemiology review and important data that were neglected (OSHA 1990). These comments will emphasize two points: 1) OSHA's conclusions regarding BD epidemiology resulted from selective interpretation of the available data; and 2) the available epidemiology studies do not suggest a dose response relationship between BD exposure and lymphopoietic cancer. In these areas, I will show that the weight-of-evidence indicates that BD is not an occupational carcinogen.

The IISRP SBR worker research program is by far the largest and most significant for the assessment of human risk from BD exposure (Matanoski 1988, Matanoski 1990). This study included 12,110 workers at eight SBR plants followed over a 40 year period. The very low mortality rates found for these workers have largely been ignored in OSHA's discussion of BD epidemiology.

Lymphopoietic cancer mortality for this large cohort was lower than or consistent with general population rates. Specific

findings were: all lymphopoietic cancers (55 observed (obs), 56.7 expected (exp), standardized mortality ratio (SMR) = 97), lymphosarcoma (7 obs, 11.5 exp, SMR = 61), and leukemia (22 obs, 22.8 exp, SMR = 96). Analyses by job category also showed low mortality rates. Importantly, leukemia mortality was not elevated for the two job category subgroups with highest potential for BD exposure -- production workers (7 obs, 6.4 exp, SMR = 111) and mechanical workers (6 obs, 8.6 exp, SMR = 70). As such, the overwhelming evidence from this study would not suggest an excess of leukemia or any other cause of death associated with BD exposure or employment in the SBR industry.

The IISRP program sponsored further research into the relationship between BD and lymphopoietic cancers - a nested case control study (Matanoski 1989). The findings and conclusions for leukemia from the case control study were markedly inconsistent with the low leukemia mortality rates found in the cohort study of the same population (as discussed above). That is, despite the lack of a leukemia excess for this cohort, the case control study found a significantly elevated odds ratio (OR) of 7.6 for BD and leukemia. This discrepancy seems to reflect, in part, a deficit of leukemia mortality among unexposed workers and more normal mortality rates among exposed workers (Cole 1990). In addition, my reanalysis of the case control data shows that the OR of 7.6 was based on a very selective analysis of the data. This is reflected by the marked decrease in the BD-leukemia OR which

results from minor changes in exposure categorization (i.e. from 7.6 to 0.9) and by the irregular pattern of ORs by exposure level. This important information was not presented or discussed in the final report of this study, but undoubtedly contributes to the discrepancy between the case control and cohort findings.

Several problems were also illustrated in the design, execution, and analysis of this study, each of which would have tended to exert a positive bias on the OR for BD and leukemia. Therefore, until these issues and the discrepancies between the SBR worker cohort and case control results are resolved, the OR estimates from the case control study should not be interpreted at face value. Rather, emphasis should be placed on finding an interpretation for the case control results that is consistent with the lack of a leukemia excess for the SBR worker cohort overall.

OSHA's review of the BD epidemiology literature appears to have selectively cited data from individual studies, creating the appearance of a relationship between BD exposure and lymphopoietic cancer. This resulted primarily from focusing on findings for one of many subgroups in specific studies and by omitting conflicting data for other subgroups (often workers doing the same or higher exposure jobs). OSHA also did not consider the possible effect of confounding factors in evaluating these studies, especially in the case of lymphopoietic cancer

findings among short-term, but not long-term, workers. These omissions precluded a scientifically balanced evaluation of the available epidemiologic evidence for BD. The consistent theme across all the BD epidemiology studies - that lymphopoietic cancer mortality rates were not elevated among long-term workers (Acquavella 1990) - was not addressed by OSHA in their epidemiology review.

OSHA developed an analysis of lymphopoietic cancer mortality by exposure level for two of the BD cohorts and concluded that there was a dose response relationship. This conclusion resulted primarily from errors in classifying workers by exposure level and by neglecting duration of employment in the exposure level analyses. I present analyses to illustrate these points. These analyses show that lymphopoietic cancer mortality was not elevated for workers with the highest BD exposures and, in fact, show a pattern of lower SMRs in higher exposed workgroups. These data are consistent with the viewpoint that BD exposure was not related to lymphopoietic cancer mortality in these studies.

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Introduction

My testimony will concentrate on the research program for which I provided scientific oversight and reanalysis: the international styrene butadiene rubber (SBR) worker cohort mortality study (Matanoski 1988, Matanoski 1990) sponsored by the International Institute of Synthetic Rubber Producers (IISRP) and the related lymphopoietic cancer nested case control study (Matanoski 1989). In addition, I will point out several factual errors in OSHA's epidemiology review and important data that were neglected (OSHA 1990). These comments will emphasize two points: 1) the available epidemiology studies do not suggest a dose response relationship between butadiene (hereafter BD) exposure and lymphopoietic cancer; and 2) OSHA's conclusions regarding BD epidemiology resulted from selective interpretation of the available data. In these areas, I will show that the weight-of-evidence indicates that BD is not an occupational carcinogen.

I. IISRP SBR worker cohort mortality study

The IISRP SBR worker cohort study included 12,110 workers who were employed at least one year at one of eight SBR facilities located in the U.S. and Canada (Matanoski 1988, Matanoski 1990). The time period covered in this update of an earlier study (Matanoski 1987) was from industry startup in 1943 through 1982. Mortality rates for SBR workers were compared with mortality rates for the U.S. general population.

A. Evidence of overall low mortality rates

The results of this study showed very low mortality rates for SBR workers. Mortality was less than expected for almost every cause of death, including: total mortality, cancer mortality, lung cancer, lymphopoietic cancers, leukemia, and lymphosarcoma

Table 1. Results of IISRP SBR worker study (Matanoski 1988)

cause of death	obs	exp	SMR	95% CI
all causes	2441	3000.8	81	78-85
all cancers	514	606.6	85	78-92
lung cancer	166	198.4	84	71-97
lymphopoietic cancer	55	56.7	97	73-126
lymphosarcoma	7	11.5	61	24-126
leukemia	22	22.8	96	60-146

B. Evidence of low mortality rates by job category

Analyses of mortality rates for the four job category subgroups (i.e., production, maintenance, utilities, and other) showed a similar pattern of low mortality rates for each subgroup. Moreover, as my subsequent analysis will demonstrate, the SMRs by job category should have been substantially lower than reported in this study due to a type of selection bias in the analysis: selective omission of workers with very low mortality rates (Acquavella 1989).

Of most interest among the job category mortality analyses were the findings for production and maintenance workers, the two subgroups with the highest exposures to BD. As reported by Matanoski (Matanoski 1988, Matanoski 1990), production workers showed significantly low total mortality (594 obs, 676.6 exp, SMR = 88, 95% CI 81-95) and cancer mortality was not elevated (124 obs, 135.4 exp, SMR = 92, 95% CI 76-109). Focusing on specific lymphopoietic cancers, production workers (blacks and whites combined) had 7 observed leukemias versus 5.3 expected (SMR = 134, 95% CI 53-277), a non significant elevation. One lymphosarcoma was observed versus 2.6 expected (SMR = 38, 95% CI 1-213).

Analyses for mechanical workers showed a similar significant deficit for total mortality (937 obs, 1036.8 exp, SMR = 90, 95% CI 85-96 - note this 95% CI was reported incorrectly as 85-106 in Matanoski 1990) and cancer mortality rates that were not elevated (198 obs, 207.4 exp, SMR = 95, 95% CI 83-110). There were somewhat fewer leukemia deaths than expected (6 obs versus 7.1 exp, SMR = 85, 95% CI 31-184), along with fewer lymphosarcomas (2 obs, 3.7 exp, SMR = 54, 95% CI 6-195), although these findings were not statistically significant.

C. Selection bias in the job category analyses

According to my re-analysis, the SMRs by job category would have been substantially lower had the investigators not omitted

2391 workers (approximately 20% of the cohort) due to partially incomplete work histories (Acquavella 1989). Approximately 75% of these omitted workers were active employees at the time of the study at one SBR plant. Matanoski acknowledged the potential bias stating:

> "The loss of person-years from the low risk group of recent, healthy active workers may have artificially inflated the SMRs related to work area" (Matanoski 1990).

Unfortunately, the mortality experience of this large omitted subgroup was not presented in the final report to help evaluate the amount of bias in the job category SMR analysis. This may be because the author, as quoted above, considered these workers to be primarily short-term, recently hired, employees who would not have contributed substantial numbers of expected deaths to the mortality analysis. However, active SBR plant workforces are actually heavily weighted with long-term employees. These workers would have contributed substantial numbers of expected deaths to the job category analysis had they not been omitted.

My calculations demonstrate that SMRs for these excluded workers showed strikingly low mortality rates: total mortality was only 38% of expected (161 obs, 422.1 exp), cancer mortality was 46% of expected (42 obs, 90.5 exp), lung cancer was 22% of expected (7 obs, 32.0 exp), lymphopoietic cancer was 57% of

expected (5 obs, 8.8 exp), and there were no leukemias (0 obs, 4.5 exp). Had these workers been included in the job category analysis, as they should have been, the reported SMRs would have been substantially lower. Thus, for example, the somewhat higher SMRs for total mortality and cancer mortality among mechanical workers compared with values for the total cohort (SMRs = 92 versus 81, 95 versus 85, respectively) give an estimate of the bias for major causes of death due to these exclusions.

Moreover, the bias from these exclusions was much greater for leukemia, since there were no deaths from this cause versus 4.5 expected among the omitted workers. Fortunately, this bias can be easily "corrected" by estimating the additional expected numbers of leukemias by job category and re-calculating the SMRs using a "corrected" expected number of deaths. For example, production workers were reported to have 7 observed versus 5.2 expected leukemias (SMR = 134). However, since production workers contributed approximately 25 percent of the expected deaths for the cohort, the expected number of leukemia deaths (i.e., 5.2) should be increased by approximately 1.1 (viz., 25% of the 4.5 expected leukemias). This yields a "corrected" SMR of 111 (i.e., 7 obs, 6.3 exp, 95% CI 45-229) - a more accurate portrayal of leukemia mortality for production workers than the SMR of 134 reported from this study. This "corrected" SMR indicates that the leukemia rate for production workers was essentially identical to the general population rate.

Likewise, for mechanical workers, the reported leukemia SMR of 85 (6 obs, 7.1 exp) is biased. Since these workers contributed 34% of the expected deaths for the cohort, the "corrected" expected number of deaths should be increased by 1.5 to yield an SMR of 70 (6 obs, 8.6 exp, 95% CI 25-152). This indicates leukemia mortality was 30% less for these workers than for the general population.

Thus, leukemia mortality for the two subgroups of SBR workers with the highest BD exposures was only 87% (a combined 13 obs, 14.9 exp, 95% CI 46%-149%) of general population rates. This provides convincing evidence that leukemia mortality is not elevated among the two workgroups with the highest potential BD exposures.

Unfortunately, it was not possible to correct the SMRs for other causes of death by job category, which would be especially useful for the all and other lymphopoietic cancer categories. The reason is that job category is unknown for all workers omitted from the job category analysis and this information is necessary to apportion observed deaths by job category in a "corrected" SMR analysis. However, it is clear that the SMRs by job category should be considered to be overestimated and that this issue must be considered when comparing the results for these workers and other BD exposed populations.

In sum, mortality rates for this large cohort of BD exposed workers are very low overall and for specific exposure subgroups. As such, the overwhelming evidence from this large cohort study would not suggest an excess of leukemia or any other cause of death associated with BD exposure or employment in the SBR industry.

II. IISRP lymphopoietic cancer case control study

Dr Cole presented testimony on the study design, results, and conclusions of the SBR worker lymphopoietic cancer nested case control study (see Cole 1990 re Matanoski 1989). In this review, he characterized the conclusions of this study - that leukemia rates were seven to nine fold higher for exposed versus unexposed workers - as strikingly inconsistent with the findings of no excess leukemia mortality in the cohort study of the same population (i.e., 22 obs versus 22.8 exp deaths, SMR = 96). He offered an explanation for these seemingly inconsistent findings and presented a supporting analysis: specifically, that unexposed workers had lower leukemia rates than the general population, while exposed workers had rates more similar to general population rates. Therefore, since nested case control studies estimate the ratio of rates for exposed versus unexposed workers, the case control findings reflected primarily a deficit of leukemia among unexposed workers, not a large excess among the workers exposed to BD. This explanation would resolve much of the

conflict between the results of the cohort and case control study - the unifying conclusion being that leukemia rates were not significantly elevated among BD exposed workers.

There are also some methodologic concerns regarding the design, conduct, and analysis of the case control study that need to be considered -- each of which would suggest that the odds ratio (OR) for BD exposure was either unreliable or biased. These include:

- selective analysis of the BD exposure data,
- inclusion of ineligible controls, and
- differential treatment of control exposure histories.

These concerns came to light during my reanalysis of the case control data, which were provided to me by the study's author.

A. Selective analysis of the BD exposure data

The most important methodologic problem with this study was the selective analysis of the BD exposure data. This selective analysis masked striking internal inconsistencies in the OR estimates related to BD exposure.

Most of the analyses presented in the study report (Matanoski 1989) categorized workers as either exposed or unexposed based on the mean of the logarithms of the BD

exposures. These analyses formed the basis for the conclusions cited from this study. The report did not mention that an analysis based on the actual, non-transformed, BD data, instead of the logarithms of the data, would have resulted in substantially lower ORs - consistent with the conclusion of no association between leukemia and BD exposure. I will illustrate this point shortly. Furthermore, as Dr Cole pointed out, other standard methods of exposure categorization, and even exposure level analyses, result in much lower estimates of the OR than the log BD analysis (Cole 1990).

Logarithmic transformation of data is frequently used with statistical procedures that require a normal distribution of the data. The rationale given in this report for employing a logarithmic transformation was "... due to the skewing of the data." That is, the BD exposure data were not normally distributed, and the investigators attempted to normalize the exposure distribution. The advisability of this transformation was questionable for two reasons. First, textbooks on case control methodology prescribe no such normality requirement for the underlying exposure data used to calculate ORs (Rothman 1986, Schlesselman 1982, Kleinbaum et al. 1982). Second, my analysis found that the logarithmic transformation did <u>not</u> produce a normal distribution of the BD exposure scores. All that was accomplished was a shift in the criterion for dichotomizing cases and controls into exposed or unexposed subgroups. In light of the

questionable justification for the logarithmic transformation, it would have been prudent, at a minimum, also to assess results for the actual, non-transformed, BD exposure data.

The following analyses illustrate the striking difference between ORs calculated based on the logarithms (as presented by the author) versus calculations based on the actual BD exposure values (my calculations). Table 2, an unmatched analysis based on the mean of the logarithms of the BD exposure data, shows a significantly elevated OR of 5.2 (95% confidence interval (CI) 1.5-18.7), suggesting a strong BD-leukemia association:

Table 2. Odds ratio based on the mean of the <u>logarithm</u> of the BD exposure scores

	exposed	not exposed	total	
cases	23	3	26	
				OR = 5.2 (1.5 - 18.7)
controls	50	34	84	

However, the same analysis based on the mean of the actual BD scores, <u>without the logarithmic transformation</u>, gives an OR of 0.8 (95% CI 0.3-2.2), indicating no BD-leukemia association (Table 3):

Table 3. Odds ratio based on the mean of the actual BD exposure scores

	exposed	not exposed	total	
cases	8	18	26	
				OR = 0.8 (0.3 - 2.2)
controls	29	55	84	

Matched case control analyses (which are more appropriate due to the matched design of this study), computed by conditional logistic regression models, show the same pattern of results:

analysis - mean log BD scores OR = 7.6 (1.6 - 35.6)

analysis - mean actual BD scores OR = 0.9 (0.3 - 2.6)

Thus, the latter ORs based on the mean log BD scores suggest that leukemia rates are 7.6 times higher among exposed versus unexposed workers, while a similar analysis based on the actual BD scores suggests leukemia rates are 10% <u>lower</u> among exposed workers. The sole difference in these two types of analyses is the arbitrary choice of an exposure value for dichotomizing workers as unexposed or exposed.

Accordingly, it is unclear why the logarithmic transformation was used in this study and why results based on

the actual, non-transformed, BD scores were not presented. The striking difference illustrated above suggests a large random error component in the reported OR of 7.6 for BD and leukemia and points out the unreliability of conclusions based on that result. As Rothman has pointed out in his text (Rothman 1986, p 135):

> "... the shift of a boundary in categorization rarely has a substantial effect on the magnitude of an estimate and then only because of a large random error component."

In light of this variability, Dr Cole pointed out that an analysis by exposure levels would be more informative and would permit the evaluation of dose response trends (Cole 1990) - a criterion often used to help differentiate causal from spurious associations. He presented such an analysis in his testimony in which he distributed controls evenly by exposure tertiles - an unbiased method of exposure categorization. These analyses revealed an irregular (and hence unlikely) exposure response pattern with a precipitous decline in the OR for the highest exposure category (i.e., ORs 1.0, 5.3 and 2.3 for the lowest, intermediate and highest exposure categories, respectively). Clearly, there was no evidence of a linear exposure response relationship. In fact, the marked decline in ORs from the intermediate to the highest exposure category again indicates a large random error component in this study. It also reinforces the unreliability of the OR estimate of 7.6, upon which the

author's conclusions from this study were based. Other methodologic issues, discussed in the following paragraphs, also contributed to the surprisingly high ORs seen in the case control study.

B. Inclusion of ineligible controls

A second important methodologic concern is the failure to adhere to the study's stated control selection procedures. These were explicitly stated in the final report (Matanoski 1989, page 8, emphasis supplied) as follows:

> "Controls were individually matched to cases on the following criteria: plant, age, hire year, employment as long or longer than the case, and survival to the death of the case."

However, my analysis found 44 (of 84) leukemia controls were not employed as long or longer than the case. After pointing this out in a letter to the investigator, I received the following reply (emphasis supplied for the critical points):

> "Controls who had been terminated could be matched to cases by duration worked within 2-3 years. (As noted, hire year was already matched in these controls.) Controls were also selected from those who worked longer than the case, and, in this situation, the termination date of the case was

assigned to those controls and measurement of exposure ended on that date." (Matanoski 1989b)

It is obvious that this explanation describes a much different control selection criterion than the original explicit language in the study report (viz. employment as long or longer than the case). As such, it suggests that there were changes in or confusion about the control selection criteria during the conduct of this study.

Moreover, even this new explanation was not consistent with the data. Twelve controls had durations of employment which were more than three years different than the respective cases. In addition, there was one control who should not have been in the study at all. The worker had been employed only six months at an SBR facility; he did not meet the one year criterion for inclusion in the base population for this study.

The presence of controls who did not meet the stated study criteria is very surprising and reduces the credibility of this study. This fact was never mentioned in the report of this study, so it is unclear how this happened and why the control selection criteria were not followed. It is also unclear how much the study results were affected by deviating from the planned control selection procedures.

C. Differential treatment of control exposure histories

A final methodologic issue was the different treatment accorded to certain control exposure histories in the estimation of BD exposures. Specifically, I refer to the procedure of truncating exposure histories for controls who worked longer than their respective cases. This procedure has the effect of overestimating the ORs in this study, as illustrated by the following hypothetical example.

In figure 1, I have represented the duration of employment for a case and his two matched controls. Exclamation points mark the dates of last employment and, for illustrative purposes, I have assumed the same date of first employment for the case and the controls. Using the procedure employed in this study, the control who worked a shorter duration than the case has his BD exposure calculated for his entire employment history. But, the control who worked longer than the case has his BD exposure calculation truncated at the case's date of employment termination, as indicated by the parenthesis in his time line.

```
----------------------------!          case

---------------------!                  control shorter

----------------------------)-----------!  control longer
```

Figure 1. Employment time lines for a case and matched controls

For illustrative purposes, assume all cases and controls worked the same job in the same plant location (i.e., had the same daily exposures) and had, on average, the same duration of employment. Then the truncation procedure employed in this study ensures that controls who worked longer than their respective cases can have at most equivalent exposure; controls who worked shorter durations than cases will (by definition) have lower exposure scores. Accordingly, this method of analysis would yield an elevated OR, even when there is no difference between case and control exposures.

Therefore, the procedure of truncating control exposure histories, as applied in the SBR case control study, would overestimate the BD-leukemia OR. The amount of this bias would increase as the difference in length of employment between cases and controls (i.e. the matching interval) increased due to the inclusion of controls with shorter and shorter, but not longer and longer, employment periods for estimating BD exposure.

D. Findings for other lymphopoietic cancers

In contrast to the findings for leukemia, findings for lymphosarcoma (OR = 0.5, 95% CI 0.1-4.2), Hodgkin's disease (OR = 1.1, 95% CI 0.2-5.2), and other lymphatic cancers (OR = 1.5, 95% CI 0.5-4.8) did not show a significant association with estimates of BD exposure. This does not contradict the previous demonstration of positive bias in the OR for BD-leukemia in this

study; rather, it means that the ORs for these other lymphopoietic cancers would have been even lower or unchanged had the exposure comparisons been based on an unbiased method.

In sum, the findings and conclusions of the case control study were markedly inconsistent with the findings of no leukemia excess in the cohort study of the same population. This discrepancy seems to reflect, in part, a deficit of leukemia mortality among unexposed workers and more normal mortality rates among exposed workers (Cole 1990). In addition, the OR estimates from the case control study have a large random error component. Several problems were also illustrated in the design, execution, and analysis of this study, each of which would have tended to exert a positive bias on the OR for BD and leukemia. As such, the case control findings are problematic to interpret and should not be interpreted at face value. Rather, emphasis should be placed on finding an interpretation for the case control results that is consistent with the lack of a leukemia excess for the SBR worker cohort overall.

III. Comments on OSHA's review of BD epidemiology

OSHA's review of the BD epidemiology literature appears to have been very selective, focusing on isolated mortality excesses for one of many subgroups (usually shorter-term workers) in specific studies, while omitting conflicting data for other subgroups and/or the overall study populations. The consistent

theme that I found across all the BD epidemiology studies - that lymphopoietic cancer mortality rates were not elevated among long-term BD exposed workers (Acquavella 1990) - was not factored into OSHA's assessment of the BD epidemiology. The selectivity of OSHA's review precluded a scientifically balanced evaluation of the available epidemiologic evidence for BD. As my testimony will indicate, such an approach would not have suggested an excess of leukemia or any other cause of death associated with employment in BD-related occupations.

A. OSHA's assessment of dose response among BD workers

OSHA has concluded that the BD epidemiologic studies consistently show increasing lymphopoietic cancer mortality with increasing proxy measures for BD exposure as derived by OSHA (called exposure values (EVs)). The basis for this conclusion was an analysis of SMRs by EVs for the BD monomer worker study (Downs 1987, Divine 1990) and the larger SBR worker study (Matanoski 1988, Matanoski 1990). A careful examination of the evidence for this conclusion shows that worker mortality does not increase by BD exposure level. Rather, the Agency's incorrect conclusion resulted, in part, from technical errors in comparing SMRs within these studies and, more so, from inconsistent exposure classification of similar workers across studies.

1. Technical errors in comparing SMRs

OSHA's evaluation of lymphopoietic cancer SMRs by EVs is prone to confounding bias since SMRs are not directly comparable between exposure groups within or across studies. As detailed in epidemiology textbooks (Rothman 1986, Checkoway 1989), comparison of SMRs is only valid when the underlying proportions of person years by stratification variables are identical (or nearly identical). To illustrate this point, Checkoway gives an example (Checkoway 1989, page 127) of two subcohorts with identical age specific death rates and ratios of observed and expected deaths, yet different SMRs (e.g., the SMR values in his example were 211 vs 242). The difference results from the different age distributions (viz. weights for age adjustment) of the two subcohorts.

This example is directly applicable to OSHA's EV analysis, since the different subgroups within the BD monomer and SBR industries are comprised of employees with different levels of seniority, skill, and prior work experience. Hence, it is almost certain that the age distributions of the exposure subgroups will be different. Therefore, any inferences about increased mortality based on patterns of SMRs and EVs may be a function of differing age or calendar year distributions for the respective worker subgroups. A more appropriate analysis would have been to compare directly the lymphopoietic cancer rates for the worker subcohorts

to see whether rates increase with increasing EV. Until this comparison is made, valid conclusions cannot be drawn.

2. Inconsistencies in exposure classification

Apart from the issue of confounding, the EV analysis erred by classifying mechanical workers inconsistently across studies and neglecting an important determinant of BD exposure - duration of employment. As detailed in the following paragraphs, when either of these two factors is considered, there is no concordance of SMRs and estimates of BD exposure.

OSHA's EV analysis classified mechanics into markedly different exposure categories in the BD monomer (Downs 1987) and the SBR worker studies (Matanoski 1988, Matanoski 1990). Mechanics were classified in the monomer study as having the highest exposure rating (ER) (4 on a 4 point scale) and occasional, but regular, frequency (F) of exposure (2 on a 3 point scale) resulting in an EV of 8 (i.e., ER times F). By contrast, mechanics in the SBR study (called maintenance workers) were judged to have the lowest ER (1 on a 4 point scale) and the least frequent exposure (1 on a 3 point scale), resulting in the lowest possible EV of 1.

This obvious inconsistency reflects a clear error in exposure classification for SBR mechanics, since mechanics in both these industries have many similar assignments which can

involve BD exposure (e.g. repair of pipes, pumps, and other equipment which contain BD). Without the unrealistically low exposure classification for SBR mechanics, there would have been no concordance of SMRs and EVs since mechanics had the lowest lymphopoietic cancer SMR (i.e., 75) of the three SBR workgroups.

The Agency's EV analysis also misclassified utilities workers in the SBR worker study (Matanoski 1988, Matanoski 1990). These workers were classified as having the potential for highest exposures among the three SBR workgroups (ER = 4) and non-routine exposure (F = 2), when, in fact, monitoring data demonstrate that the vast majority of these workers (i.e., boiler house operators, water tower operators, refrigeration operators) have negligible BD exposure (i.e., ER and F = 1). The only exception would have been pump house operators - a very small proportion of the utilities subgroup. These pump house operators were incorrectly classified as utilities workers instead of production workers (as detailed in Matanoski 1990). So, the EV analysis classified utility workers, who in fact have very limited exposure, in a higher exposure category than production or mechanical workers. This was critical to the purported correspondence of EVs and SMRs, since utilities workers had the highest lymphopoietic cancer SMR (i.e., 203) of the three SBR job categories.

Thus, if SBR mechanics and utilities workers were classified correctly, the pattern of SMRs by EVs in OSHA's Table 12 (page 32750) would have been:

Table 4. EV's and SMRs for all lymphopoietic cancers
SBR worker study (Matanoski 1990)

Relative EV	job group	SMR
1	utilities	203
2	maintenance	75
3	production	146

reflecting a lack of association between BD exposure and lymphopoietic cancer SMRs. It also bears repeating that the SMRs for all three subgroups overestimate lymphopoietic cancer mortality, as discussed previously (see section 1.C.).

3. Omission of duration employed in the EV analyses

A final significant oversight in OSHA's EV analysis is that it neglected an important determinant of BD exposure - duration of employment. It seems axiomatic that workers employed for longer periods of time in specific job categories have greater exposures than their short-term counterparts. To the extent possible, this should be factored into OSHA's EV analysis. To do otherwise is to dilute the estimation of risk for higher exposed workers (with lower exposed workers) and to omit an important

check on confounding factors among short-term employees - i.e., whether SMRs increase with increasing exposure.

An opposing argument to this viewpoint (frequently posed when overall cohort SMRs are elevated, but rates do not increase by exposure category) is based on the speculation that short-term workers who leave employment had greater exposures than short-term workers who remained in industry (viz. those who became long-term workers). This very point was recently examined by Stewart and co-workers at the National Cancer Institute in a comprehensive study of more than 26,000 workers in the formaldehyde industry. These investigators found that short-term workers held similar jobs in the plant to those held by workers who went on to become long-term employees (Stewart 1990). In the published account of this study, the authors concluded:

> "The results from the comparison of job titles suggest that workers who worked less than a year in these plants did not, in general, hold different jobs than those who went on to work for longer periods of time and the jobs did not have higher exposure levels.
>
> There was also no clear evidence that short-term workers had consistently higher levels of exposure to formaldehyde than long-term workers. In fact, in seven of the nine plants the median exposure level to formaldehyde of

short-term workers was the same or lower than that of long-term workers."

Thus, the often quoted proposition - that shorter term workers (who did not go on to become long-term workers) were preferentially assigned the highest exposure jobs - seems to have no basis in fact and has the effect of incorrectly equating the known higher mortality among transient workers (Gilbert 1982), most often a sociological phenomenon, to a "perception" of higher workplace exposures. Stewart's findings support the importance of including duration of employment in the EV analysis to assess risk accurately and to point out the potential effect of confounding factors (e.g. prior or subsequent exposures) among short-term, lesser exposed, workers.

Only the BD monomer workers study (Downs 1987, Divine 1990) presented SMRs by exposure category and duration of employment. For these workers, by dichotomizing workers at 10 years employment, the EV scale might be reformulated as follows: EV = exposure level x frequency of contact x duration of employment (employed less than 10 years = 1; employed 10+ years = 2). In that case, the EV analysis for the monomer workers (expanding Table 8 of the OSHA summary, page 32746) would show the following:

Table 5. EV's and SMRs for all lymphopoietic cancers

BD monomer workers study (Divine 1990)

Exposure - duration	Relative EV	Obs	SMR
low - less than 10 years	1	3	188
low - 10+ years	2	1	59
non-routine less than 10 years	3	7	167
routine less than 10 years	4	7	259
non-routine 10+ years	5	4	111
routine 10+ years	6	1	56

Clearly, these data show no association between EV and SMRs. Further, there is no indication of excess mortality among the longer-term employees in each exposure category (i.e., low, non-routine, and routine).

In sum, OSHA's conclusion of a consistent dose response relationship for lymphopoietic cancers across epidemiology studies resulted primarily from errors in classifying workers by exposure level and by neglecting to include duration of employment in its exposure level analyses. These omissions and errors created the appearance of a consistent trend of EVs and SMRs in the BD monomer and SBR worker studies. Proper and consistent classification of workers by exposure categories would not have shown a pattern of increasing SMRs with increasing EVs

and, in fact, would have indicated a paradoxical pattern of lower SMRs in higher exposed workgroups.

B. Selective interpretation of the BD epidemiology

Throughout the review of epidemiologic studies, OSHA presented only isolated results that might be interpreted as consistent with a BD-lymphopoietic cancer association. Specific findings that would suggest no relationship between BD and lymphopoietic cancer were omitted. Results from the available epidemiology studies should have been reviewed more completely, conflicting observations should have been detailed, and, at that point, judgments should have been made to resolve conflicting findings. The effect of omitting conflicting observations was to preclude a scientifically balanced weight-of-evidence evaluation of the BD epidemiologic data. The selective interpretation must be remedied to assess adequately human risk (or lack of risk) among BD exposed workers.

1. BD monomer workers study

In discussing the study of BD monomer workers (Downs 1987), OSHA misquoted the author's conclusions and incorrectly concluded that lymphopoietic cancer mortality increased with increasing BD exposure. Specifically, the Agency stated:

> "Nevertheless, it appears as though SMRs for ALL LHC and LSC/RCS increase as routine BD exposure increases. Downs, as

well as other researchers, has drawn inferences from this data." (OSHA 1990, page 32747)

Yet, Downs had come to exactly the opposite conclusion stating:

"The patterns in Table IX for latency and duration of employment are contrary to expectation. If a carcinogen were active in this environment, one would expect the SMRs to show a positive relationship to latency, and one would also expect the same pattern with increasing duration of employment. Neither pattern was present for either all lymphohematopoietic cancer or lymphosarcoma." (Downs 1987)

In fact, OSHA's conclusion about increasing lymphopoietic cancer SMRs with increasing exposure to BD was inconsistent with data published on these workers, as summarized below from the recent mortality update (Divine 1990). Clearly, as Drs Downs and Divine both pointed out in their published articles (Downs 1987, Divine 1990), there is no pattern of increasing lymphopoietic cancer mortality with increasing routine or non-routine BD exposure.

Table 6. Lymphopoietic cancer SMRs for BD monomer employees by exposure group and duration of employment (Divine 1990)

	Routine exposure		Non-routine exposure	
		ALL LHC		
Duration	obs/exp	SMR	obs/exp	SMR
< 10 years	7/2.7	259	7/4.2	167
10-19 years	0/0.5	0	3/1.6	188
20+ years	1/1.3	77	1/1.8	56
		LSC/RCS		
< 10 years	5/0.6	833	1/0.8	125
10-19 years	0/0.1	0	0/0.3	0
20+ years	0/0.2	0	1/0.4	250
		Leukemia		
< 10 years	1/1.2	83	5/1.7	294
10-19 years	0/0.1	0	1/0.7	143
20+ years	0/0.5	0	0/0.8	0

2. NIOSH SBR worker study

In reviewing the NIOSH SBR worker study (Meinhardt 1982), OSHA concentrated on leukemia findings in one of the two identical SBR plants studied (plant A) - where there were 5 observed versus 2.5 expected deaths. Results from the contiguous sister SBR plant (plant B) were excluded - a plant where there was no leukemia excess (1 obs, 0.99 exp).

This selective method of analyzing data was criticized previously by OSHA in its analysis of epidemiologic data in the Final Rule on Benzene (OSHA 1987). In that instance, OSHA stated:

> "... the authors noted that the decision to examine mortality separately for the two locations was not made prior to initial analysis and therefore should not be given undue emphasis. In OSHA's opinion, it seems appropriate to combine data for the two facilities because the operations through which workers were exposed to benzene were virtually identical."

The same rationale should be applied to the analysis of this study because as mentioned in the proposed BD Rule:

> "The synthetic rubber industry did not exist until 1943. At that time the federal government undertook to construct 15 plants all of which had similar design and all of which were committed to the manufacture of styrene BD rubber." (OSHA 1990, page 32747)

Therefore, the analysis of data from the NIOSH SBR worker study is contrary to the Agency's previously stated rationale for the analysis of multi-plant studies used in the Benzene Rule (OSHA 1987). The basis for this rationale is sound. That is, when plants are indeed identical and a study was designed as a multi-

plant study, it is clearly misleading to pick and choose findings from an individual plant. A combined analysis of the leukemia data for the two SBR plants would have been more appropriate and would have shown 6 observed versus 3.5 expected (SMR = 171, 95% CI 63-373), a non significant elevation.

It has been pointed out that the slight elevation for leukemia in this study could be attributed to three cases that occurred among short-term workers with very short latent periods (the three cases worked 7, 10, and 18 months with 3, 4, and 3 year latencies, respectively) (Acquavella 1989). Accordingly, the findings among short-term workers were unlikely to have been due to their limited experience in SBR manufacturing and were more likely the result of previous (confounding) exposures or personal factors. This point is supported by the lack of excess leukemias with extremely short latency in another, much larger, study of SBR workers in essentially identical work environments (Matanoski 1990). If short latency leukemias were a characteristic of BD exposure or SBR employment, they should be apparent in other similar studies. Moreover, OSHA did not address the fact that leukemia mortality was not elevated among long-term workers in this cohort - a finding most scientists consider necessary to establish a chemical as an occupational carcinogen (Doll 1985).

In other comments on this study, OSHA implied that leukemia SMRs were underestimated because several leukemias did not meet

the criteria for inclusion in the mortality analysis. Specifically, the Agency stated:

> "In plant A, five deaths from leukemia were included in the mortality analysis. Six other workers with leukemia were excluded from analysis because they did not fit the cohort definition."
>
> "For cohort B ... Three other individuals employed in the plant and diagnosed with leukemia were excluded from the analyses because they did not meet the cohort definition." (OSHA 1990, page 32747)

Meinhardt had detailed the reasons for these exclusions as: working less than the six month employment criterion, being alive at the end of the mortality followup period, and having leukemia as a contributing, but not underlying, cause of death (Meinhardt 1982). Each of these exclusions is consistent with standard epidemiologic practice for the conduct of cohort mortality studies and would not bias the SMRs reported from this study. The reasons for this are threefold. First, mortality studies do not count survivors with specific diseases because disease incidence (or prevalence) is not the focus of the study. Although incidence data would often be a preferred endpoint for analysis, incidence data were not collected for this SBR cohort and comparable

incidence statistics would not have been available for the general population.

Second, the number of leukemia deaths among workers who were employed less than six months can only be evaluated against the respective underlying population and the related number of expected deaths. This information was not available. But, even if it were available, it would have less relevance (if any) than findings among SBR workers with longer employment - i.e.. those who were included in the study.

Third, the fact that leukemia was a contributing cause of death for two workers would only be meaningful if death certificate coding practices used to establish the underlying cause of death were somehow different for SBR workers and the general population. Otherwise, mortality rates would have been comparable for workers and the general population. The fact that leukemia was a contributing cause of death for two workers (or, for that matter, for thousands of decedents in the general population) is irrelevant.

In sum, the Agency's mention of the number of excluded leukemias among SBR workers sheds no light on the findings from this study; it may only serve to bias the uneducated reader.

3. IISRP SBR worker study

As discussed previously, the IISRP sponsored study of SBR workers is by far the largest assessment of mortality patterns among BD exposed workers. As such, the SMRs for these workers are considerably more precise than are those from the other studies and should have received more emphasis in OSHA's overall assessment of BD epidemiology.

OSHA cited EPA's criticisms of this study as having insufficient latency and low power. These criticisms are incredible since the study included 12,110 workers with mortality followup over a 40 year period - an extremely large study by any measure.

To put the size of this study into perspective, the 3001 expected deaths were more than triple the number from the BD monomer study (963) (Divine 1990) and approximately seven times the number reported in the NIOSH SBR worker study (430) (Meinhardt 1982). On average, there were 20.3 and 17.5 years of followup per person among white and black workers, respectively. Also, 71% of the expected deaths occurred among subjects having at least 15 years since they were hired (i.e., latency), and 54% occurred among subjects with at least 20 years since their hire date. These observations suggest that for a large proportion of the cohort's experience, a long follow-up period for the induction of chronic diseases, including cancer, was available.

OSHA also misinterpreted three important issues regarding this study: 1) the etiologic significance of isolated findings in one very small subgroup of SBR workers; 2) the probable effect (or lack thereof) of excluding workers who did not meet the study definition (ineligible employees); and 3) the impact of incomplete rosters of workers for the early manufacturing years at four of the eight plants in this study.

a. Etiologic significance of isolated findings

The overall results of this SBR worker study show deficits of mortality from almost every cause of death (see my table 1 on page 2) reflecting the most favorable mortality patterns that I have seen in any occupational study of this scope. Importantly, there was no excess of lymphopoietic cancer, lymphosarcoma, or leukemia. None of these findings could be construed as suggestive of an occupational carcinogen. Yet, none of these data were considered in OSHA's review of this study.

Instead, the Agency concentrated on lymphopoietic cancer findings among black production workers - a small subgroup (3% of the cohort) that was poorly enumerated due to missing racial information in personnel records. SMRs for this subgroup were overestimated to an unknown degree since black decedents could be enumerated completely from information on death certificates, but the total black workforce was underestimated because racial

information was often missing from personnel records. Workers were assumed to be white when racial information was not available.

Mortality from all lymphopoietic cancers was reported to be significantly elevated for black production workers (6 obs, 1.2 exp, SMR = 507, 95% CI 183-1088), but the lack of a mortality excess for white production workers (13 obs, 11.9 exp, SMR = 110, 95% CI 58-187) was not discussed. Additional analysis shows that four of the six black production worker lymphopoietic cancer cases had very short durations of employment and very long latencies, respectively: lymphosarcoma 1.3 and 18 years, other lymphatic cancer 1.3 and 21 years, leukemia 2.0 and 32 years, and leukemia 3.8 and 30 years. Thus, the reported results were largely influenced by findings among short-term workers, but are not analogous to the short-term/short latency findings from the NIOSH SBR worker study (Meinhardt 1982).

OSHA also reported analyses of the lymphopoietic cancer subcategories and emphasized the significantly elevated leukemia SMR for black production workers (SMR = 656, 95% CI 135-1906). However, again, the Agency did not mention the lack of a leukemia excess for the much larger subgroup of white production workers (SMR = 84, 95% CI 22-215), or for production workers overall (SMR = 134, 95% CI 53-277 - corrected SMR = 111, 95% CI 45-229 as discussed previously) (see table below). Thus, the overall

picture for production workers is that leukemia mortality was essentially identical to the general population rate. OSHA's selective reporting of results for black production workers obscured this important finding.

Table 7. SMRs for production SBR workers (Matanoski 1990)

	whites			blacks			total		
cause of death	obs	exp	SMR	obs	exp	SMR	obs	exp	SMR
all causes	502	577.7	87	92	98.9	93	594	676.6	88
all cancers	105	118.9	88	19	16.5	115	124	135.4	92
all LHC	13	11.9	110	6	1.2	507	19	13.1	146
lymphosarcoma	0	2.4	0	1	0.2	532	1	2.6	39
leukemia	4	4.8	84	3	0.5	656	7	5.3	134
corrected leukemia							7	6.3	111
other lymphatic	7	3.1	230	2	0.4	482	9	3.5	260

Production workers of both races were also reported to have had elevated SMRs for the category of other lymphatic cancers (see Table 7) - a "catch-all" category which includes unspecified non-Hodgkin's lymphoma, multiple myeloma, and polycythemia vera. However, this finding cannot be taken at face value since lymphosarcomas were less frequent than expected and the "other lymphatic cancer" category frequently includes misclassified lymphosarcomas (Percy 1982). A more appropriate analysis would have computed separate SMRs for non-Hodgkin's lymphoma (including lympho/reticulo sarcomas, and unspecified non-Hodgkin's

lymphomas) and multiple myeloma. This has been pointed out previously (Acquavella 1990).

SMRs for non-Hodgkin's lymphomas could have been derived from the data already available for production workers (Matanoski 1990). Matanoski specified that four of the nine "other lymphatic cancer" deaths were non-Hodgkin's lymphomas and five were multiple myelomas. The expected number for non-Hodgkin's lymphomas could have been estimated by combining the expected number for lympho/reticulo sarcomas (2.6) and 50% of the expected number for the other lymphatic cancer category (1.7). Thus, for non-Hodgkin's lymphomas, production workers show 4 observed versus an estimated 4.3 expected (SMR = 93, 95% CI 25-238). This analysis demonstrates that non-Hodgkin's lymphoma mortality was not elevated for production workers and dispels any analogy with the lymphosarcoma findings from the BD monomer workers study (Downs 1987, Divine 1990).

Apart from the results among production workers, findings for maintenance workers - the largest exposed subcohort in this study (approximately 50% larger than the production worker subgroup) - showed very low mortality rates (see table below). Yet, these findings were not mentioned by OSHA. It bears noting that SMRs were especially low for the lymphopoietic cancers, specifically: all LHC SMR = 75, lymphosarcoma SMR = 54, leukemia (corrected) SMR = 70, and other lymphatic cancer SMR = 39. In

contrast to the findings among black production workers, black maintenance workers had no lymphopoietic cancer deaths (viz. SMRs = 0 for lymphosarcoma, leukemia, and other lymphatic cancer). Since mechanical workers comprise a large subgroup of SBR workers who have had exposure to BD, their low lymphopoietic cancer SMRs need to be factored into OSHA's interpretation of this study.

Table 8. SMRs for mechanical workers IISRP SBR worker study

	whites			blacks			total		
cause of death	obs	exp	SMR	obs	exp	SMR	obs	exp	SMR
all causes	749	859.9	87	186	176.9	105	935	1036.8	90
all cancers	173	176.6	98	23	30.8	75	196	207.4	95
all LHC	14	16.5	85	0	2.1	0	14	18.6	75
lymphosarcoma	2	3.4	59	0	0.3	0	2	3.7	54
leukemia	6	6.4	93	0	0.7	0	6	7.1	85
corrected leukemia							6	8.6	70
other lymphatic	2	4.4	46	0	0.7	0	2	5.1	39

In sum, a thorough assessment of mortality rates among production <u>and</u> mechanical workers of <u>both</u> races shows no consistent elevation of lymphopoietic cancer mortality. The findings among black production workers seem an isolated phenomenon among primarily short-term workers - a phenomenon that is not supported by the analyses for white production workers or other subgroups in this study with equal or greater potential for BD exposure.

b. Misstatements re excluding employees

The Agency's only reference to the low mortality rates found for this SBR worker cohort was to imply that the results should be questioned since a large number of workers at these plants were excluded from the study population. These exclusions were incorrectly attributed to incomplete records, specifically:

> "In these plants, more than half of the original worker population was excluded from analyses due to incomplete records." (OSHA 1990, page 32749)

This statement is not correct. Matanoski clearly stated that 58.2% of the exclusions were due to working less than the one year cohort eligibility criterion and 27.3% were due to excluding females from the study population (Matanoski 1987, Matanoski 1990). Less common exclusions were for termination before record keeping (6.9%), being hired after 1976 (5.0%), and missing birth year on employment records (2.5%). Thus, almost all of the exclusions were due to not meeting the criteria for the study population and, as such, the exclusions should not create any bias in this study.

c. Impact of incomplete rosters at four plants

OSHA also expressed concern that SMRs were underestimated because eligible cohort members could not be enumerated from

startup at four of the eight plants in this study. Specifically, the Agency stated:

> "OSHA believes that although the effect of the exclusion of 'early workers' is not known, it is reasonable to assume that the loss of their data reduces the chance of identifying relationships between BD and disease." (OSHA 1990, page 32749)

Implicit in this statement is the belief that short-term "early workers" received higher exposures than longer term "early workers." This belief was discussed in detail previously and shown to be inconsistent with comprehensive studies of this issue (see section III.A.3.). OSHA's supporting reference for this position was not an comprehensive peer reviewed publication on this topic, but a letter to the editor of Lancet (Infante 1986) advancing this opinion as a criticism of Dr Acheson's analysis of the British formaldehyde workers study (Acheson 1984). In response to this letter, Gardner and Acheson pointed out that the author was wrong in his contention, since 82% (2802) of the workers in this study had only one job and 11% (387) had only two jobs (Gardner 1986). Of those who changed jobs, Gardner showed that most (288) went to jobs of the same exposure potential with the remainder going to higher (110) or lower (188) exposure jobs. As Gardner concluded:

"There is therefore little scope for or evidence of the postulated move to lower exposures." (Gardner 1986)

Supportive of Gardner's argument, as mentioned previously, was the National Cancer Institute's extensive comparison of jobs and exposures among 26,000 long and short-term workers in the U.S. formaldehyde industry (Stewart 1990). These investigators found that short-term workers did not hold jobs with higher exposures than long-term workers.

Nevertheless, the Agency's belief, that exclusion of early workers represented a potential bias, can be evaluated directly by reviewing the SMRs for the four plants in this study where workers were enumerated from the beginning of plant operations. SMRs for these plants were contained in the final report of this study (Matanoski 1988) and in an IISRP submission to EPA in 1986 (IISRP 1986). These data (Table 9 below) show a pattern of

Table 9. SMRs for plants with complete records
SBR worker study (IISRP 1987)

cause of death	obs	exp	SMR	95% CI
all causes	1188	1567.2	76	72-80
all cancers	233	313.2	74	65-85
lymphopoietic cancer	24	35.0	69	44-102
lymphosarcoma	2	7.0	29	3-103
leukemia	10	13.5	74	36-136

extremely low SMRs including significant deficits of total mortality and cancer mortality and borderline significant deficits of lymphopoietic cancers ($p = 0.06$) and lymphosarcoma ($p = 0.06$). Leukemia mortality was only 74% of expected values. These data do not support the contention that bias from incomplete enumeration of early workers is likely to mask a relationship between BD and lymphopoietic cancers in this study. They demonstrate that OHSA's concern about exclusion of early workers is unfounded.

4. Superiority of the case control study design?

The nested lymphopoietic cancer case control study (Matanoski 1989) is mentioned briefly by OSHA along with the author's conclusion that the leukemia rate may be seven to nine fold higher in workers with BD exposure than workers without such exposure. I discussed this study and its limitations previously. Briefly, the major issue is how the reported seven to nine fold elevated leukemia rate among exposed workers was not detected in the cohort study of the same population (Matanoski 1988, Matanoski 1990) - a study which found 22 observed and 22.8 expected leukemia deaths. In fact, I know of no other nested occupational case control study with such a startling discrepancy for a chemical that is nearly ubiquitous throughout plant operations. This discrepancy casts doubt on the procedures and results of the case control study.

OSHA's only comment on this discrepancy was to claim that case control methodology is superior to cohort methodology, specifically:

> "However, it is well established that case-control studies, as opposed to cohort studies, are proper for use in testing etiologic hypotheses for specific rare diseases ..." (OSHA 1990, page 32750)

This comment reflects a lack of understanding about the epidemiologic measures that are being estimated in these related study designs. In fact, the case control design can be conceptualized as a cohort study in which the person time experience of the disease rate denominators is sampled rather than enumerated completely as in a cohort study (Rothman 1986). Nested case control studies often involve a small fraction of the workers in the parent cohort study, which may allow for a more comprehensive exposure estimation than would be possible in the larger cohort study. In that regard, it may offer the opportunity for more specificity in exposure assessment; but the so called "dilution of exposure" in a cohort study, from less specific exposure assessment, would never obscure a seven to nine fold higher rate of leukemia among workers exposed to a chemical as widely used in SBR plants as BD (Acquavella 1989).

Therefore, the two study methodologies should yield similar results (allowing for sampling variability) when applied to studies of the same SBR workforce. However, as Rothman points out, case control studies present more opportunities for bias and mistaken inference when not designed, conducted, or analyzed properly. Hence, it is not the choice of study design which should form the basis for favoring the case control or cohort study findings, but rather the quality of the design, execution, and analysis of the individual studies.

OSHA's argument about the superiority of the case control design is an inappropriate rationale to overlook the inconsistency of the leukemia results from the case control and cohort studies of this SBR workforce. To do so would ignore the fundamental relationship between these two study designs. Proper evaluation of the case control study can only be done in concert with the results of the cohort study.

5. Studies of tire manufacturing workers

OSHA reviewed findings from McMichael's study of tire manufacturing workers (McMichael 1976) and concluded that lymphatic leukemia cases tended to work longer periods in the synthetic plant - where BD exposure was possible. IISRP pointed out in previous comments to OSHA (IISRP 1986) that a subsequent study of this tire workers population by the same research group, which focused on specific workplace exposures, failed to identify

BD or employment in the synthetic rubber department as a risk factor for leukemia (Checkoway 1984). Matanoski made the same point in her review of these studies stating (Matanoski 1990):

> "In a subsequent study, these investigators associated the leukemia risk with solvent exposure only and did not mention a relationship to the SBR department."

Again, only part of the evidence was presented in OSHA's review and important data which conflicts with the appearance that BD is an occupational leukemogen was omitted. In addition, IISRP commented that this study and other studies of tire manufacturing workers (e.g., Andjelkovich 1977) were not appropriate for assessing risks from BD exposure due to the limited nature of BD related operations in this industry and the widespread use of other agents known to cause leukemia and lymphomas (IISRP 1986). These studies have nothing to contribute to our understanding of BD epidemiology.

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Future Directions in Epidemiologic Studies of 1,3-Butadiene-Exposed Workers

by John F. Acquavella*

To date, epidemiologic research on 1,3-butadiene has consisted of cohort mortality studies of workers in the styrene-butadiene rubber (SBR) and butadiene monomer industries. These studies have been extremely useful both in defining the focus on human health effects to the lymphopoietic cancers and in providing a perspective on which to evaluate the available animal models for human risk assessment. The next step for epidemiologic research will involve a lymphopoietic cancer case control approach to enable a more precise assessment of whether there is a relationship between 1,3-butadiene exposure and lymphopoietic cancer. In addition, periodic mortality updates of the 1,3-butadiene-exposed worker cohorts will be important to monitor trends in lymphopoietic cancer rates and to ensure that other cancers with long latency do not begin to show elevated rates. This paper describes an industry-sponsored program of case-control and cohort mortality update studies along with the critical elements in research design and analysis for each study. Epidemiological studies will play an important role in testing hypotheses developed from toxicological studies about potential biological mechanisms of 1,3-butadiene carcinogenesis in humans.

Introduction

In our attempt to understand the potential human health risks of exposure to 1,3-butadiene, we are quite fortunate to have available a considerable amount of toxicological and epidemiological data. In this latter regard, the update of the three available epidemiological studies (*1–3*) provides additional information on the mortality experience of workers with occupational 1,3-butadiene exposure. The purpose of this paper is to discuss future areas for epidemiological research. Some of the study areas have already been incorporated into the International Institute of Synthetic Rubber Producer's (IISRP) epidemiology program, funded jointly by the IISRP and the butadiene monomer producers. Other study areas are longer term and await developments in related scientific fields.

Perhaps the best way to set the stage for discussing future epidemiologic research related to 1,3-butadiene is to consider the present status of epidemiologic research, identify existing data gaps and important methodologic issues that need to be resolved, and plan studies to address these issues. In addition, this paper discusses anticipated related toxicological developments and points out where epidemiologic studies can contribute to refining or testing specific hypotheses.

Cohort Studies of Worker Mortality

The previous three papers are examples of historical prospective cohort (or follow-up) mortality studies (*1–3*). Cohort mortality studies begin by defining a worker population (the cohort) at a point in time (1943 for these studies) and following workers prospectively to assess each individual's vital status at the end of the study period. Death rates for workers are then compared with general population rates. Additionally, for large cohorts, there may be comparisons of rates between exposed and unexposed workers. Since a cause of death is determined for almost all decedents in the cohort, these studies allow an evaluation of death rates for workers for many causes of death. However, since cohort studies typically involve thousands of workers, assembling detailed exposure data or making exposure estimates for each worker is often impractical, limiting the potential to study exposure-disease relationships. Accordingly, these cohort studies are particularly useful to assess whether rates for many causes of death are elevated among worker populations and to generate hypotheses for further, more detailed, studies of specific occupational subgroups.

Summary of Findings

A comprehensive review of 1,3-butadiene epidemiology is beyond the scope of this paper and is the topic of the succeeding two papers (*4,5*). However, a brief summary of the available epidemiologic findings is provided, since future trends in epidemiologic research evolve from the context of our current state of scientific knowledge.

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Studies of styrene-butadiene rubber (SBR) workers and butadiene monomer employees show a generally favorable mortality profile of lower overall mortality and total cancer mortality compared to general population rates (*1–3,6–8*). Further, mortality from most cancers is less frequent than expected. However, mortality from lymphopoietic cancer [International Classification of Diseases (ICD), 8th revision, 200–209] emerges as a potential cause for concern, since each study has a subgroup of workers with an elevated mortality rate for a type of lymphopoietic cancer. These excesses generally involve shorter term workers, and there is no consistency in the lymphopoietic cancer cell types across studies. In addition, analyses of the lymphopoietic cancer deaths do not indicate elevated mortality rates among workers with longest duration of employment and/or long latency, which would be typical for an exposure-related excess. Nevertheless, these lymphopoietic cancer findings deserve further follow-up and, therefore, are the focus of the current IISRP-sponsored epidemiologic research program. Thus, for now, these cohort mortality studies have narrowed the scope of 1,3-butadiene-related human health research to lymphopoietic cancers. This is important in light of the results from the $B6C3F_1$ mouse studies that show a striking 1,3-butadiene-related excess for thymic lymphomas, but also show tumor excesses for several other organ systems (*9*).

Impact on Risk Assessment

While the cohort studies of 1,3-butadiene-exposed workers have provided an important perspective on worker mortality rates, these studies are often not useful for risk assessment modeling because exposure estimates are not available for individual workers. However, despite this limitation, the human data can be used to evaluate projected worker mortality based on risk estimates (unit risks) derived from the chronic rat (*10*) and mouse (*11*) bioassays. This analysis requires two simplifying assumptions: *a*) that workers on the average were exposed to a specific exposure level (in this example, 1, 5, and 10 ppm were used since these levels are consistent with the available monitoring data as shown in Fig. 1); and *b*) that any excess mortality would be from lymphopoietic cancer (following directly from the previous summary of findings).

For example, based on the largest published SBR workers study (*8*) to date, Figure 2 compares the observed human lymphopoietic cancer mortality (the white bar) and the mortality predicted based on the unit risk estimates from the rat (*10*) (grey bar) and mouse (*11*)

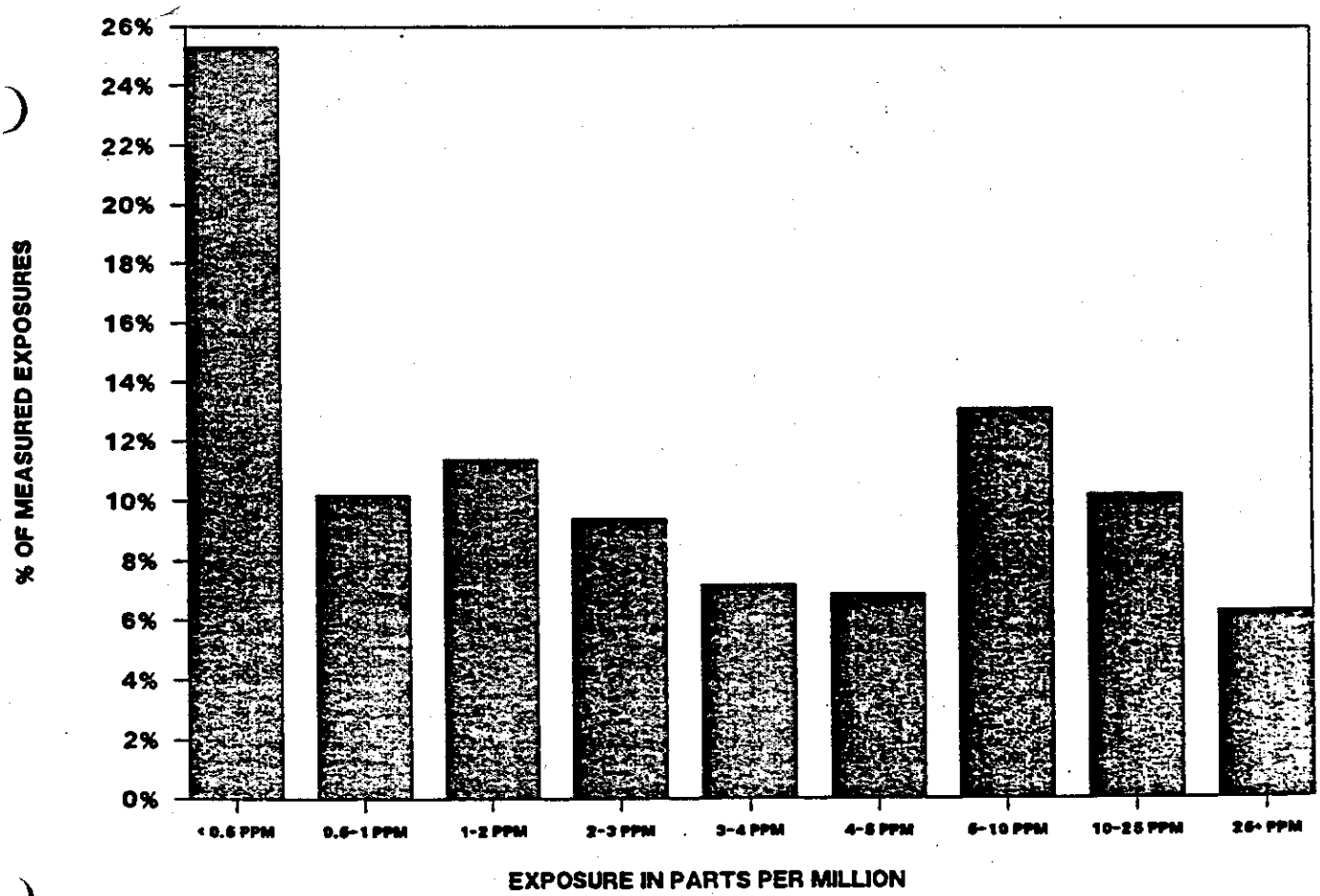

FIGURE 1. 1,3-butadiene job exposure data from all monitored jobs, 1981 to 1987.

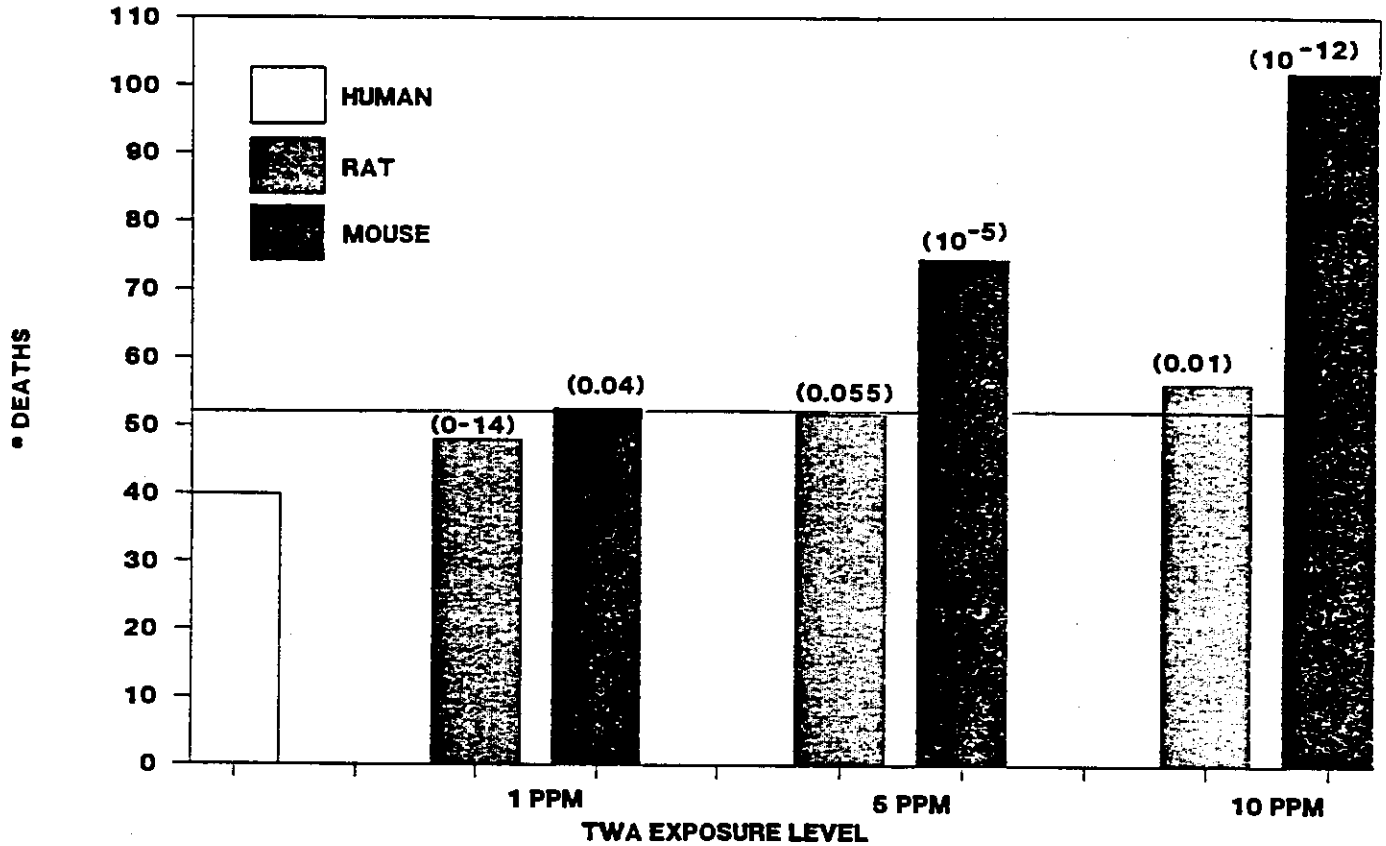

FIGURE 2. Lymphopoietic cancer among SBR workers: comparison with rodent model predictions.

(black bar) models, assuming that the average worker-exposure levels were 1, 5, and 10 ppm. The horizontal line on the graph is the level where the animal models significantly overpredict the observed human mortality. The numbers in parentheses on top of the bars are the probabilities of seeing as few or fewer deaths among SBR workers if the excess cancer risk was as great as that predicted by the animal models. From this figure, it is clear that the mouse model significantly overpredicts human mortality at average exposure levels of 1 ppm and greater levels, which current monitoring data tell us still exist in SBR facilities (*12*). Projections based on the rat model are less severe, but they still seem to overpredict human mortality at levels of 5 ppm or greater. Clearly then, to the extent that the two assumptions above are reasonable, the human data offer a perspective on the worker mortality projections, based on the existing animal models for 1,3-butadiene. In the same way, future developments from toxicological studies should be evaluated, where possible, against the available epidemiological data.

Importance of Continued Follow-up

In light of the approximate 40-year study period for each of these cohorts, the question arises: Is there anything to be gained by continuing the follow-up period for these workers? Clearly, the answer to this question is that much remains to be learned from cohort mortality studies of 1,3-butadiene-exposed workers. Specifically, until more is known about the applicability of the animal models, the continued monitoring of the workers' mortality experience will be a critical component of any future research program. In this regard, maintaining a current data base of human mortality will be important to assess temporal trends in cancer mortality, especially for cancers that may occur with long latent periods, and to aid in evaluating new leads from continued toxicological research. Accordingly, future research priorities within the IISRP include a continuation of the SBR workers mortality study soon after completing detailed studies of the relationship between lymphopoietic cancer and 1,3-butadiene exposure. Obviously, continued follow-up of the National Institute for Occupational Safety and Health (NIOSH) (*1*) and Texaco (*3*) cohorts would provide useful parallel efforts.

Four modifications should be considered to improve the data from future cohort mortality studies. First, the usefulness of the human mortality data would be greatly improved by a realignment of the lymphopoietic cancer categories used in the mortality analyses. This realignment should reflect the current thinking on the characteristics of the individual lymphopoietic cancer cell types. The three studies reported today have employed lymphopoietic cancer groups that mix potentially re-

Table 1. Lymphopoietic cancer groupings used in the existing 1,3-butadiene epidemiology studies.

Category	Includes (ICD 8[a])
Lympho/reticulo sarcoma	Lymphosarcoma (200)
	Reticulum cell sarcoma (200)
Hodgkin's disease	Hodgkin's lymphoma (201)
Other lymphopoietic tissue	Giant follicular and other lymphoma (202)
	Multiple myeloma (203)
	Polycythemia vera (208)
Leukemia and aleukemia	Lymphatic leukemia (204)
	Myeloid leukemia (205)
	Monocytic leukemia (206)
	Leukemia not otherwise specified (207)

[a]International Classification of Diseases, 8th revision.

lated and unrelated cell types (Table 1). For example, the category entitled "cancer of other lymphatic tissue" mixes giant follicular and other lymphomas, multiple myeloma, and polycythemia vera. Similarly, the leukemia category combines lymphoid, myeloid, monocytic, and leukemia not otherwise specified. A better grouping would have separate categories for the non-Hodgkin's lymphomas (lymphosarcoma, reticulum cell sarcoma, and giant follicular lymphoma), Hodgkin's disease, multiple myeloma, and each leukemia cell type. Mortality analyses presented in this way would then allow an evaluation of results within and across studies for consistency and for compatibility with advancing knowledge of biological mechanisms.

A second suggestion would be to use local mortality rates for comparisons of worker mortality. At present, interpretation of SMRs for 1,3-butadiene-exposed workers is clouded by variability that is introduced by using U.S. mortality rates as a basis for evaluating worker mortality at plants scattered throughout the U.S. and Canada. Mortality rates for U.S. states and counties and for Canadian provinces are available from several sources and should be incorporated in future mortality studies. To date, only the previously published Texaco study used local rates for their mortality analysis (*7*). The importance of using local rates was vividly illustrated in that study, as comparisons based on both U.S. and local rates showed that local general population lymphosarcoma rates were 30% higher than U.S. rates.

A third suggestion would be to present lymphopoietic cancer SMRs for various latency/duration of employment subgroups. The purpose of this suggestion is to have the authors specify which, if any, subgroups are showing elevated lymphopoietic cancer rates. At that point, data across studies could be evaluated as suggested by Doll (*13*) to see if increased risk varies appropriately with intensity and duration of exposure and time after exposure begins and ends; and is observed repeatedly in different circumstances. At present, it is impossible to apply these criteria to the 1,3-butadiene literature.

A final methodologic suggestion would be to evaluate lifetime work histories for a sample of short-term employees in each of these cohort studies. This evaluation would review work experience before and after employment in 1,3-butadiene-related occupations. Clearly, the findings of lymphopoietic cancer excesses among short-term workers suggests that possible longer employment in other industries must be considered in interpreting the results in 1,3-butadiene-related industries.

Lymphopoietic Cancer Case Control Studies

Prior to initiating another mortality update of the IISRP SBR workers cohort study (*8*), the IISRP research program is focusing on detailed studies of a potential relationship between 1,3-butadiene exposure and lymphopoietic cancer(s). The most common research design for this purpose is the nested case-control study. The term "nested" refers to the fact that cases and controls are selected from within the cohort for which mortality data are available. In contrast to cohort studies, case-control studies usually concentrate on one disease or a related group of diseases and compare the odds of previous exposure for those with the disease (the cases) versus those without the disease (the controls). Since nested case-control studies focus on a small subgroup of an occupational cohort (namely those with a specific disease and a sample of nondiseased workers), considerably more attention can be given to the data available for each study subject. This allows detailed evaluation in two critical areas: validation of lymphopoietic cancer diagnoses and estimation of historical 1,3-butadiene exposures.

Validation of lymphopoietic cancer diagnoses is extremely important for case-control studies in light of the unreliability of lymphopoietic cancer diagnoses on death certificates. Perhaps the best study on this issue to date was conducted by the National Center for Health Statistics. In this study, Percy et al. (*14*) looked at the death certificate diagnosis for more than 48,000 cancer deaths from the Third National Cancer Survey and compared this information to the primary cancer site reported on the hospital diagnosis. This analysis showed considerable underdiagnosis and misclassification of the individual lymphopoietic cancer types. For example, Table 2 shows that only 79.9% of lymphocytic leukemia deaths would have been detected from death certificate diagnoses. Further, of those specified as lymphocytic leukemias on death certificates, only 86.3% could be confirmed from hospital records. A more recent study by Gittlesohn, for the period 1968 to 1978, showed a one-third decline in lymphosarcoma and reticulum cell sarcoma as death certificate diagnoses and a corresponding doubling of the number of deaths attributed to unspecified malignancy of lymphoid tissue (*15*). Clearly then, case-control research should incorporate confirmation of the diagnoses and cell type, when possible, for each lymphopoietic cancer. Otherwise, the valid assessment of the relationship between 1,3-butadiene and the individual lymphopoietic cancer cell types will be obscured by the mixing of unrelated lymphopoietic and

Table 2. Detection and confirmation rates for lymphopoietic cancers from the Third National Cancer Survey.[a]

ICD 8[b]	Primary site	Number	Percent detected	Percent confirmed
200,202	Non-Hodgkin's lymphoma	1562	83.2	88.4
201	Hodgkin's disease	572	86.7	92.5
203	Multiple myeloma	699	96.6	98.1
204	Lymphocytic leukemia	743	79.9	86.3
205	Myeloid leukemia	1107	76.2	92.2
206	Monocytic leukemia	98	57.1	53.8
207	Other and unspecified leukemia	204	73.0	34.3

[a]From Percy et al. (*12*).
[b]International Classification of Diseases, 8th revision.

other cancers in the case group.

Equally important for case-control studies is the proper estimation of historical 1,3-butadiene exposures for cases and controls. Many of the large petrochemical companies have had collaborative epidemiology and industrial hygiene programs to assess strategies for retrospective exposure assessment. From these efforts, it has been shown that exposure estimating schemes must consider available plant monitoring data as well as plant-specific changes in engineering controls and work practices (especially use of personal protective equipment) that could have affected workplace exposures. Job titles can often be misleading, especially in interindustry studies, and should be used with caution as an indicator of worker exposure. A better approach would be to use job titles in conjunction with a detailed analysis of plant-specific monitoring data, engineering controls for specific time periods, and work practices. Once this background work is done, exposures can be estimated for each job title and cumulative exposure scores calculated for each case and control based on their work history. Whenever possible, exposure estimates should be aligned with exposure values as a guide to the scaling of exposure scores in subsequent dose-response analyses.

A lymphopoietic cancer case-control study is currently underway using cases and controls selected from the IISRP SBR workers' cohort. This study is being conducted in two phases, with phase I expected to be completed by the summer of 1988 (Mantanoski et al., unpublished report). The respective components of phase I and II case control studies are detailed in Table 3.

Phase I is using diagnostic information from workers' death certificates to select cases of lymphopoietic cancer based on either the underlying or a contributing cause of death. Exposure to 1,3-butadiene and styrene was estimated for both cases and controls in two steps. First, a dictionary of job titles was developed across all eight plants included in the cohort study. Then an industry workgroup rated the exposure potential of each job title on high/medium/low/no and 0 to 10 scales. These ratings reflected the opinions of the industry workgroup and did not employ available monitoring data. From these exposure estimates, a cumulative exposure potential score was developed for each worker as the sum of the exposure score times the time spent in each job. The analysis is currently ongoing to determine whether

Table 3. Lymphopoietic cancer case-control study.

Components	Phase I	Phase II
Case ascertainment	Death certificates	May add cases from medical record review
Case validation	No	Yes
Exposure assessment	Judgments across eight plants; no use of monitoring data, process changes, or work practices	Local personnel make estimates based on monitoring data, process changes, and work practices
Data analysis	Tests for association and dose response	Tests for association and dose response
Time frame	September 1986–June 1988	January 1989–December 1991

cases tended to spend more time in jobs judged to have higher exposure potential than did the controls.

The phase II lymphopoietic cancer case control study will require roughly 18 to 24 months for completion. In this study, medical records will be reviewed to verify diagnoses and specify cell types for all cases. This will allow evaluation of risk for specific lymphopoietic cancer cell types. In conjunction with medical record review, exposure assessment will be improved by employing all available monitoring data and the knowledge of local plant industrial hygiene and technical personnel to document changes in equipment and work practices that might have affected worker exposures.

Epidemiology Studies Suggested by Toxicological Research

The next stage of future epidemiological studies depends on advances from toxicological studies into mechanisms of 1,3-butadiene activity in animal and *in vitro* systems and on the applicability of this research to our understanding of human cancer risk. Many of the following comments will apply as much to 1,3-butadiene as they do to a number of other chemicals that are the subject of ongoing toxicological research. Such studies will ultimately arise in two areas: *a*) studies of cancer risk in populations with potentially increased susceptibility to effects of 1,3-butadiene; and *b*) correlations of biological markers of intermediate disease stages with 1,3-butadiene exposure. Of these, studies of potentially susceptible subpopulations seem most likely to occur within the next decade, so the ensuing discussion will be confined to some preliminary thoughts in this area.

By definition, a susceptible subpopulation is one that has a high prevalence of a trait resulting in an increased cancer risk. For example, a number of years ago Kellerman et al. (*16*) suggested that individuals with higher levels of aryl hydrocarbon hydroxylase (AHH) were at increased risk for lung cancer. Soon thereafter, Paigen et al. (*17*) presented data to suggest that Kellerman's

findings were a consequence of lung cancer, rather than a risk factor. However, had AHH proven to be an indicator of increased lung cancer risk, it would have proved useful for identifying susceptible individuals and would have shown obvious implications for research and cancer prevention.

As research into the mechanisms of chemical carcinogenesis develops, traits that modify cancer incidence in experimental animals will need to be evaluated for their applicability for human cancer risk assessment. If analogous mechanisms are thought to operate for humans, any worker population with a high prevalence of that trait would be a potentially susceptible subpopulation. Epidemiological studies of these populations would be useful as the ultimate test of these hypothesis, by allowing a comparison of the observed disease occurrence versus that predicted based on the experimental data.

Clearly, there will be several intermediate steps that remain to be done to assess whether potential mechanisms from experimental studies have any relevance for human populations. Provided these intermediate steps can be done, the existence of a potential biological mechanism can be incorporated directly into the planning of an appropriate epidemiologic study. For example, it seems likely that a biological mechanism suggestive of increased susceptibility among worker populations would have a multiplicative effect on human cancer incidence. Accordingly, the expectation of a multiplicative model can be incorporated into sample size calculations, in proportion to the prevalence of the trait among specific populations, to assess the number of workers necessary to address this hypothesis. Most often, this will require a smaller study population than is traditionally thought necessary for an occupational epidemiologic study.

Conclusion

In conclusion, the available butadiene monomer and SBR worker-cohort studies have been extremely useful in documenting the generally favorable mortality patterns among 1,3-butadiene-exposed workers and in pointing out the need for further, more detailed, studies focusing on lymphopoietic cancers. These cohort studies have also provided a basis for evaluating projections of worker mortality based on the available animal models. The next step for epidemiologic research will employ nested case-control studies for a more precise assessment of whether there is a relationship between 1,3-butadiene exposure and lymphopoietic cancer. Periodic mortality updates of 1,3-butadiene-exposed worker cohorts will be important to monitor trends in lymphopoietic cancer rates and to ensure that long latency cancers do not begin to show elevated rates. Finally, epidemiological studies developed from toxicological studies will play an important role in testing hypotheses about biological mechanisms of human carcinogenesis. These studies will require close collaboration between toxicologists, industrial hygienists, technical plant personnel, and epidemiologists in planning, conducting, and analyzing these studies.

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Paper

The paradox of butadiene epidemiology

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Key words: butadiene epidemiology, butadiene workers; extrapolation, methodological issues

Introduction

Toxicological studies have shown 1,3-butadiene (herafter called butadiene) to be a potent multi-site animal carcinogen with marked differences in potency across rodent species. For the most sensitive species studied (the $B_6C_3F_1$ mouse), cancers have been seen at exposures as low as 62.5 ppm (6). These experimental findings raised have concerns about cancer risk among butadiene exposed workers and increase the importance attached to the available epidemiological studies.

There have been 4 studies of butadiene exposed workers. Three were cohort mortality studies, which together involve more than 17,000 workers studied 33–43 years (5, 1, 2, 4). The fourth study is a lymphopoietic cancer case control study nested withing the largest of the 3 cohort studies. This review will focus on the consistency of the epidemiologic findings and discuss some methodological issues in extrapolating from animal to human studies.

Methodological issues – cohort studies

As a prelude to discussing the butadiene cohort studies, some general methodologic comments are necessary. All 3 studies compared worker death rates to general population rates, adjusting for age and calendar period. The resulting statistic, called the Standardized Mortality Ratio (SMR), is most often expressed as the ratio of observed to expected (O/E) deaths. The SMR can be thought of as a weighted average of O/E deaths across different strata of age, calendar time, duration of employment, and latency. The real data of etiologic importance are within the SMR – the O/E strata for workers with longest employment or highest exposure and with an appropriate latent period. When O/E results are inconsistent across strata, conclusions drawn from the SMR can be misleading. Therefore, an adequate appraisal of the butadiene cohort studies requires not only an assessment of SMRs, but also an evaluation of important O/E strata by duration of employment and latency. These latter O/E results should be consistently elevated across studies if butadiene exposure is carcinogenic to exposed workers..

MEINHARDT et al. – Styrene butadiene rubber workers study

The first published butadiene cohort study included 2,756 workers employed at least 6 months at 2 styrene butadiene rubber (SBR) plants in Port Neches, Texas (5). SMRs were calculated from 1943–1976 at one plant (plant A) and 1950–1976 at the second plant (plant B) versus United States (US) rates. Combining the results for the 2 plants, worker death rates were significantly lower than US rates for all causes of death, all cancers, and cardiovascular diseases. SMRs were less than 1.0 for most cancers, except lymphopoietic cancers (O/E = 11/8.4), due to slight (non significant) elevations of leukemia (O/E = 6/3.5) and lymphosarcoma (O/E = 4/2.5). In plant A

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among workers first employed in 1943–1945, there was a borderline significant excess of leukemia (O/E = 5/1.8).

Lymphopoietic cancer SMRs were not reported by duration of employment or job category, so it was impossible to evaluate SMRs for long term workers – a critical consideration in evaluating these findings. The authors provided a listing of the leukemia cases, which showed that 3 of the 6 cases had very limited SBR employment (0.6, 0.8, and 1.5 years, respectively) and latencies so short (3, 3, and 4 years, respectively) as to suggest etiologic factors operative before employment in these SBR plants. There were also 2 leukemias among workers employed less than the 6 months necessary for inclusion in this study (0.1 and 0.3 years). However, focusing on long term workers, the number of observed leukemia deaths appears to be consistent with the expected number. Taken together, this suggests an elevated leukemia rate in short term workers, but not among long term workers: paradoxical findings if butadiene exposure is related to leukemia in this study.

Divine – Butadiene monomer workers study

Two recent studies have examined mortality patterns among butadiene monomer (BDM) production workers at a plant contiguous to the SBR plants studied by Meinhardt et al. Only the more recent study will be reviewed, focusing on 2,582 workers employed at least 6 months between 1943 –1979 (1). Mortality followup was through 1985 and SMRs were calculated bases on U.S. rates.

Worker mortality rates for the categories all causes, all cancers, and cardiovascular diseases were significantly lower than US rates. For most cancer sites, mortality was less frequent than or consistent with expected values. However, lymphosarcoma mortality was significantly elevated (O/E = 9/3.9), although leukemia rates showed no elevation (O/E = 8/7.9).

Further analysis showed the lymphosarcoma excess was concentrated among workers in departments with potential for routine butadiene exposure (O/E = 5/0.9). The remainder of the cohort had lymphosarcoma mortality consistent with expected values (O/E = 4/3). Leukemia rates for the routinely exposed subgroup were not elevated (O/E = 1/1.8). Analyses by duration of employment for routinely exposed workers showed all the lymphosarcomas and the one leukemia occurred in workers employed less than 10 years, while no lymphosarcomas or leukemias occurred among workers employed more then 10 years (viz. SMR = 0). Again, these findings suggest, paradoxically, that lymphopoietic cancer rates were elevated among short-term, but not long-term, workers.

Matanoski et al. – SBR workers study

The largest butadiene cohort study evaluated mortality between 1943–1982 for 12,110 workers at 8 SBR plants in the US and Canada (2). For U.S. plants, eligible workers were employed for at least one year between 1943–1976, while for the one Canadian plant eligible employes worked at least 10 years or reached age 45 during employment as of 1976. Records were complete from 1943 at 4 of the plants enabling complete cohort followup, while mortality followup at the remaining plants started when records were judged to be complete (1953, 1958, 194, 1970).

For the total cohort, mortality from all causes and all cancers was significantly lower than US rates (SMRs 80 and 84 respectively). Mortality for the lymphopoietic cancers was less than expected (O/E = 55/56.7), including the component categories lymphosarcoma (O/E = 7/11.5) and leukemia (O/E = 22/22.8). Analyses of lymphopoietic cancer by duration of employment and latency did not indicate increased risk among long-term workers or for specific latent periods. Interestingly, SMRs for the 4 plants with complete record showed lymphopoietic cancer mortality substantially less than expected (O/E = 24/35), inlcuding mortality for lymphosarcoma (O/E = 2/7) and leukemia (O/E = 10/13.5).

SMRs were also presented for workers in 4 job categories: production, maintenance, utilities, and other areas. For black production workers, there was a significant excess of lymphopoietic cancers (O/E = 6/1.2), due to elevated mortality from leukemia (O/E = 3/0.5) and other lymphatic cancers (O/E = 2/0.4). White production workers showed lymphopoietic cancer

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mortality consistent with expected values (O/E = 13/11.9), with no excess for leukemia (O/E = 4/4.8) or lymphosarcoma (O/E = 0/2.4) and a slight excess of other lymphatic cancers (O/E = 7/3.1). Further analysis of the black production worker findings showed the lymphopoietic cancers were concentrated in short-term workers; 4 of the 6 deaths worked for very short periods (1.3, 1.3, 2.0, and 3.8 years respectively). SMRs for maintenance workers, in contrast to the findings for black production workers, showed lower mortality than expected for all lymphopoietic cancer subgroups for both black and white workers.

The above job category analyses omitted 2,391 workers with incomplete work histories. Roughly 75% of these workers were active employees at one plant, which had limited computerized job category data for active employees. SMRs for this subgroup show strikingly low mortality: total mortality was only 38% of expected (O/E = 161/422.1), cancer mortality was 46% of expected (O/E = 42/90.5), lymphopoietic cancer was 57% of expected (O/E = 5/8.8) and there were no leukemias (O/E = 0/4.5). Proper classification of these workers among the 4 job categories would be expected to lower the reported SMRs.

Lymphopoietic cancer case controls study

The most recent epidemiology study is a lymphopoietic cancer case control study "nested" within the largest butadiene workers cohort study (4). Nested case control studies are used frequently in industry because they involve relatively few workers, thereby enabling detailed attention to exposure assessment. Methodologically, nested case control studies are conceptualized within the framework of the base (population) cohort study. While cohort studies estimate the ratio of worker and general population death reates (the SMR), case control studies estimate the ratio of death rates between exposed and unexposed workers – called the expsosure odds ratio (OR). The OR is an unbiased estimator of the SMR if controls are selected independent of exposure. Case control studies also yield another critical measure – the exposure prevalence among controls, which is an estimate of exposure prevalence in the base cohort. From the OR and the exposure prevalence, the findings from case control and cohort studies can be related (viz. the OR and SMR).

The SBR workers lymphopoietic cancer case control study has already been described in detail (4). Briefly, cases and controls were selected from the SBR workers cohort and exposure to butadiene was estimated for each workers's entire SBR plant employment history. Estimates of the OR for butadiene exposure showed a significant OR of 9.4 for leukemia and no significant elevation for the other lymphopoietic cancers (ORs 0.5 for lymphosarcoma, 1.1 for Hodgkins's disease, and 1.5 for other lymphatic cancer). The control exposure prevalence was estimated to be approximately 60% in the leukemia analyses.

These leukemia findings (OR = 9.4, exposure prevalence of 60%), conflict markedly with the results of the base cohort study, which found no leukemia excess (O/E = 22/22.9). Specifically, assuming similar leukemia rates for unexposed workers and the general population, the number of leukemia deaths in the base cohort should be estimable from the case control results by summing [OR X exp deaths X% exposed] + [exp deaths X% unexposed]
[i.e., $(9.4 \times 22.9 \times 60\%) + (22.9 \times 40\%)$]. However, this results in an estimate of 138 leukemia deaths, when only 22 deaths were observed in the base cohort!

Accordingly, the interpretation of the case control results is problematic. At present, there are 3 possible explanations for these conflicting results: bias (viz. incomplete cohort enumeration or death ascertainment) in the base cohort study, bias in the case control study, or an extremely low leukemia rate among unexposed workers. Bias in the base cohort study could have resulted from selective omission of high risk workers (or their deaths) from the cohort – perhaps in the 4 plants with incomplete records. This possibility seems unlikely since analyses of the 4 plants with complete records showed a near significant deficit of lymphopoietic cancers. Further, to miss significant numbers of leukemias in vital status followup, literally hundreds of deaths would have to be missed for the cohort, and this would be clearly evident in the other cause of death analyses. The second possibility, bias in the case control study, could have resulted from restrictive control selection citeria, yielding an unrepresentative sample of controls with respect to butadiene exposure. This possibility can only be evaluated by a reanalysis using a new control series. The final

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possibility, that unexposed workers have a much lower leukemia rate than the general population (and exposed workers), also requires further evaluation. To the extent that these latter 2 explanations are found to be valid, the interpretation of the case control results would change in a manner more consistent with the available butadiene cohort studies.

A note on extrapolation to human studies from animal models

When data exist for animal species and humans, a notable goal is to determine whether the animal and human data show consistent results. This typically requires extrapolation from animal studies at high doses to the lower exposure levels found in the workplace. When predictions based on animal models are not significantly different than the observed human data, the results are often termed "consistent" across species.

There a many problems with this approach. Some are mechanistic, some relate to confounding factors in human studies, and some relate to the statistical criterion for consistency. These issues are too complex to be discussed in this short paper. Rather, I will focus on a single consideration – the role of sample size – and how it affects conclusions based on this extrapolation process.

Previously an assessment was published which showed that mortality predictions based on the $B_6C_3F_1$ mouse model significantly exceeded findings from the largest SBR workers study (7). At that time, the SBR workers study included 12,100 workers followed from 1943–1979, and lymphopoietic cancer mortality was 85% of expected (O/E = 40/47.1) (3). Yet, for this population, assuming average exposures of only 1 ppm (a level below what has been shown to exist in industry), the animal model predicted 52.6 deaths – a significant difference compared with the 40 deaths actually observed. However, had the cohort been smaller – for example the size workforce studied by MEINHARDT et al. (about 2,700 workers) – the SMR of 0.85 for lymphopoietic cancer would not have been statistically inconsistent with the mouse model's predictions. In that case, a potent animal model could be deemed consistent (or not inconsistent) with epidemiological data which show less mortality than expected. Clearly, qualitatively disparate results across species can be deemed quantitatively consistent solely due to the small study size of the referent epidemiologic study.

Conclusions

Epidemiologic studies show no consistent pattern of mortality among long-term butadiene exposed workers, but do show lymphopoietic cancer excesses among certain short term worker subgroups. More attention needs to be paid to possible confounding factors (e.g. other employment) among these short-term workers. The inconsistent leukemia findings between the case control and cohort studies for the largest butadiene workers cohort need to be resolved before the former study can be properly interpreted. Extrapolation from animal to human studies for butadiene seems appropriate for large worker populations, but wiht smaller studies discriminatory power is reduced, often rendering these comparisons meaningless. In the case of 1–3 butadiene, where ample human data exist, this problem can and should be avoided.

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Book Review

Mechanisms in B-Cell Neoplasia 1988

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edited by M. POTTER and F. MELCHERS (Volume 141 of the series: "Current Topics in Microbiology and Immunology"). 340 pages, with 122 figures, DM 160,–, ISBN: 3-540-50212-2. Springer-Verlag, Berlin-Heidelberg-New York-London-Paris-Tokyo-Hong Kong 1988.

Lymphomas represent neoplasias which are widely used as model in order to study mechanisms of tumorigenesis. The 6th workshop on "Mechanisms in B-Cell Neoplasia", held in Bethesda at the National Cancer Institute in 1988, dealt with new aspects in this field of cancer research and was mainly focused on oncogenes that are important in B-cell tumor formation.

The papers read at this meeting were compiled and published as volume 141 of "Current Topics in Microbiology and Immunology". In 45 articles the up-to-date knowledge on genetic mechanisms of B-cell lymphoma development is presented under the headings of "Pathogenetic Mechanisms" (concerning (1) early B-cell tumors, and (2) B-cell and plasma cell tumors) and "Studies of B-Cell Relevant Oncogenes" (c-myc, c-abl, c-myb and bcl-2). Those genes that are known targets of mutagenesis in one or more types of B-cell neoplasia are of utmost scientific interest, especially directed at recognizing their normal physiological function inclusive of the biochemical action of their products, and at understanding the role of the mutant forms in the regulation of cellular proliferation and differentiation.

It seems to he largely accepted that a single genetic alteration is not sufficient for producing malignant lymphomas but that multiple genetic changes must occur. But the suggestion may be justified as well that mutations which activate proto-oncogenes produce biological effects that do not directly transform cells from a normal to a neoplastic state. Thus, questions concerning the biological effects of pathologically activated oncogenes are now standing in the center of discussions about the significance of oncogenes and their influence on the deregulation of the cellular growth. In this context new concepts of oncogene function have recently been elaborated as also reflected in the book presented here.

In summary, the volume "Mechanisms in B-Cell Neoplasia 1988" enriches the topical literature about lymphoma generation by recent results of oncogene research. By presenting the current and very specialized knowledge on this topic and by alluding to future directions of research in this rapidly growing and developing field the book is mainly destined for experts and may serve as state-of-art and stimulation of further scientific endeavours at the same time. D. KATENKAMP, Jena

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EMPLOYMENT IN THE BUTADIENE AND STYRENE-BUTADIENE RUBBER INDUSTRIES AND LYMPHATIC AND HEMATOPOIETIC TISSUE CANCER

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6 November 1990

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SUMMARY

Several follow-up studies of styrene-butadiene rubber (SBR) and of workers in related industries have been done and contain useful information on the relation between employment in these industries and the occurrence of lymphatic and hematopoietic cancer (LHC). These investigations include 17,448 subjects with an average of 22 years of follow-up. These studies collectively reported a total of 36 observed leukemia deaths, compared with 34.1 expected. The ratio (x 100) of the observed to the expected number of leukemia deaths (known as the standardized mortality ratio or SMR) is 106, with a 95% confidence interval (CI) of 74 - 146. These null data show no association between employment in the SBR industry and this disease.

Similarly, there is little difference between the observed and expected numbers of deaths from other forms of LHC among SBR workers. These are: 20/17.8 (SMR = 112; 95% CI = 69 - 173) for lymphosarcoma and reticulosarcoma; 12/10.2 (SMR = 118; 95% CI = 61 - 206) for Hodgkin's disease; and 22/21.6 (SMR = 102; 95% CI = 64 - 154) for other forms of LHC.

Two of the studies permit an evaluation of LHC patterns by duration of employment and time since hire. For subjects with <10 years and with 10+ years of employment, the aggregate data show SMRs of 111 (35/32) and 102 (45/44), respectively. For subjects with <10 years and with 10+ years since hire, the SMRs are 83 (7 observed/8 expected LHC deaths) and 108 (73/67), respectively. Again, these data support the absence of a causal relation between employment in the SBR industry and LHC.

Only one study has evaluated directly the relation between butadiene (BD) and leukemia, and this investigation reports a positive association. However, this can not be interpreted as causal because of the high likelihood that it is due, in whole or in part, to random and systematic errors. Furthermore, no satisfactory explanation has been advanced for the marked discrepancy between the findings of this case-control study and the null results of a follow-up study, based on the same subjects.

Overall, the epidemiologic evidence relating to employment in the BD and SBR industries suggests that such employment does not increase the risk of LHC. Further, the studies show collectively that overall cancer mortality in the BD and SBR industries is lower than that of the general population.

The lung cancer mortality rates of SBR workers are lower than general population rates. None of the follow-up studies reports an excess of this cancer. The pertinent SMRs range from 66 to 90.

BACKGROUND

This report reviews epidemiologic studies pertaining to the possible relationship between employment in the butadiene (BD) and styrene-butadiene (SBR) industries and the occurrence of LHC. This focus on LHC was prompted by OSHA's suggestion that the available epidemiologic research shows mortality from these cancers to be in excess among workers in the BD and SBR industries.

Toxicology

SBR manufacturing started in the early 1940s. Workers in this industry are exposed to a number of chemicals, with concern about toxic effects centering recently on BD and styrene. The acute toxicity to the central nervous system of styrene was recognized when the SBR industry began, and a relatively low TLV of 100 ppm has been recommended by the ACGIH. In contrast, BD has low acute toxicity, and so a relatively high TLV of 1,000 ppm has been the standard for occupational exposure. During the past decade, the possible carcinogenicity both of styrene and of BD has become a concern. Both are classified by the International Agency for Research on Cancer (IARC) as "possibly carcinogenic to humans" (1). For styrene, data on carcinogenicity are judged by the IARC to be inadequate in humans and limited in experimental animals. For BD the evidence is judged by the IARC to be inadequate in humans and sufficient in experimental animals. This evidence comes from mutagenicity and genotoxicity studies (2-5), from experimental work on carcinogenicity in mice and rats (6-10) and from numerous epidemiologic studies of workers in the SBR manufacturing industry (11-22).

Butadiene, itself, is nonmutagenic in bacteria (2). However, its epoxide and diepoxide metabolites are mutagenic and genotoxic *in vitro* (2), and *in*

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vivo studies of mice have found that these reactive intermediates bind covalently to DNA in liver cells and induce chromosomal damage in bone marrow (3-5,11).

Investigations of the carcinogenicity of BD in mice have reported the induction of "lymphoma/leukemia," as well as tumors of the lungs and of several other organs (6-9). These studies also reported that susceptibility to BD-induced leukemogenesis is enhanced greatly by the presence of the murine leukemia virus.

An investigation of BD carcinogenicity in rats reported an exposure-related increase in the incidence of mammary gland, Leydig cell and thyroid follicular cell tumors (10). However, there was no evidence of hematologic effects, in contrast to the findings of bone marrow toxicity in mice (11).

It has been suggested that the species differences in BD-induced carcinogenesis are due in part to species-specific variation in the retention and metabolism of this compound. Laib et al. (12) demonstrated that mice metabolize BD at twice the rate of rats, regardless of exposure level. Further, Bond et al. (13) reported that mice retain at least two times as much BD and its metabolites as do rats and that at a given exposure level, the blood concentration of epoxybutene is two to five times higher in mice than in rats. In a further investigation, Bond and coworkers (14) found that, under exposure conditions leading to the retention of equal amounts of BD and metabolites, mice have greater tissue concentrations than do rats. Kreiling et al. (15) also observed that mice are less able than rats to eliminate epoxybutene and thus accumulate more of this reactive metabolite. Dahl et al. (16) provided

further evidence of important species differences in BD metabolism in a study which found that BD epoxide and diepoxide blood concentrations are, respectively, 592 and 40 times higher in mice than in monkeys at similar low exposure levels.

In summary, experimental studies suggest that metabolites of BD are genotoxic and cytotoxic in certain assays. However, large differences exist among animal species exist in the formation, detoxification and accumulation of toxic metabolites. Butadiene appears to be a weak carcinogen in rats, but the bone marrow is not the target organ for cancer induction or for other toxic effects. Butadiene increases the occurrence of lymphoma/leukemia in mice. However, this effect is enhanced by activation of murine retroviruses, suggesting that the presence of such viruses is important for tumor induction in mice. Thus, although toxicology studies indicate that BD is carcinogenic in rodents, these studies have no clear implications for BD's carcinogenicity for human beings.

Epidemiology - An Overview

As a branch of science, epidemiology has as its major objective the description of the causes of diseases of human beings. In the last 15 years, epidemiology has moved to a position of importance in regulation, in public policy formulation and in litigation in the health field. Thus, it is crucial to understand both the power and the limitations of epidemiology as a science.

The major strength of epidemiology is that it pertains directly to human beings. Thus, such facts as can be established by epidemiology have direct implications for public health strategies. This is not true of the results of

nearly all animal research. On the other hand, and more importantly, the great limitation of epidemiology is that it is a nonexperimental science. As such, it is highly susceptible to error. History has taught us that, usually, only epidemiologic investigations of the highest quality will prove to be of value. Moreover, it is more important in epidemiology than in other areas of the biological sciences to perceive patterns of findings within and among several independent investigations. Only in rare circumstances would it be justifiable to infer a cause-effect relationship from a small number of studies. Rather, it is usually necessary for a reasonably large number of independent studies to provide similar findings before an experienced epidemiologist would be willing to make a causal inference.

Every epidemiologic study is subject to four possible interpretations. The results may be: 1) random, 2) confounded, 3) biased and 4) valid. These four interpretations are collectively exhaustive, but they are not mutually exclusive. That is, an epidemiologic result could have elements of all four interpretations. Strangely, OSHA has put forward the idea that an epidemiologic study can have only one of two, apparently mutually exclusive, interpretations. OSHA writes, "When the p-value is smaller than some value, usually .05, we conclude that an observed result could not be due to chance alone and must therefore be due to BD exposure" (Federal Register, page 32745). It is to be hoped that this position reflects only its author's views and not those of OSHA. For, if it reflects OSHA's position, then virtually all of the pronouncements of OSHA regarding causality are fundamentally flawed. Different scientists will hold different classification systems by which epidemiologic studies may be interpreted. And, I do not represent that my own is the only acceptable system. However, I know of no system that is

restricted to the two categories, chance and causality. Any learned discussion of the interpretations of the studies under review would evaluate each study against a series of criteria, such as those listed above.

Random variation is the first factor to be considered in assessing any epidemiologic result. This quantitative consideration specifies a range of values, or "confidence interval (CI)," with which a particular study's results may be said to be consistent. The most common measure of association used in epidemiologic research is the relative rate (RR). This parameter describes the rate of disease among exposed persons relative to the rate of disease among unexposed persons. Thus, for example, a study of cigarette smoking and lung cancer may show an RR of 10 with a 95% CI of 5 to 20. This means that smokers are estimated to have 10 times the lung cancer rate of nonsmokers and that the true value of the RR probably lies in the range of RRs from 5 to 20.

Confounding means that the factor under study (for example, diet in relation to esophagus cancer) is not an actual cause of the disease but, rather, is only a marker of some true cause (for example, alcohol consumption). The word bias is not used in epidemiology in its everyday prejudicial sense. Instead, it means "in error." The sources of bias in epidemiologic research are innumerable and are both of a general type, common to virtually all forms of epidemiologic investigation, and of a specific type, unique to each individual investigation. Finally, valid means that the foregoing explanations are perceived as weak or untenable, and the study is construed as correct, or largely correct.

There are many different types of epidemiologic investigation. Each has strengths and appropriate circumstances. Similarly, each has limitations and inappropriate circumstances. All of the epidemiologic studies of SBR workers are either retrospective follow-up studies (RFS) or case-control studies.

An RFS usually relates to a group of persons, often called a "cohort," who sustained exposure to a factor of interest at some time in the past. These persons are traced up to the present day. Of course, some will have died in the normal course of events. The number of deaths from specific diseases observed among the cohort members can be compared with the number of deaths which would have been expected if they had died at the same rate as the general population. Such a comparison provides an estimate of the RR of disease, known in the context of an RFS as the standardized mortality ratio (SMR). An SMR of 100 indicates that the observed mortality rate in the study group is equal to the rate expected on the basis of the general population's experience. An SMR greater than 100 means that the rate in the study group is higher than the rate in the general population, and an SMR less than 100 indicates that the rate in the study group is lower than the rate in the general population.

RFSs are often imprecise (subject to chance, or random, error), for there may be relatively few deaths from any one particular cause. However, the major problem with RFSs is that they are prone to confounding. Employed groups usually are exposed to a myriad of factors, and it may prove impossible to identify the effects of any one factor. Further, there may be confounding by other, nonoccupational factors. Confounding is a common and major

limitation of the RFS. On the other hand, RFSs usually are not biased to a large degree.

The case-control study is an investigation of a group of "cases," who are persons with a disease of interest, and a group of "controls," who are persons without the disease of interest. The two groups generally are similar with respect to a number of factors, such as age, gender and race. The exposure history of all of the cases and of all of the controls is ascertained. Then, using statistical procedures, one compares formally the exposure history of cases with that of controls to derive an estimate of the RR, known in the context of a case-control study as the odds ratio. The odds ratio is computed as the exposure odds among the cases divided by the exposure odds among the controls.

Case-control studies may be imprecise if the frequency of exposure is low or if the frequency of exposure is quite high. Also, case-control studies often are perceived as biased for a number of reasons. One is that the controls may not be truly comparable to the cases. Another is that the ascertainment of exposure may be done with a knowledge of the subjects' case or control status. This could subconsciously influence the researcher's evaluation of the exposure.

In sum, epidemiologic study designs are complex, and the successful execution of such studies requires a considerable understanding of human biology, statistics and the specific methodologies of epidemiology. The interpretation of such research further requires a broad understanding of the kinds of interpretations available and the kinds of information that can be

used to support each. In light of these complexities, it is not surprising that consistency of results among investigations is so important in judging whether a given association is causal or noncausal.

EPIDEMIOLOGIC STUDIES

Seven epidemiologic studies have evaluated the mortality experience of workers in the SBR and closely related industries (17-28). Several of these involved subjects employed in large manufacturing complexes in the tire (17-20) and chemical (21) industries and are reviewed in Appendix A. The relevance of these studies to the issue of BD carcinogenicity in humans is uncertain because the subjects may have worked in many manufacturing processes in addition to those entailing BD exposure.

Four investigations have assessed mortality patterns among workers at SBR latex (22, 25-28) or BD (23,24) manufacturing plants. All of these involved workers with potential exposure to many chemicals in addition to BD, some of which may be carcinogenic. Therefore, in assessing the results of the epidemiologic studies, it generally is not possible to attribute observed disease excesses to a specific agent such as BD or styrene. Only one study has attempted to evaluate directly the relation between LHC and exposure to BD and styrene (28). The results of this investigation are discussed in detail later in the report. The other epidemiologic studies are reviewed first.

The studies discussed here report results for LHC as a whole, as well as for specific disease entities included in the LHC group, including leukemia, nonHodgkin's lymphoma (NHL), Hodgkin's disease and multiple myeloma. In

evaluating the possible effect of employment in the SBR industry and of exposure to BD , it is important to consider the consistency across studies of results for each specific disease, as well as for the aggregated LHC category. This is so because it is possible that each disease in the group has a different etiology. The observation of an excess of leukemia in one group of workers and of NHL in a second group of workers should not be interpreted as evidence of a common etiologic agent affecting both groups or of consistency between the two studies.

Contrary to OSHA's position, there is no basis for inferring that the category "all LHC" consists of diseases with a common etiology. It is a principle of epidemiology - and of disease investigation in general - that entities should be divided as finely as possible in order to maximize the prospect that one has delineated a homogeneous etiologic entity. Entities may be grouped for investigative purposes only when there is substantial evidence that they share a common etiology. Such evidence is usually both morphologic (based on histology) and epidemiologic. Only rarely would clinical observations justify grouping together potentially distinct entities.

The question of the etiologic heterogeneity of the LHCs is important, perhaps crucial, in the present context. I have therefore reviewed OSHA's position in some detail in Appendix B to this document. However, it is important to note here that the arguments advanced by OSHA to support its position that all LHC cancers are appropriately grouped are unconvincing.

The first argument is that, based on clinical difficulties in making distinctions, there is "general agreement" that the diseases "may represent"

stages of one disease. In fact, these entities are not distinguished clinically. The diagnosis of the individual case rests on histologic, not clinical, evidence. Further, OSHA's references (several textbooks) do not bear out the statements attributed to them. In fact, they clearly imply the reverse.

The second argument is that a gene lesion in a stem cell can cause leukemia. This is probably true. But, OSHA's inference that "Therefore, any form of leukemia, lymphoma, or possibly NHL/MMY may be possible as a result of exposure to a cancer-causing substance." is a gross overinterpretation and is not based on the publications cited.

Finally, not only are the various entities within "all LHC" heterogeneous with regard to etiology but, moreover, several of these entities are almost certainly etiologically heterogeneous even within themselves. For example, it is well recognized that both morphologically and epidemiologically, HD among young adults is quite different from HD among persons over age 50. In fact, for this particular disease, the clinical picture and treatment regimen are also different at different ages. Leukemia is another label that encompasses entities with different morphology, epidemiology and clinical courses.

On page 37748 of the Federal Register it is stated, "...OSHA is persuaded that leukemia need not have a common cell type to be significant and that the broad category of 'leukemia' provides sufficient detail for the purpose of these analyses." Such a statement identifies OSHA as singularly lacking in critical ability and as standing alone in its views.

Texas SBR Production

Meinhardt et al. (22) evaluated the mortality experience of men employed for at least six months in production-related jobs at two SBR production plants in Texas. The study included 1,662 workers with a follow-up period of 1943-1976 from "Plant A" and 1,094 workers with a follow-up period of 1950-1976 from "Plant B." The observed numbers of deaths among these men were compared with the numbers expected on the basis of the general United States (US) male population. For workers at both plants combined, there was a statistically significant 28% deficit of deaths from all cancer (56 observed/78 expected; SMR = 72; 95% CI = 54-93). SMRs for several major groups of cancer were as follows: digestive system, SMR = 58 (13/22; 95% CI = 31-99); lung, SMR = 85 (21/25; 95% CI = 53 - 130); genital organs, SMR = 110 (5/4.6; 95% CI = 36 - 256); urinary organs, SMR = 73 (3/4.1; 95% CI = 15 - 214); all LHC, SMR = 132 (11/8.3; 95% CI = 66 - 236); all other sites, SMR = 20 (3/15; 95% CI = 4 - 59).

The observed 32% increase in LHC deaths was neither large nor statistically significant. The 80% deficit of deaths from cancer of "all other sites" is a most exceptional finding but was not discussed by Meinhardt et al.

Examination of mortality patterns of LHC for workers at Plant A and Plant B revealed some differences between the two groups. Plant A workers had 5 observed/2.5 expected leukemia deaths, 3/1.7 lymphosarcoma and reticulosarcoma deaths, 1/0.9 Hodgkin's disease deaths and 0/0.8 other LHC deaths. None of these findings was statistically significant. An analysis of the subgroup of 600 men who had worked at Plant A in 1943-1945 ("a time which coincided with

process and operational changes" (22, p. 250)), found that all 5 of the leukemia deaths (1.8 expected) and all 4 of the other LHC deaths (2.5 expected) occurred in this subcohort. In contrast to these findings at Plant A, at Plant B there was only 1 observed and 1 expected leukemia death and 1 observed and 1.6 expected death from all other LHC's.

These data are more consistent with a random pattern of LHC occurrence in the two groups of workers, than with a causal association among Plant A workers. The overall results for leukemia and other LHC at Plant A were not statistically significant. Furthermore, according to Meinhardt et al., there was no "discernible pattern" of increasing LHC with longer duration of employment, as might be found if occupational exposures were responsible for cancer occurrence. In addition, for 2 of the 5 leukemia deaths at Plant A the interval between first employment and death was only about three years, an insufficient induction-survival time. Further, no excess leukemia deaths were seen among long term workers in this cohort. The reported excess among workers with extremely short follow-up without a corresponding or greater excess among long term workers (who also had longer follow-up interval), is not consistent with the action of an occupational carcinogen.

OSHA expressed the opinion that a three or four year period between first employment and death from leukemia was consistent with findings from studies of survivors of the atomic bombings and of benzene workers. However, in advancing this argument, OSHA did not address the lack of a leukemia excess among long term workers in the study and how that might affect the interpretation of a BD-leukemia association. Nevertheless, examination of the studies of atom bomb survivors and benzene workers does not support OSHA's conclusion

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that the excess of leukemia among workers with short employment and follow-up should be attributable to BD exposure. This issue warrants attention in detail.

In order to consider the follow-up (or latency) issue, some basic issues need to be kept in mind. Of course, a putative cause must precede an effect. Thus, the interval between the start of exposure and the diagnosis of disease can be characterized and may assist in understanding the action of chemical carcinogens. What is loosely referred to as the "latent" period (or induction period) actually encompasses two distinct stages: the induction period and the latent period (29). The induction period is the time during which occurs an exposure sufficient to "cause" the disease: cancer (leukemia in this instance) is then an inevitable outcome for workers who survive a sufficient time period. The true latent period is the time from causation until disease manifestation. In almost every epidemiologic study, the term "latent period" includes both the induction and latent periods - referring to the time from first employment until disease detection. In mortality studies, such as the Meinhardt study, there is also a third component. The latent period is incremented by the survival time after diagnosis. The survival time could be short or it could last many years, according to the type of disease under study (e.g. acute versus chronic leukemia). It should be remembered that occupational cohorts have a "natural" (or baseline) rate of leukemia mortality and that only mortality which exceeds the baseline should be interpreted as relevant to characterizing the latent period for a particular exposure-disease relationship.

The first example cited by OSHA are the studies of atom bomb survivors, some of whom developed leukemia as soon as 2 years after the atom bomb blast (30). Three points need to be considered to evaluate whether the findings from this cohort are analogous to the findings from the study by Meinhardt et al. (22). First, radiation is a physical agent that directly damages DNA. On the other hand, chemical agents usually require metabolic conversion to an active form prior to exerting carcinogenic affects. In general, on an exposure equivalent basis, this would reduce the effective dose and prolong the latent period for chemical exposures. Second, the earliest leukemia cases among atom bomb survivors received extremely high radiation doses - estimated to have been several hundred rem or more. These doses produced severe acute effects on the bone marrow before causing leukemia. In the SBR plants studied by Meinhardt, there is no evidence of catastrophic exposures to BD or of short-term effects on the bone marrow. Thus, the atom bomb survivor studies cited by OSHA have no utility for establishing a minimum latent period for a BD-leukemia relationship.

A second example cited by OSHA is the presence of short latent periods in studies of benzene exposed workers. Perhaps the most often cited benzene epidemiologic studies are the case studies by Aksoy (31) and the cohort mortality studies by NIOSH (32, 33), the Dow Chemical Company (21,34) and the Chemical Manufacturers Association (CMA) (35, 36, 37). Contrary to OSHA's conclusion, these studies show no evidence of leukemia excess with short latency among benzene exposed workers.

Case studies can be informative about the occurrence of a cancer hazard. However, such studies do not enumerate the total population at risk.

Therefore, the information on latency from a case series reflects the idiosyncrasies of individual persons rather than population-based characteristics that have etiologic importance. Nevertheless, the case reports cited in a recent review by Aksoy reflected chronic benzene exposure accompanied by periods of bone marrow depression prior to the development of leukemia (38). No mention was made of an excess of leukemia cases with short latent periods.

The NIOSH benzene cohort study involved 1165 pliofilm workers at two plants in Ohio (32, 33). As documented in the more recent publication, the latent periods for the leukemia decedents were 2, 3.5, 10.5, 13.5, 15.5, 17, 20, 22, and 37 years. The two leukemia cases who died less than 10 years after initial employment had very short employment and very low benzene exposures (0.1 ppm-yrs during 1 month employment, and 13.7 ppm-years during 18 months employment). Further, in contrast to the leukemias among long term, more exposed employees, neither of these leukemias was of the acute myelogenous type. Leukemia mortality was not elevated among short term workers, but was elevated among longer term workers. OSHA commented on this in the Final Rule on Occupational Exposure to Benzene (39) stating:

> "When the data was analyzed by length of employment, a significant excess in leukemia was observed among workers employed 5 or more years, but not among workers employed for less than 5 years."

Thus the data from this study do not demonstrate that benzene associated leukemias occur with short latency, but rather show a latent period of 10 or more years. It is also important to emphasize that the leukemia excess for

this cohort was marked among long term workers, a finding absent from the results of the Meinhardt SBR workers study.

Studies of 956 Dow Chemical Company benzene workers have detected five incident cases of leukemia to date (21, 34). For these cases, the latent periods were 11, 15, 15, 35, and 37 years. This further supports the view that benzene associated leukemias have at least a 10 year latent period.

Similarly, the Chemical Manufacturers Association inter-industry benzene study of 4607 workers at seven plants showed long latencies for leukemia. The seven leukemia decedents in this study had latent periods of 6.0, 14.3, 18.9, 28.6, 28.9, 29.7, and 49.4 years. The six year latency was for an individual with only intermittent and uncharacterized exposure for 1.4 years of employment. Notably, there was no indication of a leukemia excess among workers exposed to benzene less than five years (SMR = 88) and the fact that virtually all cases had a latency period of more than 14 years again suggest latent periods of at least 10 years for benzene related leukemias.

The benzene follow-up studies are consistent in showing a latent period for leukemia of at least 10 years. In each of these studies, leukemia is significantly elevated among long term workers, but not among short term workers - a finding opposite to that reported in SBR plants by Meinhardt. As such, the lack of a leukemia excess among long term SBR workers in the Meinhardt study is inconsistent with the findings from the benzene epidemiology studies and studies of other known carcinogens.

The atom bomb survivor and benzene worker studies provide a dubious basis for interpreting the Meinhardt SBR workers study. A much better comparison would be with the IISRP sponsored study of eight SBR plants (27). This study included more than 12,000 workers at eight SBR plants with mortality follow-up from 1943-1983 - a study roughly five times larger and with seven years longer follow-up than the Meinhardt study. It warrants mention that the designs and operating characteristics of all U.S. SBR plants were nearly identical (27), so the IISRP sponsored study involves conditions similar to those studied by Meinhardt.

In this larger study, there were 26 leukemias listed as the underlying or as a contributing cause of death among SBR workers. Among these leukemias, only one occurred with less than 10 years latency (4 years), six occurred with 10-19 years latency (13, 15, 15, 15, 17, 18 years, respectively), 12 occurred with 20-29 years latency, and the remaining seven occurred with 30+ years latency. There was no indication of a leukemia excess among workers with short latency in this study. Clearly, these findings suggest that the excess of leukemias with short latent periods in the Meinhardt study is atypical for SBR workers and, hence, is unlikely to be related to BD exposure from SBR manufacturing.

The concentration of leukemias among SBR workers with extremely short latency in the Meinhardt study is inconsistent with findings among other SBR workers and from studies of leukemia among benzene exposed workers. In fact, I have estimated that in the Meinhardt study the short term workers experienced a leukemia mortality rate five times higher than the rate among long term workers. This is unprecedented for known occupational carcinogens. As

such, there is little basis for concluding that the findings in the Meinhardt study are consistent with a leukemogenic effect for BD or for concluding that there is precedent from other epidemiologic studies to support such a hypothesis.

Finally, even if it were considered that the Meinhardt study showed an excess of leukemia and other LHC deaths, it would not be reasonable to attribute this to BD. Meinhardt's current measurements indicated a higher level of BD contamination in Plant B than in Plant A. There were no historical data on past exposures. However, the two factories underwent similar modernizations of processes over time and it is reasonable to assume that the relative exposures in the two factories have been about the same. It would then be reasonable to expect a higher number of cases among workers at Plant B than at Plant A if BD is the causative exposure. The absence of such a finding can not be attributed to a supposed short follow-up period among the Plant B cohort. The mean latency of the cases observed at Plant A was 17.2 years. A large number of subjects in the Plant B cohort (at least, those in the 1950-55 subcohort) were followed-up for twenty years or more and thus the period of follow-up was sufficient for some excess cases to occur. The lack of an excess strongly argues against BD being the causative agent.

In addition, in virtually all studies, the workers experienced some exposures outside the rubber industry as a result of either earlier or later employment in other industries. Since the studies did not identify the independent effects of such exposures, their contribution to the induction of cases can not be ruled out. Thus, the multiplicity of exposures makes the identification of a single substance as the causal exposure a speculation.

The discussion of the results of the BD epidemiology studies in the OSHA proposed standard fails to recognize the potential implications of the diverse findings seen in the various studies reviewed. The one common thread woven throughout all the studies discussed is the unusual finding that the majority both of leukemias and of lymphomas reported, occurred in individuals with short duration of employment and relatively early employment, i.e., they were first employed in the SBR or BD monomer industry during 1942-1945 or World War II. To dismiss these findings by claiming that such individuals must have had higher exposures does not adequately address the issue. For one thing, early in the SBR and BD monomer industry, the start-up employees were not new hires off the street--they were carefully selected, experienced petrochemical workers borrowed from area refineries and chemical plants to support a new industry vital to the war effort. They were neither young nor inexperienced, and their exposures were not necessarily high. In fact, many of the shorter duration employees were likely to have been skilled technicians needed for only a short period to train a more permanent workforce. This also implies that the prospect that these employees had a multiplicity of other chemical exposures during the majority of their work life is very great.

Divine (24) found an overlap of 122 persons between Texaco BD monomer plant workers and workers in another study of Texaco refinery, petrochemical, and research laboratory workers. It should be noted that Texaco was but one of many petroleum and petrochemical companies in the area providing workers for this wartime effort. In addition, 116 workers were employed at both the monomer plant and at the two SBR plants studied by Meinhardt (22), including one of the leukemia and one of the lymphosarcoma cases.

It is unjustified to suggest that short exposures to BD could cause an excess of deaths when no such excess is seen among persons with long duration exposures. Exposures to substances other than BD among workers who came from, and returned to, the petrochemical industry are likely to have included benzene and other organic solvents. This is not a trivial matter. The mortality patterns seen in the group of workers exposed to BD are very similar to those reported for the petrochemical industry as a whole, where elevations in various lymphohematopoietic neoplasms are seen and where mixed chemical exposures may well be contributing to the patterns found.

Further support for the argument that other chemical exposures are likely to have influenced the results seen for short duration early employees is the fact that in those studies where sufficient information is available, a comparison between short duration early employees and long duration early employees does not show evidence of a dose response.

Using the Meinhardt (22) plant A data, one can derive an estimated SMR for leukemia in employees with less than 3 years of employment in the period 1943-1945 of roughly 5 times that for longer duration employees hired during the same period. Such a result is very difficult to attribute to BD exposure.

Examining the cohort at the BD monomer plant (24), one finds that all but 9 of the LHC decedents were first employed before 1946. If one examines the SMRs for persons employed less than 10 years versus those employed 10 years or more, one finds that all of the SMRs for LHC are higher in those employed for the shorter period.

	Wartime Hires	
	Employed <10 yrs SMR	Employed >10 yrs SMR
Lymphosarcoma	370	161
Leukemia	148	112
Other Lymphoma	117	0
All LHC	169	93

Since both groups in the table above were hired at about the same time, it is reasonable to suppose that their exposures were similar and that those employed for longer periods had higher cumulative exposures to BD. Therefore, the above SMRs are contrary to any type of dose-response effect.

It has been suggested that newly hired workers are given the "dirtiest" jobs or those with the highest exposures. It is then hypothesized that persons who quit after a short employment were men with the highest exposures and the greatest adverse reactions to the exposures. If these short term workers truly had the highest exposures, then it might be reasonable for them to have higher SMRs for causes associated with exposures. A recent study by Stewart et al. (40), however, showed in another industry that exposures for workers employed less than one year were similar to early exposures for those employed greater than one year. Thus, any elevated SMRs in the group of short term workers is likely to be due to other factors such as lifestyle and other jobs.

The elevated SMRs for different lymphohematopoietic cancers in workers first employed in the BD monomer or SBR industry during World War II has been used to argue that these elevations are due to BD. It is generally acknowledged that wartime exposures were probably higher than those at later dates, and so, these higher exposures are supposed to have caused the excess LHC. However, persons with the highest overall exposures to BD, those first

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employed during the war and employed for most of their working lifetime are those with the lowest SMRs for LHC. Therefore, the results based on wartime exposures suggest that BD does not cause LHC.

Butadiene Manufacture

Downs et al. (23) studied 2,586 men employed for at least six months at the plant which manufactured BD for use at the two SBR plants investigated by Meinhardt et al. According to Divine (24), who later updated the Downs study, 116 of these men had also worked at the SBR plants. The BD production cohort was followed-up from 1943 through 1979, and its mortality experience was compared with that of the US and regional Texas general populations.

For the overall cohort, there were 122 deaths from all cancer combined, compared with 146 expected on the basis of US rates (SMR = 84) and 161 expected on the basis of regional Texas rates (SMR = 76). Forty-one of the observed cancer deaths were due to lung cancer, in contrast with expected numbers of 46 (US rates) and 62 (regional rates).

There were 7 leukemia deaths, compared with expected numbers of 5.9 (US rates) and 6.3 (regional rates). Men employed in 1943-1945 had 6 observed leukemia deaths, compared with expected numbers of 4.2 (US rates) and 4.5 (regional rates). The slight overall increase in leukemia was restricted to men with fewer than five years of employment at the plant (5 observed and about 2.8 expected) and to men with nonroutine, as opposed to routine, exposure to BD. None of these small differences was statistically significant.

For LHC other than leukemia, there were 14 observed and 8.6 - 8.8 expected deaths. This approximately 60% increase in deaths from other LHC was due mainly to an excess of lymphosarcoma and reticulosarcoma (8 observed vs. 3.4 - 4.4 expected). There was no clear pattern of an increasing excess of "lymphosarcoma" with increasing duration of employment. Of the 8 observed deaths, 6 were among men with less than 10 years of employment, compared to 1.7 expected, and 2 occurred among men with 20+ years of employment (0.7 expected). Moreover, the excess occurred primarily among men with both short (<10 years) duration of employment and with short (<10 years) induction times, whereas there was little evidence of an excess among longterm workers. The former subgroup had 4/0.33 lymphosarcoma deaths, in contrast to subjects with long duration and long induction times, who had 2 observed and about 1.4 deaths expected from this disease.

Divine (24) recently updated the study of Downs et al., extending follow-up through 1985. She found no difference between the observed and the expected number of leukemia deaths (8/7.9), indicating no excess of this disease among BD production workers. For all other LHC the SMR was 153 (95% CI = 89 - 246), based on 17 observed and 11 expected deaths. This elevated SMR was due entirely to an increase in deaths from lymphosarcoma and reticulosarcoma (9/3.9; SMR = 229; 95% CI = 104 - 435).

As in the earlier study, there was no difference between observed and expected numbers of lymphosarcoma deaths among longterm (10+ years) employees (2 observed/1.8 expected). Instead, the increase was concentrated in the subcohort of men with <10 years of employment (7 observed vs. 2.1 expected) and particularly among men with both <10 years of employment and <10 years

between first exposure and death from lymphosarcoma (4 observed vs. 0.3 expected). The presence of such a pattern detracts from a causal interpretation of the association.

Multi-plant SBR Production: Follow-up Studies

Matanoski and coworkers have completed two retrospective follow-up studies of workers at eight SBR production plants (25-27). The first of these studies (25,26) included 13,920 men employed for at least one year at any of the plants and followed-up from 1943-1970 (depending on the plant) through 1979. The second (27) extends the follow-up through 1982. Matanoski et al. (28) also have done a case-control study of 59 cases of LHC observed in the cohort of workers. The two follow-up studies report mortality patterns for the overall cohort and for subgroups specified on the basis of broad categories of last job held (production, utilities, maintenance, other). The case-control study, reviewed below, specifically evaluates exposure to BD, styrene and to certain work areas and processes.

The initial follow-up study by Matanoski et al. found no strong positive association between any type of cancer and employment in the SBR industry (25). The SMR for all cancer was 84, based on 398 observed deaths. SMRs for gastrointestinal cancer (the category that had stimulated the study) were slightly elevated in some subgroups. However, such a pattern was not apparent in the updated follow-up study, and the result is not discussed further. The lung cancer SMR was 85, based on 126 observed deaths.

For the overall cohort, observed/expected numbers of LHC deaths were 17/19 for leukemia, 5/10 for lymphosarcoma and reticulum cell sarcoma, 8/6.7

for Hodgkin's disease and 10/11 for other LHC. That is, there was an inverse association between employment as an SBR worker and mortality from LHC (40 observed/47 expected). Also, there were no notable or statistically significant excesses or deficits of these diseases within the production, utilities, maintenance or other subcohorts. Among production workers, who would have had the highest sustained exposures to BD and styrene, there were 3 leukemia deaths observed and about 4 expected. The authors stated, "if there is an increased risk of leukemia in rubber manufacture...it would not seem to be related to an exposure to styrene or butadiene" (26, p. 23).

Matanoski also reported that deaths from other LHC were increased among production workers (5 observed vs. 2.5 expected). This result is difficult to interpret for two reasons. First, the observed and expected numbers in this disease category are quite small, and the difference is not statistically significant. Second, production workers probably had a deficit of deaths from lymphosarcoma, a disease which tends to be confused with other LHC because both categories contain nonHodgkin's lymphoma. Unfortunately, Matanoski did not present SMRs for all the relevant specific forms of cancer. However, for a combined category consisting of all lymphatic cancers other than leukemia and Hodgkin's disease, there were 6 observed and 4.9 expected deaths among production workers, a null result.

The update of this study included 12,110 subjects with an average of 21 years of follow-up. The mortality experience of these subjects was generally similar to the patterns reported by Downs et al. (23) and Divine (24). There was a deficit of deaths from all cancer (SMR = 85; 95% CI = 78 - 93). The only statistically significant results for specific cancers were deficits of

oral cancer (SMR = 26; 95% CI = 8 -60) and of respiratory cancer (SMR = 84; 95% CI = 72 - 98).

Observed and expected numbers of LHC deaths were similar overall (55/57) and were 22/23 for leukemia, 8/6.6 for Hodgkin's disease, 7/11 for lymphosarcoma and 17/15 for other LHC. An increase in leukemia deaths was reported among black men. However, this result was based on only 4 observed and 1.8 expected deaths, and it is possible that the expected number of leukemia deaths is slightly underestimated because subjects whose race was unknown (including, no doubt, some blacks) were classified as white (see 27, p. 16).

Matanoski et al. also analyzed mortality patterns by work area (determined on the basis of the job each subject held for the longest time). The interpretability of these analyses is limited because almost 20% of the cohort was excluded due to missing data on job assignments and because there was no leukemia in the excluded group. Thus, contrary to OSHA's position the exclusion of these data does not explain Matanoski's null results. Rather, their inclusion would make the study even more negative. Matanoski found that observed/expected numbers of leukemia deaths were 7/5.2 for production workers, 6/7.6 for maintenance workers, 2/1.0 for utilities workers and 7/5.6 for workers in other areas. Production workers had more deaths than expected from other LHC (9/3.5). However, this excess was balanced to some extent by a deficit of lymphosarcoma (1/2.6), and the SMR for these two disease categories combined was 164 (95% CI = 79 - 302), based on 10 observed and 6.1 expected deaths among production workers. There also was an elevated SMR of 656 (95% CI = 135 - 1906) for leukemia, based on 3 observed and about 0.5 expected deaths, among subjects classified as black production workers. The subsequent

case-control study, however, found no, or even an inverse, association between employment in production and leukemia. Data on the association of other types of LHC with work areas were not reported in the latter study.

Multi-plant SBR Production: Case-control Study

The purpose of the case-control study by Matanoski et al. was to evaluate the relation between LHC and exposure to BD, styrene and specific jobs among the SBR workers included in the follow-up study described above. The case-control study included 59 cases (26 leukemia, 13 NHL, 8 Hodgkin's disease, 10 multiple myeloma and 2 other LHC) and 193 controls selected from among other cohort members.

The major finding of the study was a 7- to 9-fold increased rate of leukemia among workers exposed to BD. There was no association of leukemia with styrene, after the effects of BD were taken into account, and there was no strong association between either of these chemicals and any other type of LHC. Possible interpretations of the major, positive finding are that it is due to chance, to confounding, to some form of bias or to a causal relation between BD and leukemia. These possible explanations are discussed in detail because these are the only seemingly clearly positive results in the epidemiologic literature. Yet, they are contrary to the results of the population-based study from which they derive.

Chance - The reported association between BD and leukemia was statistically significant at the 5% probability level. However, any association can be due to chance, whether or not it is statistically significant, and several

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aspects of this association should be considered in evaluating the role of chance in the case-control study.

First, when subjects were classified as "ever versus never" exposed to BD, the RR of leukemia was 6.8, with a 95% confidence interval of 1.1 to 42. This confidence interval is wide, indicating that the estimated RR is statistically unstable or imprecise. Because of this instability, relatively small data errors, such as an error in exposure classification, could have a large impact on observed results. For example, only one case was "never exposed." Thus, if only one of the exposed cases were to be reclassified as not exposed, a reanalysis of these data would find a statistically nonsignificant RR of 3.3. Therefore, the analysis of the association between BD and leukemia, when BD exposure is considered as "ever versus never," is not convincing.

The study evaluated many associations between LHC and various exposures. Several types of analyses were done for each of four cancer sites, for BD and for styrene exposure. Also, for leukemia, analyses were done for seven job groups, as well as for BD and styrene. It would not be unusual for one of the results of this large number of analyses to be statistically significant by chance. However, on balance, all of the different types of analysis point to a positive association between BD and leukemia. Although the RRs for this association are imprecise and the magnitude of the apparent effect of BD is thus uncertain, this association probably is not due entirely to chance.

Confounding - There is no obvious source of confounding which has been overlooked or ignored in this study and which could account for the observed

association between BD and leukemia. However, certain data presented in the report suggest the possibility of confounding by unidentified factors.

First, cases and controls were on average 36 years of age when they started working at the plants under study, and they worked in the SBR manufacturing industry for an average of only about 15 years. Therefore, many of the subjects probably worked in other industries at least as long as they worked in SBR manufacturing. It is possible that subjects exposed to BD tended to have prior or subsequent occupational exposure to leukemogenic agents more often than subjects unexposed to BD and that these other unidentified exposures are responsible for the reported association between BD and leukemia. However, no data are available to evaluate this possibility.

Second, Matanoski et al. report associations between leukemia and several jobs which associations persist after controlling for BD exposure (28, tables 19 and 20). They suggest that these associations are the result of misclassification of BD exposure. However, an alternative interpretation is that there are unidentified leukemogenic exposures which are correlated with certain jobs, and that confounding by these exposures is not controlled for in analyses which adjust the BD and leukemia association for job assignments. Again, however, it is unlikely that this source of confounding could be completely responsible for the observed association of BD and leukemia.

Bias - Potential sources of bias are differential misclassification of exposure and selective inclusion of cases or of controls whose BD exposure is higher or lower than that of all eligible cases and controls. Exposure classification was done "blind", that is, without knowledge of subjects'

status as a case or a control. Therefore, it is unlikely that bias due to systematic classification errors can explain the positive results for BD and leukemia.

Matanoski et al. state that controls were matched to cases on year of hire and duration of employment. A review of the study data indicates that, in fact, 43 of the leukemia controls had shorter durations of employment than their corresponding cases, with the differences ranging from 1 to 55 months (average, 16 months), and that 37 of the controls had longer durations, with differences ranging from 1 to 241 months (average, 28 months). [If two case/control sets which appear to contain erroneous data showing that controls worked 155, 164, 166 and 241 months longer than their corresponding cases are removed from these computations, there remain 33 controls with longer duration of employment than their cases, ranging from 1 to 40 months (average, 9 months).] These differences between cases and controls in total duration of employment would have biased the study in the positive direction, perhaps substantially.

Other considerations - There are four important inconsistencies among the analyses within the case-control study and between the case-control study and the follow-up study. These inconsistencies reduce the interpretability and persuasiveness of the work by Matanoski et al. and argue against the main conclusion, i.e., that there is a strong, causal relationship between BD and leukemia.

1) In the case-control study an analysis was done to evaluate whether the association of leukemia with the "log cumulative rank" of BD and with the

cumulative log rank of styrene, with both exposures classified as continuous variables, exhibits a dose-response pattern. The presence of a dose-response pattern in an epidemiologic study is usually viewed as important evidence that an observed association is causal. The analysis done by Matanoski et al. found a positive association, of borderline statistical significance, with BD (p=.046) and with styrene (p=.054), but neither of these remained statistically significant after control for the possible confounding effect of the other. The authors attribute the weakness of the associations found in these analyses to the fact that the relation between leukemia and the log cumulative rank of exposure is nonlinear. This may be correct. Alternatively, the apparent associations observed in this set of analyses may be due entirely or in part to chance.

2) A key analysis of the case-control study classified subjects' exposure to BD and to styrene as being above or below the mean log cumulative rank for the two series of subjects combined. The RRs for BD were 5.2 (95% CI = 1.6 - 17) from a crude analysis, 9.4 (95% CI = 2.1- 23) from a matched analysis and 7.6 (p=0.01) from a conditional logistic regression analysis. The latter RR was almost unchanged after adjusting for styrene exposure, and there was no independent association between leukemia and styrene in this analysis. However, when the data of Matanoski et al. are analyzed using raw, rather than log-transformed, cumulative exposure scores, the estimated RR is 0.8 (95% CI = 0.3 - 2.2). Finally, when the same data are analyzed using the dichotomized raw exposure score categories of above vs. below the median, rather than the mean, score of controls, the crude RR is 3.3 (95% CI = 1.3 - 8.9).

The discrepancies among these analyses underscore the desirability of avoiding a dichotomous categorization of exposure in favor of finer categories. The use of finer categories also allows a further evaluation of dose-response patterns. To do this, we specified exposure categories by dividing the control distribution of cumulative scores into tertiles. We then computed the crude RR for each level of exposure, using the lowest tertile as the referent category. The results are:

Tertile	Cases/ controls	RR	(95% CI)
1	3/28	1.0	-
2	16/28	5.3	(1.5-19)
3	7/28	2.3	(0.6-9.8)

If subjects with no BD exposure are excluded, and the dose-response pattern only among exposed subjects is examined, the impression remains unchanged. The results of all such analyses are consistent with a positive association between BD and leukemia, with an irregular dose-response pattern. The association is unstable because of the small number of "unexposed" cases (3 in the tertile analysis). Matanoski et al. found a similar irregular pattern of RRs in an analysis using seven exposure levels in addition to the referent category of no exposure. The absence of a clear dose-response pattern in these analyses reduces the credibility of the observed association between BD and leukemia.

3) Matanoski and coworkers have stated elsewhere that in the SBR industry, BD exposure tends to be highest in the production area (26, p. 23). Yet, the case-control study found an inverse association between duration of employment in production and leukemia (RR=0.58, p=0.33) (28, table 19). Matanoski et al. offer no explanation for this.

4) The follow-up study on which the case-control study is based found no relation between employment in the SBR industry and leukemia (SMR = 96; 22 observed versus 22.8 expected leukemia deaths) (27). The discrepancy between this result and the strong positive association reported for BD and leukemia in the case-control study is difficult to explain and detracts seriously from a causal interpretation of the latter finding. Remarkably, Matanoski et al. do not comment on this striking discrepancy. In our view, the possible explanations include:

a) random or systematic errors in the case-control study;

b) a spuriously elevated RR in the case-control study due to a surprisingly low leukemia rate among BD non-exposed subjects and a slightly increased leukemia rate in the BD-exposed subjects;

c) a combination of some of the above factors.

It is important to recognize that the overall leukemia SMR of 96 in the follow-up study is a blend of the SMRs of a BD-exposed subcohort and of an BD-unexposed subcohort. These two exposure-specific SMRs have not been reported but can be estimated using the following assumptions:

a) 19 (88%) of the 22 leukemia deaths observed in the follow-up study were exposed to greater than the mean log cumulative rank of BD, and 3 (12%) were unexposed (these percentages are the same as reported for exposed and unexposed cases in the case-control study);

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b) the expected numbers of leukemia deaths were 13.7 in the exposed subcohort in the follow-up study (60% of the total of 22.8 expected leukemia deaths) and 9.1 (40%) in the unexposed subcohort (this is based on the fact that among the leukemia controls in the case-control study, 60% were exposed, and 40% were unexposed to BD above the mean log cumulative rank).

Application of these assumptions produces a leukemia SMR of 139 (19 observed/13.7 expected) (95% CI, 84 - 217) in the BD-exposed subcohort and an SMR of 33 (3 observed/9.1 expected) (95% CI, 7 - 96) in the unexposed subcohort. We note that the estimated excess of leukemia among exposed subjects is small and not statistically significant. In contrast, the estimated deficit of leukemia among the unexposed subjects is both large and statistically significant. We also note that if a nested case-control study were done based on these 22 cases (exposed and 3 unexposed) and 88 controls (4 per case), 60% of whom are exposed and 40% of whom are not, the crude RR would be 4.2. This result is quite similar to the crude RR of 5.2 for BD exposure reported by Matanoski et al. in their case-control study.

The above analyses are somewhat speculative, and their validity depends on the degree to which the exposure frequency of controls is representative of the exposure frequency of the total cohort. If a large deficit of leukemia deaths, in fact, were present among unexposed subjects in the follow-up study, the reason for it is unknown. Such a deficit may be due to chance, to systematic misclassification of BD exposure or to a real difference in determinants of leukemia between the unexposed workers and both the US population and the exposed workers. The last explanation - that the BD-exposed and unexposed subcohorts differ with respect to other, rather strong causes of leukemia - is

implausible. In any case, the most probable explanation for the discordance between the follow-up and case-control study results is that there was a small excess of leukemia among BD-exposed subjects in the follow-up study and a large deficit among unexposed subjects, and that the marked association between BD and leukemia in the case-control study is due primarily to the deficit of leukemia among the unexposed subcohort.

If the above explanation is correct, the positive association reported in the case-control study is noncausal and is due, instead, to random error (chance) and, perhaps, to one or more unidentified systematic errors.

FINAL REMARKS

In the aggregate, the follow-up studies of SBR workers include 17,448 subjects with an average of 22 years of follow-up (22,24,27). The combined results of these studies include 36 observed leukemia deaths, compared with 34.1 expected. The aggregate SMR is 106, with a 95% CI of 74 - 146. These null data suggest that there is no relationship between employment in the SBR industry and leukemia. Differences between the observed and expected numbers of deaths from other forms of LHC also are small: 20 observed vs. 17.8 expected deaths from lymphosarcoma and reticulosarcoma, 12 vs. 10.2 for Hodgkin's disease and 22 vs. 21.6 for other forms of LHC. Only one study has evaluated the relation between BD, per se, and leukemia. This study reports a positive association. However, the association can not be interpreted as causal because of the high likelihood that it is due, at least in part, to error.

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APPENDIX A: STUDIES IN RELATED INDUSTRIES

McMichael et al. (A-1)

This case-control study examined several types of cancer occurring in a cohort of 6,678 rubber workers during the period of 1964-1973. The study included a comparison of the detailed job histories of 51 workers who died of LHC with the histories of 1,482 controls. In one analysis, relative risks (RR) were estimated for workers employed for five or more years in the "synthetic plant," where SBR was made, in comparison to workers employed elsewhere at the rubber manufacturing complex. Elevated RRs of 6.2 and 3.9 were reported for all LHC and for lymphatic leukemia, respectively. The interpretation of these results is unclear for several reasons. Only 4 men with LHC and 1 with lymphatic leukemia had worked at the synthetic plant (A-2), and the reported elevated RRs are, therefore, quite imprecise. In addition, each of 4 cases had a different type of LHC (nonHodgkin's lymphoma, lymphocytic leukemia, chronic myelocytic leukemia and Hodgkin's disease), and Spirtas et al., in a subsequent study based on the same cases, reported a much lower LHC RR of 2.5 for employment of at least five years in the synthetic plant (A-3). Finally, two other synthetic rubber plants were later investigated by the same group of researchers, and no association with LHC was found in those plants (A-2).

Andjelkovich et al. (A-4)

This investigation evaluated the mortality experience of synthetic latex manufacturing workers as part of a study of disease patterns in the rubber industry. The number of workers included in this analysis was not stated but was apparently small. There were only 12 deaths from all causes combined (10 expected) and only 3 cancer deaths (2.2 expected) among the synthetic latex

workers. The 3 cancer deaths were due to lung cancer (0.7 expected). No LHC death was observed, compared with about 0.2 expected. [The expected number of LHC was not reported and is estimated here as 10% of the 2.2 expected total cancer deaths.]

Ott et al. (A-5)

This was a study of workers involved in the development or manufacture of styrene-based products, 391 of whom had been employed in the production of styrene-butadiene latex and thus had potential exposure to BD. No death from leukemia was observed in this group during the follow-up period, from the early 1940s to 1975. The number of leukemia deaths expected on the basis of general population mortality rates was not stated but was probably about 0.3. Thus, this study, like the investigation by Andjelkovich et al., is not very informative about the relation between BD and leukemia or other LHC.

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APPENDIX B: THE HETEROGENEOUS ETIOLOGY OF THE LYMPHATIC-HEMATOPOIETIC CANCERS

I have reviewed the relevant citations from the Federal Register (p. 32746) used by OSHA including Wintrobe's Clinical Hematology (B-1) and Dameshek and Gunz's Leukemia (edited by Henderson et al.) (B-2). Each text acknowledges the difficulty in forming a precise, categorical delineation of the lymphohematopoietic cancers (LHC), and the possible morphologic conversions of some of the diseases. However, they all accept the heterogeneous nature of these diseases in terms of etiologic, clinical and morphological variations. As emphasized by Wintrobe (p. 1453), Dameshek's suggestion that these diseases be termed "myeloproliferative or lymphoproliferative" disorders according to whether they originate from myeloid or lymphoid elements was meant only to imply a broad descriptive concept. Wintrobe categorically asserts that "the causes of these diseases may differ" (p. 1471) and contends that "few authorities would disagree concerning the recognition of chronic myeloid leukemia (CML), acute myeloid leukemia (AML), polycythaemia vera (PV), idiopathic myelofibrosis (IMF), chronic lymphocytic leukemia (CLL), acute lymphoblastic leukemia (ALL), Hodgkin's disease, the non-Hodgkin's lymphoma (NHL), multiple myeloma (MM), and macroglobulinemia as distinct entities" (p. 1449). Even a subgroup such as AML is observed to be heterogeneous both in its clinical manifestation and possible causation (B-2, p. 49).

No textbook with Jaffe and Costan as co-authors or editors could be located using computer and catalog searches, despite OSHA's reference to such a book. In Jaffe ES, Surgical Pathology of the Lymph Nodes and Related Organs (B-3), no direct reference is made to the etiology of the LHCs, but they are

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appropriately discussed as distinct entities in their histopathologies. Similarly, Costan Berard classifies and discusses the lymphomas as distinct diseases with distinct subgroups based on their varied morphology, clinical presentation and prognostic factors in his book Malignant Lymphomas (co-edited with RF Dorfman) (B-4).

Other textbooks such as Harrison's Principles of Internal Medicine (B-5), Cecil's Textbook of Medicine (B-6) and Thompson's A Concise Textbook of Hematology (B-7) discuss these diseases as distinct entities.

The recent studies using chromosomal banding cited by OSHA (p. 32746) suggest only that many of the LHCs are derived from primitive stem cells and that it is common for two or more diseases to share a common chromosomal defect. There is no indication that differentiation of a "neoplastic stem cell" is a random process or that "any form of leukemia or possibly NHL/MNY may be possible as a result of exposure to a cancer causing substance." As succinctly put by Yunis, "Although the same stem cell is believed to be affected in these three disorders (CML, ALL, ANLL), there may be additional mechanisms that affect the differentiation of the cell lineage and result in different hematologic pictures of CML, ALL, ANLL" (B-8). Thus the "multipotent leukemia precursor...responds with ordered differentiation and maturation," (B-9) and the factors determining the specific expression operate most notably in the differentiated environment (B-10).

In fact, not all LHCs are derived from the same stem cell. While the pluripotent stem cell is almost certainly the site of onset for most cases of CML and possibly for PV and IMF, it seems unlikely that AML begins in the

totipotent compartment (B-1). Recent studies even suggest the existence of marked immunological heterogeneity at the level of the leukemic progenitor cells (B-11).

The epidemiology of the LHCs also supports their etiologic heterogeneity. The causal association of ionizing radiation with LHC is established. But as was noted by Bizzozero, et al. (1966) (B-12) long ago, "at no point has the effect of radiation succeeded in the induction of chronic lymphocytic leukemia" although significantly higher rates of acute lymphocytic, monocytic and myelomonocytic, and chronic granulocytic leukemias were observed following the atomic bombings of Hiroshima and Nagasaki. This is clearly inconsistent with a carcinogen having the capability of giving rise to any form of leukemia, lymphoma or possibly NHL/MMY. Nor can the extraordinary rarity of CLL in oriental countries and persons of oriental ancestry living in other countries (B-13), the comparatively low incidence of CLL in children (B-1), the association of CGL in Down's syndrome, and the preponderance of CLL in the rare instances of familial leukemia be viewed as consistent with a homogeneous etiology. And neither can the rarity of case reports of chronic myeloid or lymphocytic leukemia following exposure to benzene and such drugs such as chloramphenicol, hexachlorocyclohexane, phenylbutazone in contrast to the frequency of occurrence of AML be reconciled with such a theory (B-14, B-15).

Indeed, all these observations, coupled with the lack of association between the rate of increase of the various LHCs (e.g., a significant increase in childhood leukemia in Israel between 1950 and 1960 while the lymphoma rate remains constant; a rising NHL incidence in the face of a stable HD incidence in the USA), point towards a heterogeneous etiology.

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The bimodal age incidence curve of Hodgkin's disease and the different sex ratio in the two age-groups is not paralleled by any other LHC. This bimodal pattern coupled with the different histologic subtypes in the two age-groups suggests that Hodgkin's disease itself consists of at least two distinct etiologic entities (B-17, B-18).

The markedly increased incidence of NHL, but not of the leukemias, in therapeutically immunosuppressed subjects points towards a varied etiology. And even within the NHLs, the different patterns of the rate of rise of the reticulum cell sarcoma and the lymphosarcomas and their varied susceptibility to chemotherapy all suggest that their differences from other LHC and from each other is more than morphologic.

It can be concluded that the LHCs are distinct diseases and that their causes are likely to be heterogeneous and multifactorial. Such causes most likely interact in a causal web leading to a lesion or transformation in the genetic material of the stem cell. The observed differences in the various forms of the cancers, in spite of the occasional transformation seen in some, has an underlying etiologic difference. As emphasized by Wintrobe, "the development of a second type of hematologic neoplasm in the absence of cytotoxic therapy has many possible explanations other than that the original disease has arisen in a stem cell compartment pluripotent for both diseases. For example, the development of a lymphoid neoplasm might reflect increased susceptibility to neoplasia because of immunosuppression or in the case of any of the diseases under discussion, this might reflect a genetic instability of stem cells in general."

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Principles of epidemiologic research	1985
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International Agency for Research on Cancer	
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Associate Editor, American Journal of Epidemiology	1982-88
Editorial Board, International Journal of Breast and Mammary Pathology	1984-
Editorial Board, Fundamental and Applied Toxicology	1984-
Editorial Board, Southern Medical Journal	1990-

Publications: See attached list.

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An Update on Mortality among Workers at a 1,3-Butadiene Facility—Preliminary Results

by Barbara J. Divine*

This is a cohort study of 2582 male workers employed at least 6 months between 1943 and 1979 at a 1,3-butadiene manufacturing facility. An earlier report on mortality through 1979 found a statistically significant deficit for all causes of death and lower than expected mortality for most of the leading causes of death. However, there was a statistically significant excess of deaths from lymphosarcoma. This report is a preliminary update of cohort information through 1985 and also a reanalysis of mortality. The all-causes standardized mortality ratio is 84 and that for all cancers is 80. These are statistically significant deficits; significant deficits were also seen for all cancer of the digestive system and all external causes of death. One additional death from lymphosarcoma was observed during the extended follow-up period giving a statistically significant standardized mortality ratio (SMR) of 229. The increase was concentrated in those employed less than 10 years and in those first employed before 1946. No increase was seen overall for leukemia (SMR = 102).

Introduction

This study reports on updated cause-specific mortality in a cohort of workers employed at a 1,3-butadiene production plant. The plant was built during World War II in Port Neches, TX, to supply 1,3-butadiene to two adjacent rubber manufacturing facilities. This cohort was the subject of an earlier report (*1*) that covered the period from the beginning of plant operation through 1979. The cohort consisted of all males who were employed at the plant for at least 6 months between the time the plant began operation in 1943 and the end of 1979.

The earlier report found a statistically significant deficit for all causes of death and lower than expected mortality for almost all the leading causes of death. The only significant excess cause of death seen was for lymphosarcoma and reticulum cell sarcoma. The cohort was divided into four exposure groups (routine, nonroutine, low, and unknown) and mortality was examined for each of the groups. The standardized mortality ratios (SMRs) for non-Hodgkin's lymphomas (International Classification of Diseases codes 200, 202, 203, and 208) were elevated in all three known exposure groups. Direct comparisons between the low exposure group and each of the exposure groups for this cause of death were inconsistent. Because of the continuing interest in determining whether 1,3-butadiene is a human carcinogen, information on the original cohort was updated through 1985.

Methods

The study population is the same as that in the earlier report (*1*). The data were obtained from the previous investigators and merged with company computerized personnel files. Company data were used to update information on those persons still employed or known to be deceased. Dates of termination were obtained for those who left after the previous study end date. Further edits were done, and two duplicate records and two females were removed from the cohort.

Information was obtained from the Social Security Administration (SSA) for those whose vital status was unknown. This included all persons not known to be currently working for the company or those noted as deceased but for whom a death certificate was not located. SSA data were complete through 1985 although a number of deaths in 1986 and 1987 were also identified. Death certificates were requested from the states identified by SSA as most likely to have the information. At the time of this report, death certificates had been received from Texas, Louisiana, Ohio, and Mississippi. All death certificates were coded for underlying cause of death by a trained nosologist according to the Eight Revision rules of the International Classification of Diseases (ICD). Information on those whose vital status was unknown after the SSA search was submitted to the

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National Death Index (NDI). The NDI records were searched for possible matches for deaths from 1979 through 1986.

The qualitative exposure classification scale developed for the previous report (*1*) was again used. There were four exposure groups: low exposure, routine exposure, nonroutine exposure, and unknown exposure. Each person was assigned to an exposure group based on his department code. Although no industrial hygiene sampling data are available for most of the period covered by the study, a review of current sampling data results supports the groupings.

Analysis of mortality was performed using Monson's computer program (*2*) to compare the observed and expected number of deaths with the U.S. white male population as the comparison group. White male rates were used because of uncertainties about the validity of the race information in the company files and because of the small number of blacks who worked at the plant. Person-years were accrued beginning when the person met the cohort eligibility criteria of 6 months of employment. Those persons with vital status unknown were assumed to be lost to study on the date last observed. Those persons who were alive as of the earlier study end-date of 12/31/79 and for whom the NDI could find no match were assumed to be alive. Persons known to be deceased but for whom no death certificate could be located were counted in the all-causes mortality but not in the cause-specific mortality results.

It should be noted that while the previous report (*1*) grouped all the non-Hodgkin's lymphomas together in the tables (ICD codes 200, 202, 203, and 208), for this report lymphosarcoma (ICD code 200) and cancers of other lymphatic tissue (ICD codes 202, 203, and 208) are reported separately.

Results

The final cohort consisted of 2582 men who had worked for at least 6 months prior to the end of 1979. Table 1 shows the demographic and other characteristics of the total cohort and of the four exposure cohorts described above. There were 74,219 person-years of follow-up, and the mean duration of survival was 28.7 years. There were 826 deaths known to have occurred in the cohort through 1985, and death certificates were obtained for all but 49 (6%) of them. There were 1708 persons still alive at the end of the study (66%) and 48 (1.9%) lost to follow-up.

Table 2 shows the SMRs for selected causes of death for the total cohort and for those employed greater than 5, 10, and 20 years. The SMR for all causes of death for the total cohort is significantly low at 84, as well as the all-cancer SMR at 80. Deficits are again seen for almost all the leading causes of death. There is an elevated SMR of 130 for lymphatic and hematopoietic cancer, which is almost entirely due to the significantly increased SMR of 229 for lymphosarcoma and reticulosarcoma. The increase for lymphosarcoma is seen primarily in those employed fewer than 10 years. The only cause of death that increases as years of employment increases is cancer of the kidney, but both the observed and expected

Table 1. Demographic, employment, and other characteristics of the study population and exposure cohorts.

Cohort	Low exposure I	Routine exposure II	Nonroutine exposure III	Unknown exposure IV	Total
Number of persons Percent of total	433 (16.9%)	705 (27.3%)	993 (38.5%)	451 (17.5%)	2582 (100%)
Mean age of entry, years	30.7	27.5	32.5	32.0	30.7
Mean year of entry	1954	1952	1951	1949	1952
Number hired between 1940–45 Percent of cohort	165 (38%)	216 (31%)	462 (47%)	223 (49%)	1066 (41%)
Mean years of employment	12.4	12.4	11.6	14.3	12.4
Number of lost to follow-up Percent of each cohort	6 (1.4%)	6 (0.9%)	26 (2.6%)	10 (2.2%)	48 (1.9%)
Number of person-years	11851.7	21423.1	27433.8	13512.8	74218.8
Number of deaths Percent of deaths in each cohort	131 (30.0%)	163 (23.1%)	361 (36.4%)	171 (37.9%)	826 (32.0%)
Number without death certificates Percent of deaths	11 (8.4%)	9 (5.5%)	24 (6.7%)	5 (2.9%)	49 (6.0%)
Mean age at death, years	64.1	59.2	64.2	65.3	63.4
Mean year of death	1973	1973	1970	1971	1971
Mean duration of survival, years	27.4	30.4	27.1	30.0	28.7

Table 2. Observed and expected deaths and SMRs for selected causes for cohort members by length of employment.

Cause of death (8th ICDA[a])	Total cohort n = 2582, P-Y = 74219				Employed > 5 years n = 1362, P-Y = 33959				Employed > 10 years n = 1096, P-Y = 24167				Employed > 20 years n = 781, P-Y = 11634			
	Observed deaths	Expected deaths	SMR[a]	95% CI[a]	Observed deaths	Expected deaths	SMR	95% CI	Observed deaths	Expected deaths	SMR	95% CI	Observed deaths	Expected deaths	SMR	95% CI
All causes	826	979.6	84	79–90	433	516.6	84	76–92	354	440.8	80	72–89	200	258.6	77	67–89
All cancers (140–209)	163	202.7	80	69–94	83	109.0	76	61–94	69	94.9	73	57–92	47	61.5	76	56–102
Cancer of buccal cavity and pharynx (140–149)	5	6.1	82	26–190	3	3.3	92	18–268	2	2.8	71	8–256	2	1.8	110	12–398
Cancer of digestive system (150–159)	39	56.3	69	49–95	16	30.2	53	30–86	15	26.1	58	32–95	11	16.0	69	34–123
Cancer of esophagus (150)	3	4.8	63	13–184	0	2.6	0	0–143	0	2.2	0	0–164	0	1.5	0	0–248
Cancer of stomach (151)	4	10.2	39	11–100	0	5.4	0	0–68	0	4.5	0	0–82	0	2.4	0	0–151
Cancer of large intestine (153)	18	18.7	96	57–152	11	10.1	109	54–195	10	8.9	113	54–208	7	5.6	124	50–256
Cancer of rectum (154)	3	6.1	49	10–144	2	3.2	62	7–223	2	2.8	73	8–262	1	1.6	63	1–353
Cancer of liver (155–156)	1	4.3	24	0–131	0	2.3	0	0–162	0	1.9	0	0–190	0	1.2	0	0–319
Cancer of pancreas (157)	9	10.9	83	38–157	3	5.9	51	10–149	3	5.2	58	12–170	3	3.3	91	18–266
Cancer of respiratory system (160–163)	57	69.8	82	62–106	30	37.9	79	53–113	24	33.5	72	46–106	17	23.5	72	42–116
Cancer of the larynx (161)	4	3.0	136	37–347	0	1.6	0	0–232	0	1.4	0	0–266	0	0.9	0	0–407
Cancer of the lung (162–163)	53	66.2	80	60–105	30	35.9	83	56–119	24	31.8	75	48–112	17	22.4	76	44–122
Cancer of bone (170)	1	0.9	114	1–635	0	0.4	0	0–837	0	0.4	0	0–1050	0	0.2	0	0–2006
Cancer of skin (172–173)	3	3.5	86	17–252	1	1.8	55	1–304	1	1.5	66	1–365	1	0.9	108	1–602
Cancer of prostate (185)	10	14.6	69	33–126	8	8.1	99	43–195	8	7.3	110	47–216	4	4.4	91	25–234
Cancer of bladder (188)	3	6.2	49	10–142	2	3.4	59	7–214	2	3.0	67	8–242	2	1.8	111	12–400
Cancer of kidney (189)	5	5.0	100	32–234	4	2.7	150	40–383	4	2.3	173	46–442	3	1.5	198	40–578
Cancer of brain and central nervous system (191–192)	4	5.7	70	19–179	3	2.9	102	20–298	2	2.4	83	9–299	1	1.5	66	1–367
Lymphatic and hematopoietic cancer (200–209)	25	19.2	130	84–192	11	10.1	109	54–195	6	8.6	70	26–152	3	5.2	58	12–169
Lymphosarcoma and reticulosarcoma (200)	9	3.9	229	104-435	5	2.0	245	79–572	2	1.7	116	13–420	2	1.0	205	23–741
Hodgkin's disease (201)	3	2.1	141	28–413	1	1.0	99	1–551	1	0.8	134	2–743	0	0.4	0	0–1028
Leukemia and aleukemia (204–207)	8	7.9	102	44–200	2	4.2	48	5–174	1	3.5	28	0–158	0	2.1	0	0–176
Other lymphatic tissue (202, 203, 208)	5	5.1	97	31–227	3	2.8	106	21–311	2	2.5	80	9–289	1	1.7	58	1–323
Benign neoplasms (210–239)	2	2.7	74	8–266	1	1.4	73	1–403	0	1.1	0	0–325	0	0.7	0	0–550
Diabetes mellitus (250)	9	13.9	65	30–123	4	7.4	54	15–139	3	6.3	47	10–138	2	3.8	53	6–192
Arteriosclerotic heart disease (410–413)	250	345.8	72	64–82	137	187.9	73	61–86	109	163.7	67	55–80	58	95.7	61	46–78
Vascular lesions of central nervous system (430–438)	63	65.2	97	74–124	31	35.4	88	60–124	27	30.7	88	58–128	10	15.9	63	30–116
Nonmalignant respiratory disease (460–519)	49	62.1	79	58–104	32	33.6	95	65–135	27	29.8	91	60–132	12	18.1	66	34–116
Pneumonia (480–486)	23	21.1	109	69–164	18	11.1	163	96–257	15	9.6	156	87–257	7	5.0	140	56–289
Emphysema (492)	7	13.6	51	21–106	3	7.7	39	8–114	3	7.0	43	9–125	2	4.3	47	5–170
Cirrhosis of liver (551)	10	22.9	44	21–80	4	12.0	33	9–86	4	10.0	40	11–103	2	6.3	32	4–115
All external causes (800–998)	64	84.1	76	59–97	31	38.6	80	55–114	20	28.2	71	43–110	12	13.8	87	45–152

[a]8th ICDA, 8th International Classification of Diseases; SMR, standardized mortality ratio; 95% CI, 95% confidence interval.

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Table 3. Observed and expected deaths and SMRs for selected causes for all cohort members by time first employed.

Cause of death (8th ICDA[a])	Employed < 1946 (war) n = 1066, P-Y = 33553				Employed ≥ 1946 (postwar) n = 1516, P-Y = 40666			
	Observed deaths	Expected deaths	SMR[a]	95% CI[a]	Observed deaths	Expected deaths	SMR	95% CI
All causes	593	693.4	86	79–93	233	286.2	81	71–93
All cancers (140–209)	106	140.9	75	62–91	57	61.8	92	70–120
Cancer of buccal cavity and pharynx (140–149)	3	4.2	72	14–211	2	2.0	102	11–367
Cancer of digestive system (150–159)	24	41.0	59	37–87	15	15.3	98	55–162
Cancer of esophagus (150)	3	3.3	91	18–267	0	1.5	0	0–250
Cancer of stomach (151)	2	7.8	26	3–93	2	2.5	81	9–293
Cancer of large intestine (153)	11	13.5	81	40–145	7	5.1	137	55–282
Cancer of rectum (154)	2	4.6	44	5–158	1	1.5	66	1–369
Cancer of liver (155–156)	0	3.1	0	0–117	1	1.1	90	1–502
Cancer of pancreas (157)	5	7.7	65	21–152	4	3.2	126	34–323
Cancer of respiratory system (160–163)	39	46.7	84	59–114	18	23.1	78	46–123
Cancer of the larynx (161)	3	2.1	146	29–426	1	0.9	112	1–624
Cancer of the lung (162–163)	36	44.2	82	57–113	17	22.0	77	45–124
Cancer of bone (170)	1	0.6	168	2–935	0	0.3	0	0–1303
Cancer of skin (172–173)	0	2.0	0	0–181	3	1.5	205	41–600
Cancer of prostate (185)	9	12.0	75	34–142	1	2.5	39	1–220
Cancer of bladder (188)	2	4.9	41	5–148	1	1.3	77	1–430
Cancer of kidney (189)	3	3.3	90	18–263	2	1.7	121	14–438
Cancer of brain and central nervous system (191–192)	2	3.3	60	7–218	2	2.4	83	9–301
Lymphatic and hematopoietic cancer (200–209)	16	12.7	126	72–205	9	6.5	138	63–262
Lymphosarcoma and reticulosarcoma (200)	7	2.6	269	108–555	2	1.3	155	17–558
Hodgkin's disease (201)	1	1.2	85	1–470	2	0.9	213	24–767
Leukemia and aleukemia (204–207)	6	5.4	112	41–244	2	2.5	80	9–287
Other lymphatic tissue (202, 203, 208)	2	3.4	59	7–212	3	1.7	174	35–509
Benign neoplasms (210–239)	2	1.8	111	12–401	0	0.9	0	0–399
Diabetes mellitus (250)	6	10.0	60	22–131	3	3.9	77	15–222
Arteriosclerotic heart disease (410–413)	176	254.6	69	59–80	74	91.2	81	64–102
Vascular lesions of central nervous system (430–438)	58	52.5	110	84–143	5	12.7	39	13–92
Nonmalignant respiratory disease (460–519)	33	47.5	70	48–98	16	14.6	109	62–178
Pneumonia (480–486)	16	16.4	98	56–159	7	4.7	148	59–305
Emphysema (492)	5	10.7	47	15–109	2	2.9	69	8–250
Cirrhosis of liver (551)	5	13.1	38	12–89	5	9.8	51	16–119
All external causes (800–998)	39	41.8	93	66–128	25	42.3	59	38–87

[a]8th ICDA, 8th International Classification of Diseases; SMR, standardized mortality ratio; 95% CI, 95% confidence interval.

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numbers of deaths are small and the confidence intervals wide. One cause of death that is significantly elevated in this update, as compared to the previous report, is ill-defined symptoms and senility. (SMR = 200).

Table 3 shows the SMRs for selected causes of death for those first employed during World War II versus those who were first employed after the war. The all-causes SMR is similar for the two groups. The SMR is increased for lymphohematopoietic cancer for both subcohorts. Almost all the excess for those first employed during the war is due to the significantly elevated SMR for lymphosarcoma (SMR = 269). For those first employed after the war, elevated SMRs are seen for lymphosarcoma, Hodgkin's disease, and cancer of other lymphatic tissue. None of these is statistically significant, and the numbers of observed deaths are small. Cancers of the large intestine and the pancreas are nonsignificantly elevated for those first employed postwar, whereas deficits were seen for those first employed during the war. There is a slight elevation for leukemia (SMR = 112) in those first employed during the war, but it is based on less than one death.

Table 4 shows the SMRs for selected causes of death for each of the exposure groups for all in the subgroup and for those employed 10 years or more in the group. All groups except the unknown exposure group show elevated SMRs for lymphohematopoietic cancer for those ever in the group. For the low exposure group, the excess is due to elevated SMRs for lymphosarcoma and Hodgkin's disease; both of these are based on two or fewer observed deaths. The overall excess disappears when the analysis is restricted to those employed 10 years or more. The routine exposure group has a statistically significant excess of lymphosarcoma (SMR = 561), which accounts for most of the lymphohematopoietic excess. All of the lymphosarcomas occurred in persons employed fewer than 10 years. Cancer of both the large intestine and kidney increased from the group ever employed to those employed 10 years or more.

Most of the excess in lymphohematopoietic cancer for the nonroutine group is due to a nonsignificant increase in leukemia (SMR = 185), which is limited to those employed less than 10 years. Only a slight increase is seen for this group for lymphosarcoma (SMR = 126). Cancer of the prostate has a nonsignificant increase for those employed more than 10 years (SMR = 146), while there is a deficit for the group overall. There is a nonsignificant elevation for stroke for the group overall, but this disappears when the group is restricted to those employed more than 10 years. Cancer of the brain shows an elevated SMR (283) for the unknown exposure group, which increases for those employed more than 10 years (SMR = 415). These SMRs are based on three or fewer observed deaths, however. Both nonmalignant respiratory disease and cirrhosis of the liver show nonsignificantly elevated SMRs for the group employed 10 years or more, as compared to those ever in the group, but neither increase is statistically significant. The increase in respiratory disease is primarily due to an increase in deaths from pneumonia (SMR = 232).

The number of observed and expected deaths by years worked and latency for all males for all deaths, all cancer deaths, all hemotopoietic deaths, lymphosarcoma, and leukemia are shown in Table 5. For all deaths the SMRs are essentially unchanged as either latency or duration increases. Similarly, no pattern with latency or duration is seen for all cancer deaths. For all hematopoietic deaths, the largest elevation is seen for those with latency and length of employment fewer than 10 years. The SMRs actually decrease with increasing employment. For lymphosarcoma, again the largest elevation is for those with the shortest employment and latency. The SMRs for leukemia increase with increasing latency but decrease with increasing length of employment.

Tables 6 through 9 show the same information for each of the four exposure groups. No overall pattern is seen for the low-exposure group other than that the SMRs for all cancers and for all hematopoietic cancer tend to decrease with increasing lengths of employment. For the routine exposure group, again no pattern is seen. The biggest excess is seen for lymphosarcoma; all deaths occurred in those employed fewer than 10 years; two were in those with fewer than 10 years latency, one in those with 20 to 29 latency, and two in those with 30 years or more of latency. The most interesting result for the nonroutine exposure group is for leukemia. Five of the six deaths occurred in those employed fewer than 10 years, and all had at least 10 years of latency although the deaths were spread out among the other latency categories. No pattern was seen for the unknown exposure group.

Discussion

The overall pattern of results for this update is essentially unchanged from the earlier report on this cohort (*1*). There was an overall reduction in expected mortality (SMR = 84) when compared with the U.S. white male population, which again was primarily due to a reduction in deaths because of arteriosclerotic heart disease (SMR = 72). The major finding is still the significantly elevated SMR for lymphosarcoma. The group with the highest risk for this cause of death appears to be those employed fewer than 10 years, first hired during World War II, and employed in a job with the potential for routine exposure. The fact that the risk does not increase with increasing length of employment (and presumably increasing cumulative exposure) must be incorporated into any theory that this excess is related to 1,3-butadiene exposure.

Although the leukemia SMR is not elevated for the cohort overall, it is nonsignificantly elevated for the nonroutine exposure group. Half of the leukemia deaths in this group occurred in persons who were first employed during the war and employed less than 5 years. Overall, six of the eight total leukemia deaths were first employed during the war. Again, the risk does not increase with length of employment. The increased risk

Table 4. Observed and expected deaths and standardized mortality ratios for selected causes for

Cause of death (8th ICDA*)	Exposure group I: low exposure ever employed n = 433, P-Y = 11852				Exposure group I: low exposure employed > 10 years n = 190, P-Y = 3995				Exposure group II: routine exposure ever employed n = 705, P-Y = 21422			
	Observed deaths	Expected deaths	SMR*	95% CI*	Observed deaths	Expected deaths	SMR	95% CI	Observed deaths	Expected deaths	SMR	95% CI
All causes	131	163.1	80	67–95	58	83.3	70	53–90	163	213.1	76	65–89
All cancers (140–209)	24	33.9	71	41–105	7	17.6	40	16–82	42	46.3	91	65–123
Cancer of buccal cavity and pharynx (140–149)	0	1.0	0	0–361	0	0.5	0	0–717	1	1.4	70	1–389
Cancer of digestive system (150–159)	9	9.4	96	44–183	2	4.9	41	5–148	9	12.0	75	34–142
Cancer of esophagus (150)	2	0.8	253	28–915	0	0.4	0	0–899	0	1.1	0	0–335
Cancer of stomach (151)	0	1.7	0	0–221	0	0.8	0	0–439	1	2.0	50	1–279
Cancer of large intestine (153)	4	3.1	127	34–326	2	1.7	119	13–430	4	4.1	99	27–253
Cancer of rectum (154)	0	1.0	0	0–366	0	0.5	0	0–706	1	1.2	81	1–452
Cancer of liver (155–156)	1	0.7	143	2–796	0	0.4	0	0–1022	0	0.9	0	0–413
Cancer of pancreas (157)	2	1.8	110	12–397	0	1.0	0	0–382	2	2.4	82	9–297
Cancer of respiratory system (160–163)	7	11.7	60	24–123	3	6.1	49	10–143	14	17.0	83	45–138
Cancer of the larynx (161)	0	0.5	0	0–747	0	0.3	0	0–1454	2	0.7	296	33–1070
Cancer of lung (162–163)	6	11.1	63	25–129	3	5.8	52	10–151	12	16.1	74	38–130
Cancer of bone (170)	0	0.1	0	0–2569	0	0.1	0	0–5740	1	0.2	516	7–2870
Cancer of skin (172–173)	2	0.6	351	39–1268	0	0.3	0	0–1354	0	0.9	0	0–413
Cancer of prostate (185)	0	2.5	0	0–147	0	1.5	0	0–246	2	2.6	77	9–277
Cancer of bladder (188)	0	1.1	0	0–351	0	0.6	0	0–623	1	1.2	84	1–467
Cancer of kidney (189)	2	0.8	241	27–871	1	0.4	237	3–1321	2	1.2	169	19–610
Cancer of brain and central nervous system (191–192)	0	0.9	0	0–391	0	0.4	0	0–877	0	1.5	0	1–242
Lymphatic and hematopoietic cancer (200–209)	4	3.2	126	34–323	1	1.6	63	1–352	8	4.5	177	76–349
Lymphosarcoma and reticulosarcoma (200)	2	0.6	321	36–1159	1	0.3	322	4–1791	5	0.9	561	181–1310
Hodgkin's disease (201)	1	0.3	294	4–1635	0	0.1	0	0–2843	1	0.6	179	2–998
Leukemia and aleukemia (204–207)	1	1.3	76	1–424	0	0.7	0	0–556	1	1.8	56	1–311
Other lymphatic tissue (202, 203, 208)	0	0.9	0	0–422	0	0.5	0	0–790	1	1.2	80	1–448
Benign neoplasms (210–239)	0	0.5	0	0–820	0	0.2	0	0–1775	0	0.7	0	0–566
Diabetes mellitus (250)	0	2.3	0	0–159	0	1.2	0	0–305	1	3.0	34	0–188
Arteriosclerotic heart disease (410–413)	43	58.1	74	54–100	23	31.2	74	47–111	48	72.5	66	49–88
Vascular lesions of central nervous system (430–438)	14	10.9	128	70–215	9	6.2	145	66–276	6	11.4	52	19–114
Nonmalignant respiratory disease (460–519)	10	10.6	95	45–174	6	5.9	103	37–223	9	12.7	71	32–135
Pneumonia (480–486)	3	3.5	85	17–248	2	1.9	104	12–374	3	4.0	75	15–220
Emphysema (492)	2	2.4	85	10–306	2	1.4	146	16–526	2	2.7	74	8–267
Cirrhosis of liver (551)	0	3.7	0	0–98	0	1.7	0	0–215	2	6.1	33	4–119
All external causes (800–998)	12	13.6	88	46–154	2	4.9	41	5–149	14	22.9	61	33–102

*8th ICDA, 8th International Classification of Diseases; SMR, standardized mortality ratio; 95% CI, 95% confidence interval.

for the category symptoms, senility, and ill-defined conditions is not readily explainable. The increase is concentrated in persons who were employed fewer than 20 years, were first employed during the war, and were not in the low exposure group. Obviously this category would decrease with more specific cause of death information from the attending physician.

Two other studies have examined mortality in cohorts exposed to 1,3-butadiene although both cohorts consisted of persons engaged in the manufacture of styrene-butadiene rubber (SBR) and so had the opportunity for multiple exposures. The first study by Matanoski and Schwartz (*3*) covered males who had worked at eight SBR manufacturing facilities in the U.S. and Canada. The overall SMR (81) was very similar to that seen for the current study. The only significant excess seen was for arteriosclerotic heart disease among black males. Risks were examined by major work areas as well as by pay grade, and no significant differences in cancer mortality for specific sites were seen. In particular, no increase was seen for lymphosarcoma (SMR = 49) or leukemia (SMR = 91).

The second study by Meinhardt et al. (*4*) covered persons employed at the two SBR plants supplied by the facility in the current study. The Meinhardt study found an elevated SMR for leukemia that was concentrated in people hired during the war. Elevated SMRs for lymphosarcoma were also seen although they were not statistically significant and were not as high as those seen in this study. One major problem with comparisons between the Meinhardt study and the current study is the overlap between the study cohorts. There were 116 persons who were employed both at the 1,3-butadiene and the SBR manufacturing facilities, including at least one of the leukemia deaths and one of the lymphosarcoma deaths. The increases for each of these populations may be exaggerated because of this.

Another report among oil refinery workers also saw an increase in a number of causes of deaths for those persons first employed during World War II (*5*). The increase was seen for many of the specific cancer sites including leukemia as well as for external causes of death. This study and the Meinhardt study show the elevation for leukemia but not for external causes of death. There are two possibilities for the wartime differences if they are real. One is that this group of people is

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all cohort members by exposure group and length of employment.

Exposure group II: routine exposure employed > 10 years n = 270, P-Y = 6450				Exposure group III: Nonroutine ever employed n = 993, P-Y = 27434				Exposure group III: Nonroutine employed > 10 years n = 419, P-Y = 8714				Exposure group IV: Unknown ever employed n = 451, P-Y = 13513				Exposure group IV: Unknown employed > 10 years n = 217, P-Y = 5008			
Observed deaths	Expected deaths	SMR	95% CI	Observed deaths	Expected deaths	SMR	95% CI	Observed deaths	Expected deaths	SMR	95% CI	Observed deaths	Expected deaths	SMR	95% CI	Observed deaths	Expected deaths	SMR	95% CI
61	85.1	72	55-92	361	408.6	88	79-98	154	189.6	81	68-95	171	194.9	88	75-102	81	82.9	98	78-121
15	19.5	77	43-127	59	83.1	71	54-92	30	39.9	75	51-107	38	39.4	97	68-133	17	17.9	95	55-153
0	0.6	0	0-604	2	2.5	80	9-290	1	1.2	87	1-483	2	1.2	167	19-603	1	0.6	182	2-1015
5	5.1	99	32-231	10	23.6	42	20-78	4	11.2	36	10-91	11	11.3	98	49-175	4	4.9	81	22-208
0	0.5	0	0-777	0	1.9	0	0-189	0	0.9	0	0-396	1	0.9	108	1-589	0	0.4	0	0-855
0	0.8	0	0-457	1	4.4	23	0-126	0	1.9	0	0-187	2	2.2	93	10-336	0	0.9	0	0-427
4	1.7	233	63-596	7	7.8	90	36-185	3	3.8	79	16-230	3	3.7	82	16-238	1	1.6	61	1-340
0	0.5	0	0-727	0	2.6	0	0-142	0	1.2	0	0-304	2	1.2	161	18-580	2	0.5	381	43-1376
0	0.4	0	0-997	0	1.8	0	0-204	0	0.8	0	0-438	0	0.9	0	0-427	0	0.4	0	0-1005
1	1.0	96	1-535	2	4.5	45	5-161	1	2.2	46	1-255	3	2.1	141	28-413	1	1.0	103	1-571
4	7.4	54	15-138	24	27.9	86	55-128	10	13.7	73	35-135	12	13.2	91	47-159	7	6.4	110	44-227
0	0.3	0	0-1266	1	1.2	83	1-462	0	0.6	0	0-644	1	0.6	174	2-965	0	0.3	0	0-1380
4	7.1	57	15-1415	23	26.4	87	55-131	10	12.9	77	37-142	11	12.5	88	44-158	7	6.0	116	46-239
0	0.1	0	0-5335	0	0.4	0	0-1010	0	0.2	0	0-2480	0	0.2	0	0-2079	0	0.1	0	0-5318
0	0.4	0	0-1036	1	1.4	73	1-408	1	0.6	166	2-923	0	0.7	0	0-553	0	0.3	0	0-1231
1	1.1	89	1-494	5	6.5	77	25-180	5	3.4	146	47-341	3	3.0	100	20-292	2	1.3	159	16-574
0	0.5	0	0-721	1	2.7	37	0-208	1	1.4	74	1-411	1	1.3	80	1-444	1	0.5	186	2-1034
2	0.5	396	45-1436	1	2.0	50	1-276	1	1.0	105	1-586	0	1.0	0	0-382	0	0.4	0	0-825
0	0.6	0	0-615	1	2.2	45	1-253	0	0.9	0	0-398	3	1.1	283	57-827	2	0.5	415	47-1497
1	1.8	56	1-310	11	7.8	141	70-253	4	3.6	112	30-287	2	3.7	55	6-197	0	1.6	0	0-225
0	0.4	0	0-1017	2	1.6	126	14-454	1	0.7	141	2-783	0	0.7	0	0-503	0	0.3	0	0-1086
0	0.2	0	0-2081	1	0.8	122	2-677	1	0.3	344	4-1915	0	0.4	0	0-907	0	0.2	0	0-2394
0	0.7	0	0-523	6	3.2	185	68-403	1	1.5	67	1-371	0	1.5	0	0-239	0	0.7	0	0-554
1	0.5	185	2-1031	2	2.1	97	11-350	1	1.0	97	1-539	2	1.0	209	23-753	0	0.5	0	0-794
0	0.2	0	0-1507	2	1.1	182	20-656	0	0.5	0	0-790	0	0.5	0	0-702	0	0.2	0	0-1702
0	1.2	0	0-306	4	5.8	69	19-176	2	2.8	73	8-263	4	2.8	144	39-369	1	1.2	84	1-468
22	30.5	72	45-109	101	145.9	69	56-84	41	71.3	58	41-78	58	69.3	84	64-108	23	30.8	75	47-1212
1	4.6	22	0-120	36	28.9	124	87-172	12	14.3	84	43-146	7	13.9	50	20-104	5	5.6	90	29-209
2	5.3	38	4-137	20	26.6	75	46-116	12	13.3	90	47-157	10	12.3	81	39-149	7	5.3	132	53-271
1	1.5	65	1-363	10	9.2	109	52-200	8	4.5	180	77-354	7	4.4	160	64-331	4	1.7	232	62-594
0	1.2	0	0-311	3	5.9	51	10-150	1	3.2	31	0-175	0	2.7	0	0-136	0	1.3	0	0-285
0	2.5	0	0-147	4	8.8	46	12-117	1	3.8	27	0-148	4	4.3	93	25-238	3	2.0	148	30-433
4	6.9	58	16-148	27	32.0	84	55-123	10	10.7	94	45-172	11	15.6	71	35-126	4	5.7	70	19-179

somehow different from those hired at other times, perhaps because they were not eligible for the draft because of health reasons. The other is that there were major differences in exposure during the war that led to the increases.

In examining work history records for an earlier study of Texaco workers (*6*), it became obvious that a number of experienced persons were lent to the 1,3-butadiene plant during the war. In fact, 122 persons overlapped between the two studies. It is very likely that similar overlaps exist with cohorts from other petroleum and chemical facilities in the area. Since most of the elevations of interest were concentrated primarily in persons with short-term employment, the question remains of where else these persons were employed and what other exposures could have lasted substantially longer than those at the butadiene facility.

As mentioned in the earlier report on this cohort (*1*), there are several weaknesses in this study. Because of unreliable race designations, conclusions based on race-specific rates could not be generated. For this reason, all comparisons were made using white male mortality rates. Second, no work histories or industrial hygiene data were available for the time period of the study. Almost half of the cohort worked fewer than 5 years and obviously spent considerable working time elsewhere with the possibility for numerous other exposures. As mentioned above, it is this portion of the cohort where the increased SMRs are concentrated. Finally, the cohort size is small, and the numbers become even smaller for the exposure group analyses.

However, the study does cover one of the largest cohorts involved solely in the manufacture of butadiene. The cohort has been followed for 43 years and includes all those with 6 months or more employment since the plant began production. The numbers with vital status unknown (1.9%) and without cause of death information (6%) are relatively small, and qualitative measures of exposures were available.

Further efforts are underway to increase the information available for this cohort. Information has been received from the NDI for those persons with vital status unknown or for whom no death certificate could be located based on SSA information. There are outstanding requests for death certificates from other state health departments, and additional death certificates

Table 5. Observed and expected deaths by years worked and latency for the total cohort.

Years worked		Latency, years 0–9	10–19	20–29	30+	Totals
All deaths						
< 10	Observed	84	98	123	167	472
	Expected	101.3	91.8	145.9	199.9	538.9
	SMR[a]	8.3	107	84	84	88
10–19	Observed	—	56	54	44	154
	Expected	—	89.0	52.6	40.6	182.2
	SMR	—	63	103	108	85
20+	Observed	—	—	76	124	200
	Expected	—	—	100	158	258.0
	SMR	—	—	76	79	78
Totals	Observed	84	154	253	335	826
	Expected	101.3	180.8	298.5	398.5	979.6
	SMR	83	85	85	84	84
Cancer deaths						
< 10	Observed	11	19	26	88	94
	Expected	13.8	16.2	30.2	47.6	107.8
	SMR	80	117	86	80	87
10–19	Observed	—	7	9	6	22
	Expected	—	16.2	9.8	7.4	33.4
	SMR	—	43	92	81	66
20+	Observed	—	—	20	27	47
	Expected	—	—	22.1	39.3	61.4
	SMR	—	—	91	69	77
Totals	Observed	11	26	55	71	163
	Expected	13.8	32.4	62.1	94.3	202.7
	SMR	80	80	89	75	80
All hematopoietic deaths						
< 10	Observed	4	3	4	8	19
	Expected	2.0	1.9	2.8	3.9	10.6
	SMR	200	158	143	205	179
10–19	Observed	—	0	3	0	3
	Expected	—	1.8	0.9	0.6	3.3
	SMR	—	0	333	0	91
20+	Observed	—	—	2	1	3
	Expected	—	—	2.0	3.2	5.2
	SMR	—	—	100	31	58
Totals	Observed	4	3	9	9	25
	Expected	2.0	3.7	5.7	7.7	19.2
	SMR	200	81	158	117	130
Lymphosarcoma deaths						
< 10	Observed	4	0	1	2	7
	Expected	0.3	0.5	0.7	0.6	2.1
	SMR	1333	0	143	3333	3333
10–19	Observed	—	0	0	0	0
	Expected	—	0.5	0.2	0.1	0.8
	SMR	—	0	0	0	0
20+	Observed	—	—	2	0	2
	Expected	—	—	0.5	0.5	1.0
	SMR	—	—	400	0	200
Totals	Observed	4	0	3	2	9
	Expected	0.3	1.0	1.4	1.2	3.9
	SMR	1333	0	214	167	229
Leukemia						
< 10	Observed	0	1	2	4	7
	Expected	0.9	0.8	1.1	1.6	4.4
	SMR	0	125	182	250	159
10–19	Observed	—	0	1	0	1
	Expected	—	0.7	0.4	0.3	1.4
	SMR	—	0	2.5	0	71
20+	Observed	—	—	0	0	0
	Expected	—	—	0.8	1.3	2.1
	SMR	—	—	0	0	0
Totals	Observed	0	1	3	4	8
	Expected	0.9	1.5	2.3	3.2	7.9
	SMR	0	67	130	125	102

[a]SMR, standardized mortality ratio.

Table 6. Observed and expected deaths by years worked and latency for the low-exposure group.

Years worked		Latency, years 0–9	10–19	20–29	30+	Totals
All deaths						
< 10	Observed	14	11	17	31	73
	Expected	15.9	12.1	22.8	29.1	79.9
	SMR[a]	88	91	75	107	91
10–19	Observed	—	5	7	12	24
	Expected	—	16.2	9.7	8.2	34.1
	SMR	—	31	72	146	70
20+	Observed	—	—	10	24	34
	Expected	—	—	19.6	29.6	49.2
	SMR	—	—	51	81	69
Totals	Observed	14	16	34	67	131
	Expected	15.9	28.3	52.1	66.9	163.1
	SMR	88	57	65	100	80
Cancer deaths						
< 10	Observed	2	2	3	18	17
	Expected	2.2	2.1	4.8	7.2	16.3
	SMR	91	95	63	139	104
10–19	Observed	—	0	1	2	3
	Expected	—	3.0	1.7	1.3	6.0
	SMR	—	0	59	154	50
20+	Observed	—	—	3	1	4
	Expected	—	—	4.4	7.2	11.6
	SMR	—	—	68	14	35
Totals	Observed	2	2	7	13	24
	Expected	2.2	5.1	10.9	15.7	33.9
	SMR	91	39	64	83	71
All hematopoietic deaths						
< 10	Observed	1	0	0	2	3
	Expected	0.3	0.3	0.4	0.6	1.6
	SMR	333	0	0	333	188
10–19	Observed	—	0	0	0	0
	Expected	—	0.3	0.2	0.1	0.6
	SMR	—	0	0	0	0
20+	Observed	—	—	1	0	1
	Expected	—	—	0.5	0.6	1.1
	SMR	—	—	200	0	91
Totals	Observed	1	0	1	2	4
	Expected	0.3	0.6	1.0	1.2	3.2
	SMR	333	0	104	161	126
Lymphosarcoma deaths						
< 10	Observed	1	0	0	0	1
	Expected	0.1	0.1	0.1	0.1	0.4
	SMR	192	0	0	0	250
10–19	Observed	—	0	0	0	0
	Expected	—	0.1	0.0	0.0	0.1
	SMR	—	0	0	0	0
20+	Observed	—	—	1	0	1
	Expected	—	—	0.1	0.1	0.2
	SMR	—	—	1000	0	500
Totals	Observed	1	0	1	0	2
	Expected	0.1	0.1	0.2	0.2	0.6
	SMR	192	0	452	0	321
Leukemia						
< 10	Observed	0	0	0	1	1
	Expected	0.1	0.1	0.2	0.2	0.6
	SMR	0	0	0	442	167
10–19	Observed	—	0	0	0	0
	Expected	—	0.1	0.1	0.1	0.3
	SMR	—	0	0	0	0
20+	Observed	—	—	0	0	0
	Expected	—	—	0.2	0.3	0.5
	SMR	—	—	0	0	0
Totals	Observed	0	0	0	1	1
	Expected	0.1	0.2	0.4	0.5	1.43
	SMR	0	0	0	188	76

[a]SMR, standardized mortality ratio.

Table 7. Observed and expected deaths by years worked and latency for the routine exposure group.

Years worked		Latency, years 0–9	10–19	20–29	30+	Totals
All deaths						
< 10	Observed	14	20	26	42	102
	Expected	18.1	18.9	35.0	56.0	128.0
	SMR[a]	77	106	74	75	80
10–19	Observed	—	6	2	3	11
	Expected	—	13.7	3.0	3.8	20.5
	SMR	—	44	67	79	54
20+	Observed	—	—	19	31	50
	Expected	—	—	23.4	41.1	64.5
	SMR	—	—	81	75	78
Totals	Observed	14	26	47	76	163
	Expected	18.1	32.6	61.4	100.9	213.1
	SMR	77	80	77	75	76
Cancer deaths						
< 10	Observed	2	6	8	11	27
	Expected	2.1	3.2	7.5	14.0	26.8
	SMR	95	188	107	79	101
10–19	Observed	—	1	1	0	2
	Expected	—	2.3	0.6	1.0	3.9
	SMR	—	44	167	0	51
20+	Observed	—	—	5	8	13
	Expected	—	—	5.1	10.5	15.6
	SMR	—	—	98	76	83
Totals	Observed	2	7	14	19	42
	Expected	2.1	5.5	13.2	25.5	46.3
	SMR	95	127	106	75	91
All hematopoietic deaths						
< 10	Observed	2	1	1	3	7
	Expected	0.4	0.5	0.7	1.1	2.7
	SMR	500	200	143	273	259
10–19	Observed	—	0	0	0	0
	Expected	—	0.3	0.1	0.1	0.5
	SMR	—	0	0	0	0
20+	Observed	—	—	0	1	1
	Expected	—	—	0.5	0.8	1.3
	SMR	—	—	0	125	77
Totals	Observed	2	1	1	4	8
	Expected	0.4	0.7	1.3	2.0	4.5
	SMR	500	143	77	200	178.7
Lymphosarcoma deaths						
< 10	Observed	2	0	1	2	5
	Expected	0.1	0.1	0.2	0.2	0.6
	SMR	2000	0	500	1000	833
10–19	Observed	—	0	0	0	0
	Expected	—	0.1	0.0	0.0	0.1
	SMR	—	0	0	0	0
20+	Observed	—	—	0	0	0
	Expected	—	—	0.1	0.1	0.2
	SMR	—	—	0	0	0
Totals	Observed	2	0	1	2	5
	Expected	0.1	0.2	0.3	0.3	0.9
	SMR	2000	0	333	667	561
Leukemia						
< 10	Observed	0	0	0	1	1
	Expected	0.2	0.2	0.3	0.5	1.2
	SMR	0	0	0	200	83
10–19	Observed	—	0	0	0	0
	Expected	—	0.1	0.0	0.0	0.1
	SMR	—	0	0	0	0
20+	Observed	—	—	0	0	0
	Expected	—	—	0.2	0.3	0.5
	SMR	—	—	0	0	0
Totals	Observed	0	0	0	1	1
	Expected	0.2	0.3	0.5	0.8	1.8
	SMR	0	0	0	125	56

[a]SMR, standardized mortality ratio.

Table 8. Observed and expected deaths by years worked for the nonroutine exposure group.

Years worked		Latency, years 0–9	10–19	20–29	30+	Totals
All deaths						
< 10	Observed	43	46	53	65	207
	Expected	45.4	39.2	57.6	76.9	219.1
	SMR[a]	95	119	94	85	95
10–19	Observed	—	34	33	22	89
	Expected	—	41.2	29.3	22.2	92.7
	SMR	—	83	113	99	96
20+	Observed	—	—	24	41	65
	Expected	—	—	37.8	58.9	96.7
	SMR	—	—	64	70	67
Totals	Observed	43	80	110	128	361
	Expected	45.4	80.4	124.7	158.0	408.6
	SMR	95	100	88	81	88
Cancer deaths						
< 10	Observed	6	6	8	9	29
	Expected	6.5	7.0	11.8	18.0	43.3
	SMR	92	86	68	50	67
10–19	Observed	—	4	7	4	15
	Expected	—	7.7	5.5	3.8	17.0
	SMR	—	52	127	105	88
20+	Observed	—	—	6	9	15
	Expected	—	—	8.4	14.3	22.7
	SMR	—	—	71	63	66
Totals	Observed	6	10	21	22	59
	Expected	6.5	14.7	25.7	36.1	83.1
	SMR	92	68	82	61	71
All hematopoietic deaths						
< 10	Observed	1	1	2	3	7
	Expected	0.8	0.8	1.1	1.5	4.2
	SMR	125	125	182	200	167
10–19	Observed	—	0	3	0	3
	Expected	—	0.8	0.5	0.3	1.6
	SMR	—	0	600	0	188
20+	Observed	—	—	1	0	1
	Expected	—	—	0.7	1.1	1.8
	SMR	—	—	143	0	56
Totals	Observed	1	1	6	3	11
	Expected	0.8	1.6	2.3	2.9	7.8
	SMR	125	63	261	104	141
Lymphosarcoma deaths						
< 10	Observed	1	0	0	0	1
	Expected	0.1	0.2	0.3	0.2	0.8
	SMR	1000	0	0	0	125
10–19	Observed	—	0	0	0	0
	Expected	—	0.2	0.2	0.0	0.3
	SMR	—	0	0	0	0
20+	Observed	—	—	1	0	1
	Expected	—	—	0.2	0.2	0.4
	SMR	—	—	500	0	250
Totals	Observed	1	0	1	0	2
	Expected	0.1	0.4	0.6	0.4	1.6
	SMR	1000	0	167	0	126
Leukemia						
< 10	Observed	0	1	2	2	5
	Expected	0.4	0.3	0.4	0.6	1.7
	SMR	0	333	500	333	294
10–19	Observed	—	0	1	0	1
	Expected	—	0.3	0.2	0.2	0.7
	SMR	—	0	500	0	143
20+	Observed	—	—	0	0	0
	Expected	—	—	0.3	0.5	0.8
	SMR	—	—	0	0	0
Totals	Observed	0	1	3	2	6
	Expected	0.4	0.6	0.9	1.3	3.2
	SMR	0	167	333	154	185

[a]SMR, standardized mortality ratio.

Table 9. Observed and expected deaths by years worked for the unknown exposure group.

Years worked		Latency, years 0–9	10–19	20–29	30+	Totals
All deaths						
< 10	Observed	13	21	27	29	90
	Expected	22.0	21.7	30.6	37.8	112.1
	SMR[a]	59	97	88	77	80
10–19	Observed	—	11	12	7	30
	Expected	—	18.1	10.7	6.4	35.2
	SMR	—	61	112	109	85
20+	Observed	—	—	23	28	51
	Expected	—	—	19.2	28.5	47.7
	SMR	—	—	120	98	110
Totals	Observed	13	32	62	64	171
	Expected	22.0	39.8	60.5	72.7	194.9
	SMR	59	80	103	88	88
Cancer deaths						
< 10	Observed	1	5	7	8	21
	Expected	3.2	3.9	5.8	8.4	21.3
	SMR	31	128	121	95	94
10–19	Observed	—	2	0	0	2
	Expected	—	3.2	1.9	1.2	6.3
	SMR	—	63	0	0	32
20+	Observed	—	—	6	9	15
	Expected	—	—	4.1	7.3	11.4
	SMR	—	—	146	123	132
Totals	Observed	1	7	13	17	38
	Expected	3.2	7.1	11.8	16.9	39.4
	SMR	31	99	110	101	97
All hematopoietic deaths						
< 10	Observed	0	1	1	0	2
	Expected	0.4	0.4	0.6	0.7	2.1
	SMR	0	250	167	0	95
10–19	Observed	—	0	0	0	0
	Expected	—	0.4	0.2	0.1	0.7
	SMR	—	0	0	0	0
20+	Observed	—	—	0	0	0
	Expected	—	—	0.4	0.6	1.0
	SMR	—	—	0	0	0
Totals	Observed	0	1	1	0	2
	Expected	0.4	0.8	1.2	1.4	3.7
	SMR	0	125	83	0	55
Lymphosarcoma deaths						
< 10	Observed	0	0	0	0	0
	Expected	0.1	0.1	0.1	0.1	0.4
	SMR	0	0	0	0	0
10–19	Observed	—	0	0	0	0
	Expected	—	0.1	0.0	0.0	0.1
	SMR	—	0	0	0	0
20+	Observed	—	—	0	0	0
	Expected	—	—	0.1	0.1	0.2
	SMR	—	—	0	0	0
Totals	Observed	0	0	0	0	0
	Expected	0.1	0.2	0.2	0.2	0.7
	SMR	0	0	0	0	0
Leukemia						
< 10	Observed	0	0	0	0	0
	Expected	0.2	0.2	0.2	0.3	0.9
	SMR	0	0	0	0	0
10–19	Observed	—	0	0	0	0
	Expected	—	0.2	0.1	0.1	0.4
	SMR	—	0	0	0	0
20+	Observed	—	—	0	0	0
	Expected	—	—	0.2	0.2	0.4
	SMR	—	—	0	0	0
Totals	Observed	0	0	0	0	0
	Expected	0.2	0.4	0.5	0.6	1.5
	SMR	0	0	0	0	0

[a]SMR, standardized mortality ratio.

have been requested based on the NDI search results. The analyses will be repeated when these data are available. In addition, the company intends to continue to update cause of death information on the cohort at periodic intervals.

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Mortality of a Cohort of Workers in the Styrene-Butadiene Polymer Manufacturing Industry (1943–1982)

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A cohort of 12,110 male workers employed 1 or more years in eight styrene-butadiene polymer (SBR) manufacturing plants in the United States and Canada has been followed for mortality over a 40-year period, 1943 to 1982. The all-cause mortality of these workers was low [standardized mortality ratio (SMR) = 0.81] compared to that of the general population. However, some specific sites of cancers had SMRs that exceeded 1.00. These sites were then examined by major work divisions. The sites of interest included leukemia and non-Hodgkin's lymphoma in whites. The SMRs for cancers of the digestive tract were higher than expected, especially esophageal cancer in whites and stomach cancer in blacks. The SMR for arteriosclerotic heart disease in black workers was significantly higher than would be expected based on general population rates.

Employees were assigned to a work area based on job longest held. The SMRs for specific diseases differed by work area. Production workers showed increased SMRs for hematologic neoplasms and maintenance workers, for digestive cancers. A significant excess SMR for arteriosclerotic heart disease occurred only in black maintenance workers, although excess mortality from this disease occurred in blacks regardless of where they worked the longest. A significant excess SMR for rheumatic heart disease was associated with work in the combined, all-other work areas. For many causes of death, there were significant deficits in the SMRs.

Introduction

The synthetic rubber industry began in the United States in 1942 under the control of the federally sponsored Rubber Reserve Company, a part of the Reconstruction Finance Corporation (*1*). The plants were operated initially by privately owned companies and later were sold by the government to private industry. In the early years, all plants manufactured styrene-butadiene rubber (SBR), although, at some time in their histories, most companies manufactured other synthetic rubbers and related materials. Initially, 15 plants were built in the U.S. and 1 in Canada—all similar in design. One additional plant with the same design was built in the 1950s and also manufactured SBR. The common features of plant design and product manufacture in these companies provided a unique opportunity for an epidemiologic study. At the start of the study in 1977, 10 of these plants were still in operation. The cohort for study was developed using the records from 8 of the 10 plants. The National Institute of Occupational Safety and Health (NIOSH) included the remaining 2 plants in their epidemiologic studies (*1*). Two of these facilities included in the original cohort ended operations in 1978 and 1984, but these plants and their workers still have been followed in the current study.

The basic method of producing synthetic rubber involves an emulsion polymerization process in which aqueous monomers of 1,3-butadiene and styrene are combined in the presence of a soap solution and an initiator system such as *p*-methane hydroperoxide and sodium formaldehyde sulfoxylate in combination with a mercaptan. When the desired level of polymerization is reached, an inhibiting agent, such as sodium dimethyldithiocarbamate, is added to the mixture to end the reaction. The unreacted monomer of 1,3-butadiene is recovered through distillation, and the styrene monomer is stripped by steam for reuse. Antioxidants, such as diphenylamine, are added to extend the life of the product. The latex is coagulated using salt and sulfuric acid. The coagulate is then washed and dried, and the crumb is prepared for shipment.

Several other chemicals may be added to SBR, such as carbon black, which is added to latex prepared for tire production, and extender oils, which change the proper-

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ties of the rubber. Other curing agents, accelerators, retarders, antioxidants, and antiozonants may be added depending on the final intended use of the latex.

All plants can be divided into work areas based on the manufacturing process. The geographic locations of work areas within the plant differ depending on specific plant layouts. The tank farm receives shipments of the monomers of styrene and 1,3-butadiene and pumps them to the various parts of the plant where needed. Located close to each other are the reactor area, where the polymerization takes place in glass-lined steel reactors, and the recovery area, wehre the unreacted monomers styrene and 1,3-butadiene are removed from the latex. The final steps in production occur in the coagulation area where chemicals that are prepared or stored in pigment preparation are added to the latex. Finishing includes all final steps such as extrusion, drying, baling, and any other steps that result in a product ready for shipping.

The plants require a large maintenance work force especially in trades, such as plumbing, pipefitting, carpentry, and welding. All plants have auxiliary utilities for operation such as power, refrigeration, and water treatment units. In addition they have laboratories involved in quality control for production and usually others for research and development. Most plants produce rubbers and plastics other than styrene-butadiene rubber. Some plants make these other products in a separate area with a specific crew; other plants use the same line where SBR is produced. Pilot plants at the facilities test new processes or changes in current methods. Some plants currently manufacture or have manufactured raw materials used in the production of SBR.

Background of Study

In 1976, two leukemia cases were reported among workers in two Texas SBR plants. The National Institute for Occupational Safety and Health (NIOSH) conducted a cohort study of the workers in these plants and reported an excess risk of leukemia in one of the two plants (*1*). The risk of leukemia in the popluation hired in 1943 through 1945 was 2.12 times higher than the risk for U.S. males, but the difference was significant only when a one-sided test was used. Several cases occurred in workers with short employment and latency and those who were first hired during World War II when the industry was starting.

Previous studies of the rubber industry had focused primarily on tire manufacture. McMichael and collaborators (*2*) however, had noted that lymphatic and hematopoietic cancers occurred more frequently among workers in the synthetic rubber manufacturing area of the plant. In a subsequent publication, these investigators associated the leukemia risk with solvent exposure only and did not mention a relationship to the SBR department (*3*).

In 1977, a cohort study of rubber workers from eight operating SBR polymer manufacturing plants was initiated. The results of this study indicated that the overall mortality of these workers was low compared to that of the general population, and no significant differences in site-specific cancer risks occurred (*4,5*). At the time the original study was initiated, styrene was a suspected carcinogenic chemical in this industry.

In recent years, toxicologic evidence focused attention on the potential carcinogenic effect of 1,3-butadiene rather than styrene (*6*). A cohort study of the mortality of workers at a 1,3-butadiene manufacturing plant demonstrated a significant risk of lymphosarcomas that was 2.35 times higher than that of the general population of the U.S. The risk appeared in workers who had routine exposures to 1,3-butadiene (*7*). They represented those employed in processing, laboratory, receiving, storage, and transport.

Since there was some toxicologic data but limited epidemiologic data regarding potential carcinogenic effects of 1,3-butadiene, it seemed worthwhile to follow-up the original cohort of SBR production workers to identify any possible risks from this chemical. From the time of the original study, the average age of the workers would have increased with a resultant increase in the number of deaths. Since the original cohort was already large, the study would have substantial power to demonstrate any risks that might exist. The present study, therefore, evaluates the total mortality of the original cohort of rubber workers for 40 years, examines the risks of specific cancers and other diseases compared to the general population, and evaluates the variation in the risks by major plant divisions.

Description of Study

The original study cohort consisted of workers in the eight U.S. and Canadian styrene-butadiene polymer manufacturing plants that were in operation in 1976. All but one of the plants had started production in 1942 and 1943 when the synthetic rubber industry began in the U.S. The original cohort was established by abstracting the personnel records of each plant and by using computerized records of current workers in one plant. Information on name, birthdate, social security number for U.S. workers, and dates of hiring and termination was abstracted for each worker. The data for all employees from all plants were combined into a common, computerized data base.

A job listing was formed that included the job titles and work areas abstracted from the individual personnel files. Tasks required for the performance of each job were defined from employment requisitions and job descriptions that were provided by the plant. The jobs were grouped into broad work areas or departments that were usually listed as part of the job in the work history of each employee. The work area groupings are the same as those that were used to determine the mortality experience of workers in the original study (*5*). At the time of this study, the jobs had not been reviewed

by company industrial hygienists to identify exposures by job and to assure that the groupings within and across plants were correct.

A review of the personal records indicated that not all plants had complete data on all employees from the beginning of plant operations. All available records were abstracted and the start of the complete cohort for follow-up was determined by identifying the earliest year from which all possible lengths of employment occurred. This was the point from which record retention was considered complete. The start of plant production, which is the beginning of the cohort, and the start of follow-up are shown for each plant in Table 1. As can be seen from these data, limited mortality information will be gained from the two plants in which the follow-up starts in 1964 and 1970 with all workers who were employed at that time and all newly hired workers. These cohorts are heavily weighted with young and new employees. However, all plants have been included in the analysis.

The total population is defined as all individuals who were hired at the plants at some time between the start of production to December 1976 for complete cohorts, or who were working at the time of complete record-keeping, or were hired after that time to the end of 1976 for plants where all records were not retained. The total number of workers included in the population was 28,937 (Table 2). The population size has changed slightly from that of the original study because of editing, which removed duplicates and corrected records. Additional information from company files resulted in vital record searches on 2604 more workers than in the first study. However, most of the added information related to short-term employees who were eliminated from the study population by definition.

Table 1. Cohort or production and follow-up start dates by plant.

Plant	Cohort start	Follow-up start
1	October 1957	1964
2	September 1943	1958
3	September 1943	1943
4	July 1943	1970
5	January 1943	1953
6	March 1943	1943
7	October 1943	1943
8	July 1943	1943

Table 2. Total workers and total cohort after exclusions (by order of exclusions).

Cohort definitions	Number
Total subjects	28,937
Exclusion variables	
Females	4,238
Worked less than 1 year	9,022
Left before complete record keeping	1,073
Hired after 1976	711
Birth year missing or wrong	411
Total exclusions	15,515
Total cohort	13,422

The study cohort for follow-up was defined as only males who had either worked 1 year and who had been hired after the start of production in the plant or had worked at anytime after record keeping was complete until the end of cohort accrual on December 31, 1976. As can be seen from Table 2, 58% of all exclusions worked less than 1 year. Most of the remaining exclusions represent female workers and those who left employment before the start of follow-up in each plant. The 13,422 employees included in the follow-up cohort were traced for vital status to the end of 1982. A total of 11,155 workers or 82.5% of the population had worked in the four plants that had complete follow-up of all employees from the start of production. The fifth plant had included a complete cohort of all workers employed from early in production in 1953 and all new employees from that time forward. Thus, the population represents most workers who started employment in these eight plants at the beginning of the industry.

Follow-up of Population

The vital status of each worker has been determined for the U.S. plants through the death notification system of the Social Security Administration and the National Death Index, as well as through follow-up by local plant beneficiary records and motor vehicle administration records. The vital status of individuals who were not working in 1982 were traced through these sources. For any individual who was identified as deceased, a death certificate was sought from the local health department. Death certificates were obtained for 97.2% of the study cohort deaths. The vital status of workers in the Canadian plant could only be determined through company records because of the high cost of a death search through Statistics Canada. However, the Canadian company has a pension and insurance plan that identifies all deaths among employees who worked 10 years or more or reached age 45 during employment. This cohort definition is similar to that of early studies of the rubber industry (*8*). For the analyses presented in this report, the Canadian population has been restricted to workers defined in this way. For known deaths in Canada, death certificates were obtained. All deaths were coded to the Eighth Revision of the International Classification of Causes of Death by a senior nosologist. A physician resolved all questions about disease diagnoses. Thus, the coding of deaths is similar for all plants.

In the previous study of this cohort, direct follow-up of a sample of the population identified previously unrecognized deaths through contact with respondents. If the fact of death was confirmed through retrieval of a certificate, the death was included. Otherwise, the subjects were counted as alive since respondents often could not provide any details about the death.

The mortality analysis included the follow-up cohort of all U.S. plants and, for the Canadian plant, those cohort members who worked 10 years or more or reached age 45 while still employed. This population is designated as the restricted cohort. As seen in Table 3, the vital status

Table 3. Vital status of restricted cohort study population followed to December 31, 1982.

Vital status	Number	Population
Living, employed in plant	2,784	23.0
Living, not in plant	6,472	53.4
Deceased	2,441	20.2
Status unknown	416	3.4
Total	12,113	100.0

was known for 96.6% of these workers. About 20% of the cohort are currently deceased. The group living "employed in plant" in this table designates those workers who were identified as still working in some plants.

Job Categories

The job dictionary created by the investigators categorized each job by five characteristics: plant division, subdivision, work area, job title, and rank whenever applicable. If the worker's history designated a job only, a division was assigned to the job or based on the usual division in which that job appeared. (Recent discussions with process engineers from the industry have indicated that a few of these work areas or division assignments may have resulted in misclassification, but it was impractical to recode the work histories at that time since the study was complete.) The work history of each employee was reviewed and the first job, the last job, and the longest job held were coded for each worker. One or more of these jobs might not be coded for an individual worker if either all or part of the employment history was missing. If a worker had no history, no job was coded. If 50% or more of the work history was missing for the period of employment, no listing of longest job could be determined. In some cases, no listing was included for the first or last job. For one plant, no work histories were available for all workers employed at the time of filming of personnel files in 1976, because the active employees were identified from a computerized roster in that plant.

Analysis

The data were analyzed using cumulative person-years of follow-up for each individual by age and calendar-time in the study cohort. These person-years were then multiplied by the appropriate U.S. rates for white and black males to determine the expected number of deaths. This represents the number of deaths that would have occurred had the workers died at the same rate as the general population. The modified life-table program available from Monson (*9*) was used for the analysis but not for significance testing.

The program allows for several modifications that eliminated any early worker experience in the industry before the start of follow-up. Since workers were included only if they had worked for 1 year, the first year of work experience was omitted from the sum of follow-up years. Workers' person-years of experience were only included from the time plant records were complete, thus avoiding the error of including the early experience of workers who continued employment until start of cohort follow-up. Since the study of the population in the Canadian plant was restricted to employees working 10 or more years or reaching age 45 years while employed, the person-years at risk prior to 10 years of employment or age 45 were omitted to meet these eligibility criteria.

The Canadian population has been compared to population rates for both Canada and Ontario. The results were similar using either comparison population, so only the analyses with Ontario rates are discussed.

The deaths among workers are compared to the reference populations using an indirect adjustment for age, race, and calendar-year of death. The results are expressed as standardized mortality ratios or SMRs that are calculated as the observed deaths among workers divided by the deaths which would have occurred, had they died at the same rate as the reference population with a similar age, race, and time distribution to that of the workers. Thus, the ratio will be 1.00 if the workers and the reference population have the same risk of dying. The usual healthy-worker effect seen in working populations is apparent for most causes of death in this cohort as well.

Statistically significant excess standardized mortality ratios (SMR) are indicated for those causes which include more than one death. Significance was based on a probability of 0.05 of less (two-tailed) using the Poisson distribution.

Results

The known racial distribution of the population was 75% white, 10% black, 15% unknown, and less than 1% other. However, the racial distribution differs by plant, depending on geographic location. Company personnel made extensive efforts to identify missing information on race from all record sources. However, 15% of the population still had unknown race and therefore were considered white in the analysis. Two plants had missing data on race for 40 to 50% of the workers. However, in only one of these plants is the omission a problem, since the population is predominantly white in all other plants with missing data. Because of the difficulty in determining an appropriate algorithm to assign race to workers without data, any worker with missing race information was assumed to be white. Death certificate data indicating race were used to update the records. Any error that this assumption has caused in the race-adjusted rates must be small since the population is predominantly white.

A total of 2,441 deaths are included in the analysis and 15.6% of them occurred in blacks. This indicates a slightly higher representation of blacks among the deaths than the 9.5% found in the total population. This difference may be due to an artificial inflation of the proportion of whites in the living white population through the inclusion of workers who have no race information or simply a higher mortality in blacks.

The cohort for analysis that restricted the Canadian population to persons with 10 or more years employment includes 12,100 workers who contributed 251,431 person-years of follow-up (Table 4). Three individuals had attained 100 or more years of age and are rejected by the analysis program. No attempt was made to alter information to force their inclusion in the analysis. The current analysis cohort is smaller than in the original study (13,608) because of data corrections and the restriction on the Canadian study population (*5*). However, death ascertainment is probably equally complete in U.S. and Canadian plants when applying this constraint.

The distribution of the population by race and entry into follow-up in the 10-year calendar periods and the median age of workers at entry for each period are shown in Table 5. A large number of workers entered follow-up at the start of the industry in plants with complete cohorts. In the next two 10-year decades, entries represent not only new employees but older workers who start follow-up at late ages because they belong to the plants with incomplete records or to the Canadian plant with the 10-year restriction on employment. Even in the first calendar period with complete cohorts for follow-up, the age at entry is older than one might expect in a newly developed industry. In fact, for the black workers in the two calendar periods 1943 through 1949 and 1970 onward, which would represent complete cohorts of all workers who started in those periods, the age at start was 9 years older for those in the early period, compared to the late time frame.

The standardized mortality ratios by 5-year calendar time periods, as presented in Table 6, are very low (SMR = 0.37; 95% CI: 0.25–0.53) in the early years of follow-up probably because of the hiring of new healthy workers and rise to 0.87 in 1980 and 1982 when the closed population that remained after 1976 is continuing in follow-up. The mortality ratios increase more in the black than the white workers by calendar period. In fact, the SMR for black workers reaches a high of 1.34 (CI: 1.01–1.75) in the final 3-year period of follow-up. However, all ratios in all time periods among white workers indicate that their mortality is low compared to that of the general population.

The standardized mortality ratios for specific causes of death for white and black workers and for the total population adjusted for age, race, and calendar time are presented in the following tables. All results are based on the restricted follow-up cohort in Canada. For the major categories of death (Table 7) the mortality ratios are generally lower than expected, based on population rates. For most causes, white workers and the total population have death rates that are significantly lower than that of the U.S., as demonstrated by the fact that the upper confidence interval is less than 1.00. The only significantly high ratios in this table are those associated with deaths from arteriosclerotic heart disease (ASHD) and the related umbrella category, circulatory system diseases, found in black workers. The ratio for ASHD is 48% higher than would be expected based on population

Table 4. Characteristics of restricted cohort population for analysis.

Population	White	Black	Total
Total cohort	10,915[a]	1,195	12,110
Total person-years of follow-up	226,474.80	24,956.20	251,431
Average survival from start of follow-up	20.75	20.88	20.8
Average age of entry into follow-up	34.08	32.82	34.0
Observed deaths	2,061	380	2,441

[a]Includes 1,767 with unknown race and 62 with other race.

Table 5. Distribution of population by year and median age of entry into follow-up.

Calendar period	White	Median age	Blacks	Median age	Total
1943–1949	2,210	33.9	418	36.3	2,628
1950–1959	3,134	34.9	246	32.7	3,380
1960–1969	3,190	30.6	268	28.8	3,458
1970+	2,381	31.3	263	27.3	2,644
Total	10,915	32.7	1,195	31.3	12,110

Table 6. All-cause standardized mortality ratios by race and calender year of death (reference population: U.S. males).

	White			Black			Total	
Calendar year	Deaths	SMR[a]	95% CI[a]	Deaths	SMR	95% CI	SMR	95% CI
1943–49	22	0.40	0.25–0.61	7	0.30	0.12–0.61	0.37	0.25–0.53
1950–54	71	0.79	0.62–1.00	17	0.54	0.31–0.86	0.73	0.58–0.89
1955–59	135	0.80	0.69–0.97	33	0.84	0.58–1.18	0.80	0.69–0.94
1960–64	203	0.76	0.65–0.86	40	0.77	0.55–1.05	0.76	0.67–0.87
1965–69	313	0.78	0.69–0.87	64	0.96	0.74–1.23	0.81	0.73–0.90
1970–74	445	0.83	0.76–0.92	76	1.01	0.80–1.26	0.85	0.78–0.92
1975–79	509	0.80	0.73–0.87	89	1.22	0.98–1.50	0.84	0.78–0.91
1980–82	363	0.82	0.73–0.91	54	1.34	1.01–1.75	0.87	0.79–0.95
Total	2061	0.79	0.76–0.83	380	0.95	0.85–1.05	0.81	0.78–0.85

[a]SMR, standardized mortality ratio; 95% CI, 95% confidence interval based on Poisson distribution.

Table 7. Standardized mortality ratios for major causes of death adjusted for age, calendar time, and race (reference population: U.S. males).

	White (10,915)			Black (1,195)			Total (12,110)	
Cause of death[a]	Observed	SMR[b]	95% CI[b]	Observed	SMR	95% CI	SMR	95% CI
All causes	2,061	0.79	0.76–0.83	380	0.95	0.85–1.05	0.81	0.78–0.85
All cancers	454	0.85	0.77–0.92	64	0.92	0.71–1.18	0.85	0.78–0.93
All infections	17	0.54	0.32–0.87	9	0.62	0.28–1.17	0.57	0.37–0.83
Nervous system disease	22	0.96	0.60–1.45	3	0.78	0.16–2.28	0.93	0.60–1.38
Circulatory system disease	1,063	0.80	0.75–0.85	214	1.18	1.03–1.35	0.85	0.81–0.89
ASHD	772	0.84	0.78–0.90	125	1.48	1.23–1.76	0.89	0.84–0.95
Vascular CNS	141	0.85	0.72–1.01	47	1.18	0.87–1.57	0.92	0.79–1.06
RHD	20	0.86	0.53–1.33	1	0.36	—	0.81	0.50–1.24
Respiratory disease	106	0.68	0.56–0.82	18	0.79	0.47–1.24	0.69	0.58–0.83
Digestive disease	66	0.51	0.40–0.65	14	0.72	0.39–1.21	0.54	0.43–0.67
Genito-urinary disease	23	0.68	0.43–1.02	7	0.58	0.23–1.19	0.65	0.44–0.93
All external causes	174	0.69	0.59–0.80	39	0.77	0.55–1.05	0.70	0.61–0.80

[a]ASHD, arteriosclerotic heart disease; CNS, central nervous system; RHD, rheumatic heart disease.
[b]SMR, standardized mortality ratio; 95% CI, 95% confidence interval based on Poisson distribution.

rates (SMR = 1.48; 95% CI: 1.23–1.76).

The SMRs for most cancer sites that included five or more deaths (Table 8), are below 1.00, indicating that the risks of these cancers in the workers are less than those of the general population. For some cancers such as those of the kidney, digestive, and lymphohematopoietic system the risks approach those of the reference population. This is somewhat unusual for workers with low overall risks. Since the current update was prompted by toxicologic data relating 1,3-butadiene exposure to development of certain tumors, further analysis has focused on cancers of related biologic systems in humans. These systems include the gastrointestinal, hematopoietic, and lymphatic systems. Extensive evaluation of these sites was conducted, even though the SMRs related to these cancers often were only slightly above 1.00.

The specific sites in the digestive system (Table 8) that seem to contribute a higher risk than others are esophageal cancers in whites (SMR = 1.34; 95% CI: 0.78–2.14), stomach cancers in all workers (SMR = 1.05; 95% CI: 0.73–1.46), cancer of the large intestine in whites (SMR = 0.93; 95% CI: 0.68–1.25), and liver cancers in black workers (SMR = 1.44; 95% CI: 0.30–4.19). The sites of cancer that are high in the hematopoietic system are Hodgkin's disease in whites (SMR = 1.31; 95% CI: 0.56–2.58), leukemia in blacks (SMR = 2.18; 95% CI: 0.59–5.60), and other lymphatic cancers in all workers (SMR = 1.11; 95% CI: 0.64–1.77). None of the ratios is significantly higher than 1.00. The number of deaths in some groups is very small. Nevertheless, it was felt on the basis of these data that further analysis by work area was warranted in order to identify specific patterns of risk. These, in turn, could suggest

Table 8. Standardized mortality ratios for selected cancers adjusted for age, calendar time, and race (reference population: U.S. males).

	White (10,915)			Black (1,195)			Total (12,160)	
Cancers	Observed	SMR[a]	95% CI[a]	Observed	SMR	95% CI	SMR	95% CI
Oral cavity	5	0.30	0.10–0.69	0	0	—	0.26	0.08–0.60
Digestive	137	0.94	0.79–1.11	21	0.92	0.57–1.41	0.93	0.79–1.09
Esophagus	17	1.34	0.78–2.14	0	0	—	1.00	0.58–1.61
Stomach	25	0.95	0.62–1.41	9	1.45	0.66–2.76	1.05	0.73–1.46
Large intestine	45	0.93	0.68–1.25	3	0.67	0.14–1.96	0.91	0.67–1.21
Rectum	11	0.70	0.35–1.24	1	0.62	—	0.69	0.36–1.20
Liver	8	0.75	0.32–1.48	3	1.44	0.30–4.19	0.86	0.43–1.54
Pancreas	24	0.83	0.53–1.23	3	0.80	0.17–2.35	0.83	0.54–1.20
Respiratory	157	0.83	0.70–0.98	20	0.93	0.57–1.43	0.84	0.72–0.98
Prostate	28	0.82	0.54–1.18	9	1.18	0.54–2.24	0.88	0.62–1.22
Bladder	11	0.72	0.36–1.28	1	0.67	—	0.71	0.37–1.24
Kidney	15	1.11	0.62–2.05	0	0	—	1.03	0.58–1.69
Brain	14	0.85	0.47–1.43	0	0	—	0.81	0.44–1.36
All lymphopoietic	48	0.92	0.68–1.23	7	1.46	0.59–3.01	0.97	0.73–1.26
Lymphosarcoma	6	0.56	0.21–1.22	1	1.32	—	0.61	0.25–1.26
Hodgkin's	8	1.31	0.56–2.58	0	0	—	1.20	0.52–2.37
Leukemia	18	0.86	0.51–1.36	4	2.18	0.59–5.60	0.96	0.60–1.46
Other lymphatic	15	1.10	0.62–1.81	2	1.16	0.14–4.20	1.11	0.64–1.77

[a]SMR, standardized mortality ratio; 95% CI, 95% confidence interval based on Poisson distribution.

that different exposures might be associated with the risks.

The total population ratios were standardized for region using Ontario rates as a comparison for the Canadian population and the U.S. rates for other areas. These ratios do not differ from those presented previously. For example, the all-cause SMR was 0.83 after adjustment for region compared to 0.81 in the previous analysis. The only differences of note were a decrease in the SMR for stomach cancer, from 1.05 unadjusted to 0.91 adjusted, and an increase in the all lymphopoietic category from 0.97 to 1.06.

Work Areas

The population was divided into four major work areas on the basis of the job held the longest. Three plant divisions, production, utilities, and maintenance, and the combination of all other work sites constituted the four groups for analysis (Table 9). These divisions are similar to those in the original study (*5*). No attempt has been made in this analysis to combine jobs or work areas by presumed exposures, although one might assume that production will include more exposures than other work sites, with the possible exception of the laboratories. The purpose of the analysis was to divide the population into major work activities in order to determine whether there are any different patterns of risk. The production grouping includes workers involved in any processes that produce the rubber and who may, therefore, have had some exposure to the basic chemicals that form the raw product. Maintenance includes workers exposed to materials related to their trades and incidental exposures to the agents used in the industrial processes. Utilities represent support facilities whose workers may have exposures to specific agents. Finally, the other-job category includes warehouse, laboratory, and administration work sites. This diverse group had workers who probably had exposures in laboratories and other workers such as administrative personnel who received no exposure.

Table 9. Work areas and jobs included in analysis by work history.

Work area	Job	
Production	Tank farm Pigment preparation or solution make-up Reactor—control meters, inspect reactors, blowdown latex Recovery—stripping styrene and butadiene Finishing Latex blending—addition of brine Coagulation—addition of sulfuric acid Washing and drying Baling and packaging Reclaiming	
Warehouse and shipping		
Utilities	Boiler house operator Water tower operator	Pump house operator Refrigeration operator
Maintenance	Boilermaker Carpenter Electrician Instrument repair Insulator Machinist Mechanic Oiler Painter Pipefitter	Sheetmetal Water gun operator or water truck driver Welder General (engineers) Millwright Mason Blacksmith Ironworkers
Laboratory and quality control		
Research and development		
Administration		
Other	Drivers Environmental controls Firemen Guards	Janitors Kitchen Medical Safety

Since a classification of longest job required complete job history records, 2391 workers (19.7%) who had gaps in their work history were omitted from these analyses. The plant that provided a computer tape of current workers in 1976 had limited job information on the active population, and this group represents 75% of all persons omitted from the work area analysis. The loss of person-years from the low risk group of recent, healthy, active workers may have artificially inflated the SMRs reported by work areas.

An examination of the specific causes of death in production workers adjusted for age, race, and time (Table 10) shows low mortality for all causes (SMR = 0.88; 95% CI: 0.81–0.95) and cancers (SMR = 0.92; 95% CI: 0.76–1.09). However, deaths from cancers of the hematologic system occurred at higher rates than expected (SMR = 1.46; 95% CI: 0.88–2.27). The ratio for black workers is 5 times higher than the mortality expected in U.S. black males and is significant (SMR = 5.07; 95% CI: 1.87–11.07). The ratio for the whites is near unity (SMR = 1.10; 95% CI: 0.58–1.87). Among the subcategories, there is no excess risk of lymphosarcoma. The ratio for Hodgkin's disease is greater than one in white workers, but it is based on only two deaths. The SMR for leukemia is 0.84 for white males (SMR = 0.84; 95% CI: 0.22–2.15), but the SMR indicates a significant excess risk in black workers, which is 6.6 times higher than expected in comparable groups (SMR = 6.56; 95% CI: 1.35–19.06). The total race-adjusted ratio is above one (SMR = 1.34; 95% CI: 0.53–2.76) but not significant. For the categories of other lymphomas that includes cases of non-Hodgkin's lymphomas as well as multiple myeloma, the ratios are high for both races and significantly high in the total population, with a 2.6-fold excess risk in the rubber workers compared in U.S. males (SMR = 2.60; 95% CI: 1.99–4.94). These cases represent four non-Hodgkin's lymphomas and five multiple myelomas according to the death certificates. On the other hand, the risk of death from digestive system

Table 10. Standardized mortality ratios of production workers for selected causes of death adjusted for age, calendar-time, and race (reference population: U.S. males).

	White (2,753)			Black (371)			Total (3,124)	
Cause[a]	Observed	SMR[b]	95% CI[b]	Oberved	SMR	95% CI	SMR	95% CI
All causes	502	0.87	0.79–0.94	92	0.93	0.75– 1.14	0.88	0.81–0.95
Circulatory System disease	254	0.89	0.78–1.01	49	1.16	0.86– 1.54	0.93	0.82–1.04
ASHD	182	0.91	0.78–1.05	29	1.47	0.99– 2.11	0.96	0.83–1.10
Vascular CNS	37	1.08	0.76–1.48	9	0.99	0.45– 1.88	1.06	0.77–1.41
RHD	5	0.93	0.30–2.16	1	0.69	—	0.99	0.36–2.14
All cancers	105	0.88	0.72–1.07	19	1.15	0.69– 1.79	0.92	0.76–1.09
Respiratory	43	1.03	0.74–1.39	6	1.14	0.42– 2.48	1.04	0.77–1.38
Larynx	1	0.57	—	0	0	—	0.49	—
Lung	41	1.03	0.74–1.40	6	1.23	0.45– 2.67	1.06	0.78–1.40
Digestive	25	0.79	0.51–1.16	3	0.56	0.11– 1.64	0.75	0.50–1.09
Esophagus	3	1.08	0.22–3.15	0	0	—	0.79	0.16–2.30
Stomach	3	0.53	0.11–1.56	1	0.70	—	0.57	0.15–1.45
Large intestine	10	0.95	0.46–1.75	1	0.96	—	0.95	0.48–1.70
Rectum	2	0.58	0.07–2.11	0	0	—	0.53	0.06–1.90
Liver	0	0	—	1	1.98	—	0.36	—
Pancreas	5	0.79	0.26–1.84	0	0	—	0.69	0.22–1.62
Kidney	5	1.66	0.54–3.88	0	0	—	1.53	0.50–3.57
All lymphopoietic	13	1.10	0.58–1.87	6	5.07	1.87–11.07	1.46	0.88–2.27
Lymphosarcoma	0	0	—	1	5.32	—	0.38	—
Hodgkin's	2	1.31	0.16–4.75	0	0	—	1.20	0.15–4.35
Leukemia	4	0.84	0.22–2.15	3	6.56	1.35–19.06	1.34	0.53–2.76
Other Lymphatic	7	2.30	0.92–4.73	2	4.82	0.59–17.62	2.60	1.19–4.94

[a]ASHD, arteriosclerotic heart disease; CNS, central nervous system; RHD, rheumatic heart disease.
[b]SMR, standardized mortality ratio; 95% CI, 95% confidence interval based on Poisson distribution.

cancers is very low in this work group (SMR = 0.75; 95% CI: 0.50–1.09) with the exception of cancer of the large intestine, which approaches unity (SMR = 0.95; 95% CI: 0.48–1.70). The only other cancer ratios which deserve comment are lung (SMR = 1.06; 95% CI: 078–1.40) and kidney (SMR = 1.53; 95% CI: 0.50–3.57)

Mortality from diseases of the circulatory system in production workers is higher than usual for a working population. Usually occupational populations of healthy workers have low mortality from this group of diseases. The excess is particularly notable for ASHD in black males where the excess is 47% above that expected (SMR = 1.47; 95% CI: 0.99–2.11).

For workers who had held their longest job in maintenance (Table 11), the all-cause SMR is 0.90 (95% CI: 0.85–1.06) and the cancer SMR is 0.95 (95% CI: 0.83–1.10). There are no excesses of hematologic neoplasms with the exception of a nonsignificant excess of Hodgkin's disease, based on only three deaths (SMR = 1.51; 95% CI: 0.31–4.41). Unlike production workers, individuals engaged in maintenance work have SMRs for cancers of the digestive system that exceed one, although they are not significant (SMR = 1.06; 95% CI: 0.81–1.35). The risk is higher in white workers (SMR = 1.11; 95% CI: 0.84–1.45) than in black workers (SMR = 0.78; 95% CI: 0.34–1.53). For white workers, mortality from stomach cancer is 66% higher (SMR = 1.66; 95% CI: 0.93–2.75), intestinal cancers 11% higher (SMR = 1.11; 95% CI: 0.66–1.75), and esophageal cancers 44% higher than expected in the general population (SMR = 1.44; 95% CI: 0.53–3.14). Three testicular cancer deaths versus 0.95 expected (SMR = 3.16) occurred in the total population, and they were all in white maintenance workers.

Both the risk of arteriosclerotic heart disease (SMR = 1.76; 95% CI: 1.36–2.33) as well as the risk of the combined category of circulatory disease (SMR = 1.38; 95% CI: 1.14–1.66) are significantly high in blacks who worked in maintenance jobs, but white workers have a SMR of only 0.91 for ASHD (95% CI: 0.81–1.03). The excess mortality from circulatory system disease probably accounts for the excess of all-cause mortality in black maintenance workers (SMR = 1.05; 95% CI: 0.91–1.21). The excess risk of ASHD in black workers appears in production area employees as well, even though the excess is not significantly high in that group. Vascular lesions of the central nervous system (CNS) occur at a slightly higher than expected rate in blacks in this population (SMR = 1.30; 95% CI: 0.83–1.94). Further investigation of the excesses of ASHD must determine the reasons for these excesses.

The 457 workers who staff the power and refrigeration plants and work in general utilities have an overall mortality ratio of 0.93 (95% CI: 0.77–1.11) (Table 12). The numbers in any disease subcategory are too small to reach firm conclusions about any risks. In general, the SMRs are higher among those specific causes that were also high for the other work groups, that is, digestive and respiratory system cancers and hematologic neoplasms.

Workers with complete job histories who were not included in the previous three work areas are combined into a single mixed group as shown in Table 13. The mortality ratios for this group of workers for all causes

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Table 11. Standardized mortality ratios of maintenance workers for selected causes of death adjusted for age, calendar-time, and race (reference population: U.S. males).

	White (2,828)			Black (443)			Total (3,271)	
Cause[a]	Observed	SMR[b]	95% CI[b]	Observed	SMR	95% CI	SMR	95% CI
All causes	751	0.87	0.81–0.94	186	1.05	0.91–1.21	0.90	0.85–1.06
Circulatory system disease	381	0.84	0.76–0.93	114	1.38	1.14–1.66	0.92	0.84–1.01
ASHD	287	0.91	0.81–1.03	67	1.76	1.36–2.33	1.01	0.90–1.12
Vascular CNS	43	0.72	0.52–0.97	24	1.30	0.83–1.94	0.86	0.66–1.09
RHD	3	0.41	0.08–1.18	0	0	—	0.35	0.07–1.01
All cancers	174	0.99	0.84–1.14	24	0.78	0.50–1.16	0.95	0.83–1.10
Respiratory	60	0.99	0.75–1.27	8	0.86	0.37–1.69	0.97	0.75–1.23
Larynx	0	0	—	0	0	—	0	—
Lung	59	1.03	0.78–1.33	8	0.92	0.40–1.82	1.01	0.79–1.29
Digestive	55	1.11	0.84–1.45	8	0.78	0.34–1.53	1.06	0.81–1.35
Esophagus	6	1.44	0.53–3.14	0	0	—	1.00	0.37–2.17
Stomach	15	1.66	0.93–2.75	3	1.04	0.21–3.04	1.51	0.90–2.39
Large intestine	18	1.11	0.66–1.75	1	0.50	—	1.04	0.63–1.62
Rectum	5	0.92	0.30–2.15	1	1.37	—	0.97	0.36–2.12
Liver	1	0.28	—	0	0	—	0.22	—
Pancreas	8	0.83	0.36–1.64	1	0.60	—	0.80	0.36–1.51
Kidney	5	1.15	0.37–2.69	0	0	—	1.04	0.34–2.43
All lymphopoietic	14	0.85	0.46–1.42	0	0	—	0.75	0.41–1.26
Lymphosarcoma	2	0.59	0.07–2.12	0	0	—	0.53	0.06–1.93
Hodgkin's	3	1.70	0.35–4.95	0	0	—	1.51	0.31–4.41
Leukemia	6	0.88	0.32–1.92	0	0	—	0.79	0.29–1.72
Other lymphatic	2	0.46	0.06–1.65	0	0	—	0.39	0.05–1.41

[a]ASHD, arteriosclerotic heart disease; CNS, central nervous system; RHD, rheumatic heart disease.
[b]SMR, standardized mortality ratio; 95% CI, 95% confidence interval based on Poisson distribution.

Table 12. Standardized mortality ratios of utility workers for selected causes of death adjusted for age, calendar-time and race (reference population: U.S. males).

Causes	Observed	SMR[a]	95% CI[a]
All causes	126	0.93	0.77–1.11
All cancers	30	1.13	0.77–1.62
Digestive cancers	8	1.05	0.45–2.06
Large intestine	4	1.61	0.44–4.13
Respiratory cancers	13	1.49	0.79–2.55
Larynx	2	5.13	0.62–8.52
Lung cancers	10	1.22	0.58–2.24
Lymphopoietic cancers	5	2.03	0.66–4.74
Leukemia	2	1.92	0.23–6.95
Other lymphatic	2	3.13	0.62–6.95

[a]SMR, standardized mortality ratio; 95% CI, 95% confidence interval based on Poisson distribution.

and cancer are low, 0.85 (95% CI: 0.79–0.92) and 0.84 (95% CI: 0.70–1.01), respectively. Only the mortality from rheumatic heart disease in white workers is significantly high with an SMR of 2.09 (95% CI: 1.08–3.65). Mortality from chronic nephritis is also high (SMR = 1.89; 95% CI: 0.61–4.40) but not significant. These sites would not be expected to be associated with industry exposures unless there is misdiagnosis since they are usually associated with an infectious etiology.

Many other ratios for this group exceed one but they are not significant, and there is no apparent pattern as noted for the other two major work areas. The SMRs that exceed one are those that were high in work areas previously reviewed, such as digestive tract cancers and stomach cancers in blacks and larynx cancers, leukemia, and Hodgkin's disease in white. The sites that have mortality ratios above expected specifically for this area are: liver cancers, with seven cases in both races (SMR = 2.24; 95% CI: 090–4.61), pancreas cancers, with ten cases also in all workers (SMR = 1.26; 95% CI: 0.61–2.32), and brain cancer in whites only (SMR = 1.25; 95% CI: 0.41–2.92). This work group needs to be redefined in order to distinguish subgroups that may have had different exposures.

An attempt to identify any association between hematologic neoplasms and the years worked in the rubber industry for the total cohort of workers is shown in Table 14. In general, these analyses show no increase in risk by duration worked or latency. The greatest number of person-years fall in the diagonal with a comparable number of years worked and years from first employment. A large proportion of current employees contribute person-years to that group, but not deaths. At most, half of this population could only contribute person-years in categories with 10 or more years of employment. Therefore, a clear trend is difficult to identify. However, the SMR is 1.34 (95% CI: 0.83–2.05) for long-term workers who have been followed for 10 years or more from first employment (latent period) and who worked 10 to 19 years.

Discussion

The total population of workers in the synthetic rubber industry show no significant excess in mortality from any specific cause of death adjusted for age, race, and time. As reported in the original study, there is a significant excess of arteriosclerotic heart disease among black employees. That group demonstrates a 45% incease in mortality over that expected based on U.S. black male rates. The significant 18% increase in all circulatory

Table 13. Standardized mortality ratios of workers in "other" category for selected causes of death adjusted for age, calendar-time and race (reference population: U.S. males).

	White (2,630)			Black (237)			Total (2,867)	
Cause[a]	Observed	SMR[b]	95% CI[b]	Observed	SMR	95% CI	SMR	95% CI
All causes	545	0.85	0.78–0.92	79	0.90	0.71– 1.12	0.85	0.79–0.92
Circulatory system disease	301	0.92	0.82–1.03	40	1.01	0.72– 1.38	0.93	0.83–1.03
ASHD	208	0.92	0.80–1.05	21	1.12	0.69– 1.71	0.93	0.82–1.06
Vascular CNS	47	1.13	0.83–1.51	12	1.38	0.71– 2.41	1.18	0.90–1.52
RHD	12	2.09	1.08–3.65	0	0	—	1.90	0.98–3.31
All cancers	105	0.80	0.65–0.96	19	1.23	0.75– 1.95	0.84	0.70–1.01
Respiratory	32	0.70	0.48–0.99	6	1.25	0.46– 2.73	0.75	0.53–1.04
Larynx	3	1.54	0.32–4.50	0	0	—	1.35	0.28–3.93
Lung	29	0.67	0.45–0.96	6	1.35	0.49– 2.93	0.73	0.51–1.02
Digestive	35	0.97	0.67–1.34	8	1.60	0.69– 3.15	1.04	0.75–1.40
Esophagus	2	0.64	0.08–2.32	0	0	—	0.50	0.06–1.78
Stomach	5	0.75	0.24–1.75	4	2.97	0.81– 7.59	1.12	0.51–2.13
Large intestines	10	0.85	0.41–1.55	1	1.00	—	0.86	0.43–1.53
Rectum	2	0.51	0.06–1.82	0	0	—	0.46	0.06–1.68
Liver	5	1.88	0.61–4.37	2	4.39	0.53–15.71	2.24	0.90–4.61
Pancreas	9	1.27	0.58–2.41	1	1.20	—	1.26	0.61–2.32
Kidney	1	0.30	—	0	0	—	0.28	
All lymphopoietic	11	0.86	0.43–1.54	1	0.94	—	0.87	0.45–1.52
Lymphosarcoma	1	0.38	—	0	0	—	0.36	
Hodgkin's	2	1.30	0.16–4.72	0	0	—	1.22	0.15–4.41
Leukemia	6	1.16	0.43–2.52	1	2.46	—	1.25	0.50–2.58
Other lymphatic	2	0.61	0.07–2.20	0	0	—	0.54	0.07–1.96

[a]ASHD, arteriosclerotic heart disease; CNS, central nervous system; RHD, rheumatic heart disease.
[b]SMR, standardized mortality ratio; 95% CI, 95% confidence interval based on Poisson distribution.

Table 14. Standardized mortality ratios age, race, and time adjusted by duration of employment and by years since first employment: lymphatic and hematopoietic cancers.

	Time from first employment (latency), years									
	< 10		10 – 19		20 – 29		30 – 39		Total	
Years worked	Observed	SMR	Observed	SMR	Observed	SMR	Observed	SMR	Observed	SMR
< 10	3	0.46	2	0.42	4	0.73	7	1.65	16	0.76
10–19			14	1.31	8	0.84	4	2.74	21	1.34
20–29					14	1.28	0	0.00	14	0.96
30–39							4	0.72	4	0.72
Total	3	0.46	16	1.03	21	1.05	15	1.02	55	0.97

disease reflects this increase in arteriosclerotic heart disease, as well as a small nonsignificant increase of 18% in deaths from vascular lesions of the central nervous system. There is no known industrial exposure that explains these excesses. How much this difference has resulted from the bias of having racial information on all deaths and not on all living workers is unknown. Since any worker with unknown race is assumed to be white, the mortality ratios for blacks are overestimated and whites are underestimated to the extent that race for unknown workers is erroneously classified. Therefore, the total SMRs are probably the most correct representation of risk.

Canadian mortality rates provided by Statistics Canada for both Canada and Ontario were used for geographic adjustment. In general, the results did not differ when this correction was added. The grouping of major disease rates differed between Canada and the U.S. mortality data. This variation and possible discrepancies in some rates made it practical to use U.S. rates for all work area analysis.

Since ratios for the total population do not provide any discrimination as to exposures of the population for analysis, a first step in examining potential work place hazards is the division of the population into large work areas. In this study, individuals were assigned to four work areas, based on longest job held. Deaths from cancers of the hematopoietic and lymphopoietic system are higher than expected in production workers with significant excesses for leukemias in black workers and other lymphomas in all workers. Although these cancers occur in excess for workers in utilities and the other miscellaneous work group, the ratios are not as high. For the large number of workers in maintenance, there is no excess risk of hematologic cancers but a higher than expected mortality for various cancer sites in the digestive tract. Production workers do not have this excess. Although the mortality for these digestive system cancers may not be significantly different from that of the reference population, the completely different cancer sites that show an excess mortality for maintenance versus production workers suggest that variation in

exposures in the two groups might be playing a role. Although it is possible that, especially for stomach cancer, ethnicity or socioeconomic factors may be playing a role and that different ethnic groups may hold different jobs in the industry, this explanation seems less likely than the attribution of the differences to exposures related to their work. These differences in cancers by work areas are also interesting because of their consistency among a limited number of sites. All hematologic neoplasms except lymphosarcoma have mortality ratios above 1.0 for production workers. The specific digestive system cancers that occur in excess in maintenance workers are esophagus, stomach, and large intestine. Workers employed in administration and the other combined work sites including laboratories have different causes of death that occur in excess than either in production or maintenance workers. Chronic rheumatic heart disease and even chronic nephritis have high mortality ratios in these workers. One might postulate that these excesses may reflect the bias of selecting workers who were not eligible to serve in the military in the early years of the industry and were employed in sedentary, nonphysically demanding jobs in administration. This observation needs to be examined further. However, the group also had a high risk of liver and pancreatic cancers that could not be explained by the same selection bias. The risk for laboratory personnel included in this group should be examined separately since they may be the source of these cancer risks. Confirmation of the information on disease outcome and work history is essential.

Missing information on total work history has forced the exclusion of 2391 workers in these tables. Many of those omitted were active workers in 1976 and are more likely to be alive than dead at the end of the study. This would mean deceased workers and their person-years represent a higher proportion of the population that is included in the calculation of expected deaths for the work area analysis, compared to the total population mortality analysis. This omission of work histories in living workers results in an all-cause mortality ratio for the combined work areas of 0.88, which, although still below one, is higher than the ratio for the total study cohort (SMR = 0.81). This higher percentage of deceased workers could result in higher cause-specific ratios by work area than those seen in the total population. However, these results would not explain the differences in risks of specific causes of death by work areas since the missing work histories for active employees would have occurred for all work areas.

Since the risks of specific causes of death such as hematologic neoplasms seem to be slightly higher than that of the general population, and, since these causes seem to be associated with different work areas, this population needs further study. The exposures by job must be identified including those related to this specific industry, such as 1,3-butadiene and styrene, as well as those associated with trades, such as welders and pipe fitters. The differences in risks for production and maintenance workers may suggest that industrial exposures are related to the cancers found in the former group, and the exposures related to a trade may be related to the digestive system cancers. An assessment of estimated levels of exposure to 1,3-butadiene and styrene for each job is necessary and should be used in a case-control study.

Summary

The mortality of workers in the styrene-1,3-butadiene polymer manufacturing industry is generally much lower than that of comparable populations. However, an excess risk of death from hematologic neoplasms, especially leukemia and other lymphomas, in production workers suggests that a further examination of exposures associated with work in this industry is warranted. The small excess of digestive system cancers among maintenance workers may represent risks associated with their specific trades or industrial exposures.

NOTE ADDED TO PROOF: In a follow-up nested case-control study in which the cases of lymphopoietic cancers were compared to an internal population of workers who did not have cancer, we found that the leukemia cases were associated with exposure specifically to butadiene (odds ratio: 9.4; 95% confidence interval: 2.1–22.9), whereas there was not a significantly increased risk associated with exposure to styrene [Matanoski et al., ILSI Monograph: Assessment of Inhalation Hazards (1989)].

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Assessment of 1,3-Butadiene Epidemiology Studies

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Positive carcinogenicity studies in mice and rats have led to concerns that 1,3-butadiene may be carcinogenic in humans under exposure conditions that have existed in occupational settings and perhaps exist today. The principal settings of interest are the styrene-butadiene rubber (SBR) manufacturing industry, which uses large quantities of 1,3-butadiene, and the 1,3-butadiene monomer industry. The potential for 1,3-butadiene exposure is highest during monomer transfer operations and is lowest in finishing areas of polymerization plants where the polymer products are processed.

Three large cohort mortality studies have been conducted in the SBR and monomer producing industries since 1980. These studies, which examined the mortality experience of over 17,000 men employed in one monomer and 10 SBR facilities, are the subject of this review. All but one of the facilites began operations during the early 1940s. The mortality experience observed within these employee cohorts is comparable to that seen in other long-term studies of men employed in the petroleum, chemical, and rubber industries for all causes of death, total malignant neoplasms, and for the specific cancers seen in excess in the toxicologic studies.

This paper discusses discrepant findings observed in more detailed analyses within individual cohorts and among employment subgroups, as well as selected limitations of the particular studies. Additional efforts to refine 1,3-butadiene exposure categories are needed. Within the context of sample size limitations inherent in these studies, there is currently inadequate evidence to establish a relationship between cancer mortality outcomes and 1,3-butadiene exposure in humans.

Assessment of 1,3-Butadiene Epidemiology Studies

Both toxicologic and epidemiologic studies have led to concerns that 1,3-butadiene may be carcinogenic in humans (*1–4*). The existing literature on this subject was reviewed by the International Agency for Research on Cancer (IARC) in 1985 (*5*). On the basis of that review, IARC concluded that sufficient evidence did exist to classify 1,3-butadiene as a carcinogen in experimental animals. IARC concluded that there was inadequate evidence for the carcinogenicity of 1,3-butadiene in humans.

The epidemiologic literature available in 1985 was limited, consisting of several studies conducted in the rubber industry and one study of two styrene-butadiene polymerization facilities (*3,4*). The rubber worker study by McMichael et al. (*3*) is mentioned because a small styrene-butadiene polymerization facility was included among the facilities surveyed. The potential for exposure to 1,3-butadiene is very low in finished rubber products manufacturing.

Since 1985, two large epidemiologic studies have been reported that specifically examined the long-term health experience of 1,3-butadiene exposed individuals (*6,7*). Given these additional studies, both of which have been updated at this symposium, it is appropriate to revisit the question of the adequacy of evidence for or against the human carcinogenicity of 1,3-butadiene.

History of the Industrial Processes Studied

The three studies that most specifically address the potential carcinogenic effects of 1,3-butadiene examined the mortality experience of employees from one Canadian and nine U.S. synthetic rubber manufacturing plants and a major 1,3-butadiene production facility (*4,6,7*). The synthetic rubber industry grew rapidly during the 1940s and matured quickly from a technological viewpoint. The dramatic growth was spurred by the threatened cutoff of natural rubber supplies during World War II (*8*). The technological maturity of the industry was advanced by the cooperative research efforts among the various producers and the U.S. government during the 1940s.

The major product of the industry was styrene-butadiene rubber polymer (SBR). The ratio of 1,3-butadiene to styrene used in manufacturing SBR was approximately 75 parts 1,3-butadiene to 25 parts

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styrene. Other synthetic rubbers were also manufactured in lesser quantities. These included polybutadiene rubber and rubbers based on polymers of 1,3-butadiene and acrylonitrile. SBR production was initiated in 1942. Production volumes reached 181,000 metric tons in 1943 and increased to 714,000 metric tons by 1945 (*9*). U.S. production peaked at about 1,398,000 metric tons in 1978 and thereafter has declined to 735,000 metric tons.

Large quantities of 1,3-butadiene were required for the synthetic rubber industry. Consequently, 1,3-butadiene production expanded in parallel with SBR production. 1,3-Butadiene has been produced by several methods including the dehydrogenation of *n*-butane, the catalytic dehydrogenation of *n*-butene, and the extractive distillation of C4 by-products from ethylene production. The latter method is principally used today because of the increased demand for ethylene.

Process Descriptions

All but one of the 10 SBR plants addressed in the epidemiologic studies had initially started production by 1943; one plant began operations in 1957. Earliest production was by batch process. Potassium persulfate and dodecyl mercaptan were used to initiate the polymerization reaction that occurred at a temperature of about 50°C (*8*). Hydroquinone was employed to stop the polymerization and *N*-phenyl-2-naphthylamine was used to stabilize the latex product.

Two major process developments occurred during the early years of SBR production. The first refinement, and perhaps the most important of the two in terms of exposure potential, was the development of a continuous feed system for the polymerization step. This increased production capacity and decreased the need for opening and closing the reactor system. By May of 1948, continuous polymerization was in operation at all of the larger plants (*10*). The second refinement was based on the discovery of polymerization initiators, such as diazothioethers that allowed the polymerization to be carried out at temperatures of 5°C and below. The first large-scale production of cold rubber, that is, SBR produced at low reaction temperatures, was carried out in February of 1948 (*10*). This development generally followed the conversion to continuous processes. For example, in one of the plants studied by Meinhardt et al. (*4*), conversion to a continuous feed system occurred in 1946, while cold rubber production began in 1949. Plant modernizations have taken place over the years, but the basic processing steps have remained unchanged.

The 1,3-butadiene production process studied by Downs et al. (*7*) was operated in an entirely enclosed system since the plant opening in 1943. The process was converted from a catalytic to an oxidative dehydrogenation process in 1975. A detailed description of the process is contained in an appendix to the published report (*7*). This plant had the highest rated capacity of any existing butadiene facility in 1945 (*9*).

Study Populations

For each of the three epidemiologic studies, eligible employees were determined from a review of personnel records at the facilities (*4,6,7*). The availability of records and criteria for selection varied across plants within studies and varied among studies. A brief summary of major selection features is given in Table 1. Minimum employment requirements ranged from 6 months to 1 year, depending on cohort. The period of observation ended between 1976 and 1985. Nevertheless, the maximum observation period for each study was at least 33 years. Cohort identification in the SBR studies extended into or through 1976, whereas cohort identification for the monomer production facility extended through 1979. Women were excluded from all three studies and nonwhite men were excluded from the smaller of the two studies of SBR employees. This latter study also excluded managerial and administrative only personnel.

Additional restrictions were necessary due to inadequacies in record systems or other problems of obtaining complete records. In cohort 2, plant B changed ownership and was not in operation for 3 years before 1950 (*4*). Prior records could not be obtained, hence cohort identi-

Table 1. Descriptions of three study cohorts used in 1,3-butadiene evaluation.

	SBR studies[a]		Monomer study
Descriptive variables	Cohort 1 (*11*)	Cohort 2 (*4*)	Cohort 3 (*12*)
Number of facilities	8	2	1
Size of population	12,110	2,756	2,582
Years of observation	1943–1982	1943–1976[b]	1943–1985
Minimum employment required	1 year	6 months	6 months
Employee exclusions	Women	Women, nonwhites, managerial, and administrative	Women
Restrictions due to incomplete records	Left-censoring 4 plants prior to 1953, 1958, 1964, 1970; in one plant employees under age 45 with < 10 years employment	Left censoring one plant prior to 1950	

[a]SBR, styrene-butadiene rubber.
[b]Preliminary vital status follow-up completed through 1982.

lation began in 1950. This cohort was thus subject to left censoring. In cohort studies left censoring occurs when otherwise qualified individuals are lost to study because of missing information at the beginning of their observation period. Right censoring results from individuals becoming lost to follow-up after qualification for the study.

In the larger SBR study, four of the facilities were similarly subject to left censoring, as employee records of former employees were not retained until 1953, 1958, 1964, and 1970, respectively (*11*). Thus, only a portion of the total worker population employed prior to these dates could be identified for study. Finally the Canadian plant was subject to an additional restriction. Since vital status follow-up could only be completed for vested employees, the Canadian subcohort was restricted to men with 10 years of employment or who had reached age 45 before termination. The population sizes for the three studies were 12,110 and 2,756 for the two SBR studies and 2,582 for the monomer plant study (*4,11,12*). These statistics reflect the studies as updated in 1988. The study populations were predominantly selected from the Gulf Coast region since the facilities for both of the smaller studies were located in Port Neches, TX, and six of the eight plants included in the larger SBR study were located in Texas (four plants) and Louisiana (two plants). However, the Texas plants contributed only about a third of the workers to the larger SBR study.

Assessment of Exposure

Industrial hygiene data were not available for the facilities covered by these studies until the 1970s. Therefore, exposure potential has been categorized based on knowledge of the substances used in the processes and their physical and chemical properties, process descriptions, job descriptions, and the limited industrial hygiene data available after 1975.

From process descriptions, it is apparent that 1,3-butadiene and styrene were the primary process materials in the SBR facilities and that 1,3-butadiene and *n*-butene were the dominant substances in monomer production. Other chemicals were generally used in much smaller quantities as initiators (potassium persulfate, diazothioethers, paramenthane hydroperoxide, sodium formaldehyde sulfoxylate, and dodecyl mercaptan), regulators (hydroquinone, *N*-phenyl-2-naphthylamine, and diphenylamine), and product modifiers (carbon black, aromatic extender oils, and other materials). Furfural was used in isolating and purifying the finished product in the 1,3-butadiene monomer facility and pilot plants in the SBR facilities may have produced smaller quantities of a variety of other synthetic rubbers.

Study participants may have worked in other company operations or have been employed elsewhere in other related work activities. This is particularly true for short-term employees. There is evidence of crossover between the 1,3-butadiene monomer facility and the two SBR facilities that it served, even though different companies owned the plants. Approximately 120 men were found to have worked in both the monomer manufacturing facility and one of the SBR plants, including one man diagnosed with leukemia (*12*).

The general approach used in the three studies to define exposure subgroups was to assign individuals to broad work area categories based on job assignment information. In the smaller of the SBR cohorts (*4*), the only subgrouping of employees was by their employment date. Those men hired prior to the end of 1945 were separately identified for analysis. This date roughly coincided with the conversion of the production process to a continuous feed operation. Job assignment data were not discussed in detail; however, the cohort was limited to nonmanagerial and nonadministrative personnel. Summary industrial hygiene data, collected at the time of the study, were presented for 1,3-butadiene, styrene, and benzene. Styrene concentrations in both plants were below 15 ppm time-weighted-average (TWA); 1,3-butadiene concentrations averaged 13.5 ppm across samples; and benzene concentrations were below 1 ppm. Benzene was not known to have been used in the facility, but monitoring was done because of an *a priori* concern regarding two leukemias at the facilities.

In the two remaining studies, work area or exposure categories were defined based on job assignments. In the larger SBR study (*11*), four categories were employed: production, utilities, maintenance, and miscellaneous (laboratory and quality control, research and development, administration, warehouse and shipping, and other plant support personnel). In the monomer facility, exposure was categorized as low (utilities, certain skilled craftsmen, office and management), routine (production workers, laboratory workers, and chemical distribution workers), nonroutine (skilled maintenance and fire department employees), and unknown (truck drivers, supervisors, and engineers). No subcategorization of production employees was provided in either study. These latter two studies addressed duration of employment and latency considerations as well as broad work areas.

The common element across the three studies is the large-scale use of 1,3-butadiene in the facilities being studied. Recognition of the presence of multiple chemical agents is indicated. However, the multiple agent issue was not considered in any proposed exposure classification. A table of 8-hr TWA exposure data for 1,3-butadiene in the SBR industry was included in the IARC review document (*5*). The observations were based on personnel samples collected from 1976 to 1981 and were presented by job assignment. Some of the measurement data may have been based on total C4 compounds present in the sample, rather than having been specific to 1,3-butadiene.

The available industrial hygiene data were examined to determine if the distributions of exposure concentrations by job corresponded to what is known about the nature of the jobs and their relationship to the SBR

Table 2. Eight-hour, time-weighted-average exposures in SBR[a] plants, 1976–1981.

Job grouping	Number of samples	Percent distribution of samples			
		0–5 ppm	5.1–10 ppm	10.1–25 ppm	25.1 ≥ ppm
1[b]	1886	97	2	1	1
2[c]	847	84	7	5	4
3[d]	794	76	8	8	8
4[e]	259	42	5	12	41

[a]SBR, styrene-butadiene rubber.
[b]Foreman, charge solution makeup, vessel cleaners, waste treatment, finishing operators, warehousemen.
[c]Lab analysts, maintenance crafts (nonroutine).
[d]Stripping-column operators, reactor operators.
[e]Tank car loaders/unloaders.

process. Since 1,3-butadiene is a very volatile gas, potential exposures would be anticipated to be highest during transfer operations and would be expected to be higher in the polymerization than in finishing areas of the plant.

The measurement data are summarized in Table 2 by four job groupings. There are readily apparent differences in the distribution of 1,3-butadiene concentrations across job groupings. For job group one, 97% of the sample readings were below 5 ppm TWA; for each job in their grouping at least 90% of the readings were below 5 ppm TWA. The jobs themselves are primarily distributed in the finishing area of the process. These included coagulation operators, dryer operators, baler and packing operations, and warehousemen. Charge solution makeup occurs before the reaction stage and thus would be expected to present a low opportunity for exposure to 1,3-butadiene. Foremen and vessel cleaners were also assigned to job group one because of the low frequency of readings over 5 ppm TWA. Foremen might be expected to spend time in both polymerization and finishing areas of the plants and to be involved in other supervisory, training, and trouble-shooting activities. Vessel cleaners are responsible for cleaning the reactors after the polymerization step has been completed. Without additional knowledge of the work practices related to this activity, it is not evident why exposure readings experienced in this job are so low, and it is not known whether exposures related to this activity were higher in earlier years.

The second job grouping included individuals assigned to nonproduction jobs who may have been exposed to 1,3-butadiene on a nonroutine basis. For example, individuals in various maintenance crafts would have worked in the SBR production areas on an as-needed basis. Similarly, quality assurance personnel may have been exposed during 1,3-butadiene sample collection and processing but not necessarily at other times during the day. What is not evident from the industrial hygiene data is whether or not sampling was performed only on those occasions when the individual was involved in butadiene-related activities.

The third job grouping included reactor and stripping-column operators. These operators were assigned to the polymerization area or the area in which unreacted monomers were removed and recovered. As indicated in Table 2, these jobs were associated with a greater opportunity for 1,3-butadiene exposure than jobs in the finishing area. The fourth job grouping was made up of jobs involving 1,3-butadiene transfer operations. Highest exposure concentrations have been measured for these jobs, consistent with expectation. The available data do not indicate whether or not respiratory protection was worn during these activities. What is also not known from these or any other extant data is the secular trends in 1,3-butadiene exposure prior to the 1970s. It does appear that the processes themselves have not changed greatly.

Findings across Studies

Cancer mortality findings for the three major cohort studies of 1,3-butadiene workers are summarized in Table 3. Observed and expected numbers of deaths and standardized mortality ratios (SMRs) are presented for selected cancer sites. In each study, the investigators had calculated expected deaths by applying age-specific U.S. death rates to the corresponding distributions of person years lived for the respective cohorts.

The SMR is frequently used to describe mortality in occupational cohorts and is calculated simply as the ratio of observed to expected deaths. For ease of presentation, the SMR has been expressed as the ratio multiplied by 100. The choice of the U.S. general or other community-based population as the referent group for an employee population has been criticized based on the recognition that initial and ongoing selection of healthy men may lead to relatively lower death rates in the occupational cohort. This issue is less critical when the period of observation is long, the follow-up of terminated employees is complete, the mortality ratios are examined relative to interval since first employment, and comparisons to the mortality experience of other employee-based cohorts are made.

Aside from the selection issue, there are two weaknesses of the SMR that need to be kept in mind when interpreting mortality findings. First, an SMR can be misleading when the implied assumption of a constant proportional hazard is not true for all population subgroups. In other words, there may be risks not reflected in the summary SMR because of the dilution of effects specific to a particular group of employees. In practice,

Table 3. Mortality findings for three 1,3-butadiene studies.[a]

Cause of death category	SBR plants				Monomer plant	
	Cohort 1 (*11*)		Cohort 2 (*4*)		Cohort 3 (*12*)	
	O/E	SMR	O/E	SMR	O/E	SMR
All causes	2441/3001	81	332/430	77	826/980	84
All cancer	518/606.7	85	56/78.1	72	163/202.7	80
Total digestive	158/169.1	93	13/22.4	58	39/56.3	69
Esophagus	17/16.9	100	NR		3/4.8	[b]
Stomach	34/32.5	105	NR		4/10.2	39
Respiratory	177/209.8	84	21/24.6	85	57/69.8	82
Brain and other central nervous system	14/17.3	81	NR		4/5.7	70
All lymphopoietic	55/56.7	97	11/8.3	133	25/19.2	130
Leukemia	22/22.8	96	6/3.5	171	8/7.9	102
Hodgkin's disease	8/6.6	120	1/1.4	[b]	3/2.1	
Lymphosarcoma	7/11.5	61	4/2.4	[b]	9/3.9	229
Other lymphatic tissue	17/15.4	111	0/1.1	[b]	5/5.1	97

[a]Abbreviations: SBR, styrene butadiene rubber; O, observed; E, expected; SMR, standardized mortality ratio; NR, not reported.
[b]Fewer than five observed and five expected deaths.

the solution to this problem is to examine and evaluate separately the mortality findings for key subgroups within the cohort before using summary measures to describe the findings. Homogeneity tests are available for quantitatively evaluating the consistency of risk ratios across strata. A second weakness is that SMRs may lack mutual comparability because each SMR is standardized to its own set of internal stratum weights. Again, this is a problem only to the extent that the assumption of a constant proportional hazard is invalid, again, the broad solution is to examine the detailed findings before using the summary measure. Similar considerations apply to the question of combining mortality data across studies.

Returning to Table 3, it can be seen that the SMRs for all causes of death were relatively consistent across the three studies, the SMRs varying between 77 and 84. Similarly, the SMRs for total cancer deaths varied between 72 and 85. The findings were also relatively consistent and unremarkable for respiratory cancer and cancer of the brain. SMRs were relatively higher for digestive system cancers in the larger SBR cohort (cohort 1) but were similar between cohorts 2 and 3. In fact, the general pattern of mortality findings was quite similar between the two Port Neches cohorts for each cause of death examined.

For cancers of the lymphopoietic system, the distribution of deaths by cancer subcategory differed notably between the larger SBR cohort and the two smaller cohorts. The largest contrast was seen for lymphosarcoma deaths where the mortality ratio for cohorts 2 and 3 combined (SMR = 206 based on 13 deaths) was more than two times that of cohort 1 (SMR = 61 based on 7 deaths). The difference was less striking when lymphosarcoma deaths were combined with deaths due to other lymphatic tissue cancers. This latter category includes multiple myeloma and other lymphoid tissue neoplasms; the other lymphoid tissue tumors are often grouped with lymphosarcoma under the rubric of non-Hodgkins lymphoma. For all lymphopoietic cancers, mortality was about 30% higher for cohorts 2 and 3 combined compared to cohort 1; however, the number of observations was small and the difference was not inconsistent with a chance occurrence. There were 36 observed and 34.2 expected leukemia deaths across the three cohorts and no remarkable differences between cohorts.

In Table 4, the distribution of observed and expected deaths due to all lymphopoietic tissue cancer is presented by length of employment in the industry and latency (interval since hire) for cohorts 1 and 3 combined. Comparable data were not available for cohort 2. The SMRs increased slightly with longer intervals since hire and decreased slightly with longer lengths of employment, but these trends are, for the most part, rather unremarkable.

Observed and expected deaths are summarized for production and maintenance employees in Table 5, again combining cohorts 1 and 3. The work area categories are

Table 4. Distribution of observed and expected deaths by length of employment and latency for all lymphopoietic cancer for cohort 1 and cohort 3.[a]

Length of employment, years	Latency, years						Total	
	<10		10–19		20+			
	O/E	SMR	O/E	SMR	O/E	SMR	O/E	SMR
<10	7/8.4	83	5/6.6	76	23/16.5	139	35/31.6	111
10–19			14/12.5	112	10/6.5	154	24/19.0	126
20+					21/25.2	83	21/25.2	83
Total	7/8.4	83	19/19.1	99	54/48.2	112	80/75.8	106

[a]Abbreviations: O/E, observed/expected; SMR, standardized mortality ratio.

Table 5. Observed and expected deaths by cause for production and maintenance employees for cohort 1 and cohort 3 combined.[a]

Cause of death category	Production		Maintenance	
	O/E	SMR	O/E	SMR
All causes	757/890	85	1298/1445	90
All cancer	166/182	91	257/290	89
Total digestive	37/49.2	75	73/83.3	88
Esophagus	3/4.8	[b]	6/7.9	76
Stomach	5/9.0	56	19/16.3	138
Respiratory	63/64.1	98	92/97.9	94
Brain and other central nervous system	3/5.6	54	5/7.4	68
All lymphopoietic	27/17.5	154	25/26.4	95
Leukemia	8/7.0	114	12/10.8	111
Hodgkin's disease	3/2.3	[b]	4/2.8	[b]
Lymphosarcoma	6/3.5	171	4/5.3	75
Other lymphatic tissue	10/4.7	213	4/7.2	56

[a]Abbreviations: O/E, observed/expected; SMR, standardized mortality ratio.
[b]Fewer than five observed and five expected deaths.

approximate since the production group in cohort 3 includes laboratory personnel and 1,3-butadiene-distribution employees. The SMRs for all causes of death and total cancer deaths are similar in both employee groups and are somewhat above the comparable SMRs for the total cohort. The stomach cancer SMR is relatively higher in the maintenance work group than in the production work group; for lymphopoietic cancer the reverse is true. The overall lymphopoietic cancer pattern reflects the differences in lymphosarcoma and cancer of other lymphatic tissue between the two groups. The SMRs for lymphosarcoma and cancer of other lymphatic tissue are 171 (based on 6 deaths) and 213 (based on 10 deaths) in the production group, and they are 75 (based on 4 deaths) and 56 (based on 4 deaths) in the maintenance group. The larger SBR cohort contributed 9 of the 10 observed deaths due to other lymphoid tissue cancers in the production group, whereas the monomer cohort contributed 5 of the 6 observed lymphosarcoma deaths in the production group. The mortality ratios for leukemia and Hodgkin's disease were nearly the same for production versus maintenance employees and did not differ from the ratios observed in the combined population of the three studies.

Discussion

The mortality experience of over 17,000 men employed in the synthetic rubber industry or in a 1,3-butadiene monomer producing facility has been examined in three large retrospective cohort studies (*4,11,12*). In each study the period of observation exceeded 30 years. Overall and total cancer mortality were rather unremarkable in the combined populations from these studies. The corresponding SMRs were comparable to those observed in similar long-term studies of men working in the petroleum, chemical, and rubber industries (*13–21*). Likewise, the SMRs for total lymphopoietic cancer and leukemia of 108 and 105 were consistent with the range of SMRs, 95 to 110 and 88 to 118, respectively, observed in other long-term occupational studies (*13–21*). These cancer sites were targeted for review because of an increased occurrence of lymphomas observed in $B6C3F_1$ mice, relative to 1,3-butadiene exposure (*1*). Hemangiosarcomas of the heart and other proliferative lesions of the lung and forestomach were also reported in the mouse bioassay.

In general, there were no remarkable mortality findings relative to the cancer sites examined for the three cohorts viewed together. Furthermore, a combined analysis of the large SBR cohort and the monomer cohort by length of employment in the facilities and interval since hire failed to provide evidence of a relationship between total lymphopoietic cancer and these factors. Thus, the overall pattern of findings does not indicate that untoward mortality effects have occurred in the studied populations. Nevertheless, an assessment of the carcinogenicity of 1,3-butadiene based on the combined data alone is less than satisfactory. There may be dilution effects across the studies since the cohorts included both production and production support personnel whose exposures to 1,3-butadiene may have been minimal and there have been no analyses based on exposure intensity.

Several discrepant findings were observed in more detailed analyses within individual cohorts and employment subgroups. Among the individual cohorts, there was an increased number of deaths due to lymphosarcoma in cohorts 2 and 3, the two Texas cohorts. For cohort 3 analyses, Downs et al. (*7*) provided mortality comparisons to both a seven-county region of southeast Texas and the general U.S. population. These data indicated that the regional death rates for lymphosarcoma were about 30% higher than the corresponding national rates. This regional difference is too small to account for the total excess of lymphosarcoma deaths observed in cohorts 2 and 3. However, when considered in the context that regional death rates for other lymphatic tissue cancers were lower for southeast Texas than for the rest of the nation, these data suggest that geographic factors play a role in the distributional differences of lymphopoietic tissue cancers seen among the cohorts.

This observation also draws attention to the question the reliability of the death certificate diagnoses within the lymphopoietic system. Detection and confirmation rates are reasonably good for major subcategories such as leukemia and non-Hodgkin's lymphoma, but are lower for more refined subcategories, for example, myeloid leukemia and monocytic leukemia (*22*). In general, difficulties in obtaining accurate diagnoses would be expected to obscure underlying relationships between exposure and disease outcome. While efforts to confirm diagnoses for deaths within the lymphopoietic cancer category may be helpful, this approach does not address the underreporting aspects of the issue.

An interesting observation in the Meinhardt et al. (*4*) study was the short latency period reported for three of the six qualifying leukemia cases and the early hire date of the cases. The interval between first hire and death due to leukemia was 3 years in two cases and 4 years in one case. Two of these decedents had first been employed in the plant prior to 1945, as had the remaining three leukemia decedents with longer intervals between hire date and death. In the remaining two cohorts, only one additional leukemia death was observed with a latency period of under 10 years. This decedent had been hired in 1976. Thus the unusual pattern observed in one cohort relative to interval since hire was not repeated in the other two studies. The larger SBR study did not provide tables to examine separately the leukemia experience of men hired during the early years of operation and therefore, early hire date could not be evaluated across studies.

Mortality comparisons between two broadly defined job activity groups, production and maintenance, identified differences in death rates for several categories. In particular, lymphopoietic cancer death rates were relatively higher among production employees; stomach cancer death rates were relatively higher among maintenance employees. Within the lymphopoietic cancer category, highest SMRs for production employees were observed for lymphosarcoma and other lymphatic tissue cancers. Because production employees may include individuals with potential 1,3-butadiene exposures less than as well as greater than the exposures of maintenance personnel, one can only speculate as to meaning of these data relative to 1,3-butadiene.

Additional efforts to refine the measures of 1,3-butadiene exposure and to develop a more comprehensive assessment of other exposures in the SBR industry would be helpful in more precisely evaluating the mortality findings from these three studies. Continuing efforts to update the existing studies are also needed. In the interim, these studies do not provide convincing evidence that links adverse mortality effects to 1,3-butadiene exposure.

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An Evaluation of the Associations of Leukemia and Rubber Industry Solvent Exposures

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Excessive leukemia mortality has appeared consistently in epidemiological studies of British and U.S. rubber industry workers. Attempts to identify causative factors have focused on exposure to benzene and other solvents. Interpretations of findings from these studies have often been influenced by expectations of a benzene/nonlymphocytic leukemia association, seen from previous work in other settings. However, data from the rubber industry studies have not been consistent with this expectation, as lymphocytic and nonlymphocytic leukemia have shown similar mortality excesses. Data from a small case-control study of lymphocytic leukemia are presented to illustrate an approach that considers multiple solvent exposures. The associations with lymphocytic leukemia risk observed for a number of solvents, most notably carbon tetrachloride and carbon disulfide, were stronger than those detected for benzene.

Key words: leukemia, rubber industry, epidemiology, solvents, benzene, occupational health

INTRODUCTION

Background

Leukemia mortality excesses have been prominent among the findings from numerous epidemiological studies conducted in the British and U.S. rubber industries. Recognition of the extensive use of organic solvents in rubber and tire manufacturing stimulated a priori suspicions that there might exist leukemia hazards in the industry. The well-documented causal association between benzene exposures and leukemia seen in the rotogravure and shoe industries [Vigliani and Saita, 1964; Aksoy and Erdem, 1978] served as a guide in this regard.

The relationship between leukemia risk and solvent exposures in the rubber industry has been, at most, indirectly inferred. The main reason has been that these investigations were retrospective designs—a common, often necessary feature of epidemiological studies of rare, chronic diseases—with either nonexistent or unsystematically obtained environmental exposure data. Nonetheless, the consistently

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observed patterns of elevated leukemia mortality rates, accompanied by apparent localization of risks to workers with the greatest potential for past solvent exposures, prompted the International Agency for Research on Cancer to conclude in a recent monograph on the rubber industry [IARC, 1982a] that there is sufficient evidence to consider the association as causal.

In this paper we review the studies of leukemia and solvent exposures in the British and U.S. rubber industires. Particular attention is paid to the specificity of this relationship, in terms of leukemia cell type, and with regard to the various solvents used in the industry. Much of the interpretation of the findings from previous research has been influenced by how well or poorly the results accord with expectations derived from studies of benzene-exposed workers in other occupational settings. In particular, the presence or absence of a benzene/acute nonlymphocytic leukemia association has been the standard for causality, despite some conflicting evidence. The implications of this approach are discussed.

In addition, we present findings from a small-scale case-control study that are suggestive of etiological associations between lymphocytic leukemia and a number of rubber industry solvents other than benzene, most notably carbon tetrachloride and carbon disulfide.

A detailed summary of the findings from the British and U.S. studies of leukemia among rubber workers is given in Table I. Selected important findings and conclusions drawn from these investigations are discussed below.

British Rubber Industry Studies

Two companion series of epidemiologic studies have been undertaken by the Employment Medical Advisory Service [Fox et al, 1974; Fox and Collier, 1976; Baxter and Werner, 1980] and the British Rubber Manufacturers' Association [Veys, 1981; Parkes et al, 1982]. Modest excesses of leukemia have been observed generally. The most direct evidence pertaining to solvent exposures was a marked excess of acute myeloid leukemia in one tire plant (SMR=714) where plastic film manufacturing, entailing benzene exposure, had occurred in past years [Baxter and Werner, 1980]. In contrast, Parkes et al [1982] discounted the likelihood of a causal association between leukemia mortality and solvents because the observed excesses occurred in years when benzene was no longer used, and there was no unusual leukemia cell type distribution.

U.S. Rubber Industry Studies

Epidemiologic research in the U.S. rubber industry has revealed fairly consistent patterns of elevated leukemia mortality rates (see Table I). While there has been considerable variability in the reported relative risks, most have been in the range of 1.5 to 3.0, and lymphocytic leukemia excesses generally have been as prominent as those from the nonlymphocytic leukemias. Cell type distinctions were not made in many of the studies, however.

Etiological associations with solvents have been examined by some investigators who used work history information to indicate the likelihood for past exposure. Several of these studies warrant comment.

Researchers from the National Institute for Occupational Safety and Health reported a Standardized Mortality Ratio (SMR) of 560 for leukemia among 748 workers engaged in rubber hydrochloride (Pliofilm) manufacturing in the 1940s

[Infante et al, 1977; Rinsky et al, 1981]. Benzene exposures were known to have occurred in the Pliofilm departments studied. An argument for a potent leukemogenic effect of benzene was offered on the basis of the estimated low exposure levels, presumed to be in compliance with existing standards, and because all seven observed leukemia deaths were either myelogenous or monocytic [Rinsky et al, 1981]. Andjelkovich et al [1976, 1977] previously had found no leukemia excess among Pliofilm workers.

McMichael et al [1974] reported a dramatic excess of lymphocytic leukemia (SMR = 764) for workers aged 40–64 in one plant. Leukemia risk was related to work experience in a number of jobs where there existed potential for solvent exposures [McMichael et al, 1976b]. An apparent dose–effect relationship between solvent exposures and lymphocytic leukemia was detected in a case-control study of 15 deaths from several plants [McMichael et al, 1975]. Specific solvents were not identified in these studies, as the exposures were considered to be mixtures of various aromatic and aliphatic compounds.

In a larger case-control study of leukemia deaths in four companies, Wolf et al [1981] confirmed the association of lymphocytic leukemia with solvent exposures in the company reported on earlier by McMichael et al [1975] but did not find similar results for the other three companies. Also, myeloid leukemia was apparently unrelated to solvent exposures.

In the case-control studies reported by McMichael et al [1975] and Wolf et al [1981], solvent exposures were inferred from occupational titles recorded on work history records, where the occupational titles were functionally homogeneous groupings of jobs, similar with respect to materials handled and machinery used [Gamble and Spirtas, 1976]. Occupational titles were useful for distinguishing jobs according to generalized solvent exposure potential; however, exposures to individual solvents could not be specified. Consequently, Arp et al [1983] devised a method to estimate worker exposures to the specific solvents encountered in the company that experienced the greatest lymphocytic leukemia excess [McMichael et al, 1974]. From company documents dating back to the 1920s, Arp created historically validated charts of annual authorized solvent usage by process area for all solvents used in order to study the association between solvents, especially benzene, and lymphocytic leukemia. The cases and controls in Arp's study were the same subjects from this company that were included in the larger case-control study [Wolf et al, 1981]. The entries from work history records and the solvent charts indicated whether workers spent time in a process area when a solvent was authorized for use. Descriptions of the job titles listed on the work histories permitted differentiation of the jobs that involved routine handling of solvents (primary exposure) from other jobs in the process which presumably involved lesser exposure to solvents (secondary exposure).

Table II shows data from Arp's study [Arp et al, 1983] of the solvent exposure history comparisons of 15 lymphocytic leukemia cases with 30 matched controls. In this analysis, workers with potential solvent exposures of at least 1 year were considered exposed. Although workers with secondary solvent exposures experienced only a slight excess of lymphocytic leukemia, the data indicated a 4.5 times greater risk among workers who routinely handled solvents (primary exposure). It is noteworthy that the exposure odds ratio for the benzene categories and the aggregate "other solvent" categories were almost identical.

TABLE 1. Summary of Rubber Industry Studies of Leukemia

Author (year)	Study characteristics	Leukemia type	Exposure	Number observed	Estimated relative risk[a]
British studies					
Fox et al (1974)	Cohort 40,867 males (1967-71)	Unspecified[b]	Overall[c]	14	SMR = 108
Fox and Collier (1976)	Cohort 40,867 males (1972-74)	Unspecified	Overall	14	SMR = 128
		Unspecified	Tire sector	6	SMR = 143
Baxter and Werner (1980)	Cohort 40,867 males (1967-76)	Unspecified	Overall	33	SMR = 98
		Acute myeloid	Tire sector	6	SMR = 134
		Acute myeloid	Plastic film	3	SMR = 714
Parkes et al (1982)	Cohort 33,815 males (1946-75)	Unspecified	Overall	31	SMR = 110
U.S. studies					
Mancuso et al (1968)	Cohort 1,877 white males (1940-64)	Unspecified	Office (ages 65+)	2	RR = 4.27
Mancuso (1975)	Cohort 8,234 males (1938-72)	Leukemia and multiple myeloma combined	Chemical plant	1	RR = 2.38
			Stock prep.	2	RR = 2.38
			Compounding and milling	5	RR = 2.35
			Maintenance and tire shop	3	RR = 1.40
			Machine shop	1	RR = 1.37
Monson and Nakano (1976b)	Cohort 13,571 white males (1940-74)	Unspecified	Overall	55	SMR = 128
			Tire building and curing	18	SMR = 150
			Raw materials processing	10	SMR = 240
Monson and Nakano (1967a)	Cohort 5,816 females 986 black males 4,727 salaried white males (1940-64)	Unspecified	Overall	9	SMR = 97
				0	SMR 0
				[illegible]	SMR [illegible]

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Monson and Fine (1978)	Cohort 13,570 white males (1940-76)	Unspecified	Calendering	4	RR = 3.6
			Rubber fabrics	4	RR = 3.5
			Tire curing	5	RR = 2.9
			Elevators	3	RR = 2.9
			Tubes	4	RR = 2.5
Delzell and Monson (1981)	Cohort 15,643 white males (1940-78)	Unspecified	Overall	68	SMR = 121
Delzell and Monson (1982)	Cohort 2,666 white males (1940-78)	Unspecified	All processing	16	SMR = 171
			Front processing	7	RR = 1.3
			Back processing	14	RR = 1.7
Delzell et al (1981)	Cohort 1,792 white males (1954-77)	Unspecified	Overall	1	SMR = 45
				3	SIR = 100
McMichael et al (1976a)	Cohort 18,903 white males (1964-73)	Unspecified	Overall	—[d]	SMR = 130
		Lymphocytic	Overall	—	SMR = 158
Andjelkovich et al (1976)	Cohort 8,418 white males (1964-73)	Unspecified	Overall	19	SMR = 147
		Lymphocytic	Overall	10	SMR = 152
		Myeloid	Overall	6	SMR = 87
		Monocytic	Overall	3	SMR = 311
Andjelkovich et al (1977)	Cohort 8,418 white males (1964-73)	Unspecified	General service	6	SMR = 246
Rinsky et al (1981)	Cohort 748 white males (1950-75)	Nonlymphocytic	Rubber Hydrochloride (benzene)	7	SMR = 560
McMichael et al (1974)	Cohort 6,678 males all races (1964-72)	Unspecified	Overall	16	SMR = 128
McMichael et al (1976b)	Cohort 6,678 males all races (1964-73)	Lymphocytic	Synthetic rubber	—	RR = 3.7
		Lymphocytic	Inspection and repair	—	RR = 3.2
		Lymphocytic	Extrusion	—	RR = 3.0
		Lymphocytic	Janitorial	—	RR = 2.6

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TABLE 1. Summary of Rubber Industry Studies of Leukemia *(Continued)*

Author (year)	Study characteristics	Leukemia type	Exposure	Number observed	Estimated relative risk[a]
McMichael et al (1975)	Case-control 17 cases 51 controls males & females all races (1964–73)	Lymphocytic	Solvents	12	OR = 3.3
Wolf et al (1981)	Case-control 72 cases 286 controls males & females all races (1964–73)	Unspecified	Solvents	8	OR = 0.8
		Lymphocytic	Solvents	5	OR = 1.6
		Lymphocytic	Solvents	4	OR = 3.2
		Myeloid	Solvents	2	OR = 1.0
		Monocytic	Solvents	0	OR = 0
		Other	Solvents	1	OR = 0.3
Arp et al (1983)	Case-control 15 cases 30 controls males & females all races (1964–73)	Lymphocytic	Benzene	3	OR = 4.5
			Other solvents	11	OR = 4.5

[a]SMR (standardized mortality ratio = obs ÷ exp × 100); SIR (standardized incidence ratio); RR (rate ratio); OR (odds ratio from case-control studies).
[b]Unspecified, refers to all types of leukemia, combined.
[c]Overall, refers to results for entire plant, ie, unspecified exposure.
[d]Cannot determine observed number of cases exposed from published data.

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TABLE II. Solvent Exposure and Lymphocytic Leukemia Relative Risk Estimates*

Exposure category	Number of cases exposed	Odds ratio	Chi square
Primary benzene	3	4.5	1.50
Secondary benzene	2	1.5	0.13
Primary other solvent	11	4.5	3.06
Secondary other solvent	8	1.6	0.41

*Data from Arp et al [1983].

NEW RESULTS FROM A STUDY BASED ON MULTIPLE SOLVENT EXPOSURES

Methods and Results

The case-control analysis of lymphocytic leukemia reported by Arp et al [1983] was extended to consider risks associated with 24 specific solvents. As before, solvents exposure charts for process areas were reconstructed from historical plant records. The determination of workers' exposure histories differed from the method used previously in that exposure codes for each solvent were assigned to the department rather than to the individual job title entries on the work history records. This simplified method of exposure coding enabled the use of a computer algorithm, and it proved to be more cost- and time-efficient for examining risks related to multiple agents than did the manual coding process used in the previous analysis, which was restricted to an evaluation of only two classes of exposure, benzene and all other solvents grouped together. The only information sacrificed was the judgmental distinction between primary and secondary exposures.

The cases in the present analysis were 11 male, hourly workers whose underlying cause of death was lymphocytic leukemia (ICD 8th Revision Code 204) who were identified from the cohort reported on by McMichael et al [1975]. These cases were also included in the studies by Wolf et al [1981] and Arp et al [1983]. Controls were a 20% age-stratified random sample of the cohort [McMichael et al, 1976b]. A total of 1,350 controls (1,280 whites and 70 blacks) was included.

Age-adjusted relative risk estimates, as approximated by the odds ratio, and associated with chi-square tests of statistical significance were computed according to procedures described by Mantel and Haenszel [1959]. Workers were considered exposed to a particular solvent if their cumulative duration of work in a solvent-related department exceeded 12 months.

Table III shows the odds ratios associated with each of the 24 solvents. As mentioned above, no distinction was made between primary and secondary exposure to benzene, which accounts for the lower relative risk estimate; however, the odds ratio (2.5) is within the range of the estimates found in the previous analysis (see Table II). Among the solvents listed in Table III, carbon tetrachloride (OR=14.8) and carbon disulfide (OR=8.7) showed the strongest associations with lymphocytic leukemia. Several other solvents were also related to leukemia mortality, and these multiple associations probably reflect simultaneous exposures to different solvents used concurrently in the industry.

Neither carbon tetrachloride nor carbon disulfide has been shown previously to be leukemogenic, and it is possible that the present findings are spurious. Nevertheless, the high odds ratios for these solvents suggest that, even if they are not etiological

TABLE III. Case-control Analysis of Lymphocytic Leukemia Risk Associated With Exposures to 24 solvents

Solvent	Number of cases exposed	Odds ratio	Chi square
Acetone	3	5.1	6.33*
Aqua ammonia	1	2.4	0.71
Benzene	4	2.5	1.59
Carbon disulfide	7	8.7	12.82**
Carbon tetrachloride	8	14.8	18.00**
Dipentene	1	1.0	0.00
Ethanol	4	2.0	1.14
Ethyl acetate	3	5.1	6.33*
Gasoline	9	4.9	3.10
Heptane	2	0.9	0.01
Hexane	7	3.8	4.32*
Isopropanol	6	1.8	0.84
Methanol	1	8.3	5.31*
Methylene chloride	0	—	0.32
Mineral spirits	1	1.5	0.15
Perchloroethylene	1	0.7	0.09
Phenol	1	0.5	0.40
Solvent A[a]	7	2.7	2.14
Special naphthas	8	2.7	1.62
Toluene	2	3.0	1.81
Trichloroethane	0	—	0.52
Trichloroethylene	2	0.8	0.07
VM and P naphthas	3	2.9	2.68
Xylenes	4	3.2	3.35

[a]Proprietary mixture of toluene and other solvents.
*$p < 0.05$.
**$p < 0.001$.

agents themselves, they are probably closely associated with the actual causative exposure(s).

The exploratory nature of this analysis and the small sample size preclude firm conclusions about specific solvent–leukemia associations. However, these findings illustrate clearly the danger of focusing exclusively on a single exposure, ie, benzene, in a multiexposure environment.

DISCUSSION

The examinations of leukemia risk factors in the British and U.S. rubber industries have proceeded sequentially from the identification of mortality excesses, relative to the generally unexposed national and regional populations, to more intensive investigations of causally related work environments and exposures within the industry. Leukemia mortality excesses of varying magnitudes have been observed in most studies. The use of solvents in various processes of rubber and tire manufacturing (eg, tire building) and the well-recognized leukemogenic potential of benzene suggest an obvious etiological association; however, the findings from many investigations of rubber worker populations do not fit especially well with a benzene-related effect. In this regard it is noteworthy that the animal data on experimental benzene

carcinogenesis also have been conflicting [Ward et al, 1975; Maltoni and Scarnato, 1979; Snyder et al, 1980; IARC, 1982b], and the reported mutagenicity results have been largely negative [Dean, 1978; IARC, 1982b]. Contradictory results in human populations are therefore not surprising.

Benzene was used in the industry as a general purpose solvent in the 1920s [Davis, 1929], but following reports of toxicity [Hunter, 1939], benzene was supplanted by toluene and other aromatic and aliphatic solvents [Mellan, 1957]. While benzene exposure still occurs in the industry, it is present primarily as a contaminant of other substances [Van Ert et al, 1980], and exposures are much lower than in the 1920s; thus it is quite likely that only small proportions of the cohorts studied were ever exposed to benzene in concentrations known to induce leukemia.

The strongest causal associations for benzene historically have been with the acute myeloid types of leukemia, including erythroleukemia [Goldstein, 1977], yet there are also case reports of lymphocytic leukemia as a sequela of benzene poisoning [Delore and Borgomano, 1929; Hunter, 1939; Vigliani, 1975]. Excessive mortality from lymphocytic leukemia has been as great as that from the nonlymphocytic variants in a number of rubber industry cohorts, a result that has prompted several investigators to discount an etiologic relationship with solvents [Monson and Fine, 1978; Parkes et al, 1982].

The NIOSH study of Pliofilm workers [Rinsky et al, 1981] perhaps offers the most convincing demonstration of a benzene/nonlymphocytic leukemia association. The importance placed on the fivefold mortality excess detected in this study is evidenced by the fact that the IARC working group [IARC, 1982b] recently used this relative risk approximation to estimate excess lifetime risk associated with benzene exposures. Even in view of the magnitude of the reported risk for nonlymphocytic leukemia associated with benzene exposure, the specificity of the association remains uncertain, as exposures to other rubber industry solvents were not evalutated in the analysis. Findings from a reanalysis of the case-control data of Arp et al [1983], that were presented in this paper, demonstrate the extent to which interpretations of seemingly causal relationships can change in light of newly considered exposure information.

Additional research into the etiology of leukemia in the rubber industry should be guided by several considerations made apparent from previous work. It should be recognized that the presumed class of causative agents, solvents, is a mixed group of compounds, of which benzene is generally a minor quantitative component. The indication therefore is to examine possible etiological relationships with other solvents.

As shown from the data presented in Table III, numerous solvents were used in the industry. Moreover, usage has varied qualitatively and quantitatively over time, and there has been considerable temporal overlap of exposures. Consequently, the identification of the specific agent(s) responsible for leukemia excesses is likely to be a complicated process requiring investigator judgement as to which solvents are likely to be leukemogenic, based on previous experimental and human observational data.

While the foregoing discussion suggests the need to investigate the potential leukemogenic effects of solvents other than benzene, we do not imply that continued research into benzene toxicity in the rubber industry should be abandoned. Rather, the scope of the research should be expanded to include assessment of effects of multiple exposures, and the interpretations of the findings should not be evaluated necessarily with respect to a benzene/nonlymphocytic leukemia association.

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WSPA
Western States Petroleum Association

March 22, 1991

Genevieve Shiroma, Chief
Toxic Air Contaminant Identification Branch
Stationary Source Division
Air Resources Board
Attn: 1,3-Butadiene
P.O. Box 2815
Sacramento, CA 95812

Dear Ms. Shiroma:

The Western States Petroleum Association (WSPA) appreciates the opportunity to comment on the preliminary draft report on 1,3-butadiene prepared by the Air Resources Board and the Department of Health Services.

In response to the preliminary draft report, WSPA would like to offer our concurrence with those comments submitted by the American Petroleum Institute (API) and the Chemical Manufacturers Association (CMA).

Please do not hesitate to contact me should you have any questions.

Sincerely,

Scott N. Folwarkow

cc: D. Henderson
G. Nelhams
L. Scott (API)
M. Wang

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II.

Air Resources Board Staff Responses to Summarized Comments on the Preliminary Draft Part A and the Executive Summary

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Air Resources Board Staff Responses to Summarized Comments on the Preliminary Draft Part A and the Executive Summary

o Chemical Manufacturers Association (CMA), March 21, 1991

Comment 1: The description of the ARB's analytical method (Appendix B) does not demonstrate that adequate steps were taken to separate butadiene from other four-carbon hydrocarbons (e.g., butene-1 and isobutane) commonly found in ambient air. Failure to differentiate between butadiene and other four-carbon hydrocarbons may result in an overstatement of butadiene levels in ambient air.

Response: As noted in the ARB's cited ambient air method (MLD 013), the chromatographic column used for the analysis is a 10' glass column packed with GP 80/100 Carbopack C/0.19% picric acid. This material affords baseline separation of all C4 isomers (see Supelco, Catalog 29, pg 51), preventing co-elution with 1,3-butadiene. The detector used in the analysis is a photoionization detector (PID) equipped with a 10.2ev lamp. This detector is not sensitive to saturated hydrocarbons, preventing interference from saturated C4 isomers. The PID detects the unsaturated C4 isomers and the separation of these is routinely checked during analysis. The PID is connected in tandem with a flame ionization detector (FID), and the signal ratio between the two detectors for 1,3-butadiene is routinely monitored to insure that accidental co-elutions do not occur during the analysis of ambient air samples.

Comment 2: The ARB protocol (Appendix B) does not provide enough information to validate the sample collection methodologies. Depending on the details of how the samples were collected and transported, the lab analysis may either understate or overstate the actual butadiene levels.

Response: Sample collection is performed at a constant flow over a 24 hour period. The ambient air is sampled using a Xontech 910A Whole Air Sampler. Prior to delivery to the field, this sampler is checked in the laboratory to insure that it is clean and free of interferences. Prior to 1990, the sample was collected in cleaned Tedlar bags, protected from light. The bags were delivered to the laboratory within 24 hours by special courier, and the contents analyzed within 24 hours of receipt. After 1990, the samples were collected in SUMMA polished stainless steel canisters and sent to the laboratory. The stability of 1,3-butadiene has been demonstrated by the ARB staff in Tedlar bags over a period of seven days and in SUMMA polished stainless steel canisters over a period of a month. Samples are never subjected to holding times in excess of this.

Extensive procedures have been developed by the laboratory and by the ARB Quality Assurance Section to determine the effects of sampling, transport, and analysis on the precision and accuracy of the data. The laboratory routinely sends "field blanks" (sample containers filled with zero gas) and "field spikes" (samples with known amounts of chemicals)

to our sampling stations. These "samples" are then returned to the laboratory for analysis to determine the effects of transportation. The Quality Assurance Section audits the laboratory quarterly with samples traceable to NIST (formerly NBS). In addition, the Section has developed a "Through-The-Probe" audit system. This requires that the sampling station probe be attached to a source of air with known chemical concentrations. The sample is taken on a normal sampling day over a period of 24 hours and sent to the laboratory. The laboratory staff are completely unaware that the sample represents an audit. Only after analysis is the staff told that the sample was not a real ambient sample. This type of audit not only tests the laboratory, but also the sampling and transport system. 1,3-Butadiene is one of the compounds included in all of the above procedures.

- Dow Chemical Company, March 21, 1991

Comment: The exposure assessment document states that the Northern California latex production facility (our Dow Pittsburg facility) emitted an estimated 6.95 megagrams (~8 tons) of 1,3-butadiene in 1984. This information was derived from emissions data reported to the US EPA in 1984 pursuant to a Clean Air Act 114 request. Since 1984, many facility improvements have been made in an effort to modernize our latex plant and reduce the emissions from the operation. Subsequent source tests and modeling of our latex plant emissions of 1,3-butadiene have provided us with a much lower estimate of emissions. We request that the 1,3-butadiene emissions estimates for the Northern California (Dow Pittsburg) Styrene-butadiene Copolymer Production Plant reflect the current estimates of 2,555 lbs/yr for process emissions and 533 lbs/yr for fugitive emissions rather than the 1984 US EPA values presently found in the exposure assessment document.

Response: The emission information submitted by the Dow Pittsburg facility has been reviewed by staff and incorporated into the revised Part A document.

- General Motors (GM), March 22, 1991

Comment 1: The preliminary draft Executive Summary and Part A fail to inform the reader that the risk from motor vehicle 1,3-butadiene emissions has been reduced dramatically over the past 20 years. The Executive Summary states that emissions of 1,3-butadiene are expected to increase in California for the next two years and then decrease as a result of recently adopted regulations. This statement is incorrect and misleading. Emissions will continue to decrease substantially due to the ARB's existing regulations to control criteria pollutants, together with the numerous new regulations focused on reducing hydrocarbon emissions. Day by day, new vehicles with low emissions of 1,3-butadiene are replacing older, higher emitting vehicles. The ARB has pointed out (1987) that the attrition of non-catalyst vehicles would reduce the 1,3-butadiene inventory 61 percent by the year 2000. New regulations adopted since that calculation was made will reduce emissions even further.

Response: On a vehicle/vehicle basis, motor vehicle emissions of 1,3-butadiene have been reduced from the emission levels of the 1960's due to control programs implemented by the ARB and its parent agencies. Staff agree that the present atmospheric concentrations of 1,3-butadiene are probably lower than those reported for Los Angeles during the 1960's (see response to Comment 18). However, a reduction in 1,3-butadiene concentration does not guarantee a reduction in risk--while concentrations may have been reduced, the number of exposed individuals (opportunities for 1,3-butadiene-induced cancers) has increased dramatically during the same time period. Also, on-road vehicles are better controlled, but there are millions more of them than there were in the 1960's. The staff projection that 1,3-butadiene concentrations will increase and then decrease has been deleted from the text. Recent model runs indicate that there will be a steady decrease in 1,3-butadiene emissions from mobile sources.

Comment 2: As Appendix A indicates, butadiene emissions are derived from the butadiene/TOG ratio, and based on "preliminary" inventories calculated by the ARB in 1987 as a "rough approximation of the true emission inventory." Unexplained "adjustments" could have introduced additional error.

Because motor vehicle 1,3-butadiene emissions have been measured directly since 1987, the use of oversimplified correction factors is outdated and could misinform the reader. The emission factors used in paragraph A and B of Appendix A should be replaced by measured butadiene emissions, or, the potential range of error should be discussed in the text. Butadiene data from the Auto/Oil Air Quality Improvement Research Program (made available to the ARB) could provide a better basis for statistically robust inventories and should be included in any estimates of current and future year inventories.

Response: The staff has developed emission factors based on measured 1,3-butadiene emissions from several in-use vehicles in California (representative of the California on-road vehicle fleet). The emission estimates for on-road vehicles found in the revised Part A document are based on the emission factors derived from the measured emissions of 1,3-butadiene from the representative vehicles.

The staff has reviewed the reports from the Auto/Oil Air Quality Improvement Research Program (AQIRP). While the Auto/Oil AQIRP provides data for many toxic substances from vehicular exhaust (including 1,3-butadiene) which is very useful for comparison with ARB's emissions data base, the staff cannot use the data to develop emission factors because the gasoline used in these experiments is reformulated and prototype. In Technical Bulletin No. 1, 2, and 3, the Auto/Oil AQIRP states that the composition of these fuels "is not representative" of fuels that are in-use or could be produced (Auto/Oil AQIRP, 1990 and 1991).

Comment 3: GM believes that a knowledge of the relative contributions of individual sources is important for better estimates of public exposure. In the 1980's, the US EPA introduced a conceptual model of total human exposure (TEAM). This model was used to compare benzene

emissions versus exposures. The following Table (please see GM's original comments for the reference) shows how significant individual sources of exposure are because the general public spends most of its time indoors.

Benzene Emissions: Inventories vs. Public Exposures
Based on Personal Monitoring

Contributions Based on:	Emission Inventories	Personal Monitoring
Autos	82%	18%
Cigarettes	1%	39%
ETS	---	5%
Industry	14%	3%
Personal & Home	3%	34%
	---	---
	100%	100%

The TEAM results suggest that corresponding corrections should be applied in estimating public exposure to 1,3-butadiene. In fact, the benzene and butadiene content of cigarette smoke is similar, while the ambient exposure to benzene is many times that of butadiene. A corresponding comparison of butadiene will probably show an even smaller contribution from motor vehicles than shown in the Table. An estimate of the sources of personal exposure to 1,3-butadiene would be more useful than the simple proportioning of the ambient inventories given in the Executive Summary.

Response: Benzene data are quite abundant, whereas 1,3-butadiene data are very limited. The TEAM data do not include 1,3-butadiene and we are not aware of any personal monitoring data for 1,3-butadiene. Due to the lack of data, accurate estimates of the contribution of various sources to personal exposure of 1,3-butadiene can not be made.

Comment 4: In response to the question "What is the the risk assessment for exposure to 1,3-butadiene?", the Executive Summary indicates that the number of potential excess cancers due to airborne 1,3-butadiene exposures ranges from 1 to 115 additional cancers per million people exposed throughout their lifetimes. Vostal et al. (1989) has predicted that when all motor vehicles are equipped with current emission controls, butadiene exposure from motor vehicles will have a minimal impact on the U.S. annual cancer incidence (0 to 0.05 cases per million urban people).

Response: At the present time, all vehicles are not catalyzed in California for a number of reasons, some of which include the steady influx of un-catalyzed vehicles from the other 49 states, vehicle emission control device tampering or failure, and California's existing fleet fraction of un-catalyzed motor vehicles. The potential excess cancer risk reported in the preliminary draft document (and to be found in the final version) is based by law (Health and Safety Code Section 39650 et seq) on current ambient estimations of 1,3-butadiene exposure.

Comment 5: The ARB should follow the recommendation of the US EPA's Science Advisory Board (SAB) that the US EPA target its environmental protection efforts on the basis of opportunities for the greatest risk reduction. The SAB recommended that the US EPA weigh the relative risks posed by environmental problems, determine if there are cost-effective risk reduction options, and then identify which of the options is the most cost-effective.

Response: The 1990 Amendments to the Clean Air Act list 189 substances (e.g., 1,3-butadiene) as hazardous air pollutants (HAPs), all of which must be evaluated by the US EPA in a risk management program. It was in this context that the SAB advised the US EPA to target their risk management efforts towards the substances (HAPs) that represent the greatest public risk.

California's risk assessment/risk management program is (by law) separate and distinct. First, substances are evaluated in an "identification" phase where it is demonstrated that there is public exposure to a substance which results in an increased public risk. The second step is the "control" phase where risk reduction measures are developed for the identified toxic air contaminants (TACs). California law (California Health and Safety Code Section 39655) requires the ARB to identify all HAPs as TACs.

Comment 6: The US EPA's SAB also recommended that the US EPA improve the data and analytical methodologies that support risk assessment and comparison, and that risk rankings should be based on the total human exposure to specific toxic agents.

Response: California Health and Safety Code Section 39650 (e) states "That while absolute and undisputed scientific evidence may not be available to determine the exact nature and extent of risk from toxic air contaminants, it is necessary to take action to protect public health." The law requires that the ARB act to protect public health. Meanwhile, staff of the ARB and the OEHHA endeavor to improve the data and analytical methodologies that support risk assessment.

Comment 7: The National Research Council came to conclusions similar to those of the SAB. They also wrote that "risk reduction strategies that address only outdoor air are only partially effective." Indoor air exposure should be addressed, and in setting priorities for reducing pollutant-exposure risk, all media and all routes of exposure must be assessed.

Response: The ARB has been given authority to identify and control outdoor TACs. The ARB staff recognize that indoor air exposures can pose a significant risk, and staff agree that consideration of indoor air and other routes of exposure are important for a complete risk assessment. Available information on indoor air, food, and water exposure has been reported in the document. Because indoor exposures can be significant, the ARB is continuing to sponsor research to develop data on indoor air exposures to TACs.

Comment 8: While many of the emission factors are given in Appendix A, the original data used to determine the emission factors for on-road motor vehicles are not identified.

Response: The revised Part A document identifies and describes the data used to develop 1,3-butadiene emission factors from motor vehicles.

Comment 9: The preliminary draft exposure document notes that updated emission factors were expected in the late summer of 1990. Since the document was issued in February 1991, the new emission factors should have been in it.

Response: The development of updated emission factors for on-road motor vehicles was not completed until July 1991. The revised Part A document incorporates this updated information.

Comment 10: Many of the organic analyses in the literature do not resolve 1,3-butadiene from butane and other C4 hydrocarbons, therefore, the estimated values for 1,3-butadiene emissions may not be accurate.

Response: The method employed by ARB staff to determine 1,3-butadiene in automotive exhaust uses gas chromatography and photo-ionization detection. Control standards are used daily to check the GC performance, as well as the ARB's quality control measures. At least 10 percent of the analysis are confirmed by a different analytical system (e.g., GC/MS), coupled with daily replicate sample analysis. The method (Appended to the revised Part A) is specific for 1,3-butadiene and excludes butane or other C4 hydrocarbons.

Comment 11: Some data bases developed during the late 1980's show that there is reasonable agreement between the pre-catalyst engine emissions of 1,3-butadiene measured by Marshall (34.6 mg/mi) and the ARB emission factor (27.9 mg/mi) for non-catalyst passenger vehicles given on page A-1 of Appendix A. Marshall demonstrated that a catalyst reduced the emissions of 1,3-butadiene by 97 percent. This is greater than the 90 percent reduction in the emission factor for gasoline-fueled light-duty passenger vehicles given in Table A-1 of Appendix A.

Vostal, Williams and Lipari reported 1,3-butadiene measurements for seven late-model GM and Chrysler passenger cars that averaged 0.46 mg/mi, with 1,3-butadiene representing 0.11 wt percent of the exhaust HC emissions.

Response: W. F. Marshall's report (referenced in the comment letter) is unpublished, however, the staff has obtained an earlier report written by G. A. Schoonveld and W. F. Marshall. Data from this report are similar to the data found in the Marshall report. Two non-catalyst and six catalyst vehicles were tested in this study. Arithmetic average emission factors for 1,3-butadiene from non-catalyst and catalyst vehicles are 58.5 mg/mile and 6.9 mg/mile, respectively (Schoonveld, G. A., and W. F. Marshall, 1990, The Total Effect of a Reformulated Gasoline on Vehicle Emissions by Technology (1973 to 1989). SAE Technical Paper Series No. 910380). Comparing these two factors, there

is an 88 percent reduction in the 1,3-butadiene emissions from non-catalyst to catalyst vehicles. Based on the emission factors developed in 1991 by ARB staff, there is approximately an 85 percent reduction of 1,3-butadiene emissions from non-catalyst to catalyst vehicles.

The report written by Vostal, Williams, and Lipari has not been published (as of August 1991), although it was presented at the 82nd Air and Waste Management Association's annual meeting in 1989. Mr. Vostal (one of the authors) has met with staff to share the data for evaluation and comparison.

Comment 12: The exposure document includes the lab results of 360 individual 24-hour samples collected at 20 toxic monitoring stations in California. Based on these results, an overall population-weighted statewide 1,3-butadiene concentration was calculated. At best it could be characterized as an overall statewide outdoor exposure, but, because people spend over 80 percent of their time indoors, it cannot be considered an overall statewide average.

Response: The "statewide exposure" reported in the document is an ambient air exposure estimate only, and does not include the contribution (which may be quite significant in the homes of heavy smokers) of indoor air exposure. The main focus of the "Part A" document is public exposure to airborne toxicants "...which may be or are emitted into the ambient air of California..." (California Health and Safety Code Section 39660 [a]). Although Health and Safety Code Section 39660.5 requires that the indoor air contribution to total exposure be evaluated, it does not require a quantitative analysis of total exposure and does not give the ARB control authority to deal with other-than-ambient exposures to toxic air contaminants. The ARB is, however, concerned with indoor exposures to TACs and is actively sponsoring research to determine sources, emissions, and concentrations of indoor TACs.

Comment 13: No description is given of the site selection criteria for the 20 monitoring sites. To aid the reader in judging the appropriateness of the sites, the selection criteria should be referenced.

Response: The ARB's monitoring sites were selected using the US EPA's Probe Siting Criteria for Ambient Air Quality Monitoring, found in Appendix E of 40 CFR Chapter 1, Part 58.

Comment 14: The procedure in Appendix B specifically calls for samples to be taken in stainless steel canisters and analyzed as soon as possible. Although the preliminary draft report indicates that the samples collected in Tedlar bags are of verifiable quality, the reasons for the difference between the written and actual procedures should be documented.

Response: The data set used to estimate the population-weighted exposure (July 1988 through June 1989) was derived from Tedlar bag-collected samples. During February of 1990, the monitoring equipment

for gas collection was converted from Tedlar bags to stainless steel canisters. At the time of the report's publication, the standard operating procedure (SOP) used for determination of 1,3-butadiene specified stainless steel canisters. To avoid additional confusion, the SOP that specifies Tedlar bags has been added to Appendix B to accompany the SOP that has been in use since February 1990.

Comment 15: The individual organic species data from the Southern California Air Quality Study (SCAQS) should be used to get a better idea of the spatial and temporal variation in ambient 1,3-butadiene concentrations so that a more refined exposure analysis can be carried out.

Response: The SCAQ Study (co-sponsored by the ARB) is one of the most comprehensive studies of its kind, however, it cannot be used for the development of a statewide ambient average exposure estimate. SCAQS samples were taken on "episode days" when the pollutants were expected to be at their highest concentrations. 1,3-Butadiene concentrations during the study ranged from below the level of detection to 17.7 ppb (39.08 micrograms/cubic meter). This range of concentrations have been added to the exposure document. Additionally, concentrations and the number of emission sources for the other 13 air basins in California are not well represented by the South Coast Air Basin. The toxic monitoring network data represents typical ambient concentrations with samples taken twice a month (during random days) to reduce the odds of overestimating average concentrations.

Comment 16: The SCAQS data can be used to verify the overall estimates of 1,3-butadiene emissions from vehicular sources by using the data to estimate the fraction of 1,3-butadiene in ambient non-methane organic carbon concentrations. In a similar data base, 1,3-butadiene represented 0.22 wt percent of the carbon in the samples. The analogous fraction of carbon in the SCAQS samples can be used to check the estimated statewide emissions of 1,3-butadiene.

Response: The estimates of emissions from various source categories found in the Executive Summary and Part A were developed using emission factors, emission inventories, source inventories, and usage information. While the documents report that motor vehicles are responsible for an estimated 94 percent of the total 1,3-butadiene emissions, staff recognize that other sources may present a more immediate compromise to public health. The risk management phase of the 1,3-butadiene project will consider all sources for their impact on the population (not just motor vehicles).

The 1,3-butadiene concentrations found in the South Coast Air Basin are not representative of those found in other air basins in the state (the SoCAB's concentrations are higher). At the present time, the ARB does not have SCAQS-like data for the other 13 air basins, so it would be difficult to determine or use an "analogous fraction of carbon" for 1,3-butadiene data comparison. If GM has available data, ARB staff would be interested in having access to it for a comparison study.

Comment 17: In order to carry out a risk assessment, the long-term trend in ambient 1,3-butadiene concentrations should be documented.

Response: The excess cancer risk estimate is based on present (not projected) levels of exposure. The purpose of the Part A document is to establish present exposure; the purpose of the "identification" report (Part A, Part B, and the Executive Summary) is to establish present risk to the public, and will be used as technical reference by the members of the ARB to determine whether 1,3-butadiene meets the definition of a toxic air contaminant. Changes in the future emissions of 1,3-butadiene will be considered in the risk management phase.

Comment 18: Measured 1,3-butadiene concentrations from the Los Angeles Basin taken during the mid 1960's averaged 2 ppbv, with some of the values exceeding 5 ppbv. The current 1,3-butadiene concentrations in Los Angeles are roughly one-quarter the size of those measured in the middle-to-late 1960's. This dramatic reduction occurred in spite of increasing population, numbers of vehicles, or vehicle miles traveled.

Response: If GM has an ambient air data base for the State of California that is statistically robust enough to determine a trend, the staff of the ARB would like to have access to it for our continued evaluation of this substance. The ARB's sampling and analysis for 1,3-butadiene is not frequent enough (~2 samples per month from December 1987 to the present) to determine a statistical trend.

Staff agree that hydrocarbon concentrations have been dramatically reduced in the Los Angeles Basin since the 1960's as a direct result of increased motor vehicle emissions control and reductions in emissions from industrial sources. This has been one of the goals of the ARB's criteria pollutants control program. The staff projection that 1,3-butadiene concentrations will increase and then decrease has been deleted from the text. Recent model runs indicate that there will be a steady decrease in 1,3-butadiene emissions from mobile sources.

Comment 19: From the emission factors and VMT shown in Appendix A for passenger cars and light- and medium-duty trucks, it can be shown that non-catalyst vehicles are estimated to emit 60 percent of the remaining 1,3-butadiene emissions even though they account for only 13 percent of the vehicle miles traveled. Clearly, any additional vehicle-related risk reduction efforts should be focused on these older, higher emitting vehicles.

Response: Staff recognizes that vehicles without working catalysts and/or emission controls for hydrocarbons are responsible for a large fraction of the motor vehicle-derived 1,3-butadiene. The risk management evaluation (that will follow the risk assessment evaluation now in progress) will consider the impact of these older, higher emitting vehicles.

Comment 20: The report does not provide the proper perspective on the risk from motor vehicle emissions of 1,3-butadiene relative to the

substantial human exposures to 1,3-butadiene from other sources such as environmental tobacco smoke (ETS).

While ETS is correctly identified in the report as a major source of 1,3-butadiene in indoor air, the draft indicates that there is not sufficient information to make a quantitative analysis at this time. The draft, however, makes reference to several data sources that can be used to provide some perspective on exposures from ETS. Using the same method employed by the US EPA to estimate the average emission rate of respirable suspended particulate matter per cigarette in sidestream smoke, one can calculate the average daily intake of 1,3-butadiene to be 46 ug. This can be compared to the average daily intake of 16.4 ug for an individual exposed to the average ambient concentration of 0.37 ppbv. Thus, the exposure from typical indoor concentrations far exceed that of typical outdoor concentrations (indoor concentrations are 2.8 times those of outdoor concentrations). GM recommends that the ARB carry out additional studies to quantitate this important exposure.

Response: The revised Part A includes exposure estimates for three hours of exposure in a bar or tavern with a heavy smoking environment. (Three hours is the average time spent in bars or nightclubs for those Californians who go to bars or nightclubs.) Residential exposure estimates are not given because data are too limited for that environment, and tavern exposures are not representative of residential indoor exposures. The revised Part A discusses all available residential data, including preliminary data from an ARB study which indicate that "typical" residential levels of 1,3-butadiene are very low. Staff agrees that further measurements are needed in a wider variety of homes and over different seasons to quantitate exposure. ARB-sponsored research in this area will continue.

Comment 21: The statement that approximately 94 percent of California's airborne 1,3-butadiene comes from motor vehicles is misleading. While motor vehicles are responsible for the bulk of outdoor emissions of 1,3-butadiene, they are responsible for a much smaller percentage of the actual 1,3-butadiene that Californians are exposed to.

Response: 1,3-Butadiene exposure may be greater indoors than outdoors for individuals exposed to a heavy smoking environment. The exposure experienced by those individuals, however, does not represent a typical exposure to 1,3-butadiene. Data are inadequate to draw a conclusion regarding the relative contributions of different sources of 1,3-butadiene exposure at this time.

- <u>International Institute of Synthetic Rubber Producers, Inc.</u> (IISRP), March 21, 1991

Comment: The preliminary draft "Part A" exposure assessment document contains old emissions data for Styrene-Butadiene Copolymer Plants. To our knowledge, the only polymer plants using butadiene in California are the two SB latex plants referenced in the text. Both plants have provided government authorities with more current emissions data than that contained on Page A-11 of your preliminary draft.

Response: The emissions data from the two facilities [reported in compliance with the Air Toxics Information and Assessment Act (AB 2588)] has not been forwarded to the ARB staff by the respective air pollution control districts. The Northern California facility provided the AB 2588 data to ARB staff, and the exposure document has been modified to include this information. The Southern California facility has not provided ARB staff with recent emissions data. If 1,3-butadiene is declared and listed as a toxic air contaminant by the ARB, the updated emissions information for both facilities will be used during the risk management phase.

- Western States Petroleum Association (WSPA), March 22, 1991

Comment: WSPA concurs with the comments submitted by the American Petroleum Institute (API) and the Chemical Manufacturers Association (CMA).

Response: API and CMA had no comments regarding Part A; please see the OEHHA responses to comments made by API and CMA regarding Part B.

III.

Office of Environmental Health Hazard Assessment Staff Responses to Summarized Comments on the Preliminary Draft Part B and the Executive Summary

OFFICE OF ENVIRONMENTAL HEALTH HAZARD ASSESSMENT (OEHHA)*
STAFF RESPONSES TO PUBLIC COMMENTS
ON THE DRAFT PART B AND EXECUTIVE SUMMARY
RELEASED FEBRUARY 1991
OF THE TECHNICAL SUPPORT DOCUMENT (TSD) FOR THE
PROPOSED IDENTIFICATION OF 1,3-BUTADIENE
AS A TOXIC AIR CONTAMINANT

Comments from the American Petroleum Institute (API)

1. Comment: This commenter participated in the development of, and subscribes to, the comments submitted by the Chemical Manufacturers Association (API, letter [signed by Terry F. Yosie] dated March 22, 1991).

Response: Comment noted.

2. Comment: The document's heavy reliance on studies performed on the B6C3F1 mouse leads to an overestimate of human risk. Data provide evidence that mice are uniquely sensitive to the carcinogenic effects of butadiene: mice retain larger doses of butadiene, metabolize butadiene more rapidly, and detoxify the metabolites more slowly than other species. Thus, the human risk estimation should be based on an alternate species, or the mouse data should be adjusted to account for this hypersensitivity (API letter).

Response: The draft document relied on a NTP (1984) study performed on the B6C3F1 mouse to obtain a best estimate but also employed data from the Owen et al. (1987) rat study to construct a range of plausible potency values for extrapolation to humans. The revised document uses the newer B6C3F1 mouse data of Melnick et al. (1990) for the recommended best value. When considering studies in test systems which differ markedly in estimates of risk, State of California guidelines explain that "since humans vary widely in sensitivity and some individuals are likely to be as sensitive as the most sensitive animal species, a common procedure is to use the most sensitive system as the basis for extrapolation" (Department of Health Services, *Guidelines for Chemical Carcinogen Risk Assessments and Their Scientific Rationale*, 1985, p. C-22). Following these guidelines, OEHHA staff used the most sensitive site, sex, strain and species for the document's cancer risk assessment.

The guidelines reprint this point from the U.S. Inter-Agency Regulatory Liaison Group: "Use of data from less sensitive species is justifiable only if there are strong reasons to believe that the most sensitive animal model is completely irrelevant to any segment of the exposed human population" (*Guidelines*, p. C-22). There are no strong reasons to believe

*Note: Pursuant to the Governor's Reorganization Plan No. 1, which created the California Environmental Protection Agency and the Office of Environmental Health Hazard Assessment (OEHHA) within the Agency, effective July 1991, OEHHA has acceded to the functions of the Department of Health Services in the "AB 1807" Toxic Air Contaminants program (Health and Safety Code Sec. 39650 *et seq.*). DHS staff prepared the draft TSD Part B (health effects document) addressed by the public comments summarized here, and OEHHA staff prepared the responses presented here.

that the B6C3F1 mouse is irrelevant to humans. Pharmacokinetic and tissue distribution studies (Laib et al. 1990, Bond et al. 1986) have not revealed species differences capable of explaining the observed target site carcinogenic responses in rats and mice (Melnick and Huff 1991). Thus the butadiene carcinogenic process is not adequately explained by the metabolic data obtained to date. In addition, some available epidemiological data on occupational BD exposures indicate that employment in industries with butadiene exposure is associated with lymphatic and hematopoietic cancers. These are similar to some of the cancers observed in the mouse studies. OEHHA staff consider the available mouse data to be relevant to human cancer risk assessment and an appropriate basis for the best estimate of human cancer risk.

Comment from the ARCO Products Company (ARCO)

Comment: The document does not contain a "best value" unit risk estimate as does the formaldehyde report released for comment the same month. The DHS should determine a "best value" unit risk estimate for 1,3-butadiene (ARCO, letter [signed by David A. Smith] dated March 22, 1991).

Response: The draft document gave a "best estimate" of cancer potency near the top of page 1-6. This estimate was 0.32 $(ppm)^{-1}$. A new best value, based on a new mouse study (Melnick et al. 1990), is presented in the revised document.

Comments from the Chemical Manufacturers Association (CMA)

1. Comment: The DHS risk assessment for 1,3-butadiene overstates the potential human cancer risk by a substantial margin. The DHS should make adjustments in its risk assessment to reflect more realistically butadiene's potential risks to humans (CMA, letter [Signed by Geraldine V. Cox, Ph.D.] dated March 21, 1991, Executive Summary of comments, p. 1, and comments, pp. 1-2).

Response: In view of known variations in sensitivity to carcinogens and the epidemiological evidence that butadiene (BD) may cause lymphatic cancers in humans similar to those seen in the mouse, it is impossible to conclude that risk estimates based on mouse data must be overestimates. Newer mouse data (Melnick et al. 1990) allow a potency estimate based on an individual tumor site. This revised best value for human cancer potency is similar to the previous best estimate but is based on the single most sensitive site and sex of the new bioassay, the female lung.

2. Comment: The document should emphasize that, while butadiene is a potent carcinogen in the B6C3F1 mouse, it is only a weak carcinogen in the rat (CMA Executive Summary, p. 1).

Response: Section 3.5 of the document quotes the rat bioassay report (Owen et al. 1987) which stated, "the evidence suggests ... that [butadiene] is a weak oncogen to the rat." In addition, the document's quantitative risk estimation clearly indicates that BD is a much weaker carcinogen in the rat than in the mouse. Nevertheless, BD is carcinogenic in rats, and at

multiple sites: in the bioassay, both high dose males and mid and high dose females had significantly elevated numbers of animals with multiple tumors.

3. Comment: Recent data indicate that the mouse is uniquely sensitive to the carcinogenic effects of butadiene. Therefore, the rat is a more appropriate model for human risk assessment. The rat should be used by the DHS to derive its "best estimate of risk." The DHS quantitative risk calculations do not fully recognize the limited relevance of the mouse data for human risk assessment (CMA Executive Summary, p. 1, and comments, pp. 2, 18, 19-20, 31-32).

Response: The document provides a detailed analysis of the rat bioassay data including a lengthy time-to-tumor analysis (Weibull-multistage). While in most cases, extrapolations in time and dose gave potency values (for dose) similar to those obtained with dose only, they gave potencies for benign and malignant mammary tumors that were considerably higher. Nevertheless, the B6C3F1 mouse is the most sensitive test system, and has thus been used for the "best estimate" of cancer potency. It is not clear that the mouse is uniquely sensitive to butadiene carcinogenesis, relative to humans, however. See the response to API comment 2, above.

4. Comment: The Hazleton rat bioassay results, with certain adjustments, should be used to calculate the "best estimate" of human cancer risk (CMA Executive Summary, p. 1, comments, pp. 20, 31-32).

Response: There are no data which clearly show that humans respond more like rats to butadiene exposure in terms of carcinogenicity. The rat study used two high dose levels, 1000 and 8000 ppm. The best estimate in the revised document is based on the Melnick et al. (1990) study which used 5 dose levels, ranging from 6.25 to 625 ppm. Also, the rat study was not as well documented as the mouse studies. Consequently, the Melnick et al. (1990) study is the most relevant to extrapolation to environmental levels. Thus, based on this information and state guidelines, the Hazleton rat bioassay (Owen et al. 1987) results should not be used to generate the "best estimate" of human cancer risk. See the response to API comment 2, above.

5. Comment: If the risk assessment continues to rely on the mouse data, it should exclude the lymphomas, and should adopt certain recommended adjustments to reflect species differences in butadiene metabolism and mechanism of action (CMA Executive Summary, p. 1, and comments, pp. 18, 26-30).

Response: The revised document includes a best value based only on the most sensitive site, i.e., lung tumors in female mice reported by Melnick et al. (1990). The commenter suggests that the potencies be adjusted for reactive metabolite levels. For extrapolating from mice to humans, the commenter recommends a 590-fold adjustment based on a recent publication, Dahl et al. (1990). The epoxides referred to in the comparison are not necessarily the only metabolites associated with carcinogenicity. There is no apparent correlation between tissue epoxide concentrations and tumorigenic response. Furthermore, the 2-hour peak levels of epoxide referred to by the commenter are quite similar among the species studied

when expressed per unit surface area (body weight raised to the 2/3 power). For more information, see the responses to CMA comments 19-24, below.

6. Comment: The risk estimate should be adjusted to reflect species differences in the blood levels of the monoepoxide (CMA Executive Summary, p. 1).

Response: Available data show little or no correlation between tissue concentrations of epoxide metabolites and observed incidence of carcinogenic lesions (see Sections 2 and 4 and the work of Laib et al. 1990, and Bond et al. 1986). As indicated in Table 2-4 and accompanying text, butadiene metabolites were found in highest concentration in tissues not associated with tumorigenicity. Modeling of BD metabolites as shown in Chapter 4 also indicates high concentrations in tissues not associated with carcinogenicity. Specifically, metabolites are found in among the highest concentrations in the bladder, small intestine, and kidney, sites not associated with tumor development. The lung, heart, and mammary gland have among the lowest levels of metabolites and yet are important sites of cancer. Furthermore, the relative distribution of metabolites does not explain either the lower sensitivity of rats to butadiene or the different sites of carcinogenicity in the two species. For example, the estimates and measurements for mammary tissue are not much different between the two species and the difference in response between the two species is not easily explained on the basis of metabolite levels. In view of these facts, a whole body (continuous internal) dose is the most appropriate for low dose extrapolation. For comparison, the document also provides risk estimates based on applied and metabolized dose measures.

7. Comment: The DHS does not explore alternative risk modeling approaches which would reflect interspecies differences in response to butadiene. The DHS should consider an alternative risk analysis prepared by Shell Oil Company. This alternative risk assessment uses the preliminary NTP II data described in the DHS document but not used in the risk assessment. It illustrates how certain biological data can be incorporated and demonstrates that the predicted risk is much lower when all of the data are used. This commenter is providing the Shell alternative risk assessment for consideration by the DHS and ARB (CMA Executive Summary, pp. 1-2, and comments, pp. 2, 30).

Response: The document's risk assessment has been revised to use published results of the second NTP mouse study ("NTP II"; Melnick et al. 1990). OEHHA staff considered the alternative risk assessment prepared by Shell Oil. This assessment is for occupational exposures, and is presented in insufficient detail for a full evaluation.

8. Comment: The DHS analysis should consider recent primate data reported by Dahl et al. and Sun et al. (CMA [comments], p. 2).

Response: OEHHA staff have considered these data and do not find them to be of use in the quantitative risk assessment. The data indicate some differences between rodents and monkeys. However, the data do not sufficiently explain the differences in tumor production between rats and mice to justify their use in risk assessment. Although data on BD metabolism in primates (especially humans) could be useful for human health

risk assessment, OEHHA staff do not believe existing data are sufficient for this purpose. See also the response to CMA comment 11, below.

9. Comment: The DHS should have interpreted the second NTP mouse study to have established a NOEL for reproductive effects of 20 ppm, rather than a LOAEL of 6.25 ppm. The DHS should recognize that no reproductive effects were observed at levels as high as 8000 ppm in the Hazleton rat bioassay (CMA, p. 5).

Response: The 6.25 ppm level is reported as a LOAEL since at that dose gonadal atrophy was reported in the female mice. A sentence regarding the Hazleton rat study has been added to the document, as suggested by this comment.

10. Comment: In light of the unique sensitivity of the mouse to the reproductive effects of butadiene exposure, the absence of reproductive effects in the rat should take precedence in determining butadiene's reproductive risk to humans (CMA, p. 5).

Response: Neither the Hazleton rat bioassay nor the NTP mouse bioassays were designed to be reproductive studies. The absence of effects in the Hazleton study does not preclude the possibility of reproductive effects in rats. The document is merely reporting observed results. Major conclusions are not drawn with regard to the reproductive or developmental toxicity of butadiene, due to the limited nature of the testing that has evaluated these endpoints.

11. Comment: Since butadiene is genotoxic only when metabolically activated, species differences in butadiene uptake and metabolism must be considered when evaluating the results, and relevance to human risk assessment, of rat and mouse bioassays. The DHS has not evaluated all the relevant data on butadiene pharmacokinetics and metabolism, most notably from a 1990 report by Dahl et al. (CMA, pp. 8-14).

Response: Current data does indicate that metabolic activation is necessary for mutagenicity. In preparing the revised document, OEHHA staff considered the data from the Dahl et al. report and data from additional relevant publications. The document discusses the genetic toxicity of butadiene and its metabolites in Section 3.4. Although mutagenicity in routine assays requires metabolism to reactive metabolites, the available data on the pharmacokinetics of BD and its metabolites do not sufficiently account for observed differences in tumor incidences among test systems. Risk estimates based on metabolized dose estimates form part of the range of risks presented in the document.

The data of Dahl et al. (1990) and Sun et al. (1989) show similarities in qualitative aspects of BD metabolism between rodents and monkeys. Dahl et al. (1990) gave normalized BD uptake values of 3.30, 0.46 and 0.52 μmole/hr/10 ppm/kg for the mouse, rat, and monkey, respectively. However, if these values are expressed in terms of a surface area approximation (body weight raised to the 2/3 power), the values are 0.99, 0.35, and 0.94 μmole/hr/10 ppm/kg$^{2/3}$, respectively. Such surface area corrections are often found to give more reliable interspecies comparisons of carcinogenic

responses and presumably would also apply to carcinogenesis-related metabolism.

12. Comment: In preparing its "best estimate" of human cancer risk, based on bioassay results in B6C3F1 mouse, the DHS made no adjustments for interspecies differences in butadiene uptake, retention or metabolism. The predicted cancer potency factor, 0.32 per 1 ppm, substantially overstates the potential human risk associated with butadiene (CMA, p. 18).

Response: DHS and OEHHA staff considered uptake, retention and metabolism data prior to selecting continuous internal dose as the best dose measure for use in risk assessment. Risk estimates based on a pharmacokinetic, metabolized dose adjustment are also provided for comparison. Although the draft document's predicted cancer potency factor is based on certain health protective assumptions and uses a upper 95% confidence bound, it does not clearly overstate the potential human risk associated with butadiene. Likewise, based on our current knowledge, the new "best estimate" potency factor given in the revised document does not substantially overstate the human cancer risk associated with BD exposure.

The continuous internal dose measure used in the document is based on ^{14}C-butadiene uptake, metabolism and retention in mice and rats (see Section 4.2.4). Thus, adjustments were made with regard to differences in these parameters. The methodology used to calculate internal dose was first employed by the U.S. Environmental Protection Agency, in 1985.

13. Comment: Part B states without adequate explanation that the quality of the mouse bioassay data is superior to that of the rat bioassay data. The DHS thus determined that the mouse provides the best estimate for the upper bound of plausible excess human risk. However, there are no differences between the quality or reliability of the NTP mouse study and the Hazleton rat bioassay which would warrant assigning greater weight to the mouse data. More importantly, available evidence demonstrates that the B6C3F1 mouse is uniquely sensitive to the carcinogenic effects of butadiene. With similar exposures, mice achieve higher levels of reactive butadiene metabolites than rats or primates. Cytogenetic and bone marrow effects have been seen in the mouse but not the rat or primate. As primates are more representative of humans than mice, the B6C3F1 should be viewed as an inappropriate model for human risk assessment; the Sprague-Dawley rat should be considered to be a more relevant model. Thus, the DHS should use the data from the Hazleton rat bioassay to derive its best estimate of the human cancer risk associated with exposure to low levels of butadiene (CMA, pp. 19-20, 8-17).

Response: The discussion in Part B of the relative quality of the mouse and rat bioassay data has been expanded (see Section 4.2.9 of the revised document).

Although certain cytogenetic and bone marrow effects were not seen in rats, the effects seen in mice do not necessarily indicate that mice are uniquely sensitive to the carcinogenic effects of butadiene. Butadiene does induce cancer in rats, and the non-cancer effects may not be required for carcinogenesis. In addition, the TSD Part B risk assessment is now based on lung tumors in mice, rather than cancer of the hematopoietic system and

other significant tumors. Extensive testing has been conducted with mice while the study of rats, monkeys, and other species (including humans) is extremely limited. Thus, we cannot conclude that humans respond similarly to rats but not mice in terms of butadiene genotoxicity, and the available data do not lead us to adjust the risk assessment for an interspecies difference in sensitivity. Risk assessment guidelines suggest that risk estimates be based on data from the most sensitive site, sex, strain and species, unless there are strong reasons to believe that this model is irrelevant (see the response to API comment 2, above).

The second mouse study (Melnick et al., 1990) is clearly superior to the previous mouse study in terms of design, dosing regimen, and levels chosen. An increase in tumors was found at the 6.25 ppm dose level, 100-fold below the levels considered in the rat study. The rat study employed two dose levels (1000 and 8000 ppm) and was not reported in as great detail as the two mouse studies. The recent mouse bioassay best allows a clear evaluation of carcinogenicity at individual tumor sites.

OEHHA staff acknowledge that in certain kinetic experiments, primates may have appeared more similar to rats than to mice. The reverse appears to be the case with the data of Dahl et al. (1990), however (see the response to CMA comment 11, above). Nevertheless, the limited available primate data do not clearly indicate that the rat bioassay data are more valid than the NTP II mouse data for assessing the carcinogenicity of BD in humans. As discussed in the quantitative risk assessment section of the document, the available metabolism data do not adequately explain the observed species sensitivity or site selectivity of carcinogenic response.

14. Comment: The risk estimates in the document that are based on rat data should be adjusted by excluding the mammary carcinomas (as well as the mammary fibroadenomas) from the total tumor incidence because their incidence is not significantly elevated in the 1000 and 8000 ppm dose groups. Their incidence is actually lower in the low dose group than in the control group. The risk estimate including the mammary carcinomas appears to overstate the risk by a factor of two (CMA, pp. 21-22).

Response: The risk estimates from the rat data are based on mammary carcinomas, thyroid follicular cell tumors, and zymbal gland tumors. While the mammary carcinomas alone are not significantly elevated in the high dose group, the sum of carcinomas and adenomas shows a dose-related highly-significant difference. The tumor potency for the combined mammary adenomas and carcinomas is similar to the other potencies estimated for the rat as shown in Table 4-16. As noted elsewhere (see the response to CMA comment 2 above), the multiplicity of mammary and other tumors is also a concern. Both exposure groups of females had a greater proportion of animals with multiple tumors. The adenoma and carcinoma incidences appear related to butadiene exposure. For these reasons, mammary carcinomas were included in a total significant tumor incidence count.

15. Comment: Using the internal concentration of butadiene as the measure of dose is more appropriate than using the external concentration in inhaled air. However, since DNA-reactive and mutagenic metabolites of butadiene are the probable ultimate carcinogens, it would be more

meaningful to use blood levels of the primary metabolite, 1,2-epoxybutene-3, for the measure of dose (CMA, pp. 22-23).

Response: A number of possible dose measures were considered in the draft document (Section 4.2.4) and the continuous internal dose, based on the uptake data of Bond et al. (1986), was judged the most reliable. The available metabolic data do not provide a consistent measure for carcinogenicity. The production of tumors in multiple organs and lack of correlation with tissue metabolite residues suggests the use of a dose measure that is applicable to the whole animal. The absence of human metabolic data on butadiene prevents the application of the metabolic information in a reliable manner. In view of the current state of knowledge of tumor incidences and BD metabolite concentrations, OEHHA staff believe that continuous internal dose is the most reliable measure. See the response to CMA comment 6 above.

16. Comment: Although the DHS rejected the use of a risk estimate based on pharmacokinetics primarily because it considered only the monoepoxide and not diepoxide butane (DEB) or other metabolites, crosslinking studies demonstrate that the mouse obtains high levels of DEB, so using only the monoexpoxide levels would not underpredict the actual human risk (CMA, p. 23)

Response: The "metabolized dose" approach in the document used the model of Hattis and Wasson (1987). This model evaluates the internal exposure to butadiene's chief metabolite, BMO (the monoepoxide). The model given for comparative purposes in the document is based on estimated chemical-specific constants (i.e., octanol/water, oil/water, lung/blood, KVRG (kidney vessel-rich group)/blood, muscle/blood, liver/blood and blood/air partition coefficients). For OEHHA staff to consider using a PBPK model to generate the best estimate of BD's carcinogenic potency, the model should have some experimentally determined chemical specific values (e.g., an octanol/water partition coefficient). More elaborate models including other BD metabolites are under development by various researchers, but are not yet available for review and evaluation.

In addition, it is unclear whether monoepoxide production is a sufficiently accurate dose measure since at higher experimental doses, significant amounts of the monoepoxide are exhaled. Improved pharmacokinetic models currently being developed may produce better dose measures for human dose estimation when more data become available. That the metabolized dose approach used only monoepoxide concentrations was not the primary reason it was rejected for generating the best estimate of risk. The continuous internal dose measure based on retained (radiolabel) equivalents of BD and metabolites is thought to be the best current measure of delivered dose. If both the monoepoxide and diepoxide are responsible for carcinogenicity of butadiene and their impact is additive, the metabolized dose risk estimate based on monoepoxide alone would not have resulted in an underprediction of human risk. However, if the predicted pharmacokinetic dose of monoepoxide greatly exceeded the *effective dose* of epoxide, an underestimation of risk could have occurred. Since the metabolism of butadiene in humans is not known, the impact of metabolism on actual human risk is not known either.

17. Comment: The DHS risk assessment based on the internal concentration of 1,3-epoxybutene-3 [the monoepoxide, BMO] as the measure of dose in the rat should be adjusted downward to reflect species differences in monoepoxide levels. If we accept the very reasonable assumption that humans metabolize butadiene in the same manner as monkeys, humans should be approximately 40 fold less sensitive to the carcinogenicity of butadiene than rats. To avoid underestimating the risk, the DHS might adjust the risk factor by a factor of 20. The resulting risk level should be presented by the DHS as its "best estimate" of upper bound cancer risk to man (CMA, p. 24).

Response: The commenter provides no human data supporting this assumption. Interspecies scaling of many carcinogens indicates that humans are 7-13 times *more* sensitive than mice, using conversions based on body weight raised to the 2/3 or 3/4 power. The data of Dahl et al. (1990) indicate that the BD equivalents retained after 2-6 hours are approximately the same for the mouse and the monkey when expressed in terms of body weight raised to the 2/3 power. Therefore it would be wholly inappropriate to apply any 20-fold correction to mouse-based estimates of human cancer potency. The presence of metabolized dose in various tissues in the mouse or rat does not correlate with carcinogenicity. Consequently either available metabolized dose information is not applicable to carcinogenicity or the sensitivity of the tissue to the tissue dose is a significant factor. For either case, application of a 20-fold factor, as suggested by the commenter, is inappropriate.

18. Comment: The document's rat-based risk estimation should (at a minimum) be adjusted to reflect underestimation of the internal dose. To estimate the internal dose corresponding to a given concentration of inhaled butadiene, the DHS relied on data that ignore both the metabolism and excretion of butadiene during the exposure period, as well as the potential for further metabolism after six hours. Pharmacokinetic modeling shows that these data underestimate the actual internal dose in rats by a factor of approximately 4.5 (CMA, p. 25).

Response: The pharmacokinetic modeling referred to by the commenter is that of Hattis and Wasson (1987). This modeling addresses "metabolized dose" estimation rather than "internal dose" estimation. As noted in an appendix to the commenter's submittal (Appendix 1 of Appendix G, p. 2), this modeling addresses "the amount of BD processed." The draft document did evaluate an adjusted pharmacokinetic dose measure derived using modeling similar to that of Hattis and Wasson, and referred to this measure as "metabolized dose." Thus, here the commenter has apparently confused metabolized dose estimation with internal dose estimation.

The document's "internal dose" estimation is based on the amount of radiolabel retained in animals after six-hour exposure to radiolabeled butadiene. Hattis and Wasson (1987) offered pharmacokinetic modeling in part as a response to risk assessments that use this information. They noted that use of the radiolabel retention information could lead to either underestimation or overestimation of what they called the "delivered," or "biologically effective" dose (the dose of active metabolites) (Hattis and Wasson 1987, Summary, pp. S-1 to S-2); the DHS/OEHHA document refers to

this type of measure as "metabolized dose." The usefulness for risk assessment of each type of dose measure is discussed in the document.

The internal dose estimates used in the document are based on the data of Bond et al. (1986). As mentioned above, they represent the radiolabel retained in, but not excreted from, rats and mice at the end of six-hour inhalation exposures to radiolabeled butadiene. Data from Bond et al. (1986) also show that part of the dose retained at six hours is recoverable from the carcass 65 hours later. The radiolabel bound to the carcass presumably represents butadiene that has been metabolically activated (as BD itself is not likely to bind to tissue). Thus the Bond et al. (1986) data reflect, rather than ignore, metabolism and excretion of butadiene during the exposure period.

The second NTP mouse study (NTP II), used lower doses than the first. The human cancer potency estimate (q_1^*) in the revised document is based on NTP II data. At lower doses, there is less difference between risk assessment results using internal dose measures and those using (pharmacokinetic) metabolized dose measures. The appendix to the commenter's submittal notes that "Hattis and Wasson did not model the low dose situation pertinent to NTP-II" (Appendix 1 of Appendix G, p. 2). Nevertheless, the revised document does evaluate the NTP II data with respect to the pharmacokinetic model of Hattis and Wasson.

See also the response to CMA comment 23, below.

19. Comment: If the DHS continues to rely on data from the 1984 NTP mouse study for the "best estimate" of human cancer risk, the estimate should be adjusted downward to reflect significant species differences in butadiene metabolism and mechanism of action (CMA, pp. 26-30).

Response: The revised document presents a new best estimate of human cancer risk which is based on the more recent NTP II mouse study (as reported by Melnick et al. 1990). Regarding the specific species differences listed by the commenter, see the responses to the 5 comments immediately below.

20. Comment: The MuLV retrovirus plays a critical role in the expression of malignant lymphoma in B6C3F1 mice. Data indicate that the bone marrow is a target organ of butadiene toxicity in the B6C3F1 mouse, but not the rat or primate. The DHS should exclude malignant lymphomas from the total significant tumor incidence in B6C3F1 mice used for risk estimation. In doing so, the DHS would be following the lead of the U.S. Occupational Safety and Health Administration. The unique sensitivity of the mouse bone marrow and other important differences between man and the B6C3F1 mouse make the mouse's malignant lymphoma an endpoint of questionable relevance to potential human cancer risk (CMA, pp. 26-28).

Response: The new risk estimates presented in the revised document are not based on lymphomas. Rather, they are based on lung tumors in female B6C3F1 mice. Thus this issue is no longer central to the risk assessment.

21. Comment: Blood levels of 1,3-epoxybutene-3 provide a much more meaningful dose estimate than the internal concentration of butadiene as

used by the DHS. Data indicate that humans should be 590-fold less sensitive to butadiene's carcinogenicity than are mice. Using the approach of allowing only 50 percent of this 590 fold difference, the risk estimate would be adjusted downward by a factor of 295 (CMA, p. 28).

Response: As noted above (see the response to CMA comment 6), available data show little or no correlation between tissue concentrations of epoxide metabolites and observed incidence of carcinogenic lesions. The commenter did not provide scientific support for the particular quantitative technique suggested here. Given the available data, OEHHA staff believe that the whole body continuous internal dose measure used in the document is still the most appropriate for low dose extrapolation. Adjustments such as the 590- or 295-fold adjustment suggested by the comment are not warranted due to the poor relationship between relative tissue doses and carcinogenic responses.

22. Comment: Even if the DHS decides to rely on the internal concentration of butadiene rather than the blood levels of the monoepoxide for the risk estimation, the risk estimates should be reduced to reflect underestimation of the retained (animal) dose and interspecies differences in butadiene absorption and retention (CMA, pp. 28-30).

Response: As suggested by the response to CMA comment 18, the continuous internal dose measure used in the risk assessment reflects the retained dose in mice and rats. No underestimation of dose retention has been shown. Species-specific data regarding absorption and retention of BD show that mice and monkeys are similar when the data are compared in terms of body weight raised to the 2/3 power, a typical interspecies comparison (see the response to CMA comment 11, above). Empirical data sufficient to support the adjustments suggested by the commenter are not currently available. See also the responses to the following 2 comments.

23. Comment: Pharmacokinetic modeling demonstrates that the measured dose of butadiene retained after six hours (this measure is relied on in the DHS risk assessment), underestimates the actual internal dose in mice by a factor of two. Therefore, the risk estimate should be reduced by 50 percent (CMA, p. 29).

Response: The pharmacokinetic modeling referred to by the commenter is that of Hattis and Wasson (1987), and the response to CMA comment 18 also applies to this comment. The commenter appears to assume that the metabolized or pharmacokinetic dose is the best measure of delivered toxic dose despite the fact that at higher experimental doses, the primary epoxide product of BD metabolism is partially exhaled. The continuous internal dose based on retained BD equivalents is more reflective of the effective dose.

24. Comment: The DHS has assumed that the absorbed fraction of butadiene is the same for mice and humans; this does not take into account data showing that at 10 ppm the mouse retains approximately 6.3 fold more butadiene than the monkey. Because the humans are more closely related to the monkey than the mouse, this retention data should be used to adjust the mouse data-based risk assessment downward by a factor of approximately six (CMA, pp. 29-30).

Response: The internal dose estimate is based on exposures of 0.08 ppm which is 100-fold below the level given in the comment. As exposure concentration increases, there are greater differences in inhaled butadiene between species. Thus the difference at 10 ppm may not be important at environmental levels. Primate data on butadiene absorption were not available to DHS when the draft document was assembled. The 1990 data of Dahl et al. show that the mouse retains more BD equivalents per unit body weight than the monkey. However, when these interspecies data are compared in terms of body weight raised to the 2/3 power, the values are nearly identical (i.e., 0.99 μmole/hr/10 ppm/kg$^{2/3}$ for the mouse, and 0.94 for the monkey). Thus these data do not clearly indicate a need to adjust the mouse data-based risk assessment. The revised (OEHHA) risk assessment uses body weight raised to the 2/3 power as the basis for interspecies extrapolation of the rodent cancer data to man.

25. Comment: It is misleading to assign no weight to relevant data on species differences regarding butadiene metabolism and blood levels of butadiene metabolites. When the DHS risk assessment is adjusted to account for these species differences, it becomes apparent that the present DHS risk assessment overpredicts the risk to man by one to four orders of magnitude (CMA, pp. 31-32).

Response: The adjustments suggested by the commenter are not supported by sufficient data. Although the available data are in part relevant to the issue, they do not conclusively demonstrate the need for any particular adjustments. No dose estimation data have been shown to fit the available cancer bioassay data better than the continuous internal dose data used in the document. With regard to specific adjustments suggested by the commenter, see the responses to CMA comments 17-24. With regard to the selection of the appropriate species data for use in risk assessment, see the response to API comment 2, above.

26. Comment: Care must be used in interpreting the NTP II mouse study findings on ovarian atrophy because they are preliminary and unaudited, and the histopathology narratives are limited. The draft DHS document concludes that a NOEL was not established and that 6.25 ppm was the LOAEL. However, a scientist has noted that ovarian atrophy in the 6.25 and 20 ppm groups occurred at the end of the animals' reproductive life and should not be regarded as "reproductive" effects. In addition, the mouse is uniquely sensitive to the effects of butadiene exposure and exhibits effects which are not observed in the rat. This scientist has concluded that 20 ppm should be considered a NOEL for ovarian atrophy in the B6C3F1 mouse (CMA, pp. 32-33).

Response: OEHHA staff disagree with the conclusions suggested by the commenter. This is explained in the responses to CMA comment 9 and API comment 2, above.

27. Comment: The health assessment should also highlight the absence of reproductive effects (ovarian or testicular atrophy) in the Hazleton rat bioassay by Owen et al. at lifetime exposure levels as high as 8000 ppm. This study should take precedence in determining butadiene's reproductive risks (CMA, p. 33).

Response: The Hazleton rat bioassay was not a reproductive study. However, mention of the absence of a positive reproductive finding in this study has been added to the document. See the responses to CMA comment 9 and API comment 2, above.

28. Comment: The review of butadiene should consider two additional documents, a Chemical Industry Institute of Toxicology report and a Society of Toxicology meeting poster presentation (CMA, letter [signed by Elizabeth J. Moran, Ph.D.] dated June 18, 1991)

Response: OEHHA staff have considered these documents, but have not included reviews of them in the technical support document at issue here since they are preliminary or abstracted work. OEHHA staff do not believe the information affects the outcome of the risk assessment.

Comments from the General Motors Corporation (GM)

1. Comment: The document should provide proper perspective on the risk from motor vehicles relative to the much larger risks from indoor sources of risk such as environmental tobacco smoke. The document does not put the risk from present and future ambient concentrations of 1,3-butadiene into the proper perspective (GM letter [from S.A. Leonard] dated March 22, 1991, and comments, p. 1).

Response: The ARB has not asked OEHHA staff to evaluate other risks as part of the technical support document for the proposed identification of butadiene as a toxic air contaminant. Comparative risk evaluations need not be included in TSDs prepared for the Toxic Air Contaminants Program. Although such information might be of interest to both scientists and non-scientists, the task of Part B of the butadiene TSD is to assess the risks posed by butadiene in ambient air.

2. Comment: The document should better portray the limitations and uncertainty associated with the range of unit risk factors developed for 1,3-butadiene (GM letter, and comments, p. 1).

Response: Accurate and easily understood descriptions of limitations and uncertainties should be conveyed by any risk assessment document. The draft TSD Part B did address specific limitations and uncertainties of the chosen risk assessment approach, in Section 4. Part B's Summary also conveyed the uncertainty associated with the range of unit risk factors with statements such as this: "... community exposure to ambient BD at currently detected levels (0.0004 to 0.0018 ppm) could be associated with up to 1 to 576 additional lifetime cancers per million exposed individuals" (p. 1-7, emphasis omitted).

3. Comment: The Executive Summary indicates that the number of potential excess cancers due to airborne 1,3-butadiene exposure ranges from 1 to 115 additional cancers per million people exposed throughout their lifetimes. The phrase "upper limit" should be added so that this range of potential cancers is put in the correct perspective for decision makers and the public, who may otherwise perceive the risk from exposure to ambient 1,3-butadiene as a major threat to public health (GM [comments], p. 3).

Response: This range is based on upper confidence limits from various risk modeling approaches. The sentence in the Executive Summary has been modified as suggested.

4. Comment: The "weight of evidence approach" should be used in each portion of the risk assessment, and the best estimate of the potential risk as well as the highest plausible risk should be presented (GM, p. 4).

Response: Staff use "weight of evidence" primarily in the hazard identification process (for example, in identifying butadiene as a genotoxin and carcinogen). For the risk assessment, staff consider all available scientific data in developing the range of risks. Staff identified the best value for risk assessment on the basis of the best study, the most scientifically justifiable approach, and public health judgement.

5. Comment: It is disappointing that the document concludes (on page 4-33 of Part B) that the risk assessment cannot rely at present on the pharmacokinetic estimates of metabolized dose for estimating human health risks. Considerations of pharmacokinetic principles in the disposition of 1,3-butadiene in the human body should be an integral part of the hazard identification and would significantly improve the final risk estimates (GM, pp. 8-9).

Response: Consideration of pharmacokinetic principles was an integral part of the risk assessment. However, as discussed in the response to CMA comment 16, current pharmacokinetic data are not sufficient to provide a more accurate prediction of human risk.

6. Comment: GM scientists and the scientific community at large are not absolutely certain that the ambient concentrations of 1,3-butadiene "may cause or contribute to an increase in mortality or illness, thereby posing a potential hazard to human health." The inherent uncertainties in the risk estimates are important caveats to this characterization. Clarification of cancer-producing mechanisms is an important step before final decisions are made on the real public health hazard of a chemical (GM, pp. 9-11).

Response: OEHHA staff are required to make a determination based on existing available data. As noted in the responses above, there are many uncertainties in the risk assessment of butadiene. However, the genotoxicity of BD metabolites and the in vivo formation of DNA adducts provides a plausible mechanism for BD carcinogenesis. There is still a poor correspondence between measured or predicted concentration of BD and its epoxide metabolite(s) in a target organ and subsequent tumor incidence in that organ. The pharmacokinetics investigations of BD conducted to date while representing a step in the right direction do not yet shed light on the pharmacodynamics of the carcinogenic process at the target tissue level much less at the molecular level. Butadiene has been shown to be a carcinogen in laboratory animals. It is present in California air. A threshold for the carcinogenic effect has not been identified. Consequently, butadiene exposure poses some risk. The TSD Part B provides an estimate of that risk. The risk estimate characterizes a hazard that butadiene poses to human health.

7. Comment: Part B's conclusions and assumptions regarding threshold mechanisms are premature in view of uncertainties. The text on page 4-3 recognizes that thresholds for saturation of detoxification enzymes have been proposed, but fails to mention this possibility when the non-threshold action of butadiene is assumed in the cancer potency estimates (GM, p. 9).

Response: The genetic toxicity of BD metabolites and the formation of DNA adducts in vivo support a non-threshold approach to the low dose extrapolation. As noted in the response to GM comment 6, many questions remain regarding the mechanism of oncogenic action of BD and its metabolites. However convincing evidence that a threshold exists is not available. Biological processes such as detoxification are not in themselves sufficient to prove a claim for the presence of a threshold. Such processes function on molecular interactions and chemical equilibria. As such processes are better defined they can be incorporated into risk assessments. Although they may lower risk estimates, they are not likely to present relevant finite thresholds.

8. Comment: Not all scientists would agree that the evidence for chemical (genotoxic) carcinogenicity of butadiene in animals is sufficient. First, the in vitro genotoxicity of butadiene has not been verified in vivo on laboratory rats. Second, criteria for animal carcinogenicity tests are currently under review by the U.S. National Academy of Sciences. Third, the incidence of butadiene-induced leukemia varies widely among species and even among different strains of mice (B6C3F1 vs. NIH Swiss mice), indicating that the tumors in the B6C3F1 mouse may have been induced by activation of endogenous leukemogenic retroviruses (MuLV) rather than by the direct chemical action of butadiene. This represents an important argument for a non-specific, threshold-producing effect of butadiene based on secondary tumor-regulating mechanisms. Since thymic lymphomas represent the most substantial tumor response in mice, this factor could significantly influence the cancer risk and potency calculations derived from the animal data (GM. pp. 9-10).

Response: OEHHA staff consider that genotoxicity is a presumptive rather than a confirmed mechanism for BD oncogenicity. Other mechanisms may exist. Recent data that NIH Swiss mice (without endogenous ecotropic virus) are also susceptible to BD induced thymic lymphomas and that this neoplasm is independent of activated retrovirus (Irons 1990). Furthermore, the finding of activated K-RAS oncogene in liver and lung neoplasms and in lymphomas of BD exposed mice raises new concerns about BD's carcinogenic mechanism since K-RAS in the most commonly detected oncogene in human tumors (Goodrow et al. 1990). Both the U.S. Environmental Protection Agency and the International Agency for Research on Cancer have concluded that there is sufficient evidence for carcinogenicity of butadiene in animals. Finally, butadiene produces tumors in many tissues and organs. The revised risk assessment is based on lung tumors, not leukemia or lymphomas.

9. Comment: Evidence regarding the variation in butadiene-induced leukemia incidence among different species and strains of mice questions the DHS conclusion that the mouse bioassay is superior to the rat data and the best for estimating plausible excess cancer risk to humans (GM, p. 10).

Response: Butadiene has been tested for carcinogenicity primarily in one mouse strain (B6C3F1); data from other strains (see, e.g., Irons et al. 1986b) are less useful for risk assessment. The superiority of the Melnick et al. (1990) B6C3F1 mouse study is based on the quality of the study in terms of its design, dose levels used, number of dose groups, and detail of reporting. In addition, risk assessment guidelines indicate that risk estimates should be based on data from the most sensitive site, sex, strain and species, unless there are strong reasons to believe that this model is irrelevant. See also the responses to API comment 2 and CMA comment 13.

10. Comment: The chemical carcinogenicity of 1,3-butadiene is challenged by the statement that "the epidemiological evidence of human carcinogenicity is inadequate" in spite of high occupational exposures and numerous studies. A greater emphasis on the existing uncertainties would therefore be more appropriate than the "finding" reported by the DHS of butadiene carcinogenicity in humans (GM, p. 10).

Section 1.1.3.2 of Part B lists an assumption that only 16 to 17 percent of a low inhaled dose is absorbed by animals, while the same text presents calculations that have apparently been based on the assumption that humans retain 100 percent of the inhaled butadiene. If the absorption factor of 17 percent were used for human exposure as in other animals, the resulting dose would be approximately six times lower (GM, p. 10).

Response: The context of the quote from the draft document, "the epidemiological evidence of human carcinogenicity is inadequate," was consideration of the adequacy of data *for use in quantitative risk assessment*. The quantitative estimates of cancer risk are based on animal data and linearized multistage model extrapolation to achieve low dose cancer potencies. The potencies in continuous internal $dose^{-1}$ units of $(mg/kg\text{-}d)^{-1}$ were converted by species specific conversion factors to external ppm^{-1} units and corrected for intercurrent mortality if necessary. The comment about the inhalation dose at 0.0004 ppm on p. 1-10 is correct. On page 5-5 of the draft document the conversion factor for 17% absorption in humans is given as 0.11 mg/kg-d/ppm. Humans were assumed to absorb 17% of the dose, the same as rats and mice at low concentrations. Thus 0.0004 ppm would translate to a dose of 4.4×10^{-5} mg/kg-d.

11. Comment: DHS staff selected the mouse bioassay data as superior to that of the rat but did not list supporting evidence (GM, p. 11).

Response: See the responses to CMA comment 13 and API comment 2.

12. Comment: DHS staff arbitrarily accept that exposure in humans results in the same absorption rate and handling of the inhaled 1,3-butadiene concentrations as in animals (GM, p. 11).

Response: Staff did accept that human exposure results in the same absorption rate and handling of inhaled 1,3-butadiene as in animals. This assumption is based upon lack of adequate data in humans, and OEHHA staff do not consider it arbitrary.

13. Comment: The assumptions used result in a considerable level of uncertainty in the predicted public health effects and may produce a substantial error in the risk estimates (GM, p. 11).

Response: The comment is correct. Most health risk estimates have a considerable level of uncertainty. See the response to GM comment 6.

14. Comment: Certain uncertainties are not adequately emphasized in the conclusions of Part B. Rather than stating (as on page 5-1) that the average ambient level of butadiene in California could be associated with up to 1 to 128 additional cancers per million lifetime exposed individuals, it would be more appropriate to state that the exposures may result "in up to 0 to 1 or 0 to 128 additional cancer cases per million exposed depending on the selected animal model" (GM, p. 11).

Response: The risk estimate does not predict a zero level of risk. An analysis of the lowest level of risk was not conducted and is generally not part of such a risk assessment. It may be true that the lower bound of risk is zero, but the words "up to" in the document indicate that the given range represents upper bound risk estimates. See also the responses to GM comments 2 and 3.

Comments from the
International Institute of Synthetic Rubber Producers, Inc. (IISRP)

1. Comment: The document does not cover certain recent reports, including five articles published in the June 1990 issue of *Environmental Health Perspectives* (IISRP letter [from William E. Tessmer] dated March 21, 1991, pp. 1-2, and comments, p. 1).

Response: OEHHA has incorporated the findings of more recent studies into the current version. See the response to CMA comment 11 as well.

2. Comment: The DHS and ARB should closely review certain analyses of the available epidemiology studies offered in testimony before the U.S. Occupational Safety and Health Administration. These analyses show that the large available worker exposure data base demonstrates that butadiene is not a human cancer risk at current occupational exposure levels, and, therefore, also not at lower environmental levels (IISRP letter, p. 2, and comments, p. 1).

Response: OEHHA staff have reviewed the available published data on BD cancer epidemiology and differing analyses of the studies. While these data are insufficient to support a quantitative estimate of cancer risk in man they do provide supporting evidence of a possible association between BD exposure and elevated mortality rates from lymphatic and hematopoietic cancers, particularly among black workers exposed to BD in BD or SBR production facilities during World war II.

3. Comment: Studies of tire manufacturing populations are essentially irrelevant for the assessment of butadiene, as butadiene is not liberated during tire manufacturing and is not a solvent used in such plants. Although at least one of these studies (McMichael, 1976) had a small

subcohort of workers employed in a "synthetic plant" where styrene butadiene rubber (SBR) was made at times, it is unclear whether there was butadiene exposure. There was, however, opportunity for exposure to numerous confounding factors, and the investigators have attributed the elevated leukemia rates to "solvent exposure only" (IISRP comments, pp. 1-2).

Response: Four cohorts of workers from tire manufacturing plants are reviewed in the document. For two of these, those studied by McMichael et al. (1976) and Andjelkovich et al. (1976, 1977), the document only presents results from workers who actually worked in areas of plants in which rubber is synthesized. In these areas, butadiene exposure is likely to occur. In discussing the two other studies, by Monson and Fine (1978) and Ott (1980), results from occupational title groups (OTGs) not involved with rubber synthesis are not reported or used to confirm any association of butadiene with cancers. However, OEHHA staff were not able to confirm that the synthetic plant OTG was the only one in which butadiene exposure occurred. Data from tire manufacturing plants are consistent with results of studies of SBR plants and a butadiene production facility. McMichael et al (1976) found elevated rates of leukemia among workers from several OTGs. These investigators related their findings to an earlier case-control study of leukemia in this cohort in which lymphatic leukemia was strongly associated with solvent exposure. They concluded that the elevated SMR for leukemia may have been due to solvent exposure in certain OTGs, but they did not put the synthetic plant OTG in this category, indicating that they felt butadiene exposure may represent an independent risk for leukemia.

As noted in Section 3.6 of the revised document, several studies (including McMichael et al., 1976; Andjelkovich et al., 1976; Andjelkovich et al., 1977; Monson and Fine, 1978, and Ott et al. 1980) are suggestive of increases in leukemia incidence in cohorts with multiple types of chemical exposures, but the increases cannot be definitely attributed to 1,3-butadiene exposure. See also the response to IISRP comment 2, above.

4. Comment: The document makes reference to a variety of potential health effects from butadiene exposure (including respiratory disease, cardiovascular disease, and various non-lymphopoietic cancers), yet there is general agreement that the only issue of concern relates to lymphopoietic cancers (IISRP, p. 2).

Response: OEHHA staff have reviewed the findings of each study and evaluated the studies overall for consistency of results. The revised document emphasizes the cancer endpoints. From the viewpoint of environmental exposure, the chief concern is cancer. This concern is not limited to the lymphopoietic system. While epidemiological studies of limited numbers of exposed workers indicate an association with lymphatic and hematopoietic cancers, it is uncertain whether other cancers (e.g., lung) might also be induced in larger exposed population(s).

5. Comment: The elevated SMRs for lymphopoietic cancers in SBR and butadiene production involve short-term workers and not longer-term workers employed during the same time periods. To many scientists this does not suggest a risk from butadiene exposure, but rather the role of confounding factors among short-term workers. All long-term workers were, at one time,

short-term workers. It is most logical to place greatest emphasis in evaluating butadiene on the lack of elevated mortality rates for long term workers. The relevant data do not support the position that butadiene is a human carcinogen (IISRP, pp. 2-3).

Response: The stop-exposure studies of Melnick et al. (1990) in mice indicate that short-term high exposures to BD may result in greater tumor incidences than longer-term lower exposures. In view of these data in animal studies, it is difficult to assume that cancer among short-term exposed workers is due to confounding factors.

It is also possible that short-term workers are more likely to have worked in a plant during World War II when exposure to BD was more intense. If work during this era placed a worker at greater risk then an effect of job duration might not be observed. However, McMichael et al. (1976) compared rates for those with 2 versus 5 years work in a given OTG, and did find an effect of job duration in many OTGs including the synthetic plant group where butadiene exposure was most likely.

6. Comment: Part B incorrectly characterizes several studies of tire manufacturing or other non-SBR workers as studies of SBR workers. Specifically, on page 3-37 reference is made to the McMichael (1976) study as a study of workers at a single SBR plant. This is a study of workers at a large tire manufacturing plant. On page 3-39, mention is made of the Ott (1980) study as a study of workers at Dow Chemical SBR plants. This was a styrene-based products study, not a study of SBR workers: only 391 of the 2,904 workers were employed manufacturing styrene butadiene latex, while the rest were employed in polystyrene and benzene alkylation related units (IISRP pp. 3-4).

Response: OEHHA staff have corrected the parts of the document in which tire manufacturing plants were referred to as SBR plants. The revised document specifically reports findings in synthetic plant workers (who were likely to manufacture SBR). The revised document refers to Ott's study as an investigation designed to look at the health effects of styrene, and notes that only 391 of the 2,904 workers were employed in SBR manufacturing. However, OEHHA staff were not able to confirm that the synthetic plant OTG was the only one in which butadiene exposure occurred. OEHHA staff acknowledge that McMichael et al. (1976) focused on workers in a single large tire manufacturing plant. However as McMichael et al. explained in the introduction to their report, "because of the high temperatures involved in several of the processes, there are many chemical reaction byproducts produced, most of which remain unidentified." The cancer epidemiology section (Section 3.6) of the draft document stated that "the studies reviewed could not assign any increased risk specifically to exposure to 1,3-butadiene." The revised cancer epidemiology section notes that several studies are "suggestive of an increase in leukemia incidence in [cohorts] associated with multiple types of chemical exposures, however, the increase cannot be definitely attributed to 1,3-butadiene exposure."

7. Comment: The discussion of the McMichael (1976) study gives confidence intervals (CI) as 99.9 percent CIs. This method of CI calculation is atypical and suggests more precision than is warranted for the SMRs. Hence the CIs given for this study are misleading. For this study it would be

clearer to give the observed numbers so that readers can make their own judgments about the precision of McMichael's estimates (IISRP, p. 4).

Response: The 99.9% CIs did not need to be reported and so have been removed from the current version of the document. McMichael et al. did not provide observed numbers in their report. It is important to note that the numbers presented in this study should not be interpreted as standardized mortality ratios. Instead, they are relative risks estimated from odds ratios that denote the risk of >5 years work in a particular OTG, among those workers with a particular cancer.

8. Comment: The review of the Ott (1980) study and its implications for butadiene is misleading because it neglects to mention Ott's finding of no leukemias among the 391 styrene-butadiene latex workers. The four leukemias mentioned were unrelated to the latex operations (IISRP, pp. 4-5).

Response: See the response to IISRP comment 6. OEHHA staff have incorporated the finding of no leukemias among the 391 styrene-butadiene workers into the document. However, staff were not able to confirm that butadiene exposure did not occur in other OTGs.

9. Comment: On page 3-41, the document mentions the hematological effects study by Checkoway and the conclusion that the results were suggestive of possible biological effects of unknown clinical consequence. Other points should also be mentioned: There was no significant difference between the two groups in this study, and the values for the tank farm workers were normal. The tank farm group consisted of only 8 workers, while the comparison group had 145. In the small group, one person's values could have created a slight difference in the group's mean values. Several variables, including smoking, body size, fasting, alcohol, and exercise, which affect hematological parameters were not controlled for in this study (IISRP, p. 5).

Response: The document has been modified to mention Checkoway and Williams' (1982) statement that the small number of tank farm workers precluded reliable statistical tests of significance, and to include several caveats regarding interpretation of their findings.

10. Comment: Evaluation of hematological effects might be extended to consider the available data on SBR- and butadiene monomer-worker mortality from non-malignant diseases of the blood and blood-forming organs. For the largest SBR workers study, the data show fewer deaths observed than expected for the total cohort (5 vs. 7.2) and for mechanical and process workers (2 vs. 4.1) (IISRP, pp. 5-6).

Response: The numbers cited in the comment are from an unpublished report by Matanoski and coworkers. A subsequent published report by Matanoski et al. (1990) did not present these numbers, which are small in relation to the malignant blood disease mortality in this cohort (Matanoski et al. 1990). OEHHA staff find it unnecessary to include these findings in the TSD. As suggested by IISRP comment 4, epidemiological studies of cancer are of more relevance to the butadiene TSD risk assessment than studies of non-cancer mortality in humans. The document has thus been revised to

emphasize the studies of cancer mortality. Although occupational studies found some increased and decreased mortality rates for diseases other than cancer, the document does not focus on these findings, since the chief environmental concern is cancer.

11. Comment: On page 3-44, in discussing the Downs study, the document concludes that the authors found elevated SMRs for the lymphopoietic system regardless of how the cohort was subdivided. This conflicts with Downs' conclusion that certain findings "for latency and duration of employment are contrary to expectation. If a carcinogen were active in this environment, one would expect the SMRs to show a positive relationship to latency, and one would also expect the same pattern with increasing duration of employment. Neither pattern was present for either all lymphohematopoietic cancer or lymphosarcoma." (IISRP, p. 6)

Response: The statement which reads "the authors found elevated SMRs for the lymphopoietic system regardless of how the cohort was subdivided", has been removed. The document now contains results of an updated analysis of the cohort by Divine (1990) which do show findings for workers overall, those with >6 months work from 1943-1945, and those with routine exposure. These results lend strong support to the proposed association of butadiene and lymphatic and hematopoietic cancers. The document notes that latency and duration of exposure were not related in a dose-response fashion to cancer excesses. See also the response to comment 5 in reference to latency and duration of exposure.

12. Comment: The conclusion of the epidemiology review (page 3-45) that there is strong evidence for a butadiene-lymphopoietic cancer association conflicts markedly with other reviews on the topic. This conclusion is inconsistent with the known lack of elevated mortality for long-term workers in butadiene-related industries, which is a situation unprecedented for an occupational carcinogen (IISRP, p. 6).

Response: Two recent reviews referenced in the document have concluded that there is evidence for a butadiene-lympopoietic cancer association. OEHHA staff consider the epidemiological data to be consistent with animal studies but insufficient to quantitatively assess risks resulting from environmental exposures to airborne butadiene. With reference to the epidemiological studies and their deficiencies, Section 3.6 of the draft document concluded that "the studies reviewed could not assign any increased risk specifically to exposure to 1,3-butadiene." The revised Section 3.6 notes that several studies are "suggestive of an increase in leukemia incidence ... however, the increase cannot be definitely attributed to 1,3-butadiene exposure."

Public health agencies do not require epidemiological proof of carcinogen-induced fatalities to develop prudent and scientifically justifiable estimates of environmental or occupational health risks. There currently exists an overwhelming weight of evidence that 1,3-butadiene is a carcinogen. Epidemiological studies generally lack adequate power to detect carcinogenic responses to suspect carcinogens. Furthermore, human populations are generally exposed to confounding factors. In most cases, occupational data are not available to confirm the positive results of an animal bioassay. Animal carcinogens for which human carcinogenicity data

is inadequate that have been identified as toxic air contaminants include methylene chloride, trichloroethlyene, ethylene dibromide, ethylene dichloride, ethylene oxide, chloroform, dioxin and carbon tetrachloride.

Comment from the Western States Petroleum Association (WSPA):

Comment: This commenter concurs with the comments submitted by the American Petroleum Institute and the Chemical Manufacturers Association (WSPA, letter [signed by Scott N. Folwarkow] dated March 22, 1991).

Response: Comment noted.

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PART C ADDENDUM

PUBLIC COMMENTS AND ARB/OEHHA STAFF RESPONSES ON THE SRP VERSION DRAFT OF THE 1,3-BUTADIENE "IDENTIFICATION" REPORT

Prepared by the staffs of the Air Resources Board
and the Office of Environmental Health Hazard Assessment

March 1992

This document has been reviewed by the staff of the Air Resources Board and approved for publication. Approval does not signify that the contents necessarily reflect the views and policies of the Air Resources Board or the Office of Environmental Health Hazard Assessment, nor does mention of trade names or commercial products constitute endorsement or recommendation for use.

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I.

Comment Letters Received on the October 1991
SRP Version Draft of the 1,3-Butadiene Report

CMA

CHEMICAL MANUFACTURERS ASSOCIATION

Gordon D. Strickland
Vice President-Technical Services

December 6, 1991

Ms. Genevieve Shiroma, Chief
Toxic Air Contaminant Identification Branch
Stationary Source Division
Air Resources Board
Attn: 1,3 Butadiene
P.O. Box 2815
Sacramento, California 95812

Re: The Scientific Review Panel Version of the Report to California Air Resources Board on the Proposed Identification of 1,3-Butadiene as a Toxic Air Contaminant

Dear Ms. Shiroma:

The Butadiene Panel of the Chemical Manufacturers Association is pleased to submit the enclosed comments on the Scientific Review Panel version of the report to the California Air Resources Board on the Proposed Identification of 1,3-Butadiene as a Toxic Air Contaminant. The Panel consists of the major U.S. producers and some users of butadiene.

The Panel has previously reviewed and commented on the Preliminary Draft Report for this proposal. The Panel notes with appreciation that several changes to the Report have been made in response to the earlier comments. However, the Panel continues to believe that the quantitative risk estimates contained in the Health Assessment document (Part B of the Report) overstate the potential human cancer risks by a wide margin.

Please direct any questions that you may have regarding these comments to Dr. Elizabeth J. Moran, Manager of the Butadiene Panel, at (202) 887-1182.

Sincerely,

Gordon D. Strickland

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2501 M Street, NW, Washington, DC 20037 202-887-1388 Panafax 202-887-1237 Telex 89617 (CMA WSH)

STATE OF CALIFORNIA
AIR RESOURCES BOARD
STATIONARY SOURCE DIVISION

COMMENTS OF THE CHEMICAL MANUFACTURERS ASSOCIATION BUTADIENE PANEL ON THE SCIENTIFIC REVIEW PANEL VERSION DRAFT REPORT ON THE PROPOSED IDENTIFICATION OF 1,3-BUTADIENE AS A TOXIC AIR CONTAMINANT

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December 6, 1991

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EXECUTIVE SUMMARY

The Butadiene Panel of the Chemical Manufacturers Association has reviewed the Scientific Review Panel (SRP) version of the draft report to the California Air Resources Board (CARB) on the Proposed Identification of 1,3-Butadiene as a Toxic Air Contaminant. This report was prepared by the staffs of CARB and the Office of Environmental Health Hazard Assessment (OEHHA). The Panel has previously reviewed and commented on the Preliminary Draft Report for this proposal. The Panel notes with appreciation that several changes to the Report have been made in response to the earlier comments. However, the Panel continues to believe that the quantitative risk estimates contained in the Health Assessment document (Part B of the Report) overstate the potential human cancer risks by a wide margin. In particular, the Panel offers the following comments and recommendations:

1. OEHHA's "best estimate" of the human potency or slope factor for butadiene of 0.37/ppm, based on lung tumors in female mice, is inconsistent with the results of butadiene epidemiology studies. This can be demonstrated by comparing the number of observed cancer deaths in various epidemiology studies with the number of deaths that would be predicted based on a potency slope of 0.37/ppm.

2. OEHHA's reasons for basing its best estimate of human cancer risks on the B6C3F1 mouse data are not valid. The available data on butadiene metabolism and mechanism of action indicate that the B6C3F1 mouse is uniquely susceptible to the carcinogenic effects of butadiene and not an appropriate model for human risk assessment. The rat provides a better model for human risk assessment.

3. The use of body surface area scaling for the quantitative risk estimates is not appropriate for butadiene. Body surface area scaling implies that the rat would be more sensitive on a mg/kg basis to butadiene than the mouse. However, the mouse and rat cancer bioassays show that this clearly is not the case for butadiene. Surface area scaling is inconsistent with the substantial body of data that has been developed on butadiene metabolism in mice, rats, monkeys and humans.

4. OEHHA should use the internal concentration of the reactive butadiene monoepoxide for the measure of dose. OEHHA's criticisms of the available data on species differences in metabolism of butadiene are overstated. These data can and should be used to

develop more realistic estimates of butadiene cancer risks for humans.

5. If CARB is unwilling to use butadiene metabolism data for its quantitative risk estimates, then CARB at least should use these data qualitatively. Specifically, CARB should acknowledge that: (i) Its "best estimate" of likely excess cancer deaths from exposure to butadiene in ambient air is an upper bound estimate based on numerous conservative assumptions that are derived from general risk assessment guidelines, rather than butadiene-specific data. (ii) The available data on butadiene metabolism and mechanism of action indicate that the B6C3F1 mouse is uniquely susceptible to the carcinogenic effects of butadiene. The quantitative risk assessment therefore may overstate human cancer risks by a substantial margin, and perhaps by several orders of magnitude. (iii) The number of excess human cancer deaths from exposure to butadiene in ambient air in California actually may be zero.

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INTRODUCTION

The Butadiene Panel of the Chemical Manufacturers Association appreciates this opportunity to comment on the Scientific Review Panel (SRP) version of the draft report to the California Air Resources Board (CARB) on the Proposed Identification of 1,3-Butadiene as a Toxic Air Contaminant. The Panel consists of the major domestic producers and some users of butadiene. A list of Panel member companies is attached as Appendix I.

The Butadiene Panel submitted comments to CARB in March 1991 on the Preliminary Draft Report on the proposal to identify butadiene as an air toxic. These earlier comments are included in Part C to the SRP version of the draft report. The Panel's current comments address the Health Assessment document prepared by the Office of Environmental Health Hazard Assessment ("OEHHA"), which is Part B of the SRP version of the draft report. This Health Assessment document contains a new quantitative risk estimate based on the second mouse bioassay sponsored by the National Toxicology Program (NTP-II).

CARB has provided only a thirty-day period for submission of comments on the SRP version of the draft report. This was not sufficient time for preparation of comprehensive comments on the new NTP-II risk assessment. (We recommend that CARB provide at least sixty days for comment whenever a new quantitative risk assessment is presented.) Accordingly, these comments address the Panel's primary reasons for believing that OEHHA's "best estimate" of butadiene cancer risk overstates by a substantial margin the likely human cancer risks from exposure to butadiene in ambient air.

At the outset, the Panel wishes to commend OEHHA for including in the Health Assessment a generally complete presentation of the toxicology literature on butadiene, including elements of relevant comparative metabolism. OEHHA also is to be commended for presenting a range of risk estimates based on mouse and rat tumor data sets. The recognition that butadiene epoxide metabolites are the putative mutagenic and carcinogenic moieties is an important component of the data set. The Butadiene Panel agrees with OEHHA's presentation of this concept in the document.

The Panel feels strongly that OEHHA should use more of these data for its "best estimate" of likely human cancer risks for butadiene. The "best estimate" of cancer risk selected by OEHHA is an upper bound estimate based on several "worst case" assumptions that are implicit in generic cancer risk assessment guidelines. The collective use of these worst case assumptions to arrive at a potency slope value of 0.37/ppm is not realistic and does not provide a reasonable basis for estimating likely excess human cancer deaths from exposure to butadiene in ambient air.

The alternative risk assessments which are included in the OEHHA health assessment document demonstrate the range of potential risk estimates which may be derived when different assumptions are applied. The available evidence on butadiene metabolism and mechanism of action provides strong support for the use of alternative data sets, such as the rat bioassay and comparative metabolism, to arrive at the best estimate of risk. The Panel believes use of the complete data set would produce more plausible and scientifically supportable estimates of human cancer risks.

Many of these issues were addressed at length in the Panel's earlier comments. The Panel also addressed many of these butadiene risk assessment issues in documents recently submitted to OSHA in connection with ongoing rulemaking proceedings regarding occupational exposures to butadiene. These documents include statements by Stuart Z. Cagen, Ph.D., of Shell Oil Company, and Thomas B. Starr, Ph.D., of Environ Corporation, in response to a NIOSH quantitative risk assessment based on the second NTP mouse study, and a statement by Michael G. Bird, Ph.D., of Exxon Biomedical Services, Inc., that summarizes the most recent butadiene metabolism data. Copies of these materials are included as appendices to these comments. These statements provide additional support for the points presented in these comments.

I. THE "BEST VALUE" POTENCY SLOPE OF 0.37/PPM IS NOT CONSISTENT WITH THE AVAILABLE EPIDEMIOLOGY DATA.

One way to assess the plausibility of OEHHA's "best estimate" of human cancer risks is to compare the observed cancer rates in epidemiology studies of butadiene-exposed workers with the number of cancer deaths that would be predicted from OEHHA's "best estimate." Such a comparison has been performed by Thomas B. Starr, Ph.D., of Environ Corporation. See Environ (1991a) (attached as Appendix II). Dr. Starr's analysis shows that the observed cancer rates in epidemiology studies on butadiene-exposed workers are not consistent with the cancer rate which is predicted using the 0.37/ppm potency slope.

Indeed, Dr. Starr's analysis shows that the probability of OEHHA's "best estimate" being correct is infinitesimally small -- 6.9×10^{-44} -- based on all cancers and assuming the production workers used in Dr. Starr's analysis were exposed to 10 ppm butadiene. While this exposure assumption seems reasonable (Environ 1991, Acquavella 1991), even if one assumes average workplace exposure levels were only 2 or 1 ppm, Dr. Starr's analysis still shows that OEHHA's "best estimate" is highly improbable (probabilities are 3.8×10^{-17} and 1.0×10^{-5} for 2 and 1 ppm, respectively). Similar results were obtained in a consistency check performed by Acquavella (1991) (addressing the NIOSH NTP-II risk assessment -- see Part 3 of Appendix III attached hereto).

OEHHA also has performed a consistency check of its quantitative risk assessment. However, this consistency check is based only on the maximum likelihood and upper bound potency estimates (0.0168 and 0.089 per ppm, respectively) derived from the incidence of malignant lymphomas in male mice in NTP-I. No justification is presented for excluding entirely OEHHA's "best estimate" of 0.37/ppm from the consistency check.

It also should be noted that, whereas OEHHA's "best estimate" is based on lung tumors in female mice, none of the epidemiology data suggest that the lung is a target organ in humans. Indeed, in every butadiene epidemiology study conducted thus far, a deficit in lung tumors has been observed. Acquavella (1990, 1991). This fact should be acknowledged in OEHHA's discussion of the epidemiology studies.

Additionally, the Panel believes OEHHA's analysis and interpretation of the butadiene epidemiology studies (pages 3-20 to 3-28) are flawed in several critical respects. These points are addressed at length in Appendix IV to these comments, which is devoted exclusively to epidemiology issues. Acquavella (1991a).

II. OEHHA'S REASONS FOR PREFERRING THE MOUSE DATA OVER THE RAT DATA FOR THE "BEST ESTIMATE" OF HUMAN CANCER RISKS ARE NOT VALID.

OEHHA cites several reasons for preferring the mouse data for its "best estimate" of excess cancer risk. These pertain largely to the fact that the mouse study was repeated at lower doses and obtained consistent results, and reportedly the mouse data are available in greater detail, allowing more in-depth analysis, compared to the rat data. See p. 4-24. These asserted justifications do not provide a valid basis for preferring the mouse data over the rat data for the best estimate of human cancer risks. Similar arguments have been advanced by NIOSH, and these arguments are addressed in Cagen (1991) (attached as Appendix V).[1]

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[1] OEHHA also cites "suggestions from limited epidemiological observations that butadiene exposure may be associated in humans with lymphatic and hematopoietic cancers" as a reason for choosing the mouse data for its best estimate of risk. See pp. 4-27. The epidemiology data do not provide any basis for preferring the mouse data over the rat data. In addition to the Panel's disagreements with OEHHA's evaluation of the epidemiology studies (see Acquavella (1991a) attached as Appendix IV), the increased incidence of lymphocytic and hematopoietic cancers in B6C3F1 mice has been observed only at relatively high doses and may well reflect the presence of an endogenous retrovirus present in the B6C3F1 mouse but not in humans. See Bird (1990). The

A more thoughtful and objective approach would be to acknowledge that all three cancer bioassays are valid and adequate for quantitative risk assessment, although the results seen in the two species tested differ dramatically. The objective for the risk assessor, then, is to choose the species which provides the best model for human risk assessment. The Butadiene Panel believes that the rat provides the better model because of greater similarities between the rat and human in the metabolism of butadiene, and because of the demonstrated unique susceptibility of the B6C3F1 mouse to butadiene-induced toxicity. See the Panel's March, 1991 comments, at pp. 18-21.

The available metabolism data, including human in vitro data, demonstrate clearly that the mouse produces more active epoxide metabolite(s) and detoxifies these metabolites less efficiently than rats, monkeys, and humans. Thus, there is a greater tendency for toxic butadiene metabolites to accumulate in mouse tissues. See Bird, 1990 (submitted with earlier Panel comments); Bird, 1991 (Appendix VI); Cagen, 1991 (Appendix V); Environ, 1991 (Appendix VII). Most notable is the mouse's relative inability to detoxify the metabolites via epoxide hydrolase with the resultant accumulation of putative mutagenic/carcinogenic metabolites. This is evident from the observed differences in urinary metabolites (Henderson et al. 1991), the interspecies differences in metabolism (Csanady and Bond 1991), and subsequent blood levels of circulating metabolites. Dahl et al. (1991). The collective evidence demonstrates clearly that the mouse is at an unusual disadvantage because of its propensity to accumulate higher levels of butadiene reactive metabolites, compared to rats, monkeys, and humans. Cagen (1991).

In addition, the evidence clearly demonstrates that the mouse is more susceptible than other species, including primates, to the bone marrow toxicity of butadiene. Butadiene effects bone marrow stem cell development and induces cytotoxicity in the mouse. Liederman et al. (1986); Irons et al. (1986). Such bone marrow toxicity has not been seen in the rat (Owen et al. 1987; Cunningham et al. 1986), the primate (NTP 1989), or man. Checkoway and Williams (1982). See Acquavella (1991a) in Appendix IV for additional discussion of the study by Checkoway and Williams.

If OEHHA continues to rely on the mouse data, it should acknowledge that this choice is not based on greater quality or reliability of the mouse data compared to the rat data. Rather, OEHHA's choice of data set is based on generic quantitative risk assessment guidelines that dictate a preference for the most

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relevance of these tumors for an assessment of potential human cancer risks from exposure to part per billion levels of butadiene in ambient air is extremely doubtful.

sensitive species. In the case of butadiene, however, the weight of the available evidence demonstrates that the most sensitive species is not the best model for human risk assessment.

III. SURFACE AREA SCALING IS NOT APPROPRIATE FOR BUTADIENE.

"Routine" scaling adjustments based on surface area are not justified when compound-specific comparative species information is available. The butadiene data set, considered as a whole, strongly indicates that interspecies scaling on a surface area basis is inappropriate.

Several papers that have studied appropriate interspecies scaling options have concluded that the use of compound specific data is preferred:

> O'Flaherty (1989) at page 597: "In the absence of specific information bearing on the metabolism and toxicity of the chemical, the 0.75 power of body weight dose conversion is a reasonable approach. . . ."

> Travis and White (1988) at page 124: "The National Academy of Sciences and Anderson point out that scaling should depend on the kinetic behavior of the particular compound and mechanism of toxicity . . . the 3/4 [0.75] power may be the most appropriate interspecies scaling factor for use in risk assessment of direct acting compounds. Further analysis will be needed to determine the appropriate scaling factors for compounds that are activated by metabolism."

An EPA-sponsored report prepared by Clement Associates made quantitative comparisons of carcinogenic potency in animals and humans for 23 chemicals for which suitable animal and human data were available. The study concluded that the "use of mg intake/kg body weight/day method for animal-to-human extrapolation generally causes risk related doses (RRDs) estimated from animal and human data to correspond more closely than other methods evaluated . . ." EPA (1987); see also Allen et al. (1991).

Considering what is now known about the toxicity and metabolism of butadiene in a variety of species, it is imperative to use the compound specific information to arrive at the most scientifically-based scaling factor when extrapolating to humans. For example, because it is universally accepted that epoxide metabolite(s) are the "direct acting" toxicants, scaling based on a 3/4 power of body weight might be used only after adjustments are made to account for species differences in the formation and deactivation of these toxic metabolites. Recent data of Csanady and Bond show that human tissues can detoxify the butadiene

monoepoxide (BMO) nearly 20 times faster than the tissues derived from the mouse. These data are supportive of prior work in illustrating the extraordinary capacity for the mouse to produce and retain the toxic monoepoxide metabolite(s).

Body surface scaling implies that larger species (man, rat) would be more sensitive on a mg/kg basis to butadiene than the mouse. This is not consistent with the existing data on butadiene. The clear evidence -- including both metabolism and direct tumor data -- is that the mouse is the most sensitive species tested.

To illustrate this point further, the data from the OEHHA risk assessment demonstrate that the potency slope for lung tumors in the female mouse predicts a risk of 0.37/ppm, but the "worst case" potency slope derived from the rat data predicts a risk of 0.0036/ppm. Based on these data, the mouse is 100 times more sensitive than the rat. Scaling the mouse data using body surface scaling to predict a rat risk would go in the exact opposite direction. Thus, the bioassay results confirm that the body surface scaling assumption is not valid.

IV. OEHHA SHOULD USE THE INTERNAL CONCENTRATION OF THE REACTIVE BUTADIENE MONOEPOXIDE FOR THE MEASURE OF DOSE (INSTEAD OF ABSORBED BUTADIENE).

The Butadiene Panel continues to believe strongly that OEHHA should use the internal concentration of the reactive butadiene monoepoxide for the measure of dose.

All butadiene risk assessment documents, including the current OEHHA draft risk assessment, that discuss the metabolism and toxicity of butadiene clearly and correctly implicate the reactive butadiene epoxide metabolite(s) as the putative mutagenic and carcinogenic species. These metabolic schemes, without exception, point to the butadiene monoepoxide as the first metabolic product. It follows, therefore, that any pharmacokinetics model utilizing BMO would be superior to one based on external air or absorbed levels of butadiene. The fact that a full physiologic model has not yet been developed that includes the butadiene diepoxide (DEB) does not justify the preference of butadiene to BMO.

OEHHA has expressed concern that use of BMO for the measure of dose would not allow for consideration of the diepoxide or other epoxide metabolites. See p. 4-6. However, the evidence suggests that the mouse is also unusual in its ability to produce DEB. This is demonstrated by the known greater accumulation of BMO in the mouse, which is requisite for production of DEB, as well as recent *in vitro* evidence from CIIT that actual amounts of DEB are far greater in the mouse. Data by Csanady and Bond (1991) indicate that butadiene metabolism is six times higher in mice than in humans or rats, and the subsequent removal of the mutagenic epoxide is 4-fold more rapid in humans

than in rodents. In man, BMO is predominantly and rapidly metabolized by epoxide hydrolase to non-DNA reactive 1,2-dihydroxy but-3-ene. In contrast, the major BMO metabolic pathway in the mouse is slower and results in the formation of the mutagenic diepoxide. Thus, by using BMO for the measure of dose and disregarding species differences in DEB levels, OEHHA would likely overstate human cancer risks. (The basis for OEHHA's concern about "other epoxide metabolites" is unclear; the BMO and DEB are the only butadiene metabolites that have been implicated in the extensive studies of butadiene mechanisms of action.)

OEHHA also has expressed concerns regarding the adequacy of the available metabolism data, particularly the data reported by Sun et al. (1989) and Dahl et al. (1990). OEHHA appears to make three main points: (1) available data do not show a correlation between tissue concentrations of epoxide metabolites and observed incidences of carcinogenic lesions; (2) the data do not sufficiently account for observed differences in tumor incidences between species; and (3) the data allegedly demonstrate similarities between mice and monkeys. See OEHHA responses to the Panel's earlier comments (OEHHA comments nos. 6, 8, 11 and 22 at pages C-4 through C-11). The first two comments reflect unreasonable expectations of the OEHHA staff; it is not realistic to expect the data on species differences in metabolism to account for all differences in tumor incidences and locations with mathematical precision. The lack of perfect data on butadiene metabolism should not be used as a justification for disregarding the enormous body of evidence on butadiene metabolism and mechanism of action, all of which weighs heavily in favor of using BMO as the measure of dose. OEHHA's third point, concerning alleged similarities between the mouse and monkey data in Dahl et al. (1990), also is unfounded. This "similarity" is based on inappropriate scaling techniques (see comments above).

Instead of looking for reasons not to use the metabolism data, OEHHA should recognize that, when adjustments are made for metabolized dose and/or blood levels of epoxides across species, the risk estimates based on the mouse and rat tumor data become reasonably consistent. This fact suggests that use of the metabolized dose reduces uncertainties in the quantitative risk estimates. A demonstration (used here as an example) of this is presented in Table 1 below:

TABLE 1

	RAW POTENCY (unadjusted) (2 ppm)	ADJUSTMENT* METABOLISM (epoxide)
NIOSH MOUSE (FEMALE)	5.97/100	0.1/1000
OSHA RAT (FEMALE)	0.29/100	0.07/1000

* Adjustment to account for difference in the production of the butadiene monoepoxide in blood: factors of 590 in mice and 40 in rats (from Dahl et al. (1990) using primate data).

(From Cagen (1991)).

V. CARB SHOULD MAKE GREATER QUALITATIVE USE OF THE AVAILABLE DATA ON BUTADIENE METABOLISM AND MECHANISM OF ACTION.

If CARB is unwilling to use the available data on butadiene metabolism and mechanism of action for its quantitative risk assessment, then CARB at least should make greater use of these data for a qualitative assessment of the likely human cancer risks.

As already demonstrated, CARB's "best estimate" of the human cancer risks associated with exposure to butadiene is inconsistent with the available epidemiology data. CARB's "best estimate" is an upper bound estimate based on numerous worst case assumptions that are derived from general quantitative risk assessment guidelines, rather than butadiene-specific data. The available data on butadiene metabolism and mechanism of action, including data in the mouse, rat, monkey, and human, provide strong evidence that the B6C3F1 mouse is uniquely sensitive to the carcinogenic effects of butadiene. CARB's quantitative risk estimate, based on lung tumors in the mouse, therefore may overstate human cancer risks by a substantial margin. This should be expressly acknowledged whenever CARB presents its "best estimate" of human cancer risks.

In this regard, of greatest concern is the following statement in CARB's Executive Summary: "An estimated 3,936 1,3-butadiene-induced cancers statewide (based on the best value x 30 million people) are expected to occur at average ambient concentrations." This statement implies a level of precision which cannot reasonably be attributed to the risk estimate. If such a statement is made, it should be accompanied by an explicit recognition that: (1) This estimate is intended to be a conservative upper bound estimate of possible excess human cancer deaths. (2) CARB's "best estimate" is not necessarily the most plausible estimate. (3) CARB's "best estimate" is not consistent

with the results of available epidemiology studies. (4) Actual excess human cancer deaths from ambient levels of butadiene may be zero.

CONCLUSION

For the reasons presented in these comments and the supporting attachments, the Panel urges OEHHA to base its "best estimate" of butadiene human cancer risks on the rat bioassay, without surface area scaling, and using the internal concentration of the monoepoxide for the measure of dose. The Panel believes this approach would produce a more plausible "best estimate" of butadiene cancer risks. At the very least, OEHHA should expressly recognize the limitations of its own risk assessment methodology. Specifically, OEHHA should acknowledge that its current "best estimate" of butadiene cancer risks is an upper bound estimate that probably overstates human cancer risks by a wide margin. OEHHA should expressly state in the Executive Summary that the number of excess human cancer deaths from exposure to butadiene in ambient air may be zero.

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APPENDICES TO THE
COMMENTS OF THE
CHEMICAL MANUFACTURERS ASSOCIATION
BUTADIENE PANEL
ON THE SRP VERSION OF THE DRAFT REPORT ON
PROPOSED IDENTIFICATION OF 1,3-BUTADIENE
AS A TOXIC AIR CONTAMINANT

I. Member Companies of the CMA Butadiene Panel

II. Comparison of Predicted and Observed Butadiene Human Cancer Risks -- Preliminary Comments on OEHHA Risk Assessment, prepared by Thomas B. Starr, Ph.D., of Environ Corporation

III. OSHA Post-Hearing Testimony of John F. Acquavella, Ph.D.

IV. Epidemiology Comments on CARB's Health Assessment, prepared by John F. Acquavella, Ph.D.

V. Statement of Stuart Z. Cagen, Ph.D., in Response to NIOSH Risk Assessment

VI. OSHA Post-Hearing Testimony of Michael G. Bird, Ph.D.

VII. Comments of Environ Corporation on NIOSH Risk Assessment (prepared by Thomas B. Starr, Ph.D.)

APPENDIX I

APPENDIX I

BUTADIENE PANEL MEMBER COMPANIES

Amoco Chemical Company

American Petroleum Institute

Chevron Chemical Company

Dow Chemical USA

E.I. duPont de Nemours and Company

Eastman Kodak Company

Exxon Chemical Company

Lyondell Petrochemical Company

Mobil Chemical Company

Oxy Petrochemical, Inc.

Quantum Chemical Corporation

Shell Oil Company

Texaco Chemical Company

Union Carbide Corporation

APPENDIX II

APPENDIX II

COMPARISON OF PREDICTED AND OBSERVED BUTADIENE HUMAN CANCER RISKS -- PRELIMINARY COMMENTS ON OEHHA RISK ASSESSMENT

Prepared by Thomas B. Starr, Ph.D.
Environ Corporation
December 6, 1991

OEHHA has called attention to the fact that the U.S. Environmental Protection Agency Carcinogen Assessment Group (CAG) implemented a consistency check for its "point" estimate of lifetime butadiene cancer risk using the Meinhardt et al. (1982) and Matanoski et al. (1982) studies of worker mortality in the styrene-butadiene rubber (SBR) industry. U.S. EPA (1985). In that consistency check, CAG combined its "point" estimate of butadiene potency with estimated exposure levels and the sample sizes of different worker groups to generate predicted numbers of extra deaths attributable to butadiene exposure assuming that the estimate of butadiene potency was accurate.

Fewer deaths were observed than were predicated in all but one of the six groups that CAG analyzed. U.S. EPA (1985). For example, in the study by Matanoski et al. (1982), for the worker group whose jobs last held were in the production category, there were significantly fewer observed deaths from lymphopoietic cancer than CAG's "point" estimate of butadiene potency predicted (11 deaths observed versus 20.6 predicted). CAG nevertheless concluded that its "point" estimate was not inconsistent with the observations, provided one assumed that the observed deficits in cancer deaths were due to an under ascertainment of deaths by the Matanoski et al. (1982). However, there was no basis for this assumption.

Nevertheless, the approach CAG used to evaluate the consistency of its risk estimates with epidemiologic observations is useful in objectively assessing the plausibility of different cancer potency estimates. Results from such quantitative comparisons of butadiene potency estimates have been reported previously by ENVIRON (1986, 1990 and 1991) and Acquavella (1990 and 1991). In each case, cancer potency estimates based on the mouse data have been shown to be inconsistent with the observations in the epidemiology studies.

While OEHHA also undertook such a comparison, it did so only for the maximum likelihood and upper bound potency estimates (0.0168 and 0.089 per ppm, respectively) based on malignant lymphoma incidence among male mice in the first NTP bioassay. NTP (1984). OEHHA did not perform a consistency check for its "best estimate" of cancer potency, 0.37/ppm, based on lung tumors in female mice in NTP-II. OEHHA also did not perform a consistency check for its "best" rat-based risk estimate (0.0098 per ppm), which was derived from the incidence of multiple significant tumors among female rats in the Hazelton rat study.

HLE (1981); Owen et al. (1987). It would have been far more appropriate to perform a consistency check against epidemiologic observations using these "best estimates" of butadiene cancer potency derived from the rat and mouse studies.

Tables 1 and 2 present the calculated probabilities of observing as few or fewer deaths from any cancer, respiratory tract cancer, or lymphopoietic cancer as were actually observed among the 3,124 SBR production workers as reported by Matanoski et al. (1990), assuming that the true cancer risks arising from this group's butadiene exposures were equal to the risks predicted with OEHHA potency estimates of 0.0098 (rat "best estimate") and 0.37 (mouse "best estimate") for 10, 5, 2 or 1 ppm butadiene, but with the exposure only for 10 or 50 working years and with follow up only for 21 of the 50 remaining years of life. As has been discussed elsewhere (ENVIRON, 1986, 1990, and 1991; Acquavella, 1990 and 1991), butadiene exposure levels in the SBR industry probably averaged 10 ppm or higher for these workers, so the predicted numbers of deaths appearing in Tables 1 and 2 most likely understate the extent of any inconsistencies between the observed number of cancer deaths and OEHHA's predictions.

Nevertheless, it is clear from Table 1 that the predicted risks obtained with OEHHA's "best" mouse-based potency estimate (0.37 per ppm) are altogether inconsistent statistically with the observed numbers of deaths in this SBR worker group for all cancers, respiratory tract cancers, or lymphopoietic cancer, even with exposure levels as low as 1 ppm. Many more cancer deaths should have occurred were OEHHA's "best" estimate actually correct. In contrast, as Table 2 indicates, OEHHA's "best" rat-based potency estimate yields predicted numbers of cancer deaths that are not demonstrably inconsistent with those observed.

It is important to note that significant inconsistencies between observed and OEHHA-predicted cancer deaths are readily apparent despite Matanoski et al.'s acknowledged understatement of the expected number of deaths in the absence of butadiene exposure (Matanoski et al. 1990), and despite not having considered the maintenance workers, who were also relatively heavily exposed, in this comparison. Had the subgroups of production and maintenance workers been combined appropriately, the inconsistencies between observed and OEHHA-predicted cancer deaths in this larger group would have been even more extreme than those presented in Table 1.

The epidemiologic observations thus indicate that OEHHA has significantly overstated the human cancer risks arising from butadiene exposure. In fact, OEHHA's "best" mouse-based estimate is altogether inconsistent with the actual observations in the production workers studied by Matanoksi et al. (1990), even assuming that butadiene exposures averaged about 1 ppm. While the epidemiologic data are not sufficiently powerful to categorically reject predicted risks as small or smaller than the estimate of 0.0098 per ppm which OEHHA derived from the HLE rat

bioassay, they are also entirely consistent with the far smaller risks that are predicted from a full and proper utilization of the butadiene absorption, retention, and metabolism differences that are now known to exist between rodents and primates. See Environ (1990, 1991).

TABLE 1

Consistency Check of CARB BD Cancer Risk Estimates

Predicted Versus Observed Cancer Deaths
Among 3,124 Production Workers (Matanoski et al., 1990)
Assuming a BD Cancer Potency of 0.37 per PPM
Lifetime Continuous Equivalent Exposure

	All Cancers	Respiratory Tract Cancers	Lymphopoietic Cancers
Observed Deaths	124	49	19
Predicted Deaths with 10 ppm	348.7	259.8	225.8
p-value	6.9×10^{-44}	6.2×10^{-58}	4.1×10^{-71}
Predicted Deaths with 5 ppm	242.2	83.9	119.3
p-value	3.8×10^{-17}	7.3×10^{-23}	4.3×10^{-30}
Predicted Deaths with 2 ppm	178.4	89.6	55.5
p-value	1.0×10^{-5}	2.0×10^{-6}	1.3×10^{-8}
Predicted Deaths with 1 ppm	157.1	68.3	34.3
p-value	0.0036	0.0088	0.0033

TABLE 2

Consistency Check of CARB BD Cancer Risk Estimates

Predicted Versus Observed Cancer Deaths
Among 3,124 Production Workers (Matanoski et al., 1990)
Assuming a BD Cancer Potency of 0.0098 per PPM
Lifetime Continuous Equivalent Exposure

	All Cancers	Respiratory Tract Cancers	Lymphopoietic Cancers
Observed Deaths	124	49	19
Predicted Deaths with 10 ppm	141.5	52.6	18.6
p-value	0.074	0.341	0.597
Predicted Deaths with 5 ppm	138.7	49.8	15.8
p-value	0.113	0.492	0.826
Predicted Deaths with 2 ppm	137.0	48.1	14.1
p-value	0.142	0.589	0.919
Predicted Deaths with 1 ppm	136.4	47.6	13.6
p-value	0.154	0.617	0.939

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APPENDIX III

UNITED STATES OCCUPATIONAL SAFETY AND HEALTH ADMINISTRATION

DOCKET NO. H-041

SUPPLEMENTAL WRITTEN TESTIMONY

of

JOHN F. ACQUAVELLA, PhD

on behalf of

THE INTERNATIONAL INSTITUTE OF
SYNTHETIC RUBBER PRODUCERS, INC.

on

OSHA'S PROPOSED STANDARD
FOR 1,3-BUTADIENE

NOVEMBER 26, 1991

SUPPLEMENTAL WRITTEN TESTIMONY
OF JOHN F. ACQUAVELLA, PhD
ON THE OCCUPATIONAL SAFETY AND HEALTH ADMINISTRATION'S
PROPOSED STANDARD FOR 1,3-BUTADIENE

I am pleased to provide, on behalf of the International Institute of Synthetic Rubber Producers ("IISRP"), additional evidence and commentary on the health aspects of the Occupational Safety and Health Administration's ("OSHA's") proposed standard for 1,3-butadiene. My supplemental testimony addresses three distinct sets of issues.

Part 1 addresses the issue of short-term versus long-term workers, and responds to new arguments on this issue in recent comments that have appeared in the OSHA butadiene docket. This part of my supplemental testimony also provides additional information that I was asked to supply at the hearings.

Part 2 responds to the discussion of epidemiological issues in the posthearing comments on my testimony submitted by the National Institute for Occupational Safety and Health ("NIOSH").[1] As this part of my supplemental testimony demonstrates, several of the statements made by NIOSH regarding my reanalysis of the lymphopoietic cancer case-control study are misleading or in error.

Part 3 provides an epidemiological perspective on the NIOSH risk assessment. This part of my supplemental testimony

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[1] See Posthearing Comments of the National Institute for Occupational Safety and Health on the Occupational Safety and Health Administration's Proposed Rule on Occupational Exposure to 1,3-Butadiene (Sept. 27, 1991) (Ex. No. 90).

shows that projections from the NIOSH model are totally irreconcilable with the epidemiologic evidence for butadiene-exposed workers and therefore do not provide a valid estimate of the human health risks from butadiene exposure.

These three parts of my supplemental testimony along with the attached Appendices provide new information to assist OSHA's evaluation of the epidemiological data on butadiene. This information provides additional support for my overall conclusion, which I expressed at the hearing and in my written testimony, that the butadiene epidemiologic studies show favorable results that do not support a conclusion that butadiene causes cancer in humans.

UNITED STATES OCCUPATIONAL SAFETY AND HEALTH ADMINISTRATION

DOCKET NO. H-041

SUPPLEMENTAL WRITTEN TESTIMONY OF JOHN F. ACQUAVELLA, PhD ON THE OCCUPATIONAL SAFETY AND HEALTH ADMINISTRATION'S PROPOSED STANDARD FOR 1,3-BUTADIENE

Part 1: Short-Term vs. Long-Term Workers and Additional Submissions for the Record

NOVEMBER 26, 1991

SUPPLEMENTAL WRITTEN TESTIMONY
OF JOHN F. ACQUAVELLA, PhD
ON THE OCCUPATIONAL SAFETY AND HEALTH ADMINISTRATION'S
PROPOSED STANDARD FOR 1,3-BUTADIENE

Part 1:
Short-Term vs. Long-Term Workers and Additional Submissions for the Record

During my OSHA hearing appearance, I was asked to supply further information by several members of the OSHA panel. In addition, new comments on the issue of the relative risks and exposures to short-term versus long-term workers have appeared in the butadiene docket. This supplemental testimony provides the requested information and addresses new issues in the recent comments.

SHORT-TERM vs. LONG-TERM WORKERS

In my OSHA testimony, I reviewed the epidemiologic evidence indicating that 1,3-butadiene workers did not show elevated cancer rates. For all three cohorts studied, cancer mortality was significantly lower than U.S. rates. Lymphopoietic cancer mortality, the focus of concern for OSHA, was not elevated overall or in long-term workers. Only some inconsistent findings in small short-term subgroups were found. The lack of elevated mortality rates for long-term workers does not support the hypothesis that butadiene is a carcinogen even at historical exposure levels in U.S. industry.

Dr. Landrigan submitted a March 6, 1991, letter to the docket (Ex. No. 82) arguing that "[s]ome short-term workers may

actually accrue greater total exposure than long term workers." This hypothesis is offered as an explanation for the lack of excess mortality among long-term workers and for the non-homogeneity of findings for short-term workers.

As indicated in my oral testimony, there is no evidence suggesting that short-term workers in the butadiene industry had higher exposures than their fellow workers during the same years who then continued in the industry. Indeed, there is the impression among industry supervisory personnel that short-term workers would have worked frequently in unskilled positions with fewer exposure opportunities, such as for example, baling rubber or conducting other non-process related manual work.

Dr. Landrigan's analogy is based on studies that involve worker exposure to metals in the form of respirable particles. This analogy is flawed for several reasons.

First, unlike volatile chemicals, certain metals can reside for long periods in the lung; hence, a short-term high-exposure results in continued exposure (at the target organ) as long as the metal is retained. The same cannot be said for butadiene, which is quickly metabolized and eliminated. Dr. Landrigan has not presented any examples that relate to chemical exposures (especially for volatile chemicals like butadiene), and I am aware of none.

Second, the issue of confounding exposures and differences in smoking and other lifestyle factors must be considered when comparing mortality rates for short-term versus long-term workers.

Third, in the studies cited by Dr. Landrigan, long-term workers show excess lung cancer. Indeed, this is true of virtually every established occupational carcinogen. In contrast, for butadiene, as pointed out at the OSHA hearings, workers who had long employment (even in highly exposed production and maintenance jobs) show no excess mortality from lymphopoietic or any other cancers.

Fourth, the cited studies, when read carefully, actually are inconsistent with Dr. Landrigan's argument. I describe each below:

Ott et al. (1974) found an increasing association for lung cancer with duration of employment and cumulative exposure to chemicals. For example, Ott (p. 253) reports that 12% (16/138) of decedents were lung cancers among those employed less than one year, while 27% (12/45) were lung cancers among those employed more than one year. While these proportions are not directly comparable (due to presumed age differences), these data, when properly age-adjusted, would almost certainly be contrary to the hypothesis of a greater effect in shorter term workers.

Infante et al. (1980) concentrated on a subgroup of beryllium workers whose disease state qualified them for the beryllium registry. No duration of employment analyses were presented, but I presume that the citation of this study relates to the lung cancer excess among these characterized with acute disease (primarily those with acute bronchitis or pneumonitis or,

in the absence of a clear diagnosis, those whose illness occurred within one year of initial exposure), but not those characterized with chronic disease. Two points detract from this argument. First, the acute group is not a short-term worker subgroup - only those few workers with unclear diagnostic information are characterized as acute based on short-term employment. Second, as Infante pointed out, the extremely high mortality from non-malignant respiratory disease for the chronic group (presumably due to high case fatality as a consequence of extremely high exposures) was so extensive (21% of deaths) as to preclude the subsequent development of lung cancer (see p. 40). A similar phenomenon is known to have occurred among early asbestos workers. Furthermore, other studies have shown excess lung cancer in long-term beryllium workers.

Wagoner et al. (1980) looked at a cohort of 3055 beryllium workers at a plant in Pennsylvania. SMRs were very similar for those employed less than or more than five years, especially for those with long latency (SMRs of 187 and 174, respectively, for workers with 25+ years latency). Wagoner pointed out that the destruction of work records in 1968 precluded updating employment histories from 1968-1975 (the study end date), so workers tended to be classified incorrectly into shorter employment durations (see p. 29). Hence, in this study there is little difference in findings between short-term and long-term workers; and there is an acknowledged bias toward underestimating duration of employment.

Thun et al. (1985) studied 602 white males at a cadmium recovery plant. In the abstract to this paper, the authors state clearly that there were no lung cancers among those employed less than two years. Later, in an analysis excluding workers with arsenic exposure (Table 4 and related text), the authors relate that workers employed less than two years had 0 observed versus 3.87 expected, while SMRs were approximately 200 for those employed 2-9 years, 10-19 years, and 20+ years. Again, these findings are markedly different than those seen for butadiene workers -- where there is no mortality excess for long-term workers.

Interestingly, the authors also provide evidence that exposure was indeed higher for longer term workers (see p. 332) based on cadmium biomonitoring. For workers employed less than two years, approximately 30% had urinary cadmium levels of 20 μg/l; while that level was exceeded for 81% of the total population (thus, the exclusion of those employed less than two years from the total population result would demonstrate that more than 81% of longer term workers had urine levels of at least 20 μg/l). Hence, it is clear that longer term workers had more cadmium exposure than short-term workers. The same is almost certainly true for butadiene in monomer and polymer production.

In sum, there is no precedent for occupational carcinogenic effects being seen only in short-term workers or for short-term workers having higher exposures than long-term

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workers. Such a position is unprecedented and has no scientific basis.

ADDITIONAL SUBMISSIONS FOR THE RECORD

During my testimony I was asked to submit several items for the record. Accordingly, attached as Appendices to my supplemental testimony are:

(1) My July 24, 1989, letter to Dr. Matanoski requesting information on control selection in the case-control study she authored with Dr. Santos-Burgoa (Appendix A).

(2) Dr. Santos-Burgoa's dissertation, which provides some detail on the case-control study not included in the Final Report already in the OSHA docket (Appendix B). Regarding the protocol for the study, I checked my files and with my colleagues on the Epidemiology Subcommittee and found no indication that a copy was ever provided to IISRP.

(3) Four articles that have been referenced in the OSHA proceeding:

(a) H. Checkoway et al. (1984). "An Evaluation of the Associations of Leukemia and Rubber Industry Solvent Exposures." Am. J. Indus. Med. 5:239-249 (Appendix C);

(b) H. Checkoway & T. Williams. (1982). "A Hematology Survey of Workers at Styrene-Butadiene Synthetic Rubber Manufacturing Plant." Am. Indus. Hyg. Assn. J. 43:164-169 (Appendix D);

(c) A. Smith & L. Ellis. (1977). "Styrene Butadiene Rubber Synthetic Plants and Leukemia." J. Occup. Med. 19:441 (letter to editor) (Appendix E); and

(d) R. Rodger et al. (1987). "Factors Influencing Haematological Measurements in Healthy Adults." J. Chron. Dis. 40:943-47 (Appendix F).

(4) Information indicating, as I testified, that many of the workers at the butadiene monomer facility built during World War II were borrowed from existing petrochemical facilities in the area, including:

(a) A pamphlet written in the late 1970s by the Port Neches Butane Products Co., about the butadiene monomer plant now run by Texaco and studied by Drs. Downs and Divine (Appendix G); and

(b) Examples of a few employee records from this plant indicating prior employment in the parent company petrochemical facilities (Appendix H).

I have also ascertained the answers to two questions posed to me at the hearing about the SBR industry:

(1) The dates of operations for styrene butadiene rubber (SBR) plants in North America are shown in Table 1 below:

Table 1: North American SBR Plants

	ORIGINAL COMPANY NAME	SITE	START-UP YEAR
✓	National Synthetic Rubber Corp.[1]	Louisville, KY	1943
✓	Copolymer Corp.	Baton Rouge, LA	1943
✓	Firestone	Lake Charles, LA	1943
	Firestone[2]	Port Neches, TX	1943
✓	General Tire[3]	Baytown, TX	1943
✓	General Tire	Odessa, TX	1957
	BF Goodrich[4]	Louisville, KY	1942
✓	BF Goodrich[5]	Borger, TX	1943
	BF Goodrich[6]	Port Neches, TX	1943
✓	Polymer Corp.[7]	Sarnia, Canada	1943
✓	Goodyear	Houston, TX	1943
	Goodyear[8]	Torrance, CA	1943
	Goodyear	Beaumont, TX	1961
	U.S. Rubber Co.[9]	Naugatuck, CT	1942
	U.S. Rubber Co.[10]	Institute, WV	1943
	U.S. Rubber Co.[11]	Los Angeles, CA	1943

✓ Plant included in Matanoski study.

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1 Now American Synthetic Rubber Company.

2 Now part of Ameripol Synpol (North plant).

3 Sold to Ashland Oil, subsequently closed in 1977.

4 Discontinued SBR production in 1947.

5 Sold to Phillips Petroleum, subsequently closed in 1984.

6 Now part of Ameripol Synpol (South plant).

7 Now Polysar Rubber Corporation.

8 Sold to 3M and then Shell, subsequently closed in 1969.

9 Now Uniroyal Chemical Company, discontinued SBR production in 1974.

10 Sold to Union Carbide in 1955, SBR production discontinued shortly thereafter.

11 Sold to Goodyear, 3M and then Shell, subsequently closed in 1969.

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(2) As the above list indicates, almost all the operating SBR plants in 1976 were included in the Matanoski cohort study. The only exceptions were the BF Goodrich plant and Firestone plants in Port Neches, which were not included in the Matanoski study. However, these two plants now form the Ameripol Synpol facility and comprised the study population in the Meinhardt study.

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M. Thun et al. (1985). "Mortality Among a Cohort of U.S. Cadmium Production Workers -- An Update," J. Nat'l Cancer Inst. 74:325-333.

J.K. Wagoner et al. (1980). "Beryllium: An Etiologic Agent in the Induction of Lung Cancer, Nonneoplastic Respiratory Disease, and Heart Disease Among Industrially Exposed Workers." Envtl. Res. 21:15-34.

UNITED STATES OCCUPATIONAL SAFETY AND HEALTH ADMINISTRATION

DOCKET NO. H-041

SUPPLEMENTAL WRITTEN TESTIMONY OF JOHN F. ACQUAVELLA, PhD ON THE OCCUPATIONAL SAFETY AND HEALTH ADMINISTRATION'S PROPOSED STANDARD FOR 1,3-BUTADIENE

Part 2: Response to NIOSH Posthearing Comments on Acquavella Testimony Regarding the Case-Control Study

NOVEMBER 26, 1991

SUPPLEMENTAL WRITTEN TESTIMONY
OF JOHN F. ACQUAVELLA, PhD
ON THE OCCUPATIONAL SAFETY AND HEALTH ADMINISTRATION'S
PROPOSED STANDARD FOR 1,3-BUTADIENE

Part 2:
Response to NIOSH Posthearing Comments on Acquavella Testimony Regarding the Case-Control Study

NIOSH (NIOSH 1991) has offered comments on my reanalysis of the lymphopoietic cancer case control study by Matanoski et al. (Acquavella 1990). Several of these comments are misleading or in error.

In my written OSHA testimony, I commented on the unusual analysis -- based on log butadiene rather than actual butadiene exposures -- presented by the authors of the lymphopoietic cancer case control study (Matanoski et al. 1989). Specifically, I made two points about basing the analysis on the mean log butadiene score:

(1) Textbooks on case control methodology prescribe no distributional requirement (i.e., normality) for the underlying exposure data used to calculate an odds ratio ("OR") (Rothman 1986; Schlesselman 1982; Kleinbaum et al. 1982); and

(2) The unnecessary logarithmic transformation did <u>not</u> produce a normal distribution of the butadiene exposure scores and only served to change the cutpoint for calculating the OR. Therefore, it would have been prudent,

at a minimum, also to report the OR for the actual, non-transformed, butadiene exposure data. The respective ORs were 7.6 (mean log exposure) and 0.9 (mean actual exposure). Clearly, the choice of cutpoint determines the interpretation of the findings from these dichotomous analyses.

NIOSH has taken issue with these criticisms. First, they mention (p.2, lines 6-9) that I did not give the normality statistics to support my statement that the log butadiene data were not normally distributed. The normality statistics for the 110 exposure values (SAS version 6.03, 1988) are:

Actual butadiene data: Shapiro-Wilk statistic (W) = 0.773, p value < 0.0001, skewness 1.62, kurtosis 2.13.

Log butadiene data: Shapiro-Wilk statistic (W) = 0.805, p value < 0.0001, skewness 0.975, kurtosis 0.344.

Clearly, neither distribution is normal or near normal.

Second, NIOSH suggests (p.2, lines 9-12) that a normal distribution of the actual exposure data was implied in my analysis based on the mean of the actual butadiene exposure data. This is simply not true. As stated previously, there is no requirement for normality of exposure data in case control studies. The sole difference in analyses based on the actual or log transformed butadiene data is an arbitrary choice of an exposure value for dichotomizing workers by exposure. NIOSH made this very point subsequently (p.2, lines 13-15):

"The choice of cutpoints for categorizing exposures in epidemiologic analyses relies, to some extent, on judgments for which there are no generally agreed-upon principles."

My reason for presenting the analysis based on the actual butadiene data was that it illustrated the variability in the estimation of the OR in the case control study (Matanoski et al. 1989) -- a point which should have been a major focus of discussion for the authors of this study and/or scientists charged with evaluating this study. Such variability hindered the estimation of the ratio of leukemia rates associated with butadiene exposure. Yet, this issue was not discussed at all by the authors of the study or by OSHA.

Third, the NIOSH authors imply that the variability seen in the ORs for the log transformed versus the actual data analyses (7.6 versus 0.9) is typical in case control studies (p.2, lines 13-16):

"It is well recognized that the choice of cutpoints may <u>dramatically</u> alter the findings, and that appears to be true in this case." (emphasis added)

I believe that this statement is clearly misleading. While there are situations where a slight change in cutpoint results in a modest change in the OR, I am not aware of any previous situation where changing the dichotomization point slightly, as illustrated in my analysis, changes the OR from a value indicative of a

strong association (7.6) to one indicative of no association (0.9). In my testimony, I quoted from Rothman's textbook on this issue (Rothman 1986, p.135):

> "... the shift of a boundary in categorization rarely has a substantial effect on the magnitude of an estimate and then only because of a large random error component."

In the absence of examples that demonstrate OR variability analogous to that seen in the Matanoski et al. case control study, NIOSH's opinion on this point is not credible.

NIOSH mentions the presence of a significant linear trend with butadiene exposure in a categorical analysis as strong evidence of a relationship between butadiene exposure and leukemia (p.2, lines 26-28 and lines 40-42). It is noteworthy that such a trend is not seen in analyses based on evenly balanced tertiles, quartiles, or quintiles (viz. exposure categories based on equal numbers of controls or cases and controls). For example, Dr. Philip Cole in his testimony gave ORs for three evenly balanced exposure levels (1.0, 5.3, 2.3) that did not indicate a linear relationship with dose (Cole 1990). One noteworthy property of the categorical analysis cited by NIOSH is that the 26 cases and 84 controls were apportioned unevenly into seven exposure levels, and the individual point estimates in many of the categories were based on so few cases and/or controls as to be unreliable. Again, it is the choice of exposure cutpoints that dictates the presence or absence of a significant relationship in this case control study.

NIOSH also advocates the use of a continuous butadiene exposure score in logistic regression as a more powerful test of linear trend and points out that such an analysis produced a significant coefficient for butadiene exposure (p.2, lines 28-35). However, such an analysis is considered to be inappropriate in most epidemiologic situations since it carries the inherent assumption that each exposure increment multiplies the OR by a constant factor (see Greenland 1979; Rothman 1986). Such an assumption is rarely ever appropriate and, in this case, is clearly inconsistent with the leukemia mortality seen in the base population for the case control study (22 observed, 22.8 expected) and with Dr Cole's exposure level analysis.

Finally, NIOSH mentions that overmatching in the case control study may have resulted in an underestimate of the OR for butadiene exposure. However, Dr. Cole demonstrated in his OSHA testimony (Cole 1991) that the case control odds ratio of 7.6 (along with the 60% control exposure prevalence) is incompatible with the lack of any leukemia excess in the cohort study for this worker population (i.e., 22 observed, 22.8 expected). Specifically, he presented the following data:

Leukemia deaths predicted in the SBR workers cohort study from the case control results; odds ratio = 8

% cohort exposed	predicted deaths in cohort study
25%	63
50%	103
60%	119

(adapted from Cole 1991)

The incompatibility would be even greater for the higher OR, which presumably would have resulted in the absence of overmatching.

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J. Acquavella. (1990). "Direct Testimony re Butadiene Epidemiology, OSHA Hearings on 1,3-Butadiene." (Nov. 5, 1990) (Ex. No. 34-4, Vol. I, App. A).

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UNITED STATES OCCUPATIONAL SAFETY AND HEALTH ADMINISTRATION

DOCKET NO. H-041

SUPPLEMENTAL WRITTEN TESTIMONY OF JOHN F. ACQUAVELLA, PhD ON THE OCCUPATIONAL SAFETY AND HEALTH ADMINISTRATION'S PROPOSED STANDARD FOR 1,3-BUTADIENE

Part 3: An Epidemiologic Perspective on the NIOSH Risk Assessment for Butadiene

NOVEMBER 26, 1991

SUPPLEMENTAL WRITTEN TESTIMONY
OF JOHN F. ACQUAVELLA, PhD
ON THE OCCUPATIONAL SAFETY AND HEALTH ADMINISTRATION'S
PROPOSED STANDARD FOR 1,3-BUTADIENE

Part 3:
An Epidemiologic Perspective on the NIOSH Risk Assessment for Butadiene

NIOSH has recently completed a risk assessment based on tumor incidence data from a low dose butadiene $B_6C_3F_1$ mouse bioassay (Melnick et al. 1990). Based on the most sensitive target site in the $B_6C_3F_1$ mouse - lung tumor incidence - the resulting estimate of risk at 2 ppm for 45 years exposure was 597 excess cancers per 10,000 workers per lifetime - or 5.97 per 100 (Dankovic et al. 1991). This prediction does not even vaguely resemble actual human experience in butadiene-related industries where, on an industry-wide basis, all cancer and lung cancer mortality rates are significantly lower than general population rates and lymphopoietic cancer rates are similar to general population rates (Acquavella 1990; Cole 1990).

The discrepancy between the NIOSH model and worker experience is best illustrated by the mortality findings for the Texaco subcohort of 1,066 butadiene monomer workers first employed before 1946 (Divine 1990). These workers were employed at, or soon after, industry startup and had experience working during World War II when overtime was extensive and exposure conditions resulted in higher exposures than were likely to result from recent plant operations. These workers have been followed for mortality outcomes from first employment during the

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period 1943-45 through the end of 1985 (an average 41.5 years for living workers) -- almost a lifetime followup period for risk assessment purposes. Fifty-six percent of these workers are deceased (Divine 1990). Average duration of employment for these workers was 12.4 years (B. Divine, personal communication).

I will evaluate the NIOSH risk projections for these Texaco workers in terms of all cancers, lung cancer, and lymphopoietic cancers. These cancer sites were selected for the following reasons:

(1) All cancer - Butadiene was associated with malignancies of various organs in the $B_6C_3F_1$ mouse studies (Huff et al. 1985; Melnick et al. 1990) and could be presumed by some to have an analogous effect on workers.

(2) Lung cancer - The lung was the most sensitive cancer site in the recent $B_6C_3F_1$ mouse study (Melnick et al. 1990), and one could argue that inhalation of butadiene and metabolism in the lung is qualitatively similar across species.

(3) Lymphopoietic cancer - This site was seen in excess at 200 ppm and higher doses in the $B_6C_3F_1$ mouse studies (Huff et al. 1985; Melnick et al. 1990), and there has been debate about butadiene and lymphopoietic cancers in the epidemiologic literature.

The findings for these cancer sites in the Texaco study are listed in Table 1.

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Table 1
Mortality Findings for Texaco World War II Subcohort

	observed	expected	SMR	95% CI
All cancer	106	140.9	75	62-91
Lung cancer	36	44.2	82	57-113
Lymphopoietic	16	12.7	126	72-205

It is clear upon inspection that these data conflict with the NIOSH risk assessment. There is a significant 25% deficit of all cancer mortality, and lung cancer and lymphopoietic cancers are only slightly lower or higher than general population rates, respectively. The following analyses quantify the extent of the conflict.

The NIOSH excess risk projection of 5.97 per 100 is based on 2 ppm exposure for 45 years or 90 ppm years. Past exposures are not documented for the Texaco workers, but we can assume that exposures exceeded the proposed OSHA standard of 2 ppm and the current Threshold Limit Value (TLV) of 10 ppm since 1000 ppm was the workplace exposure limit during the early decades of this industry. However, actual exposures were maintained well below 1000 ppm to minimize the danger of fires or explosions. Therefore, it seems reasonable to evaluate risk projections from the NIOSH model for a range of average exposures from a maximum of 20 ppm (i.e., 10 times the proposed OSHA

standard or 2 times the current TLV) to a minimum of 2 ppm with an intermediate value of 10 ppm.

The calculation of predicted cancers for these workers takes the general form:

predicted excess cancers + expected cancers

The actual calculation is as follows:

[(average ppm X years employed)/90 ppm years] X 1066 workers X (5.97 excess cancers/100 workers)] + expected cancers = predicted cancers

In addition, a correction factor should be incorporated into the calculation to compensate for the slightly less than lifetime followup (e.g., 41.5 years/45 years) for the 44% of the Texaco work force who were still alive at the end of the study follow-up period (correction factor = (1 -((41.5/45) X (0.44)) = 0.97).

Thus, for all cancers assuming a 20 ppm average exposure, the predicted number of cancers equals:

[((20 ppm X 12.4 years employed)/90 ppm years) X 1066 workers X (5.97 excess cancers/100 workers) X (0.97)] + 140.9 expected deaths = 311.0 predicted cancers

Predicted cancers at 20 ppm, 10 ppm, and 2 ppm are given in Table 2 along with the probabilities of seeing the observed number of deaths or fewer.

Table 2
Predicted Deaths for Texaco Subcohort

A. All Cancers (actual Texaco data = 106 observed)

ppm	NIOSH predicted	probability of observed or fewer deaths
20	311.0	1.9×10^{-41}
10	226.0	4.0×10^{-19}
2	157.9	7.3×10^{-6}

B. Lung Cancer (actual Texaco data = 36 observed)

20	214.3	2.3×10^{-51}
10	129.3	2.7×10^{-22}
2	61.2	3.5×10^{-4}

C. Lymphopoietic Cancer (actual Texaco data = 16 observed)

20	182.8	3.3×10^{-57}
10	97.8	1.3×10^{-24}
2	29.7	4.5×10^{-3}

These analyses illustrate that the cancer predictions from the NIOSH risk assessment model are totally inconsistent with human experience in a high exposure, long followup subgroup of butadiene workers. For example, assuming these workers averaged 10 ppm butadiene exposure, the NIOSH risk assessment model predicts 226 deaths from all cancers, but only 106 were

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observed. The probability of seeing only 106 deaths or fewer when 226 are predicted is 4×10^{-19}. This incredibly small probability is a measure of how well the risk assessment model fits the data it was intended to predict. (Probabilities less than 0.05 indicate that the model does not provide an adequate fit to the data.) Predictions at 10 ppm for lung cancer and lymphopoietic cancers show similar lack of fit: lung cancer - 36 observed, 129.3 predicted, probability = 3×10^{-22}; lymphopoietic cancers - 16 observed, 97.8 predicted, probability = 1×10^{-24}. Predictions at 2 ppm -- which assume that exposures 30 to 40 years ago did not exceed the current OSHA proposed standard -- still overpredict mortality from all cancer (probability = 7.3×10^{-6}), lung cancer (probability = 3.5×10^{-4}) or lymphopoietic cancers (probability = 4.5×10^{-3}). Thus, these analyses provide a high degree of empirical evidence that the NIOSH risk assessment model greatly overestimates risk for workers exposed to 1,3-butadiene.

The reason for the lack of correspondence between the NIOSH risk projections and actual human experience was discussed extensively in the OSHA hearings -- the $B_6C_3F_1$ mouse is hypersensitive to the carcinogenic effects of 1,3-butadiene (<u>see</u> Bird 1990; Hinderer 1990; Starr 1990). Accordingly, risk assessments based on the $B_6C_3F_1$ mouse data do not provide a valid basis to evaluate the risk for workers at or near the proposed OSHA standard. An alternative approach is clearly warranted.

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APPENDIX IV

APPENDIX IV

EPIDEMIOLOGY COMMENTS ON CARB'S HEALTH ASSESSMENT

Prepared by
John F. Acquavella, Ph.D.
December 6, 1991

CARB's review of the epidemiology has incorporated many of the comments previously submitted by industry representatives. However, CARB's current evaluation of the butadiene epidemiologic data selectively highlights positive findings, neglects important negative findings, and the overall interpretation relies on a number of unproven assumptions. These assumptions include a uniform reduction in butadiene exposures across industry after 1945, a lack of correlation between employment duration and cumulative exposure, and a biological consistency of varied lymphopoietic cancer findings across studies due to clinical similarity and/or misdiagnosis on death certificates. CARB also evaluates the recent lymphopoietic cancer case control study (Matanoski et al. 1989) exclusive of the markedly conflicting findings from the base cohort study (Matanoski et al 1990) and misrepresents the hematological effects findings among styrene butadiene rubber (SBR) workers (Checkoway and Williams 1982). Finally, CARB continues to give undue attention to studies of tire manufacturing populations, in which solvents, not butadiene, have previously been associated with elevated rates of lymphatic leukemia (McMichael et al 1976, Andjelkovich et al. 1977, Monson and Fine 1978, Checkoway et al. 1984).

Exposure before/after 1945

CARB asserts that exposures were reduced substantially throughout the SBR industry after 1945 and argues that elevated lymphopoietic cancer rates among World War II workers are more important for assessing carcinogenicity than trends in lymphopoietic cancer rates by duration of employment. CARB's hypothesis about exposures may or may not be true, but since it is a key argument in their evaluation of the epidemiologic data it should be evaluated critically along with the relevant studies.

Exposures before and after 1945 (for 20 or so years) are unknown. Workplace exposure monitoring for butadiene began in the 1970s. Thus, there are no exposure measurements to verify a substantial reduction in exposures after 1945, though this concept is intuitively appealing due to polymerization process changes at some, if not all, of the SBR plants. CARB should verify that process changes occurred immediately after 1945 at all SBR plants if this is a key assumption for their interpretation of the epidemiologic data. However, even if the implementation of process changes reduced exposures for polymerization areas of all SBR plants from 1946 onward, it is unlikely that there would have been a corresponding reduction in exposure for several of the high exposure jobs in non polymerization areas including: monomer loading/unloading, polymer sampling, maintenance activities, laboratory analysis, etc. In addition, there were no corresponding process changes in

the only butadiene monomer plant studied to date (Divine 1990, B. Divine, personal communication). Thus, CARB's hypothesis of a uniform exposure reduction across all jobs in industry is possibly only valid for workers employed in SBR polymerization. CARB should reconsider their interpretation of the epidemiology data under a more limited exposure reduction scenario and excluding monomer production workers.

Second, CARB's conclusion of a carcinogenic effect among workers employed prior to 1946 is not consistent with the available epidemiologic data. For example, Matanoski et al. did not see elevated lymphopoietic cancer rates in their World War II subcohort (Matanoski et al. 1988). In Meinhardt et al., the results were internally inconsistent. Only very short term workers hired before 1946 showed a leukemia excess. Specifically, two of the five leukemia decedents in Plant A had extremely short employment durations and times from first employment to death (e.g. employment periods of 6 months and 1.5 years and induction/latency periods of 3 years for both decedents), while there was no accompanying excess in their long term co-workers. The latter group would have had the high exposures characteristic of World War II operations and opportunity for subsequent exposures from continued employment. The pattern of very short latency leukemias does not appear to be of etiologic importance since it has no analogy in the other much larger SBR workers study (Matanoski et al. 1990), where only one leukemia death occurred within 10 years of first employment (an employee hired

in the 1970s). So, the Meinhardt findings are, at a minimum, internally inconsistent for workers employed before 1946. Similarly, the Divine study (Divine 1990) of butadiene monomer workers did not have a lymphosarcoma excess in long term World War II workers since there were no lymphosarcoma deaths in any worker employed more than 10 years in this study, and there was no leukemia excess among World War II workers. Thus, the varying short term worker findings, in the absence of findings among their long term colleagues, suggest the possibility that confounding exposures, not butadiene, were the responsible etiologic factors.

The Texaco World War II subcohort findings are important because they represent workers with the longest follow-up across studies (41.5 years) and who averaged 12.4 years employment (Divine, personal communication). Findings for these workers provide an important perspective, particularly for concerns about all cancer and lung cancer from butadiene exposure. As indicated in the table below, these workers had a significant deficit of all cancers and a low rate of lung cancer. Similar findings are seen in Meinhardt et al. (1982) and Matanoski et al. (1990).

Mortality findings for Texaco World War II subcohort

	observed	expected	SMR	95% CI
all cancer	106	140.9	75	62-91
lung cancer	36	44.2	82	57-113
lymphopoietic	16	12.7	126	72-205
lymphosarcoma	7	2.6	269	108-555

leukemia	6	5.5	112	41-244
other lymphatic	2	3.4	59	7-212

from Divine 1990

These data demonstrate that all cancer and lung cancer are not likely outcomes of long term occupational exposure to butadiene. The lymphopoietic cancer findings for these workers are essentially null, with the exception of lymphosarcoma and, as mentioned previously, the lymphosarcoma excess is confined (paradoxically) to short term workers.

CARB mentioned that the lymphopoietic cancer findings from the Matanoski et al. cohort study (Matanoski et al. 1988 and 1990) are less useful because the omission of early workers (viz. World War II workers) at four plants with incomplete records might have obscured a carcinogenic effect of butadiene exposure. This concern can be evaluated by reviewing the SMRs for the four plants in this study with complete records - plants where the "early workers" have been completely enumerated. SMRs for these plants were presented for this study (Matanoski 1988). These data show a pattern of extremely low SMRs including significant deficits of total mortality and cancer mortality, and borderline significant deficits of lymphopoietic cancers ($p = 0.06$) and lymphosarcoma ($p = 0.06$). Leukemia mortality was only 74% of expected values.

SMRs for plants with complete records				
cause of death	obs	exp	SMR	95% CI
all causes	1188	1567.2	76	72-80
all cancers	233	313.2	74	65-85
lymphopoietic cancer	24	35.0	69	44-102
lymphosarcoma	2	7.0	29	3-103
leukemia	10	13.5	74	36-136

from Matanoski 1988

These data do not support CARB's contention that exclusion of early workers from four of the eight plants in this study is likely to mask a relationship between butadiene and disease in this study.

Varying lymphopoietic cancer findings across studies

CARB dismisses the criticism that variation in health endpoints across studies detracts from a causal interpretation for butadiene and lymphopoietic cancer. This issue has been discussed in detail by Cole (Cole 1990) at the OSHA hearings and CARB should consider and respond to his specific criticisms on this matter before adopting this viewpoint.

CARB's basis for disregarding the heterogeneity of the lymphopoietic cancer findings seems to be comments by Matanoski et al. (1990) and Landrigan (1990) that the individual lymphopoietic cancers are related tumors (viz. butadiene may cause all types of lymphopoietic cancer) and that there is diagnostic overlap and changing nomenclature over time. These

points are logically inconsistent with the findings for butadiene workers and they need to be evaluated critically.

First, if butadiene was a common etiologic factor for several or all lymphopoietic cancers, it is unlikely that there would be marked variability in findings across studies. Rather, there would tend to be a general lymphopoietic cancer excess for all sites rather than excesses and deficits of specific lymphopoietic cancers across studies. Such is not the case.

The second issue, diagnostic overlap, is not a credible explanation for the epidemiologic findings since it also occurs in the general population - the comparison population for the butadiene cohort studies. So, for example, a doubling of the multiple myeloma rate among butadiene workers with some misdiagnosis would be compared to the general population rate also impacted by misdiagnosis. As long as the misdiagnosis is relatively comparable for workers and the general population, the SMR should not be affected greatly.

Third, diagnostic variability differs by type of lymphopoietic cancer (see table below) as illustrated by a comparison of cancer incidence and death certificate data for cases from the Third National Cancer Survey 1969-1971 (Percy et al. 1982). Therefore, diagnostic variability will have little effect on lymphopoietic cancers with a high percent confirmation.

Confirmation rates for lymphopoietic cancers on death certificates -- based on the Third National Cancer Survey

primary site	#	% confirmed
Non-Hodgkin's lymphoma	1562	88.4
Multiple myeloma	699	98.1
Lymphocytic leukemia	743	86.3
Myeloid leukemia	1107	92.2
Monocytic leukemia	98	53.8
Other & unspecified leukemia	204	34.3

from Percy et al. 1982

Finally, most of the lymphopoietic cancer deaths in these studies happened within the last 10-15 years of the respective study periods, and diagnostic specificity has improved with time.

Taken together, these points would argue that the relationships of the lymphopoietic cancers or the extent of misdiagnosis is unlikely to be a suitable explanation for the heterogeneous lymphopoietic cancer findings seen in the butadiene epidemiologic literature.

Interpretation of the lymphopoietic cancer case control study

The findings from the SBR workers lymphopoietic cancer case control study (Matanoski et al. 1989) are irreconcilable with findings for the cohort study of the same population (Matanoski et al 1990). CARB should evaluate these two related studies together to determine whether the strong leukemia/butadiene

association reported in the case control study can be rationalized with the lack of a leukemia excess in the base cohort study. In addition, two reanalyses of this study were presented at the OSHA hearings which showed markedly different findings than those presented by the authors (see Acquavella 1990, Cole 1990). Two issues were identified as critical in interpreting this study: 1) the selective analysis presented by the authors; and 2) the incompatibility of the case control and base cohort findings.

The analysis of the case control study was quite selective and masked striking internal inconsistencies in the odds ratio (OR) estimates related to butadiene exposure (Acquavella 1990). Most of the analyses presented in the study report (Matanoski 1989) categorized workers as either exposed or unexposed based on the mean of the logarithms of the butadiene exposures. The conclusions from this study were based on these analyses. The authors did not mention that an analysis based on the actual, non-transformed, butadiene data, instead of the logarithms of the data, would have resulted in substantially lower ORs - consistent with the conclusion of no association between leukemia and butadiene exposure. Furthermore, even exposure level analyses at the highest exposure level resulted in lower estimates of the OR than the mean log butadiene analysis (Cole 1990).

The rationale given in this report for employing a logarithmic transformation was "... due to the skewing of the data." That is, the butadiene exposure data were not normally

distributed and the investigators attempted to normalize the butadiene exposure distribution. The advisability of this transformation was questionable since textbooks on case control methodology prescribe no such normality requirement for the underlying exposure data used to calculate ORs (Rothman 1986, Schlesselman 1982, Kleinbaum et al. 1982). Further, the logarithmic transformation did not produce a normal distribution of the butadiene exposure scores (Acquavella 1991) as indicated by the following normality statistics:

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actual butadiene data: Shapiro-Wilk statistic (W) = 0.773, p value < 0.0001, skewness 1.62, kurtosis 2.13

log butadiene data: Shapiro-Wilk statistic (W) = 0.805, p value < 0.0001, skewness 0.975, kurtosis 0.344

from Acquavella 1991

All that resulted from the logarithmic transformation was a slight variation of the cutpoint for dichotomizing cases and controls. Thus, it would have been prudent, at a minimum, also to report results for the actual, non-transformed, butadiene exposure data (and to discuss any inconsistencies).

The results of analyses based on the actual butadiene data conflict markedly with the analyses based on the logarithmic data. Specifically, the two analyses yielded the following:

OR $_{\text{mean log butadiene score}}$	= 7.6 (1.6 - 35.6)
OR $_{\text{mean actual butadiene score}}$	= 0.9 (0.3 - 2.6)

(from Acquavella 1990)

The striking difference between analyses suggests a large random error component in the reported OR of 7.6 for butadiene and leukemia and points out the unreliability of conclusions based on that result.

In light of this variability, Cole conducted an exposure level analysis with controls distributed evenly by exposure tertiles - an unbiased method of exposure categorization (Cole 1990). These analyses revealed an irregular exposure response pattern with a precipitous decline in the OR for the highest exposure category (i.e., ORs 1.0, 5.3 and 2.3 for the lowest, intermediate and highest exposure categories, respectively). The marked decline in ORs from the intermediate to the highest exposure category again indicates a large random error component in this study.

Exposure level analyses were presented by Matanoski et al. (Matanoski et al. 1989) although they received less emphasis then the dichotomous analyses. In fact, the authors reported a significant linear trend with butadiene exposure in a categorical analysis. This categorical analysis apportioned the 26 cases and 84 controls unevenly into seven exposure levels and the

individual point estimates in many of the categories were based on so few cases and/or controls as to be unreliable. A trend by exposure level was not seen in analyses based on evenly balanced tertiles, quartiles, or quintiles (viz. exposure categories based on equal numbers of controls or cases and controls as from Cole 1990). Again, it seems that the choice of exposure cutpoints dictates the presence or absence of a significant relationship in this case control study.

The authors also used a continuous butadiene exposure score in logistic regression and found a borderline significant trend. However, such an analysis has long been considered to be inappropriate in most epidemiologic applications since it carries the inherent assumption that each exposure increment multiplies the OR by a constant factor (see Greenland 1979, Rothman 1986). Such an assumption is rarely ever appropriate and, in this case, is clearly inconsistent with the leukemia mortality seen in the base population for the case control study (22 observed, 22.8 expected).

Conflict between the cohort and case control study

The lymphopoietic cancer case control study (Matanoski et al. 1989) was nested within the cohort study of 12110 SBR workers (Matanoski et al. 1990). Lymphopoietic cancer mortality for this large cohort was lower than or consistent with general population rates. Specific findings were: all lymphopoietic cancers (55 observed (obs), 56.7 expected (exp), standardized mortality ratio (SMR) = 97), lymphosarcoma (7 obs, 11.5 exp, SMR = 61), and

leukemia (22 obs, 22.8 exp, SMR = 96). Analyses by job category also showed low mortality rates. Importantly, leukemia mortality was not elevated for the two job category subgroups with highest potential for butadiene exposure -- production workers (7 obs, 6.4 exp, SMR = 111) and mechanical workers (6 obs, 8.6 exp, SMR = 70) (corrected figures as in Acquavella 1990).

Against this backdrop, the case control study reported an OR of 7.6 based on the mean of the logarithm of case and control butadiene scores and 60% of the control population was categorized as exposed. Cole demonstrated in his OSHA testimony (Cole 1991) that the case control odds ratio of 7.6 (along with the 60% control exposure prevalence) is incompatible with the lack of any leukemia excess for this worker population (i.e. 22 observed, 22.8 expected). Specifically, he presented the following data:

leukemia deaths predicted in the SBR workers cohort study from the case control results; odds ratio = 8

% cohort exposed	predicted deaths in cohort study
25%	63
50%	103
60%	119

adapted from Cole 1991

As the last row in this table indicates, if the case control OR of 7.6 (Cole used 8.0 for the OR in his testimony) is valid and 60% of the control population is exposed, there should have been 119 leukemia deaths in the cohort study [i.e. (7.6 x 60% of the leukemia expected (22.9)) + (1.0 x (40% x 22.9))] when there were only 22. Allowing for lower exposure prevalences among controls (note the exposure prevalence of controls is representative of the exposure prevalence in the base population conditional on the matching factors - see Miettinen 1985) still yields estimates of leukemia mortality far in excess of that seen in the cohort study. Therefore, until these conflicts between the SBR worker cohort and case control results are resolved, the OR estimates should not be interpreted at face value. Rather, emphasis should be placed on finding an interpretation for the case control results that is consistent with the lack of a leukemia excess for the SBR worker cohort overall.

Checkoway and Williams hematological effects study

CARB mentioned that Checkoway and Williams attributed hematological abnormalities to butadiene exposure in a cohort of SBR workers (Checkoway and Williams 1982). This statement is simply not true since, as Checkoway mentioned, the values for the tank farm workers, the highest exposed group, were all within the normal range. Checkoway and Williams also concluded there was no significant difference between the two exposure groups (i.e. tank farm versus other) in this study. Therefore, CARB's citation of this study is misleading and the conclusion of a relationship

between hematological abnormalities and butadiene exposure is not supported by the available study.

Data on SBR worker subgroups in Matanoski et al. 1990

CARB presented a selected review of the occupational subgroup analyses in the cohort study by Matanoski et al. (1990). Neglected were the findings for white production workers and the data for white and black mechanical workers. These findings are important because process and mechanical workers have frequent opportunity for butadiene exposure according to an industrial hygiene review of the industry (Fajen et al. 1990). The findings

SMRs for mechanical workers by race

	whites			blacks			total		
cause of death	obs	exp	SMR	obs	exp	SMR	obs	exp	SMR
all cancers	173	176.6	98	23	30.8	75	196	207.4	95
all lymphopoietic cancer	14	16.5	85	0	2.1	0	14	18.6	75
lymphosarcoma	2	3.4	59	0	0.3	0	2	3.7	54
leukemia	6	6.4	93	0	0.7	0	6	7.1	85
corrected leukemia*							6	8.6	70
other lymphatic	2	4.4	46	0	0.7	0	2	5.1	39

from Matanoski et al. 1990
* from Acquavella 1990

for mechanical workers (see table above) are particularly important because these workers have had opportunity for intermittent peak exposures and CARB has drawn an analogy between the peak exposure mouse studies (Melnick 1990) and findings from

epidemiologic studies.

From this table, it is obvious that mortality rates for cancers, and specifically lymphopoietic cancers, are not elevated among mechanical workers. SMRs were especially low for the lymphopoietic cancers, specifically: all lymphopoietic cancer SMR = 75, lymphosarcoma SMR = 54, leukemia (corrected) SMR = 70, and other lymphatic cancer SMR = 39. In contrast to the findings reported for black production workers, black maintenance workers had no lymphopoietic cancer deaths (viz. SMRs = 0 for lymphosarcoma, leukemia, and other lymphatic cancer). Therefore, since mechanical workers have intermittent high exposures to butadiene, these data are inconsistent with CARB's analogy to the high exposure/short-time mouse studies by Melnick et al. (1990).

CARB mentions the significantly elevated lymphopoietic cancer mortality and leukemia mortality for black production workers (6 obs SMR = 507, 95% CI 183-1088; 3 observed, SMR = 656, 95% CI 135-1906), but the corresponding lack of lymphopoietic and leukemia excesses among white production workers was not emphasized (13 obs, SMR = 110, 95% CI 58-187; 4 observed, SMR = 84, 95% CI 22-215). Production workers of both races were also reported to have had elevated SMRs for the category "other lymphatic cancers" - a "catch-all" category which includes unspecified non-Hodgkin's lymphoma, multiple myeloma, and

SMRs for production SBR workers by race

	whites			blacks			total		
cause of death	obs	exp	SMR	obs	exp	SMR	obs	exp	SMR
all cancers	105	118.9	88	19	16.5	115	124	135.4	92
all lymphopoietic cancer	13	11.9	110	6	1.2	507	19	13.1	146
lymphosarcoma	0	2.4	0	1	0.2	532	1	2.6	39
leukemia	4	4.8	84	3	0.5	656	7	5.3	134
corrected leukemia*							7	6.3	111
other lymphatic	7	3.1	230	2	0.4	482	9	3.5	260

from Matanoski et al. 1990
* from Acquavella 1990

polycythemia vera. A more appropriate analysis of this data would have shown that there was not an overall lymphoma excess for these workers. Based on data presented by the authors it has been estimated that all lymphoma mortality was consistent with expected values (4 observed, 4.3 expected, SMR = 93, 95% CI 25-238), while multiple myeloma was somewhat elevated (5 observed, 1.7 expected, SMR = 294, 95% CI 95-686) (see Acquavella 1990 for details). This analysis again highlights the inconsistency of lymphopoietic cancer findings across studies.

Studies of Tire Manufacturing Workers

It was mentioned previously to CARB that studies of tire manufacturing populations are essentially irrelevant for butadiene since butadiene in not liberated during tire

manufacturing, and it is not a solvent used in those plants. CARB has chosen to disregard this comment and we state it again for the record. Only one of these studies had a small subcohort of workers employed in the "synthetic plant" where styrene butadiene rubber (SBR) was made at times (McMichael 1976). However, even for these workers, it is unclear whether the synthetic plant resembled production SBR plants (in design and relative production volume), whether there were typical butadiene exposures, and there was acknowledged opportunity for exposure to numerous potential confounding factors in this study (e.g. benzene and other solvent exposure, other elastomeric ingredients). Finally, further studies by this research group attributed the elevated leukemia findings to solvent exposure, not butadiene (See Checkoway 1984). As Matanoski (1990) stated on this issue "In a subsequent study, these investigators associated the leukemia risk with solvent exposure only and did not mention a relationship to the SBR department." (referring to Checkoway 1984). CARB should not confuse the evaluation of butadiene by discussing tire manufacturing studies in detail, but rather should focus on studies of SBR workers and butadiene monomer workers.

Interpretation of findings from Ott et al.

CARB's interpretation of the SB latex workers findings from Ott et al. reflects an unwillingness to accept negative findings from any study. In this study, it is clearly stated that there were no leukemias among 391 SB latex workers. CARB's contention

that this finding is not reliable because "OEHHA staff were not able to confirm that this OTG was the only one in which butadiene exposure occurred" evokes a biased perspective on this study. CARB did not apply an similar concern to the interpretation of the synthetic plant findings in McMichael et al. (1976). However, even if there were other butadiene exposed workers in this study, it would not detract from these SB latex worker findings. They stand as reported.

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APPENDIX V

OSHA DOCKET NO. H-041

INFORMAL PUBLIC HEARING ON THE PROPOSED OSHA STANDARD ON OCCUPATIONAL EXPOSURE TO 1,3-BUTADIENE

WRITTEN STATEMENT

OF

STUART Z. CAGEN, PH.D.

November 26, 1991

COMMENTS ON NIOSH QUANTITATIVE
RISK ASSESSMENT FOR 1,3-BUTADIENE

by

STUART Z. CAGEN[1/]

INTRODUCTION

At the request of the Butadiene Panel of the Chemical Manufacturers Association (CMA), I have reviewed the risk assessment on butadiene prepared by NIOSH (1991). I have been assisted in this review by other members of the CMA Butadiene Toxicology Research Task Group, as well as by other toxicologists and risk assessment experts at Shell Oil Company and other Butadiene Panel member companies. I would like to gratefully acknowledge their assistance.

The NIOSH risk assessment document identifies most of the qualitative and quantitative information that now exists with respect to the carcinogenicity of butadiene. Unfortunately, NIOSH has utilized only a portion of these data for its quantitative risk estimates, and therefore is presenting an incomplete statement regarding risk. It is difficult at this time or perhaps any time to expect risk assessments to have perfect data in the form of directly useable in vivo human data. Yet, it is important to use all available information to establish a most likely case and show a range of probable outcomes. The risk manager (OSHA) needs to be informed of this range of probable outcomes, and in order to do this it is important that risk assessments use all of the available information. This is consistent with the objectives of OSHA; the preamble to the proposed butadiene standard (August 10, 1990, 55 FR 32763) states: "When pharmacokinetic or metabolic data are available, these data should be used to estimate internal dose. By using all available information, the uncertainty associated with estimating risks across species can be reduced." We agree with this statement and urge that risk analyses like the one provided by NIOSH utilize as much current metabolic and mechanistic data as possible in order to arrive at estimates of risk to humans.

COMPLEX NATURE OF BUTADIENE RISK OPTIONS: Butadiene presents a challenge to the risk assessor and risk manager because of the numerous choices available from which risk values can be calculated. There are several epidemiology studies as well as three valid rodent bioassays. From the epidemiology side the results are largely negative, although it might be recognized that within the total epidemiology data set there are isolated anomalies that run counter to conventional dose-response principles or are

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1/ Senior Toxicologist at Shell Oil Company and member of the CMA Butadiene Panel Toxicology Research Task Group. A copy of Dr. Cagen's curriculum vitae is attached.

statistical artifacts (also see comments of IISRP for more detailed analysis of the NIOSH presentation of epidemiology). In addition, on the surface, there seems to be some confusion regarding the "most appropriate" data set from which risk can be calculated from rodent studies. Clearly, the mouse oncogenicity studies would raise great concern over statistically significant increases in lung tumors at exposure levels as low as 6.25 ppm (NTP-II). Yet there is also a high dose lifetime rat oncogenicity study and extensive mutagenicity and metabolism data on butadiene and related compounds that aid in characterizing the risk. Inclusion of all of the available information in the calculation of risk options is important to the goal of deriving useable and credible values.

SUMMARY OF COMMENTS: We find the NIOSH document to be reasonable in its presentation of most of the pertinent literature relating to the toxicity, carcinogenicity, metabolism and pharmacokinetics of 1,3 butadiene. However, we disagree with the risk assessment approach of NIOSH in utilizing only a small portion of this information in arriving at its calculated workplace risk. Indeed, the analysis presented by NIOSH concludes with a numerical representation of the risk (597/10,000) which might be viewed as an extreme value at one end of a family of estimates. NIOSH has limited the options by rejecting much information and in so doing is giving an incorrect and misleading accounting of likely human cancer risk. Insufficient arguments are given by NIOSH for rejecting the direct use of the rat tumor data and for not using appropriate multi-species pharmacokinetic data to establish a reasonable biological estimate of human risk.

Specific areas covered in detail include:

* NIOSH risk assessment rejected certain data with the implication that the information that does exist is insufficient for any use. These data include:

  Rat oncogenicity study (IISRP).
  Species specific genotoxicity.
  Species specific metabolism.

* NIOSH risk assessment used allometric scaling of body weight to the 0.75 power on the basis of empirical grounds that were derived for compounds other than butadiene. Butadiene specific information should be used, with different results.

* More appropriate treatment of mouse and rat tumor data, including information with regard to metabolism, such as the more reasonable use of internal dose of butadiene epoxide(s) as a dose measure, would significantly reduce the calculated risk.

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I. NIOSH RISK ASSESSMENT REJECTED CERTAIN DATA WITH THE IMPLICATION THAT THE INFORMATION THAT DOES EXIST EXIST IS INSUFFICIENT FOR ANY USE

A. Rat Oncogenicity Study (IISRP)

Although the NIOSH risk assessment did not explain in great detail NIOSH's reasons for rejecting the rat oncogenicity study, there were three identifiable comments which seem to indicate that NIOSH considered the study as not useful:

> On page 7 NIOSH states that: ". . . Although the study was completed in 1981, it was not formally reported until six years later (Owen, et al, 1987) . . .."
>
> On pages 12-13 NIOSH states (albeit with respect the EPA rejection of the rat study): ". . . the study (at that time) had not been peer-reviewed or published, lacked complete pathology information, allowed larger contaminant concentrations of dimerized butadiene than the mouse study, and exposed rats to concentrations greater than that producing metabolic saturation . . .."
>
> On page 17 NIOSH states that: ". . . This data set is preferable to either the Hazleton rat bioassay data (Owen, et al, 1987), or the first (high dose) mouse bioassay (NTP, 1984), because the new data set includes exposures at a concentration (6.25 ppm) close to the proposed OSHA standard of 2 ppm. The fact that the 1984 NTP data included very high exposure concentrations leads to difficulties in extrapolating the effects to low concentrations since the biologically effective doses were probably not directly proportional to the ppm exposure concentration due to metabolic saturation and possible depletion of glutathione . . .."

These reasons are insufficient justification for using only the new tumor data in mice for calculation of cancer risk. Indeed, the more recent NTP sponsored bioassay has not been peer reviewed, and should be the data set most vulnerable on grounds of insufficient review. The IISRP rat oncogenicity study has been peer reviewed and published and is considered to be a valid bioassay. For further clarification of methodological issues relating to this study (including a description of the procedures used for controlling concentrations of dimerized butadiene, which procedures were clearly more effective than those employed in the NTP-I mouse study) please review the testimony of Dr. Robert Hinderer.

Note also that in its justification of the preferred data set from the new mouse study, NIOSH (on page 17) emphasizes that: "biologically effective" doses were disproportionate in the earlier high dose mouse and rat studies. Although it might be reasonable to prefer the new mouse study to the old one, the results in rats

still provide valuable information, even preferable information, particularly because metabolism of 1,3 butadiene to active metabolites in humans (in vitro) and nonhuman primates (in vitro and in vivo) is much more like rats than mice (also see below). Clearly, if the issue NIOSH wished to pursue (on page 17) was the matching of reasonable "biologically effective" doses, then rat tumor data would be the most appropriate of the available data.

The NIOSH risk assessment, on page 46, summarizes the results of their assessment and compares the results to the prior OSHA assessment. In the end, the NIOSH value is within a factor of two (when an adjustment is made to allow for comparable scaling procedures) of the "high dose" mouse study (NTP-I). Although this result might illustrate limitations in existing extrapolation methodologies, it nonetheless demonstrates (with life shortening adjustments) for butadiene that there is no advantage of having a study with doses "close to the proposed OSHA standard of 2 ppm". The primary issue is the appropriateness of the species being modeled, not the dose. Although corrections need to be made to account for known species differences in metabolism of butadiene (see below), useable and equally valid risk values for mice could have been derived from the high dose mouse study - NTP-I. In a similar sense, the IISRP sponsored rat bioassay cannot be ignored on the grounds that the doses used were too distant from the proposed standard. At a minimum, this dichotomous species response -- high dose mouse yields large tumor response while higher dose rat yields a small tumor response -- must be fully included in a scientifically based risk assessment. Indeed, the rat study should get preference because, as discussed below, butadiene metabolism in humans more closely resembles the metabolism profile in rats as compared to mice.

B. Species Specific Genotoxicity

On page 11: NIOSH gives only a brief accounting of the current evidence for genotoxicity. Although this particular information does not have direct bearing on the final risk calculations, important species differences in the genotoxicity of 1,3 butadiene should be reviewed. It is suggested that the testimony submitted by Dr. Michael Bird be reviewed. The evidence supports the notion that mice are much more susceptible than any other species to genotoxic events produced by 1,3 butadiene either in vitro or in vivo. A more balanced presentation would illustrate to the risk manager (OSHA) that mice are extraordinarily susceptible to genotoxic events produced by 1,3-butadiene, and therefore risk estimates based exclusively on the mouse tumor response would overstate the true risks (also see below).

C. Species Specific Metabolism

As is freely acknowledged by NIOSH, and emphasized extensively by CMA (see testimony of Dr. Michael Bird), the mouse is extraordinary in its ability to produce and retain toxic epoxide metabolites of butadiene. It is commendable that NIOSH fully

captured much of the latest literature in this regard (Kreuzer, et al, 1991; Dahl, et al 1991; Csanady and Bond, 1991). However, it is important that this information also is captured in the quantitative treatment of the tumor and subsequent risk data. Although the optimal situation would be to have all required information available prior to conducting formal risk analyses, this is seldom the case, and it is often necessary to use whatever information is currently present. In a qualitative sense this is in agreement with OSHA (see preamble to proposed standard, August 10, 1990, 55 FR 32763) and NIOSH (page 17; NIOSH, 1991; "biologically effective doses"). These clear qualitative factors should be translated into reasonable species extrapolations acknowledging that the mouse is several times (perhaps several orders of magnitude) more sensitive than other species, including humans. It is recommended that the pre-hearing comments submitted by Shell Oil Company and ENVIRON (on behalf of the CMA Butadiene Panel) be reviewed and considered; these include incorporation of metabolism information into the risk analysis.

Even if the available metabolism data do not permit a complete pharmacokinetics treatment of the data for risk estimation, the available information is substantial enough for guiding the choice of species to be modeled for estimating human risk. The latest in vitro data, which were derived with several human tissue samples, indicate that the rat is the better species to model.

II. NIOSH RISK ASSESSMENT USED ALLOMETRIC SCALING OF BODY WEIGHT TO THE 0.75 POWER ON THE BASIS OF EMPIRICAL GROUNDS THAT WERE DERIVED FOR COMPOUNDS OTHER THAN BUTADIENE. BUTADIENE SPECIFIC INFORMATION SHOULD BE USED, WITH DIFFERENT RESULTS

In justifying the application of 0.75 scaling, the NIOSH risk assessment (on pages 24, and again on pages 47 - 49) references several rather generic papers that deal with this topic (O'Flaherty, 1989; Travis et al, 1990; Travis and White, 1988). Although these papers are based on a reasonable treatment of the data base those authors selected, alternative analyses can also be presented. An EPA sponsored report (INVESTIGATION OF CANCER RISK ASSESSMENT METHODS by Clement Associates for the EPA, 1987; EPA/600/6-87/007d) [copy attached] makes quantitative comparisons of carcinogenic potency in animals and humans for 23 chemicals for which suitable animal and human data exist. One conclusion of the study was: ". . . use of mg intake/kg body weight/day method for animal-to-human extrapolation generally causes risk related doses (RRDs) estimated from animal and human data to correspond more closely than other methods evaluated . . .. " (also see Allen, B.C., Crump, K.S. and Shipp, A.M. Risk Analysis 8(4):531-544, 1988 [copy attached]; and Goodman, G., and Wilson, R. Environmental Health Perspectives 94:195-218, 1991) [copy attached].

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More importantly, when considering butadiene, NIOSH does not even respond to their own calculation of a contradiction to the allometric scaling approach. On page 49, the document reviews the apparent inconsistency of 0.75 scaling when rat/mouse kinetic constants for butadiene and the monoepoxide metabolite of butadiene are scaled together (referencing data of Kreiling). This implies that 0.75 scaling is NOT appropriate with respect to butadiene. Further, it is clear that, with respect to mice and rat, the scaling should go in the exact opposite direction.

Further, the argument presented by NIOSH with regard to in vitro data, on page 48, would also support mg/kg scaling (at least), especially with regard to mouse data. Here NIOSH reviews the data of Csanady and Bond where it has been shown that tissues derived from the mouse produce 5-6 times more monoepoxide in vitro than tissues from humans. This would make the mouse MORE susceptible not less, as would be required to justify scaling to the 0.75 power. Moreover, recent data of Csanady and Bond (CIIT Activities, Volume 11 No.2, February 1991) show also that human tissues can detoxify the butadiene monoepoxide nearly 20 times faster than the tissues derived from the mouse. These data are clear and supportive of prior work (see below) in illustrating the extraordinary capacity for the mouse to produce and retain the toxic monoepoxide metabolite(s). The kinetics of these results do not even support mouse to human scaling on a straight mg/kg body weight basis, much less on a 0.75 power factor basis.

If 0.75 scaling were appropriate the implication would be that the larger species (man, rat) would be more sensitive on a mg/kg basis to butadiene that the mouse. This is clearly not the case when examining any of the existing data on butadiene. The clear evidence - be it metabolism data or tumor data directly - is that the mouse is the most sensitive species tested. It is noteworthy that the papers cited above and by NIOSH (that is: O'Flaherty, 1989; Travis and White, 1988) state clearly that compound specific data is preferred:

> O'Flaherty: page 597: ". . . IN THE ABSENCE OF SPECIFIC INFORMATION BEARING ON THE METABOLISM AND TOXICITY OF THE CHEMICAL, the 0.75 power of body weight dose conversion is a reasonable approach . . .. "
>
> Travis and White: page 124: ". . . The National Academy of Sciences and Anderson point out that scaling should depend on the kinetic behavior of the particular compound and mechanism of toxicity . . . the 3/4 [0.75] power may be the most appropriate interspecies scaling factor for use in risk assessment of DIRECT ACTING COMPOUNDS. Further analysis will be needed to determine the appropriate scaling factors for compounds that are activated by metabolism."

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Considering what is now known about the toxicity and metabolism of butadiene in a variety of species, it is imperative to use the compound specific information to arrive at the most scientifically based scaling factor when extrapolating to humans. For example, because it is universally accepted that epoxide metabolite(s) are the "direct acting" toxicants, scaling based on a 3/4 power of body weight might be used only after adjustments are made to account for species differences in the formation and de-activation of these toxic metabolites.

Species specific metabolism data (please review prior testimony of Dr. Michael Bird and below) clearly show the unique properties of the mouse with respect to activation of butadiene to toxic metabolites as well as deactivation of these epoxides to less toxicologically important moieties. A similar trend in results of toxicology results should also not be ignored. As a simple example, one can look at the data from the risk assessment prepared by the EPA in 1985 (USEPA: Mutagenicity and Carcinogenicity Assessment of 1,3 Butadiene, August 1985). The EPA calculated that the risk estimate (unit cancer risk) from the female and male mouse is about 8 or 200 times higher than those corresponding to the female or male rat, respectively. Scaling the mouse data using 0.75 scaling to predict a rat risk would go in the exact opposite direction. Thus, with respect to the experimental toxicology findings, the 0.75 scaling assumption clearly is not valid.

III. MORE APPROPRIATE TREATMENT OF MOUSE AND RAT TUMOR DATA, INCLUDING INFORMATION WITH REGARD TO METABOLISM (I.E., THE MORE REASONABLE USE OF INTERNAL DOSE OF BUTADIENE EPOXIDE(S) AS A DOSE MEASURE), WOULD SIGNIFICANTLY REDUCE THE CALCULATED RISK.

A. Comment On The Discarding Of Data By NIOSH

The NIOSH risk assessment states (on page 20): "Exposure concentration was chosen in preference to measures of internal dose, such as butadiene concentration or butadiene monoepoxide concentration, due to the lack of reliable measurements of these concentrations in mice, and the absence of any measurements in humans." On pages 52 and 53, the document goes on to justify this decision in light of an apparent inconsistency of Bond data to those of Kreiling and the difficulty of NIOSH in accepting data of Dahl and Bond because the cyrogenic trapping method might be too nonspecific. In addition, the NIOSH risk assessment, on pages 5 and 6, is critical of studies of Bond, et al (1986) and Dahl, et al (1990, 1991) because of apparent inconsistencies (blood butadiene levels higher at 2 and 4 hours in 7 ppm exposed mice than in 70 ppm exposed mice) or difficulties in relating 2 hour anesthetized monkey data to 6 hour unanesthetized rodent data.

In developing the argument regarding the cyrogenic trapping procedure of Dahl and Bond, NIOSH suggests that the method "might not distinguish 1,3 butadiene from other 1,3 butadiene metabolites." Indeed, even if this were to be a valid point, the results of Dahl are still useable in that the higher blood levels of 1,3 butadiene monoepoxide or "other metabolites" in the mouse over the rat and primate still implies that the production and retention of monoepoxide and other metabolites are much higher in mice. Since it is universally accepted that all metabolism of 1,3 butadiene MUST begin with the production of the first monoepoxide, it might matter little whether those blood levels represent monoepoxide or subsequent (similarly volatile) metabolites. Other issues raised by NIOSH -- those of rather minor inconsistencies in specific data sets, or the relative importance of anesthesia on butadiene metabolism, are addressed in the post-hearing testimony of Dr. Michael Bird. Most importantly, it must be remembered that the data set AS A WHOLE is internally consistent.

In particular: in vitro metabolism, in vivo metabolism in several laboratories (those of Kreiling, Bolt, Dahl, Bond, and all of their co-workers), and data concerning genotoxicity and carcinogenicity clearly set the mouse apart as a species extraordinarily sensitive to the toxicity of butadiene because of an extraordinary capacity to produce and maintain toxic epoxide metabolite(s). TABLE 1 (attached) summarizes a portion of the existing metabolism data base.

B. Use Of Physiologically-Based Pharmacokinetics

On page 5 of their risk assessment, NIOSH is critical of the direct use of the Bond data to estimate retention of butadiene because no attempt was made to estimate the extent to which butadiene metabolites were excreted in the breath during the six hour exposures. Although the NIOSH-sponsored study of Hattis and Wasson addressed this very point, on page 15 of the NIOSH assessment, NIOSH claims that not enough validation has been performed to warrant direct use of the results. Nonetheless, the Hattis and Wasson report concluded (among other things) that human absorption and metabolite formation at low doses is much lower than assumed by EPA (in their prior risk assessment). The Hattis and Wasson study concluded that there was an underestimation of actual dose received by mice and rats by factors of 2 and 4.5, respectively; this would result in a reduction in calculated risk.

C. Adjustments To Risk Values According To Known Differences In Butadiene Metabolism Across Species

Because of the significant amount of information now available from multiple laboratories concerning species differences in the metabolism and toxicity of butadiene, it is appropriate to utilize this information and incorporate these factors into the selection of the species to model and the calculation of risk. Although the NIOSH risk assessment mentions many of these results

the only one that is used to calculate risk is the second mouse oncogenicity study (NTP-II).

It is possible to make all of the data more compatible by demonstrating that adjusted risk values reduce the rat/mouse differences. The adjustment factors, derived from the metabolism/pharmacokinetics data base, explain in a quantitative way why the mouse would be expected to be more vulnerable than any other species, including man. One demonstration of the use of these adjustment factors has been submitted by Shell Oil Company and it is recommended that their risk assessment be reviewed. It is also recommended that the pre-hearing comments regarding the quantitative treatment of risk by ENVIRON also be reviewed.

A demonstration (used here as an example) of this can be made here:

	RAW POTENCY (unadjusted) (2 ppm)	ADJUSTMENT[2/] METABOLISM (epoxide)
NIOSH MOUSE (FEMALE)	5.97/100	0.1/1000
OSHA RAT (FEMALE)	0.29/100	0.07/1000

D. Comments By ENVIRON On The NIOSH Risk Assessment

Additional comments on the NIOSH risk assessment have been prepared by ENVIRON (on behalf of CMA). These comments illustrate quantitatively the impact of NIOSH's choice of the external concentration of butadiene as the measure of delivered dose on the estimate of risk. A table is presented to illustrate the impact on the NIOSH risk estimate if data on uptake, retention and metabolism are used as the basis for a more scientifically supportable measure of delivered dose. The approaches urged by ENVIRON produce a more plausible range of likely cancer risks. ENVIRON also illustrates the inconsistency between the NIOSH estimate of risk and the results of epidemiology studies.

2/ Adjustment to account for differences in the production of the butadiene monoepoxide in blood: factors of 590 in mice and 40 in rats (from Dahl, et al, 1990 using primate data).

CONCLUSION

In conclusion, we would like to reiterate the following key points with respect to the NIOSH risk assessment.

1. Generically risk assessments can and should use all of the available information to establish a most likely estimate along with a range of possible outcomes. Perfection of data need not be a prerequisite to using it.

2. For butadiene, this includes recognizing the marked species difference in tumor response between rats and mice, and the large body of comparative metabolism, pharmacokinetics, and genotoxicity data which includes studies using non-human primates and human tissue. These data consistently indicate the B6C3F1 mouse to be a uniquely susceptible species and the rat as a preferable model for extrapolation to humans.

3. NIOSH has chosen to essentially ignore this substantial database in favor of generic, default assumptions. This approach has lead to a particularly conservative risk assessment which is inconsistent with the available evidence (including worker mortality experience) and may be properly viewed as the conservative end of a range of possible risk estimates. A more credible risk assessment would account for the available butadiene-specific data (e.g., rat bioassay, internal dose, etc.).

4. The approximate order of magnitude difference between the NIOSH and OSHA risk assessments is <u>not</u> primarily due to the more recent dose-response data from NTP II. Rather, it is due primarily to NIOSH's chosen methodology, particularly their decision to scale using body weight to the 0.75 power.

5. NIOSH's scaling assumption is improper because 1) compound-specific data, when available, is preferable and 2) available butadiene-specific data are inconsistent with this scaling assumption (e.g., it predicts the rat would be more sensitive than the mouse).

For these and other reasons expressed in these comments, we believe the NIOSH risk assessment document overstates the likely human cancer risks from workplace exposure to butadiene.

TABLE 1

KNOWN SPECIES DIFFERENCES IN THE METABOLISM OF 1,3 BUTADIENE

AUTHOR(S)	STUDY TYPE	SPECIES MOUSE	COMPARISONS RATS	PRIMATE	AMOUNT THE MOUSE IS MOST SENSITIVE*
KREILING	IN VIVO	X	X		10
SCHMIDT/LOES	IN VITRO	X	X	X (MAN)	60
CSANADY/BOND	IN VITRO	X	X	X (MAN)	60
DAHL	IN VIVO	X	X	X	590

* = Based on the data in the study, the value represents the magnitude of measured difference in the amount of toxic epoxide metabolite(s) present in the mouse, when compared to other species. The in vitro data of Schmidt and Loeser as well as of Csanady and Bond are calculated based on the 3-fold greater activity of the mouse to produce the monoepoxide metabolite and a 20-fold greater activity of the human to detoxify this metabolite (3 X 20 = 60).

Because these were obtained from differing experimental situations, the numerical values would not be expected to be the same. Nonetheless, all of the values clearly show that the mouse produces and retains reactive epoxide metabolite(s) greater than other species. Of the data shown, the results of Dahl should be considered to be the most directly useable for risk assessment purposes because less extrapolation is needed from the experimental setting.

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Contributor to Second International Biological Reactive Intermediate Meeting, Guilford, England, July 1980.

Lecture in Industrial Toxicology in Practical Toxicology Principles, The Center for Professional Advancement, Houston, Texas, March 1981.

Guest Lectures at: The Medical College of Wisconsin, University of Texas Health Science Center-Dallas, University of Texas Health Science Center-Houston, Northwestern Medical Center, The Upjohn Company.

Member 1982-1983, Society of Toxicology Technical Committee

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Co-organizer/Founder, Society for Risk Analysis Lone Star Chapter; Tres. 1988.

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APPENDIX VI

INFORMAL PUBLIC HEARING ON THE PROPOSED OSHA STANDARD ON OCCUPATIONAL EXPOSURE TO 1,3-BUTADIENE

SUPPLEMENTAL TESTIMONY

OF

MICHAEL G. BIRD, PH.D.

November 26, 1991

POST HEARING SUPPLEMENTAL STATEMENT OF DR. MICHAEL BIRD ON KEY INTERSPECIES DIFFERENCES IN METABOLISM AND CYTOGENICITY OF 1,3-BUTADIENE

My name is Dr. Michael Bird, and I submit this post-hearing statement on behalf of the 1,3-Butadiene Panel of the Chemical Manufacturers Association. This statement supplements my previous written testimony as well as my oral testimony at the hearings on January 18, 1991 in Washington, DC. I take this opportunity to focus on points asked of me and others, by the OSHA Panel relating particularly to the interspecies differences in the pharmacokinetics and metabolism of 1,3-butadiene. However, key new data introduced at the OSHA hearings and in subsequent scientific publications support and further define the position I presented from two of the three endpoints I discussed, metabolism and cytogenetics.

My testimony showed that there are major quantitative differences between species in the formation and clearance of the reactive metabolites of butadiene and that on this basis, the mouse would be an overly sensitive model for man. New data (Csanady and Bond, 1991) indicate that the key difference in metabolism between rodent and man is the predominant and rapid metabolism of butadiene monoepoxide by epoxide hydrolase in man to non-DNA reactive 1,2-dihydroxy but-3-ene; this is in contrast to the slower but major contribution of the cytochrome P-450 in the mouse to metabolize the monoepoxide to a DNA-reactive and cross linking agent, the butadiene diepoxide. The urinary metabolite profiles for mouse and primate also reflect this difference (Henderson unpublished, Sabourin, et al. 1990). Ki-ras oncogene activation and/or MULV retrovirus activation appear as secondary to the initiating effects of the circulating mono- and diepoxides in the mouse. Perhaps linked to this is the new finding that like the rat,

micronuclei or chromosomal changes are not present in primates exposed to 8,000 ppm butadiene for up to two hours. These changes are seen in the mouse and possibly the hamster.

These and other data to be discussed subsequently continue to show that because of species differences in metabolism, a carcinogenic response in humans would not be expected to occur at current occupational exposures (10 ppm TWA or less).

Supplemental Answers to Questions Raised During Cross-Examination:

1. Data Derived from Primates

Primates are preferred in advanced pharmacokinetic and toxicokinetic studies because enzyme profiles and metabolic capacities are similar to human and because anatomical and physiological parameters (respiratory rate, blood flow) are more closely matched. Examples of the use of primates as models in pharmacological and toxicological testing are given in Table I. Primates would be used even more extensively but for limitations in availability, difficulties in handling and animal rights considerations.

Within primates, there are examples of metabolic pathways not replicated in other members including man. For example, a Cercopithecus species monkey converts endogenous purines to allantoin and not to uric acid as occurs in the chimpanzee and man. The metabolic reactions in the Rhesus and the cynomolgus monkeys have been

determined and include, as is the case for man, the Phase I (oxidative, reductive and hydrolytic) and Phase II (synthetic) reactions known to be involved in butadiene metabolism.

As a general comment, there are some metabolic reactions which are restricted in their occurrences to primates and hence the appropriate model for man should be sought from anthropoid or promazine species. One example of a pathway present in man and in primate but not in nonprimate mammals is that of conjugation by glutamine rather than glycine for acetoacetic acids such as phenylacetic acid. For a meaningful metabolic comparison, not only is the pathway to be similar but so too should the rate of metabolism. In this respect, the monkey has a similar rate of metabolism to man for plasma half-lives of xenobiotics of less than 30 hours (as is the case of butadiene and its epoxide metabolites).

In vivo inhalation studies in the primate by Dahl, et al. (1990 and 1991) show similar qualitative and quantitative production of reactive epoxide metabolites of butadiene as found in in vitro primate and human tissue preparations (Schmidt and Loeser 1985; Kreuzer, et al. 1991), <u>but different</u> from mouse (Bond, et al. 1986). The same is true for urinary metabolite data (Sabourin, 1990; Henderson 1991 unpublished) where the absence of a metabolite II mercapturic acid of butadiene epoxide in the primate, but its presence in the mouse, indicates a detoxification pathway in the primate which is not utilized in the mouse.

The above generic examples of the use of the primate and the specific data for butadiene metabolism indicate that the primate is an acceptable model for man.

2. Anesthesia/Respiration Rate

The studies of Dahl, et al. (1990 and 1991) used a light anesthesia of ketamine/halothane and subsequently pentobarbital during the experimental period. Respiration data including breathing frequency and tidal volumes during exposure were determined. It was from these data and from the knowledge of the minute volume of the resting, unexposed cynomolgus monkey (obtained from ITRI, Lovelace) that the 15-20% percent reduction due to anesthesia was calculated and mentioned in my oral testimony. Dr. Henderson (1991) states that the effects of this anesthesia in dogs results in a decrease in minute volumes of about 25%. She notes that the same or similar changes for the monkey could not account for the 4-10 fold lower uptake of butadiene in the monkey compared to the rodent.

Of additional note is the observation from data of Dahl, et al. (in press) that a 2 hour exposure of the primate to 8000 ppm butadiene decreased the minute volume significantly in comparison to comparable exposures of 310 ppm and 10 ppm. This demonstrates that the anesthesia used is light enough so as not to mask the CNS depressive effects of butadiene on respiration anticipated at such high exposures.

3. 2 Hour Exposure for the Primate

Dr. Henderson's team (which conducted the primate and rodent studies) confirm in their post hearing comment (Henderson 1991) that a 2 hour period is sufficient to obtain steady state for the blood in any species if not for fat retention. My examination of the mouse and rat data from Bond, et al. (1986), specifically of the quantitative levels of volatile metabolites at 2, 4, and 6 hours, showed that the concentration of any specific metabolite was similar across the time points (within experimental variation), indicating that near steady state had been achieved by 2 hours. Although not stated, in Table 6 of the Dahl paper (1991), the data from the 2 hour (not 6 hour) time points of Bond, et al. (1986) are used in comparison with the 2 hour primate data, thus providing comparability.

4. Normalization of Total Uptake

It might be expected that the ratio of species differences in metabolites distilled from the blood (Dahl, et al. 1991) would not be maintained when normalized for total uptake since elsewhere (Table 5) in this paper data show that the blood of mouse and rat contain more free butadiene than the primate. This may be partly due to the greater uptake of butadiene in the rodent, but also the primate and to a lesser extent the rat, have carbon dioxide as an excretory pathway which does not occur to the same extent in the mouse. Hence, the free butadiene in the blood of the rodent is available for metabolism to reactive metabolites, which means that the total uptake divisor is larger in these rodents because of the free butadiene. This results in apparently lower proportional metabolite concentrations and

consequently reduces or reverses the ratio of metabolites across species. Hence, I believe the ratio and the supporting data are consistent with marked interspecies differences and with the greater formation of reactive epoxide metabolites in the mouse compared to rat or to primate.

5. Enzyme Induction

Studies by Bond, et al. 1988 (also Dahl, et al. 1990) report that repeated exposure does not induce metabolism of butadiene in rodents. As is the case for butadiene dimer (Smith, et al. 1990), it is believed that the metabolism of butadiene is through cytochrome P-450IIE. Styrene amongst others, is known to be metabolized through cytochrome P-450IIE (Guengerich, et al. 1991) and would, therefore, be a competitive substrate for butadiene upon coexposure. There is some evidence (Dahl, et al. 1990) to suggest that like propylene, butadiene may deactivate cytochrome P-450 and partially inhibit its metabolism. The kinetics and possible induction by butadiene of epoxide hydrolase and of glutathione conjugation (both detoxification pathways) is being examined at ITRI Lovelace in research sponsored by the CMA Butadiene Panel.

6. Lorenz Data

These data (Lorenz, et al. 1984) show interspecies differences in specific enzymes in subcellular preparations of rodent and human lung and liver exposed to 1-chloro 2,4 dinitrobenzene (not butadiene or its

metabolites). While specific enzyme activities can vary accordingly to substrate, the ratios derived from the Lorenz data that I presented in my testimony are largely consistent with the interspecies differences in cytochrome P-450 and epoxide hydrolase activities described in in vitro studies for mouse, rat, primate, and man by Schmidt and Loeser (1985). More extensive work by the Chemical Industry Institute of Toxicology (Csanady and Bond 1991) is defining better the complexities of the in vitro system with the conclusions that the mouse retains significantly more of the epoxide once formed than man.

7) Human Variability/Sample Size

A reasonable criticism of the human metabolism data for butadiene has been its dependence on limited samples (in each case one human subject for Schmidt and Loeser [1985] and Kreuzer, et al. [1991]). Wistuba, et al. (1989) report that the differences in optical isomers of aliphatic alkene epoxidation by human liver microsomes <u>of four individuals</u> were negligible, but the optical isomers of the butadiene monoepoxide in man were more similar to the mouse than to the rat. While there is no information available as to the relationship of the optical isomeric "R" and "S" forms to their ability to be formed through isozyme activity, kinetic studies in human, mouse, and rat tissues have been conducted by Csanady and Bond (1991). These in vitro studies show a greater formation of butadiene monoepoxide formation in the mouse than man, and once formed, the monoepoxide in man is quickly detoxified with negligible formation of the diepoxide; in mouse the monoepoxide remains longer while being in part metabolized to the

reactive diepoxide. This is consistent with the finding of significantly higher circulating blood levels of mono and diepoxide in the mouse compared to that of primates when similarly exposed to 1,3-butadiene (Dahl, et al. 1990). To minimize any inter-individual variability in isozymes, detailed kinetic studies using human liver and lung tissues from 12 humans are being completed at CIIT (Csanady) and at the GSF-Institute for Toxicology (Kreuzer).

8) Epoxybutene Metabolism

Data from Bond, et al. (1986) show that epoxybutene metabolism in the mouse becomes saturated at 500 ppm and that the mouse is unable to remove this active metabolite. While saturation levels may not be reached in practice, species differences in the rate of expoxybutene metabolism are important at exposures lower than 500 ppm. Csanady and Bond (1991) show that human liver tissue metabolizes epoxybutene significantly faster than mouse or rat forming the non DNA-reactive 1,2-dihydroxy-3-butene. Clearance in the mouse of the reactive epoxybutene takes longer, and when it occurs, produces the butadiene diepoxide and the 1,2 dihydroxybut-3-ene.

9) Oncogenes

The exact role of oncogenes in malignancy is unknown, but studies by Leigh, et al. (1990) indicate that at least for the Ki-ras oncogene, this occurs later in the transformation process. In order to become activated to a transforming gene, a genetic event has to occur; for ras

oncogenes this can be a point mutation or an overexpression or amplification. Either event could be initiated by a reactive metabolite such as the diepoxide of butadiene which can form cross links with DNA. Hence, I see Ki-ras oncogene as a part of the mechanism for the observed cancer and not the cause, and its activation as being entirely consistent with the presence of DNA-reactive metabolites in the mouse.

10) Pharmacokinetic Data on 4-Vinylcyclohexene (VCH)

The species differences in pharmacokinetics and metabolism of 4-vinylcyclohexene (butadiene dimer) have been described in pages 36-39 of my previous testimony to these OSHA hearings. There is close analogy between VCH and butadiene metabolism and demonstrable species differences between the mouse and the rat as the mouse has a greater capacity than the rat to convert the parent VCH to reactive intermediates. Intraperitoneal injection (800 mg/Kg) of VCH resulted in 41 nmol/ml of the monoepoxide (VCH - 1,2-epoxide) in the blood of mice 2 hours after injection, whereas the blood concentration of VCH-1,2-epoxide was less than 2.5 nmol/ml for the rat (Smith, et al. 1990a).

In vitro studies (Smith, et al. 1990b) showed a 6.5-fold greater rate of epoxidation in the mouse liver than that in rat liver microsomes. In further studies, these authors associated the VCH epoxidation with certain cytochrome P-450 isozymes and went on to show that, although cytochrome P-450IIB catalyses VCH epoxidation in both

cytochrome P-450IIB in rats than mice per amount of protein. The lower concentration of cytochrome P-450IIB in the female rat is, at least, partially responsible for the lower rate of VCH epoxidation in the rat and may well be the explanation for the lack of ovarian toxicity in female rats exposed to VCH.

Recently, Smith and Sipes (1991) assessed the ability of microsomes obtained from human liver to metabolize VCH to epoxides. VCH-1,2-epoxide was the major metabolite, while the rate of VCH-7,8-epoxide formation was about 6-fold lower, and in some cases, was below the limit of detection. There was no dramatic difference in the rate of VCH epoxidation obtained from male and female humans. However, the rate of VCH-1,2-epoxide formation by female human hepatic microsomes was 13- and 2-fold lower than the rate of VCH-1,2-epoxide by female mouse and rat hepatic microsomes, respectively. Hence, as cytochrome P-450IIA and cytochrome P-450IIB account for the majority of VCH bioactivation in the mouse liver, and these isozymes are present to a lesser extent in the rat, then the results of these studies suggest again that rats are the more appropriate animal model for extrapolation of animal data to humans for butadiene dimer and for 1,3-butadiene.

New Data Demonstrating Species Differences:

In addition to the above new data by Smith and Sipes, others (Bond and Csanady 1991) have provided further in vitro data supporting the species differences in metabolism of butadiene and of butadiene monoepoxide in both the liver and lung of the $B_6C_3F_1$ mouse, Sprague Dawley rat, and the human.

Using samples from 12 different human livers, they report that butadiene metabolism as represented by V_{max}/Km ratio is about 6 times higher in mice than in humans or rats and that the subsequent removal of the mutagenic epoxide is 4 fold more rapid in the human than in the rodents. For the human, this removal represents the conversion to inactive 1,2 dihydroxbut-3-ene while in the mouse, the active butadiene diepoxide is formed as well.

Bond and Csanady also reported that the metabolic contribution of human lung microsomes in the metabolism of butadiene is negligible. In the mouse lung, significant metabolism of butadiene monoepoxide occurs. Their interpretation of these data is that differences in the rates of metabolic activation of butadiene in target tissues may be a critical factor in tissue and species sensitivity to butadiene carcinogenesis. An abstract describing their further studies to substantiate and extend these findings has been accepted for presentation at the Society of Toxicology Annual Meeting, February 1992.

Other in vitro data have become available from the Inhalation Toxicology Research Institute in Albuquerque, New Mexico. The data are from a preliminary report (ITRI 1991), an initial phase of the Chemical Manufacturers Association Research Program on Butadiene. Total butadiene metabolism was measured in microsomal preparations from liver and lung samples from humans and mice. Higher butadiene metabolism was found in mouse liver than in human liver; the ratio of activity in the mouse liver compared to human liver is consistent with that reported by Schmidt and Loeser (1986).

The Chemical Manufacturers Association, in conjunction with the American

Petroleum Institute, has begun a four year research program to increase the understanding of the mechanism of action and species differences in response. Four research centers with extensive previous involvement and expertise in butadiene research are involved. At the Inhalation Toxicology Research Institute, the original interspecies studies at this Institute (Bond, et al., 1986; 1988 and Dahl, et al. 1990; 1991) are being extended to include determinations across species of tissues and blood after both single and repeated exposures of butadiene to rodents and primates. Such studies will augment the available internal dose data for risk assessment.

Other data from this Institute (Dahl personal communication) shows the lack of micronuclei induction or chromosome effects in the blood of primates immediately following or 3 days after a 2 hour exposure to 8,000 ppm butadiene; this is in contrast to the significant response seen in the mouse under similar exposure conditions. The University of Colorado's part of the CMA program is aimed at understanding the marked species differences in susceptibility leukemia. Comparative studies are characterizing the cell-specific metabolism and fate of butadiene metabolites in purified lines of mouse and human bone marrow stem cells. The University of North Carolina is identifying and measuring any specific mutations that occur in mouse, rat and human cells in culture and will assess the significance of such changes.

The Chemical Industry Institute of Toxicology (CIIT) is generating a physiologically-based pharmacokinetic model to improve the prediction of cancer risk and which will incorporate much of the data being generated both at CIIT and at the other centers. Specific details of this ongoing program are contained in an abstract (Bird, et al.) presented at the International

Symposium on the Health Effects of Gasoline in November 1991 at Miami, Florida. While data from this program are preliminary, the initial work to date using these various approaches/end points continues to show that the sensitivity of the mouse to butadiene is in large part due to that species particular metabolism.

Since the OSHA hearings, publications by Smith, et al. (1991) and by Roberts, et al. (1991) have demonstrated clear metabolic differences between mouse and rat for the metabolism of butadiene dimer and acrylonitrile respectively. These differences are based on the differences in specific isozymes of cytochrome P-450 which exist between these species. The findings are analogous to the metabolism differences described here and previously for butadiene. I believe the data discussed in this post hearing testimony, as well as the responses to OSHA about the previous data, clearly indicate that the mouse is not predictive of the effects of butadiene in man based on both the metabolism and cytogenetic information currently available.

MGB:jms

November 22, 1991

Table 1

Examples of the Use of Primates as Models in Pharmacological and Toxicological Testing

	Species	Drug
Carcinogenesis	Rhesus, cynomolgus, green and owl monkeys, bushbaby and tree shrew	3-Methylcholanthrene, 2-acetamidofluorene, aflatoxin B, methyl-nitrourea, cyclamate, saccharin, N-nitrosodiethylamine
Treatment of neo-plastic disease	Rhesus and owl monkeys	Prednisolone, vincristine, cytosine arabinoside, cyclophosphamide
Teratogenesis	Rhesus and cynomolgus monkeys Baboon	Thalidomide, testosterone, norethindrone, progesterone, aspirin, meclizine, chlorocyclizine, aminopterin, Thalidomide
Drug dependence	Rhesus monkey Chimpanzee Baboon	Cocaine, amphetamine, opiates, barbiturates Δ^9-Tetrahydrocannabinol Δ^9-Tetrahydrocannabinol
CNS pharmacology	Rhesus monkey Squirrel monkey	Benzodiazepines, meprobamate, chlorpromazine Chlorpromazine, haloperidol
Extrapyramidal toxicity	Rhesus monkey Pig-Tailed monkey	Phenothiazines, reserpine Reserpine
Neurotoxicity	Rhesus monkey	INH, methyl fluoroacetate
Hemotoxicity	Rhesus monkey Baboon	Thiotepa, vincristine, chloramphenicol, nitrogen mustard, 6-mercaptopurine, cycloguanil, cyclophosphamide, S-dichlorovinyl-L-cysteine, methotrexate, chloroethylnitrosurea Chloramphenicol
Immunosuppression	Rhesus monkey	Oxisuran
Gastro-intestinal tract	Rhesus monkey Patas monkey	Thiazides, KCl, calcium gluconate, myalex Myalex
Jaundice	Rhesus monkey Patas monkey	Myalex
Ototoxicity	Pigailed monkey Cynomolgus monkey	Salicylates, kanamycin
Corneal irritation	Rhesus monkey	Anionic, cationic and nonionic detergents
Contraceptive development	Chimpanzee, rhesus, African green, pigtailed and squirrel monkeys, lemur, bushbaby	Estrediol, ethinylestradiol, mestranol, progesterone, megestrol, chlormadinone

From Smith and Caldwell (1977)

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November 22, 1991

APPENDIX VII

OSHA Docket No. H-041

COMMENTS ON THE NATIONAL INSTITUTE FOR OCCUPATIONAL SAFETY AND HEALTH QUANTITATIVE RISK ASSESSMENT FOR 1,3-BUTADIENE

Prepared for

The Chemical Manufacturers Association
Washington, DC

Prepared by

Thomas B. Starr, Ph.D. & Principal
ENVIRON® Corporation
Arlington, Virginia

26 November 1991

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I. INTRODUCTION

A. Background and Purpose

In 1986, at the request of the Chemical Manufacturers Association (CMA), ENVIRON conducted a detailed assessment of the potential risks to workers from 1,3-butadiene (BD) exposure. The final report of that effort (ENVIRON 1986) is now part of the U.S. Occupational Safety and Health Administration (OSHA) docket for BD. When OSHA announced the public hearings regarding its new proposed rule for BD (OSHA 1990), CMA again approached ENVIRON for assistance with the risk assessment issues raised by occupational BD exposures. ENVIRON prepared a critical review of certain aspects of OSHA's preliminary risk assessment for BD (ENVIRON 1990), commenting specifically on the extent to which certain assumptions and procedures used by OSHA to quantify internal doses and potential human cancer risks in relation to airborne BD concentration were consistent with the substantial body of scientific information regarding BD toxicity and carcinogenicity in laboratory animals and humans. ENVIRON also recommended improved methods for quantifying potential human cancer risks where the approach taken by OSHA appeared deficient in light of current knowledge regarding BD's mechanisms of toxic action.

When the National Institute for Occupational Safety and Health (NIOSH) recently released its own quantitative assessment of these potential risks (Dankovic et al., 1991), CMA requested that ENVIRON critically evaluate similar aspects of the NIOSH report. This document describes ENVIRON's observations and conclusions to date regarding NIOSH's quantitative risk assessment for BD. In reaching our conclusions, we have relied extensively on the detailed technical evaluations of BD toxicology, carcinogenicity, and epidemiology that have been prepared independently by Bird (1990), Bolt (1990), Cagen (1991), and Acquavella (1991).

B. Summary of Principal Conclusions

ENVIRON's principal finding is that there is a strong, scientific basis for concluding that NIOSH's estimates of cancer risk actually overpredict human cancer risks from BD exposure by a substantial margin. This basis is comprised of four distinct elements.

First, the NIOSH assessment assumed that BD uptake would be both complete (i.e., 100%) and identical in rodents and primates. However, data recently published in the peer-reviewed literature indicate that neither BD uptake nor its retention following exposure is complete or the same in rodents and primates when both are exposed to the low concentrations of concern to NIOSH and OSHA. Specifically, data obtained by Bond et al.

(1986) and Dahl et al. (1990, 1991) indicate that BD retention by cynomolgus monkeys immediately following exposure is approximately 10-fold less per unit body weight than is the retention by mice at low airborne BD concentrations. Thus, assuming that BD retention in humans is comparable to that observed in these nonhuman primates, one can also reasonably conclude that humans would experience about 10-fold lower cancer risks than would identically exposed mice. It is important to note that the US EPA has already accepted rodent BD retention data as providing a more relevant measure of exposure than airborne BD concentration for risk assessment purposes (EPA, 1985; Cote and Bayard 1990). A similar conclusion has also been reached by California's Air Resources Board in its recent health assessment of BD (CARB, 1991).

Second, additional data from the same laboratory studies show that cynomolgus monkeys take up (i.e., inhale, absorb, metabolize, and excrete) more than 30-fold less BD per unit body weight than do similarly exposed mice at airborne concentrations of 10 ppm or less. Thus, assuming that the uptake of BD by humans is comparable to that observed in monkeys, one can reasonably conclude that humans would experience about 30-fold lower cancer risks than would identically exposed mice.

Third, the same studies have revealed the existence of significant quantitative differences between species in the metabolism of BD to toxic intermediates. Specifically, BD metabolism occurs much more rapidly in mice than in monkeys. In fact, the mouse exhibits remarkably efficient metabolism of BD to 1,2-epoxybutene-3 even when compared to the rat. Most importantly, Dahl et al. (1990, 1991) demonstrated that blood levels of this highly DNA-reactive and mutagenic intermediate of BD metabolism are over 500-fold lower in monkeys than in mice when both are exposed to low airborne BD concentrations. Thus, using the blood concentration of 1,2-epoxybutene-3 in monkeys as the relevant measure of "delivered" dose, one can reasonably conclude that humans would experience over 500-fold lower cancer risks than would identically exposed mice.

Finally, the findings from epidemiologic studies of BD-exposed workers are fully consistent with the far smaller estimated risks that can be derived by using the measured blood concentrations of 1,2-epoxybutene-3 in monkeys for interspecies extrapolation. While NIOSH has characterized the epidemiologic evidence as being consistent with the findings from the mouse bioassays, direct quantitative comparisons of predicted cancer deaths with observe cancer mortality confirm the near certainty of NIOSH's having significantly overstated the human cancer risks arising from BD exposure.

ENVIRON has therefore concluded that the 1,2-epoxybutene-3 concentrations in the blood of BD-exposed monkeys and rodents provide the best measures of "delivered" dose that are presently available. These data, and the related mechanistic data regarding BD uptake

and retention, indicate clearly that direct use of airborne BD concentrations in risk calculations leads to substantial overstatement of the estimated human cancer risks from BD exposure.

In its risk assessment, NIOSH was highly critical of these data, citing a number of potential methodological and interpretative difficulties with them. NIOSH concluded that these data did not provide valid and relevant measures of BD exposure. Instead, NIOSH employed airborne BD concentrations directly for its risk computations, and, in addition, used the ratio of species body weights raised to the 3/4 power to determine "equivalent" BD doses in humans. This generic scaling factor may be appropriate for interspecies risk extrapolation in certain cases, specifically when chemical-specific data regarding uptake, distribution, and metabolism are unavailable. NIOSH's inappropriate use of this scaling factor served to further increase its estimates of human cancer risk by an approximately 6.5-fold factor.

To achieve a balanced and objective view of the potential human cancer risks posed by BD exposure, NIOSH and OSHA must give full and proper consideration to the mechanistic data regarding interspecies differences in the uptake, retention, and metabolism of BD. In the preamble to its proposed BD standard, OSHA (1990) has stated that such data should be employed for risk assessment purposes:

> "When pharmacokinetic data or metabolic data are available, these data should be used to estimate internal dose. By using all available information, the uncertainty associated with estimating risks across species can be reduced."

Use of these data will clearly yield far lower human cancer risks than NIOSH has estimated. Since the data regarding blood levels of 1,2-epoxybutene-3 provide the best measures of "delivered" dose that are presently available, it is ENVIRON's principal recommendation that NIOSH and OSHA base their estimated human cancer risks on these measurements rather than airborne BD concentration. If instead NIOSH and OSHA continue to base their estimates on calculated amounts of BD absorbed and/or retained, then they must fully and properly account for the differentials in BD uptake and retention that are known to exist between primates and rodents. In either case, a full and proper use of the available mechanistic data regarding BD uptake, retention, and metabolism is certain to yield more accurate estimates of the human cancer risks posed by BD exposure.

II. THE NIOSH APPROACH

NIOSH's "best" estimate of human cancer risk from occupational exposure to BD was derived from a one-stage Weibull time-to-tumor model fit to the prevalence of female B6C3F1 mouse alveolar-bronchiolar neoplasms in certain of the treatment groups from the National Toxicology Program's second BD inhalation bioassay (Melnick et al., 1990). As NIOSH noted, the tumor incidence data from this study must still be regarded as preliminary, since final review of the pathologic evaluations has yet to be completed.

NIOSH chose to employ the female mouse lung tumors for developing their "best" risk estimate because this was the most sensitive site, i.e., because other neoplastic endpoints in male or female mice produced lower estimates of human cancer risk. They also elected to treat these tumors as incidental. In other words, no animal was presumed to have died prematurely from these tumors. Other neoplasms for which NIOSH generated alternative risk estimates included male and female lymphomas and hemangiosarcomas of the heart (both fatal tumor analyses), as well as male and female squamous cell carcinomas of the forestomach, combined Harderian gland adenomas and adenocarcinomas, and combined hepatocellular adenomas and carcinomas (all incidental tumor analyses). In addition, estimates were developed for female mouse mammary gland adenocarcinomas and ovarian granulosa cell neoplasms (both incidental tumor analyses).

NIOSH also elected to disregard the tumor responses in the highest treatment group (625 ppm) from the Melnick et al. (1990) study during the model-fitting process, citing the "strikingly nonlinear" response in this group and the known sublinear metabolism of BD in mice at such high concentrations (Laib et al., 1988). NIOSH did, however, explore the impact of including this high dose group in their analyses. For the female lung tumors, the estimates with the high dose group included are approximately 30% higher than those obtained without it. An alternative analysis in which the female lung tumors were treated as rapidly fatal was also performed, and this again yielded somewhat higher estimates than did the incidental analysis which NIOSH preferred. NIOSH also considered several higher order multistage Weibull models (up to three-stage), but for female lung tumors, these appeared to collapse back to the simpler one-stage form during the model fitting process.

In effecting the extrapolation of risk from mice to humans, NIOSH assumed that both mice and humans would exhibit 100% uptake of the inhaled BD dose. They noted, however, that "Any non-zero value of percent uptake gives an identical final result, provided that uptake is the same in mice and humans." NIOSH also elected to employ a 3/4 power body weight ratio to adjust absolute BD doses (in mg/day) to "equivalency" between the two

species. In other words, NIOSH assumed that humans would experience an approximately 6.5-fold ($(70/.0398)^{1/4}$) greater risk than mice if both species received BD doses throughout life that were identical on a mg/kg/day basis.

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III. IMPLICATIONS OF MECHANISTIC DATA FOR HUMAN CANCER RISK

A. Data Regarding Retention of BD

In a recent report, Dahl et al. (1990) published findings that are highly relevant to the amounts of BD that are retained by rodents and primates following inhalation exposure. B6C3F1 mice and Sprague-Dawley rats had been previously exposed identically to 7 ppm BD for 6 hours (Bond et al., 1986). Male monkeys (Macaca fascicularis) were therefore exposed for 2 hours to 10 ppm BD for interspecies comparison purposes. When Dahl et al. normalized the amount of retained BD to the different species body masses and expressed retained BD on a per 10 ppm-hour basis, the measured BD retention rates were determined to be 5.27, 0.46, and 0.52 μmol/kg/hr/10 ppm in the mouse, rat, and monkey respectively. It should be noted here that the mouse retention value was incorrectly reported to be 3.3 μmol/kg/hr/10 ppm as a consequence of an typographical error in a previous paper (Bond et al., 1986) (personal communication from Dr. Alan Dahl).

It is clear from these observations that these species would not absorb and retain the same BD dose per unit body mass if they were identically exposed to the same airborne BD concentration. Rather, the mouse would retain a BD dose that is approximately 11-fold higher (5.3/0.46) than that retained by the rat, and 10-fold higher (5.3/0.52) than that received by the monkey. It is therefore reasonable to conclude on the basis of BD retention differences that rats and monkeys would be at significantly less risk of developing cancer than mice if all were identically exposed to the same airborne BD concentration because they would absorb and retain significantly less BD per kilogram of body mass than do mice.

Because the human species is much more closely related, both anatomically and physiologically, to the monkey than the mouse, it is entirely reasonable to expect that humans would also retain significantly less BD than would identically exposed mice. Indeed, were this the only relevant information available regarding BD disposition in different species, it would argue that the rat provides a better animal model than the mouse for evaluating human cancer risk from BD exposure.

The presence of the endogenous MuLV retrovirus in the B6C3F1 mouse and its activation by BD exposure raises additional questions with regard to the relevance to human cancer risk of any carcinogenic response in this mouse strain. While it is known that the retrovirus plays a significant modulating role in the incidence of thymic lymphoma (Irons, 1987), it is also possible that activation of the retrovirus by BD influences the incidence of other tumor types as well. Consequently, even though NIOSH has based its "best" estimates of risk on the incidence of lung neoplasms among female mice, there is still great uncertainty

regarding the relevance of these estimates to the potential human cancer risks from BD exposure, especially in quantitative terms. ENVIRON therefore continues to believe that scientifically defensible risk estimates can and should be derived from the Hazleton Laboratories rat study (HLE, 1981). Indeed, such estimates are likely to be less uncertain than any that could be obtained from either of the NTP mouse bioassays, even with appropriate corrections for the known high to low dose and interspecies differences in BD pharmacokinetics.

It is important to note here that in contrast to the NIOSH assessment, OSHA has relied on mouse BD retention data in generating its "best" estimates of risk (OSHA, 1990). EPA has also accepted the BD retention data reported by Bond et al. (1986) as providing a more relevant measure of exposure for risk assessment purposes than airborne BD concentration (EPA, 1985; Cote and Bayard 1990). A similar conclusion has also been reached recently by California's Air Resources Board in its health assessment of BD (CARB, 1991). NIOSH should therefore also adjust its human cancer risk estimates downward by an approximately 10-fold factor at a minimum so as to properly account for the smaller retention of BD by primates relative to the mouse. Clearly, it is altogether appropriate for NIOSH to make full and proper use of this important information in developing its "best" estimates of risk based on either the mouse or rat data.

B. Data Regarding BD Uptake

Very recently, additional highly relevant information regarding the uptake of BD by rodents and primates has been published in the peer-reviewed literature. Specifically, Dahl et al. (1991) exposed male cynomolgus monkeys (Macaca fascicularis) for 2 hours to 10, 310, and 7760 ppm of ^{14}C-labeled BD and estimated BD uptake as a percentage of the amount of BD inhaled. Uptake was determined by combining all radiolabeled materials excreted in urine and feces as well as via exhalation (other than BD itself) (only the residual ^{14}C that still remained in the monkey's bodies at the end of the 96 hour postexposure collection period was not included). Thus, the new Dahl et al. (1991) uptake data provide additional comprehensive measures of inhaled and absorbed BD doses. At approximately 10 ppm, monkey uptake was determined to be only 2.9% of inhaled BD, or 0.13 nmol/min kg^{-1} ppm^{-1}.

For comparison purposes, Dahl et al. also estimated BD uptake in Sprague-Dawley rats and B6C3F1 mice by graphical interpolation between the data points presented in Figure 4 of the earlier report by Laib et al. (1988). Dahl et al.'s BD uptake estimates for approximately 10 ppm exposures were 2.8 nmol/min kg^{-1} ppm^{-1}, or 15%, for rats, and 5.2

nmol/min kg^{-1} ppm^{-1}, or 12% for mice.

BD uptake in rodents may also be estimated directly from Table 1 of the Laib et al. (1988) report, which provides estimates of BD metabolized by rats and mice under the conditions of the two earlier bioassays (HLE, 1981 and NTP, 1984). It is important to note that because uptake is saturable, these estimates for high dose conditions are likely to understate rodent uptake at the lower BD doses of concern to NIOSH and OSHA. Using Laib et al.'s estimate of 140 μmol/kg per hour for rats exposed to 1000 ppm, we obtain an estimated uptake rate of 2.3 nmol/min kg^{-1} ppm^{-1}, or 12.7% of the BD inhaled. Using Laib et al.'s corresponding estimate of 165 μmol/kg per hour for mice exposed to 625 ppm, uptake is 4.4 nmol/min kg^{-1} ppm^{-1}, or 9.3% of the BD inhaled.

These estimates demonstrate clearly that BD uptake is nowhere near 100% in rats, mice, or monkeys, in contrast to the assumption made by NIOSH. The rat has the highest uptake of these three species, but that rate is nevertheless still only about 13% of the BD inhaled. Furthermore, if one compares these percentages across species, there is a significant difference between the rodents and the primate, with the monkey (2.9%) exhibiting 3-fold less uptake than the mouse (9.3%) and 4-fold less than the rat (12.7%).

The contrast between uptake in monkeys and mice is even more dramatic when it is expressed per unit body weight: BD uptake in the monkey (0.13 nmol/min kg^{-1} ppm^{-1}) is more than 30-fold smaller than that in the mouse (4.4 nmol/min kg^{-1} ppm^{-1}). Thus, assuming that BD uptake in humans is comparable to the BD uptake observed in monkeys, one can reasonably conclude that humans would experience more than 30-fold (4.4/0.13) lower cancer risks than mice when both are exposed identically to BD. Even allowing, as NIOSH did, for the difference between bioassay and occupational exposure regimens, simple correction for the differential in BD uptake percentages between mice and monkeys would lead to more than 14-fold lower estimated risks than NIOSH has projected with the generic 3/4 power body weight scaling rule for extrapolating estimated cancer risk between species.

C. Data Regarding DNA-Reactive BD Metabolites

The metabolism and pharmacokinetics of BD have been described in considerable detail by other commenters (see particularly the testimony before OSHA and additional posthearing comments of Dr. Michael Bird as well as ENVIRON's earlier BD reports (ENVIRON 1986, 1990)). Here we only summarize certain critical facts that appear to be highly relevant to the quantitation of estimated human cancer risk from BD exposure.

The first step in BD metabolism, oxidation of BD to the monoepoxide 1,2-epoxybutene-3, was first demonstrated with hepatic microsomes over ten years ago by

Malvoisin et al. (1979). Subsequently, these investigators demonstrated further oxidation in vitro of 1-2-epoxybutene-3 to 1,2:3,4-diepoxybutane, as well as the epoxide hydrolase-mediated reduction of 1,2-epoxybutene-3 to 3-butene-1,2-diol, followed by second oxidation step yielding 3,4-epoxy-1,2-butane-diol. The 1,2-epoxybutene-3 intermediate is a monofunctional alkylating agent, while 1,2:3,4-diepoxybutane is bifunctional and is known to form DNA-DNA crosslinks. Both of these metabolites of BD are DNA-reactive intermediates that also show mutagenic activity in bacterial and other test systems, while BD per se does not. Thus, both may play critical roles in the carcinogenicity of BD. It is therefore especially important to establish accurately the quantitative relationships between airborne BD concentrations and corresponding internal "delivered" doses of these BD metabolites for the relevant species.

An extensive series of studies by Bolt and colleagues (c.f., Laib et al. 1990) has confirmed the production of 1,2-epoxybutene-3 following in vivo BD exposure, and further established the saturable character, at least at high airborne BD concentrations (> 1000 ppm) of the initial conversion of BD to this monoepoxide in both rats and mice. These investigators also determined that at lower airborne BD concentrations, where linear pharmacokinetics prevail, mice metabolize BD to 1,2-epoxybutene-3 at about twice the rate as do rats. In addition, they determined that the subsequent metabolism of 1,2-epoxybutene-3 is saturable in mice at a far smaller rate (350 μmol/kg/hr) than in rats (> 2600 μmol/kg/hr). Taken together, these findings imply that internal concentrations of 1,2-epoxybutene-3 should reach much higher levels in mice than in rats when both are identically exposed to airborne BD. In fact, Laib et al. (1990) have concluded that the limited 1,2-epoxybutene-3 detoxication capacity of mice relative to that of rats is a critical determinant of the higher susceptibility of mice to BD-induced carcinogenesis. Knowledge of internal concentrations of 1,2-epoxybutene-3 is thus essential to the development of accurate estimates of the cancer risks posed by BD exposure.

The study of Bond et al. (1986) and the more recent studies by Sun et al. (1989) and Dahl et al. (1990 and 1991) have both developed significant new information regarding quantitative differences among species in the internal concentrations of several BD metabolites. The Sun et al. (1989) and Dahl et al. (1990 and 1991) studies are of particular interest, since they report comparative measurements of blood levels of the mutagenic 1,2-epoxybutene-3 obtained not only in mice and rats, but also in monkeys, following inhalation exposures to BD for 2 hours.

Specifically, B6C3F1 mice had been previously exposed to 7 and 70 ppm BD, while Sprague-Dawley rats had been exposed to 70 ppm BD (Bond et al., 1986). In addition, cynomolgus monkeys were exposed to 10 ppm BD (Sun et al., 1989; Dahl et al., 1990 and

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1991). These investigators determined that monoepoxide levels in the blood of mice and rats identically exposed to 70 ppm BD were 28.6 ± 0.7 and 5.7 ± 0.6 pmol/ml/ppm respectively (blood epoxide levels were normalized by airborne BD concentrations, i.e., expressed per ppm BD, so as to permit direct comparisons even when the different species were not identically exposed). Thus, at 70 ppm BD, mice exhibited approximately 5-fold higher 1,2-epoxybutene-3 levels in their blood than did rats. Even more importantly, in the concentration range of interest to NIOSH and OSHA, mice exposed to 7 ppm BD exhibited 85.7 ± 14.3 pmol/ml/ppm of 1,2-epoxybutene-3 in their blood, while monkeys exposed to 10 ppm exhibited only 0.16 ± 0.05 pmol/ml/ppm (Sun et al. (1989) Table 1). It must be noted here that the monkey blood level was inadvertently reported as 0.13 pmol/ml/ppm in Dahl et al. (1990). This error was corrected in the Dahl et al. (1991) report.

The findings from these studies indicate that mice developed approximately 536 ± 190-fold higher blood concentrations of 1,2-epoxybutene-3 than did similarly exposed monkeys. Thus, making the reasonable assumption that humans metabolize BD in the same manner as monkeys, the Sun et al. (1989) and Dahl et al. (1991 and 1991) results imply that humans should be approximately 536-fold less sensitive to BD's carcinogenicity than are mice.

A similar conclusion can be drawn from a comparison of the rat and monkey blood epoxide levels observed by Sun et al. (1989) and Dahl et al. (1990 and 1991). Specifically, rats exposed to 70 ppm exhibited 5.7 ± 0.6 pmol/ml/ppm blood epoxide, while monkeys similarly exposed to 10 ppm exhibited only 0.16 ± 0.05 pmol/ml/ppm blood epoxide, as noted above. Again, provided that humans metabolize BD in the same manner as monkeys (as has been indicated by the limited data reported by Schmidt and Loeser (1986)), these data show that humans should be more than 35 ± 11.7-fold less sensitive to BD's carcinogenicity than are rats.

When EPA conducted its risk assessment for BD (EPA, 1985), the best data available regarding internal doses of BD or its metabolites were the preliminary estimates of retained BD percentages obtained from a Lovelace Institute report (NTP 1985) that had not yet been published in the peer-reviewed literature. As was noted previously however, OSHA also utilized this preliminary information in constructing its estimates of human cancer risk. The new data of Sun et al. (1989) and Dahl et al. (1990 and 1991) regarding internal 1,2-epoxybutene-3 concentrations now provide a much superior alternative measure of "delivered" dose even relative to the updated BD retention data. This highly reactive and mutagenic epoxide metabolite is far more likely to be responsible for the carcinogenicity of BD than is BD per se. It is therefore strongly recommended that NIOSH and OSHA both utilize these new pharmacokinetic data in extrapolating their risk estimates from mice and rats to humans.

A summary of the approximate impacts on risk estimates of using the different measures of "delivered" BD doses discussed in this and preceding sections is provided in Table 1.

TABLE 1

Approximate Impact of Different Measures of "Delivered" Dose on NIOSH's Best Estimate of Cancer Deaths Per 10,000 Workers Exposed for a Working Lifetime to 2 ppm BD

NIOSH Current "Best" Estimate	597.
Adjusted for Retention	58.9
Adjusted for Uptake	11.6
Adjusted for BD Metabolism to 1,2-epoxybutene-3	1.1

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D. 3/4 Power Body Weight Scaling Between Species

As was noted previously, ENVIRON's principal conclusion is that the extensive pharmacokinetic data regarding species differences in BD uptake, distribution, and metabolism provide valid, useful, and highly relevant measures of BD exposure for risk assessment purposes. Nevertheless, NIOSH rejected use of these data and elected instead to employ airborne BD concentrations directly for its risk computations, calculating BD uptake assuming 100% absorption irrespective of the species being considered. In addition, NIOSH used the ratio of species body weights raised to the 3/4 power to extrapolate from rodent BD doses to "equivalent" BD doses in humans.

This particular allometric scaling rule has been suggested as plausible on empirical (Travis and White, 1988) and theoretical (O'Flaherty, 1989 and Travis et al., 1990) grounds (the latter specifically for direct acting carcinogens that do not require metabolic activation, as does BD), but only when specific pharmacokinetic data regarding chemical distribution and disposition are not available.

In fact, the available comparative data regarding BD uptake and metabolism across species directly contradict the predictions of this empirical scaling rule. Because rats and monkeys are much larger than mice, the 3/4 power body weight scaling rule predicts that these species should be more sensitive to BD than mice. Yet, as NIOSH has itself noted, exactly the opposite is the case: rats and monkeys are both markedly less sensitive to BD than mice, first because mice are remarkably efficient in metabolizing BD to 1,2-epoxybutene-3, and second, because they are remarkably inefficient at detoxifying this highly DNA-reactive intermediate of BD metabolism. This issue is discussed in considerably greater detail in the comments of Bird (1991), Bolt (1990), and Cagen (1991). The clear implication of the well-established interspecies differences in BD uptake and metabolism is that the 3/4 power body weight scaling rule is altogether inappropriate for use in interspecies extrapolation of estimated BD cancer risks.

IV. COMPARISON OF PREDICTED AND OBSERVED HUMAN RISKS

In its 1990 assessment, OSHA expressed concern that by relying upon the female mouse heart hemangiosarcoma data for its "best" estimate of risk, it might be underestimating BD's carcinogenic potential. Indeed, it called attention to the fact that its "best" risk estimate of 128/10,000 at 10 ppm BD was lower than almost all of the other estimates that had previously been derived from the first NTP mouse bioassay. Now NIOSH has produced "best" estimates of human cancer risk that are significantly higher than OSHA's, and it has called attention to the "consistency" of the increased incidence of lymphopoietic neoplasms seen in certain epidemiologic studies of exposed worker populations with the high lymphoma incidence observed in the two mouse bioassays. NIOSH did not however undertake a quantitative comparison of its risk estimates with existing human cancer mortality data. Such a comparison can prove useful in objectively assessing the plausibility of NIOSH's "best" estimates of human cancer risk from BD exposure.

EPA (1985) described a consistency check for its "point" estimate of lifetime cancer risk using the Meinhardt et al. (1982) and Matanoski et al. (1982) studies of worker mortality in the styrene-butadiene rubber (SBR) industry. Results from similar quantitative comparisons have also been reported previously by ENVIRON (1986 and 1990) and Acquavella (1990). For example, Table 2 presents the probabilities of observing as few or fewer deaths from any cancer, respiratory tract cancer, or lymphopoietic cancer as were actually observed among the 3,124 SBR production workers as reported by Matanoski et al. (1990), assuming that the true cancer risks arising from this group's BD exposures were equal to the NIOSH "best" estimates for 10, 5, 2, or 1 ppm BD, but with exposure only for 10 of 50 working years and with followup only for 21 of the 50 remaining years of life. As has been discussed previously (ENVIRON, 1986 and 1990; Acquavella, 1990), BD exposure levels in the SBR industry probably averaged 10 ppm or higher for these workers, so the predicted numbers of deaths appearing in Table 2 will most likely understate the extent of any inconsistencies between the observed number of cancer deaths and NIOSH's predictions.

Nevertheless, it is clear that NIOSH's "best" risk estimates are altogether inconsistent statistically with the observed numbers of deaths in this SBR worker group from any cancer, respiratory tract cancer, or lymphopoietic cancer, even with exposure levels as low a 1 ppm. Similar conclusions were reached by Acquavella (1991) in his objective comparison of NIOSH "best" estimates of risk with the observed cancer mortality in the World War II Texaco subcohort described by Divine (1990).

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It is important to note in this regard that the significant inconsistencies between observed and NIOSH-predicted cancer deaths are readily apparent despite Matanoski et al.'s acknowledged understatement of the expected number of deaths in the absence of BD exposure (Matanoski et al., 1990), and despite not having considered the maintenance workers from their study in this comparison. Had the subgroups of production and maintenance workers been combined appropriately, the inconsistencies between observed and NIOSH-predicted cancer deaths in this larger group would have been even more extreme than those presented in Table 2.

The epidemiologic observations thus indicate that NIOSH has significantly overstated the human cancer risks arising from BD exposure. In fact, the NIOSH "best" estimates are altogether inconsistent with the actual observations in the Matanoski et al. (1990) production workers even assuming that BD exposures averaged about 1 ppm. While the epidemiologic data are not sufficiently powerful to categorically reject predicted risks as small or smaller than OSHA's previous "best" estimate, they are also entirely consistent with the far smaller risks that are predicted by full and proper utilization of the BD absorption, retention, and metabolism differences that are now known to exist between rodents and primates.

TABLE 2

Consistency Check of NIOSH BD Cancer Risk Estimates
Based on Observed Cancer Deaths Among
3,124 Production Workers
(Matanoski et al., 1990)

	All Cancers	Respiratory Tract Cancers	Lymphopoietic Cancers
Observed Deaths	124	49	19
Expected Deaths with 10 ppm	205.3	116.5	82.5
p-value	2.5×10^{-10}	7.4×10^{-13}	3.2×10^{-17}
Expected Deaths with 5 ppm	172.7	83.9	49.9
p-value	1.8×10^{-5}	1.1×10^{-5}	3.2×10^{-7}
Expected Deaths with 2 ppm	151.4	62.6	28.6
p-value	0.0025	0.012	0.015
Expected Deaths with 1 ppm	143.9	55.1	21.1
p-value	0.0084	0.040	0.082

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Genevieve Shiroma, Chief
Toxic Air Contaminant
Identification Branch
California Air Resources Board
P.O. Box 2815
Sacramento, California 95812

Dear Ms. Shiroma:

Attached, please find General Motors comments on the California Air Resources Board revised draft report titled Proposed Identification of 1,3-Butadiene as a Toxic Air Contaminant, dated October 1991. General Motors comments focus on the following three points:

- The report does not properly inform the reader that the risk from motor vehicle 1,3-butadiene emissions has been reduced dramatically over the past 20 years and will continue to decrease substantially over the next 20 years from fuel and vehicle regulations already on the books.

- The report does not provide the proper perspective on the risk from motor vehicles relative to the much larger risks from indoor sources, particularly that of both smokers and nonsmoker from cigarette smoke and environmental tobacco smoke; and

- The report should better portray the limitations and uncertainty associated with the range of unit risk factors developed for 1,3-butadiene.

If you have any questions concerning these comments, please contact J. M. Heuss of my staff at 313-947-2787.

Sincerely,

Samuel A. Leonard, Director
Automotive Emission Control

000740

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SAFETY BELTS SAVE LIVES

GENERAL MOTORS COMMENTS ON PROPOSED IDENTIFICATION OF 1,3-BUTADIENE AS A TOXIC AIR CONTAMINANT BY THE STATE OF CALIFORNIA SRP VERSION DRAFT, OCTOBER 1991

General Motors (GM) has reviewed the October 1991 draft prepared by the Staffs of the Air Resources Board and the Office of Environmental Health Hazard Assessment and offers the following comments. While some of the recommendations provided by GM on the preliminary draft (SM-2059, letter from S. A. Leonard to Genevieve Shiroma, March 22, 1991) have been incorporated in the new draft, GM is still concerned that the risk from present and future 1,3-butadiene concentrations is not put into the proper perspective. First, the limitations and uncertainty associated with the range of unit risk factors developed for 1,3-butadiene should be better portrayed to the reader, especially in the Executive Summary. Second, additional detail should be added to inform the reader that the risk from motor vehicle 1,3-butadiene emissions has been reduced dramatically over the past 20 years and will be reduced substantially over the next 20 years from fuel and vehicle regulations already on the books. Third, additional perspective should be added on indoor exposures, in particular to the substantial exposures of both smokers and nonsmokers to direct cigarette smoke and environmental tobacco smoke (ETS).

Comments on Executive Summary

Risk Assessment. GM is concerned that the uncertainties and limitations of the risk calculations in Part B are not put in the correct perspective for the reader. The Part B Summary refers to "theoretical human risks associated with a continuous lifetime exposure to butadiene in ambient air." Unit risks based on rat and mouse inhalation studies varied by two orders of magnitude. Therefore, Part B indicates that "community exposure to ambient butadiene at currently detected levels could be associated with an upper limit of 4 to 666 additional lifetime cancers per million exposed individuals. While some of these important caveats to the risk calculation are put in the Executive Summary, there is still a statement that "An estimated 3,936 1,3-butadiene-induced cancers statewide are expected to occur at average ambient concentrations." Because statements like this can be taken out of context, the words "upper limit" should be included in each reference to the risk.

GM is concerned that there is such a wide range (nearly two orders of magnitude) in the upper limit risk based on different animal

models. The large uncertainty in the upper limit risk means that there is the same large uncertainty in judging the need for and cost-effectiveness of any additional measures to reduce the risk. It is, therefore, extremely important to establish which animal model more closely approximates the risk to humans. A better understanding of the mechanism or mechanisms of 1,3-butadiene carcinogenicity is thus of paramount importance. The reader of the Executive Summary should be clearly informed of the wide range in the upper limit risk, and the reasons for it.

GM is concerned that the upper limit risk calculations in Part B do not provide an assessment of the actual risks that Californians experience as they are exposed to 1,3-butadiene in their daily lives. A knowledge of the relative contributions of individual sources is important for better estimates of public exposure. In the 1980's, the U.S. EPA introduced a new conceptual model of total human exposure (TEAM) and used it to compare benzene emissions vs. exposures. The analysis indicated that although motor vehicles emitted 82 percent of benzene emissions, they were responsible for only 18 percent of individual exposures based on personal monitoring.[1] The TEAM study showed how significant individual sources of exposure are because the general public spends most of its time indoors.

GM reiterates its recommendation that as ARB evaluates the risk from exposure to 1,3-butadiene and other airborne toxics, it consider the findings and recommendations from several important reports and reviews by eminent scientific groups. In particular, EPA's Science Advisory Board (SAB) has recently recommended that the Agency should target its environmental protection efforts on the basis of opportunities for the greatest risk reduction.[2] In order to set priorities, the SAB indicated that the Agency "must weigh the relative risks posed by different environmental problems, determine if there are cost-effective opportunities for reducing those risks, and then identify the most cost-effective risk reduction options." Further, the SAB recommended that EPA improve the data and analytical methodologies that support risk assessment and comparison, indicating that risk rankings should be based on total human exposure to specific toxic agents.

A recent National Research Council (NRC) review of human exposure assessment for airborne pollutants came to similar conclusions.[3] The NRC committee wrote that "risk reduction strategies that address only outdoor air are only partially effective. Such strategies need to be modified to better address the importance of indoor exposures." To set priorities for reducing risk to potentially harmful pollutants, the committee indicated that all

media and all routes of exposure must be assessed.

In response to such recommendations, a high-level interagency working group has been formed at the federal level to coordinate risk assessment practices across different federal agencies, under the auspices of the Office of Science and Technology Policy (OSTP) Federal Coordinating Council on Science, Engineering and Technology. The goal is to improve, harmonize, and minimize the uncertainties in risk assessment. Among the activities is a review of the 1985 OSTP Document on Carcinogenicity.

Under Section 206 of the Clean Air Act, the U.S. Environmental Protection Agency is carrying out a study of the need for controlling emissions of mobile-source related toxics including 1,3-butadiene. A workshop to outline the research needs for 1,3-butadiene and other mobile-source toxics will be part of that effort. GM urges the ARB to participate in and coordinate its efforts with these federal efforts to improve, harmonize, and reduce the uncertainty in risk assessment.

As ARB enters the risk management phase of its consideration of 1,3-butadiene and other airborne toxics, GM recommends that decisions be made based on improved methodologies for risk assessment that account for the real exposures that Californians experience as they move about in their daily life rather than on theoretical upper limits for exposure scenarios that are not realistic.

Emission Trends. The question "Are emissions of 1,3-butadiene expected to increase in the state?" should be restated as "What are past, current, and expected future emissions of 1,3-butadiene?" The answer to the question should document the significant emission reductions that have occurred over the past 20 years and are expected over the next 20 years. On page 573 of Part C, in response to the earlier GM comments on this issue, the statement is made that "Staff agree that hydrocarbon concentrations have been dramatically reduced in the Los Angeles Basin since the 1960's as the direct result of increased motor vehicle emissions control and reductions in emissions from industrial sources." This statement should be part of the Executive Summary, not relegated to page 573 of Part C.

Evidence that current ambient concentrations are only a fraction of the ambient concentrations that existed 20 years ago should be included in Part A. Altshuller, et al.[4] measured individual hydrocarbons in several hundred samples collected in Los Angeles over several months in the fall of 1967 and reported 1,3-butadiene

concentrations averaging 2 ppbv with 10 percent of the values exceeding 5 ppbv. Similarly, the average 1,3-butadiene concentration in 218 samples analyzed by the Los Angeles Air Pollution Control District in 1965 was 2 ppbv.[5] Thus, the current 1,3-butadiene concentrations in Los Angeles are roughly one-quarter of the concentrations measured in the middle-to-late 1960's. This dramatic reduction in ambient concentrations occurred in spite of increasing population, numbers of vehicles or vehicle miles travelled and is a clear indication of the success of the emission controls on motor vehicles.

As noted in the comments on Part A, the continuing reductions in emissions anticipated with current regulations should be quantified and summarized in the Executive Summary. In particular, the 26 to 29 percent reduction in emissions from gasoline-powered motor vehicles anticipated when Phase 2 gasoline is introduced should be highlighted. The combination of dramatic reductions to date and continuing reductions over the next 20 years provides important information for the reader that puts the risks from ambient exposures in a temporal perspective.

Indoor Exposures. In response to the question "What about indoor exposure to 1,3-butadiene?," the Executive Summary indicates that indoor air may be the major route of exposure to 1,3-butadiene for those individuals exposed to a heavy smoking environment. Based on the available data it is clear that indoor air <u>is</u> the major route of exposure to 1,3-butadiene for smokers as well as those exposed to a heavy smoking environment and that exposure to environmental tobacco smoke (ETS) <u>is</u> a major route of exposure to 1,3-butadiene for Californians. An estimate of the average daily intake of 1,3-butadiene from ETS is made in the comments to follow; it is 2.8 times the daily intake from the population-weighted statewide average ambient 1,3-butadiene concentration. GM recommends that the answer to this question include comparisons of the average daily intake of 1,3-butadiene from smoking, typical exposures to ETS, heavy exposure to ETS, and exposure to ambient air.

The statement that non-smoking residential exposures may typically be close to the statewide average ambient exposure is not supported by the data provided. The analytical technique used in the Woodland study did not have sufficient sensitivity to discriminate between residential exposures above, at, or below the statewide ambient average concentration. Therefore, the statement should be removed and replaced with one that acknowledges that there is not enough information to make a quantitative comparison between ambient exposures and non-smoking residential exposures.

Comments on Part A Exposure Assessment

Emissions. In previous comments (SM-2059), GM recommended that data from the Auto/Oil Air Quality Improvement Program (AQIRP) be used to provide a better basis for statistically robust mobile source inventories. Instead, ARB staff used unpublished data[6] to estimate that 1,3-butadiene emissions from catalyst-equipped vehicles are 0.59 weight percent of Total Organic Gas (TOG) emissions. GM continues to believe that it is important to include the AQIRP data, especially because a portion of the data was obtained from vehicles operating on the "industry average gasoline" that ARB has accepted for use in calculating reactivity adjustment factors. The extensive AQIRP data on industry average gasoline for both current and older vehicle fleets demonstrate that 1,3-butadiene is a smaller percentage of TOG than the unpublished ARB data indicate. For example, the average wt. percent of 1,3-butadiene for current (1989) catalyst-equipped vehicles is 0.38 percent of TOG and for older (1983-1985) catalyst-equipped vehicles it is 0.34 percent.[7] The massive additional data on reformulated gasolines also suggests that 1,3-butadiene is between 0.3 and 0.4 wt. percent of TOG rather than 0.59, adding substantial credence to use of the AQIRP database. Inclusion of the AQIRP data would lower the estimate of emissions from catalyst-equipped vehicles substantially.

GM made a recommendation in its March 1991 comments that can help test the accuracy of the mobile source inventory. GM pointed out that:

> "There is another way that the SCAQS data can be used -- to verify overall estimates of 1,3-butadiene emissions from vehicular sources. The SCAQS data can be used to estimate the fraction of 1,3-butadiene in ambient non-methane organic carbon concentrations. In a similar data base involving several hundred samples collected in 1987 in 32 cities, Lonneman found that 1,3-butadiene represented 0.22 wt percent of the carbon in the samples.[8] The analogous fraction of carbon in the SCAQS samples can be used to check the estimated statewide emissions of 1,3-butadiene."

GM recommends that early morning SCAQS 1,3-butadiene/TOG ratios be analyzed to shed light on the ARB's estimated inventory.

The staff response (on page 567 of Part C) to GM's earlier recommendation to use the AQIRP data neglects to mention the industry average gasoline data and indicates that the gasolines used in AQIRP are prototype. However, the ARB's recent Phase 2

gasoline regulatory package used the AQIRP data to estimate that a 26 to 29 percent reduction in 1,3-butadiene emissions will be associated with Phase 2 gasoline.[9] Thus Phase 2 gasoline, because it will be introduced throughout the state and used throughout the vehicle fleet, will substantially reduce statewide 1,3-butadiene emissions.

The anticipated success of phase 2 gasoline in reducing the emissions of and risk from 1,3-butadiene raises the possibility that additional changes to gasoline may provide cost-effective reductions in 1,3-butadiene emissions. As Part A correctly points out, 1,3-butadiene is not a significant component of gasoline, rather it is formed during the combustion of other components. Based on the known mechanism of hydrocarbon oxidation at temperatures representative of the blowdown and exhaust processes in an engine,[10] 1,3-butadiene should be produced principally by hydrogen atom abstraction from a saturated carbon atom on a straight-chain alkene. Dryer and Brezinsky[11] provide evidence for such a mechanism in experiments that show that 1,3-butadiene is a significant intermediate oxidation product on n-octane but not of its isomer 2,2,4-trimethlypentane. Another route to butadiene could involve decomposition of a butene. 1,3-butadiene can also be formed from the partial oxidation of aromatic hydrocarbons. Venkat, et al.[12] have shown that butadiene is present as an intermediate in the oxidation of benzene, toluene, and ethylbenzene.

GM recommends that information on the formation of 1,3-butadiene be included in Part A, both in terms of the chemical mechanisms involved and the experimental findings from the AQIRP that have identified the effects of changing variables such as olefins, aromatics, T90, etc. An understanding of the mechanisms of 1,3-butadiene formation may lead to further composition changes that can materially reduce 1,3-butadiene.

There is an additional small source of 1,3-butadiene emissions that should be included in the inventory. The draft report alludes to the possibility that 1,3-butadiene may be released to the environment as tires wear, but indicates that there is not enough information to support or deny the theory. In contrast, Cadle and Williams[13] positively identified 1,3-butadiene and five other monomers and dimers of styrene-butadiene rubber copolymers as gaseous emissions from tire wear. Although the emission rate of 1,3-butadiene was below 0.1 mg/km/tire in average wear conditions, this small emission should not be neglected. The results presented by Cadle and Williams lump 1,3-butadiene and isoprene emissions together, but 1,3-butadiene was positively identified in additional

chromatographic separations.

Emission Projections. The text correctly indicates that 1,3-butadiene emissions are expected to steadily decrease through 2010 with the current regulations. However, it would be useful to provide a quantitative estimate of the decrease to put the risk from current and expected future exposures in perspective. The recent Phase 2 gasoline regulatory package included estimates of future trends in emissions of Volatile Organic Compounds, showing a reduction from 1400 tons per day in 1987 to 260 tons per day in 2010 for on-road gasoline-powered motor vehicles.[14] With such substantial reductions anticipated from existing regulations, the accompanying 1,3-butadiene reductions should also be substantial.

Ambient Exposures. GM continues to believe that the individual organic species data from SCAQS should be analyzed in terms of spatial and temporal variability to provide input for more refined exposure analyses. The staff comments (Part C, page 572) indicate that the risk management phase of the 1,3-butadiene project will consider all sources for their impact on the population. In order to properly account for the population exposure to 1,3-butadiene, spatial and temporal differences in ambient concentrations as well as indoor concentrations will need to be taken into account.

Along the same lines, GM is concerned that ambient exposures from urban monitoring is used to characterize the total population of California. A portion of the population resides in more rural locations where the 1,3-butadiene concentrations are expected to be below typical urban levels. If this is taken into account, the statewide average ambient exposure and accompanying risk would be reduced somewhat.

Indoor Concentrations. GM is encouraged that the current draft includes additional information on exposures to 1,3-butadiene from indoor sources such as environmental tobacco smoke. However, the statement that "it appears reasonable to assume that residential exposures ... may typically be close to ambient levels" is not supported by the data provided. Because the detection limit used in the Woodland study (0.54 ppb) is significantly above the statewide average ambient exposure, no statement about whether residential exposures are above or below ambient can be made. GM strongly encourages ARB to carry out a study of residences, offices, and commercial spaces using a technique that has a detection limit similar to that of the Woodland pilot study (0.05 ppb). Such measurements, in conjunction with estimates of smoking, would go a long way toward establishing the exposures that

Californians experience during the 80 percent or so of the time they are indoors as well as the sources of those exposures.

The draft indicates that there is not sufficient information to make a quantitative analysis of total human exposures at this time. Nevertheless, the draft includes reference to several data sources that can be used to provide some perspective on the exposures from ETS. Obviously, for the 30 percent of persons over 18 that are current smokers, the exposure to 1,3-butadiene from smoking overwhelms that from any other sources. For the roughly equal portion of the population that are former smokers, their lifetime exposure to 1,3-butadiene has probably also been dominated by their smoking experience. For non-smokers, the exposure to ETS is not insignificant. Survey information referenced in the preliminary draft report indicated that Californians are exposed to ETS approximately 2.6 hours each day on the average. Because this is (probably) self-reported exposure, it represents exposure over some threshold. In addition, the draft indicates that about four percent of California residents reported attending bars and nightclubs. Lofroth, et al.[15] have estimated that the inhaled dose in two hours in such conditions is in the range of 18 to 32 ug/exposure. For comparison, the daily inhaled dose from the statewide ambient concentration reported in the draft is 16.4 ug. Lofroth, et al. also report measurements of the airborne yield of 1,3-butadiene from sidestream smoke of 400 ug/cigarette. When one considers that roughly 70 billion cigarettes are smoked in California each year -- for the most part indoors -- the potential for significant exposure of non-smokers to 1,3-butadiene from ETS is apparent.

While GM agrees that there is not sufficient information to make a complete, quantitative analysis of the risk from outdoor vs. indoor sources at this time, GM submits that there <u>is</u> sufficient information to estimate total daily intake of 1,3-butadiene from ETS -- or passive smoking as it is also called -- when the information in Lofroth, et al. is combined with the extensive body of existing information on the exposure to ETS. Estimates of the typical daily intake of various toxic constituents of cigarette smoke for both active and passive exposure were provided as an attachment to SM-2059 (Part C, page 357, Table C-3 from Appendix C of EPA's May 1990 review of the health effects of passive smoking).[16] While it is recognized that exposure to ETS varies widely due to differences in the rate of smoking, types of cigarettes smoked, room volumes, and ventilation rates in indoor environments, the EPA calculated a "typical" exposure condition using representative values of the composition of both mainstream and sidestream cigarette smoke from the NRC assessment of the

health effects of ETS.[17] The typical exposures in Table C-3 are consistent with the average concentrations of several airborne components of ETS measured in real indoor settings.

Using the NRC reported value for the average emission rate of respirable suspended particulate matter (RSP) per cigarette in sidestream smoke, 26 mg, EPA calculated a daily intake for passive exposure of 3 mg. Using the same methodology for 1,3-butadiene, with an emission rate of 400 ug per cigarette, one can calculate the average daily intake of a passive smoker to be 46 ug. This can be compared to an average daily intake of 16.4 ug for an individual exposed for 24 hours to the average ambient concentration of 0.37 ppbv (0.82 ug/m^3). Thus the typical exposure to 1,3-butadiene from passive smoking exceeds that of typical outdoor concentrations. GM recommends that calculations of 1,3-butadiene exposure from smoking and from passive exposure to ETS be included in Part A and in the Executive Summary to provide perspective for the reader.

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3. Human Exposure Assessment for Airborne Pollutants: Advances and Opportunities, Committee on Advances in Assessing Human Exposure to Airborne Pollutants, Board on Environmental Studies and Toxicology, Commission on Geosciences, Environment, and Resources, National Academy Press, 1991.

4. A. P. Altshuller, W. A. Lonneman, F. D. Sutterfield, and S. L. Kopczynski, "Hydrocarbon Composition of the Atmosphere of the Los Angeles Basin," Environ. Sci. Technol., 5, 1009 (1971).

5. U. S. Department of Health, Education, and Welfare, National Air Pollution Control Administration, "Air Quality Criteria for Hydrocarbons," Publication No. AP-64, Table 3-4, page 3-8, March 1970.

6. ARB, 1991a. Internal ARB memorandum from K. D. Drachand to Terry McGuire regarding butadiene emission factors, July 17, 1991, Mobile Source Division, El Monte, CA.

7. The exhaust mass emission data are presented in AQIRP Technical Bulletin No. 1, December 1990; the 1,3-butadiene data are presented in Technical Bulletin No. 5, June 1991.

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II.

Air Resources Board Staff Responses to Summarized Comments on the SRP Version Draft Part A and the Executive Summary

Air Resources Board Staff Responses to Summarized Comments on the SRP Version Draft Part A and the Executive Summary

- **Chemical Manufacturers Association (CMA)**, December 6, 1991.

Comment: The ARB staff only provided a 30-day period for submission of comments on the SRP Version of the draft report. This was not sufficient time for preparation of comprehensive comments on the new risk assessment. We recommend that whenever a new risk assessment is presented, the ARB staff provide at least 60 days for comment.

Response: The public and industry have been given a number of opportunities to comment on the proposed identification of 1,3-butadiene as a toxic air contaminant. The initial public request for information was made in January 1988. 1,3-Butadiene was entered into the identification process in September 1988. Based on information from the scientific literature and the public request, the ARB and OEHHA staffs prepared an initial draft of the 1,3-butadiene report. This report was released to the public in January 1991 for a 30-day review period. In March 1991 the ARB and OEHHA staff conducted a public workshop on the document. Based on the comments received during the first comment period and the workshop, the report was revised and the best value was lowered. In November 1991 the report was re-issued for another 30-day comment period and was submitted to the SRP on January 3, 1992. If the SRP finds the 1,3-butadiene report acceptable, the final version of the report will be issued for a 45-day public comment period before the Board hearing.

- **General Motors (GM)**, December 6, 1991

Comment 1: The SRP Version of the draft report does not properly inform the reader that the risk from motor vehicle 1,3-butadiene emissions has been reduced dramatically over the past 20 years and will continue to decrease substantially over the next 20 years from fuel and vehicle regulations already on the books. While the text correctly states that 1,3-butadiene emissions are expected to steadily decrease through 2010 with the current regulations, it would be useful to provide a quantitative estimate of the decrease to put the risk for past, current and future exposures in perspective.

Response: California's toxic air contaminant program, as mandated by Health and Safety Code Section 39650 et seq., requires that the ARB identify and control substances that "may pose a present or potential hazard to human health." The purpose of the substance evaluation by staff of the ARB and OEHHA during the identification phase is to determine present exposure and risk. Furthermore, a quantitative estimate of future exposures cannot accurately be made because of the number of variables involved. For example, information obtained through the US EPA's SARA 313 program indicate that 1,3-butadiene usage in 1991 has increased by 55 percent over 1990 usage. That increase may or may not result in increased emissions to the atmosphere, and staff do not know if the usage increase will continue. Additionally, economic factors are having an impact on the replacement of older vehicles (that emit more 1,3-butadiene than newer vehicles) in California's passenger vehicle fleet.

Comment 2: Evidence that current ambient 1,3-butadiene concentrations are only a fraction of the ambient concentrations that existed 20 years ago should be included in Part A and the Executive Summary. The continued reductions should be quantified and summarized in the documents, in particular those resulting from the 26 to 29 percent reductions in emissions from gasoline-powered motor vehicles anticipated when Phase 2 gasoline is introduced.

Response: As discussed in the response to comment 1, the focus of the "identification report" is on present risk. Presently, California's air is being impacted by non-Phase 2 gasoline combustion products. Future motor vehicle emissions of 1,3-butadiene are expected to be reduced per vehicle as a result of Phase 2 gasoline usage, however, there will continue to be additional vehicles introduced to California as the population increases.

Comment 3: The Executive Summary question "are emissions of 1,3-butadiene expected to increase in the state?" should be restated as "what are past, current, and expected future emissions of 1,3-butadiene?." The answer to the new question should document the significant emission reductions that have occurred over the past 20 years, and are expected over the next 20 years. Staff responses to the earlier GM comments on this issue should be part of the Executive Summary, not relegated to page 573 of Part C.

Response: Please see staff responses to comments 1 and 2.

Comment 4: GM recommends that information on the formation of 1,3-butadiene be included in Part A, including the chemical mechanisms involved and the experimental Auto/Oil Air Quality Improvement Program findings that have identified the effects of changing fuel variables. An understanding of the mechanisms of 1,3-butadiene formation may lead to further fuel composition changes that materially reduce 1,3-butadiene.

Response: Information on the formation of 1,3-butadiene during combustion of petroleum fuels will be added to the Board Version of Part A. The experimental Auto/Oil data has not been added to Part A, however, that data will be used by staff during the risk management phase if 1,3-butadiene is identified by the Board as a toxic air contaminant.

Comment 5: The draft report states that 1,3-butadiene may be released to the environment as tires wear, but indicates that there is not enough information to support or deny the theory. Cadle and Williams have positively identified 1,3-butadiene and five other monomers and dimers of styrene-butadiene rubber copolymers as gaseous emissions from tire wear. Although the emission rate for 1,3-butadiene/isoprene was below 0.1 mg/km/tire in average wear conditions, this small emission should not be neglected.

Response: Based on the information found in Cadle and Williams' study of automobile tire emissions (S. H. Cadle and R. L. Williams, Gas and Particle Emissions from Automobile Tires in Laboratory and Field Studies, J. Air Pollut. Control Assoc., 28, 502, 1978.), the Part A has been

revised to state that 1,3-butadiene is emitted to the environment as tires wear. A statewide emission estimate for 1,3-butadiene from tires has not been developed for the Part A since 1,3-butadiene and isoprene emissions were quantified together in the laboratory study. The ARB staff will continue to investigate tires as a source of 1,3-butadiene emissions.

Comment 6: The US EPA's Science Advisory Board has recently recommended that the US EPA target its environmental protection efforts on the basis of opportunities for the greatest risk reduction. The US EPA were told that they should weigh the relative risks posed by different environmental problems, determine if there are cost-effective ways to deal with the problems, and then identify the most cost-effective risk reduction options. Additionally, risk rankings should be based on total human exposure to specific toxic agents.

Response: Staff has previously responded to this comment in Part C, page 569, paragraphs 2 and 3. The response: "The 1990 Amendments to the Clean Air Act list 189 substances (e.g., 1,3-butadiene) as hazardous air pollutants (HAPs), all of which must be evaluated by the US EPA in a risk management program. It was in this context that the SAB advised the US EPA to target their risk management efforts towards the substances (HAPs) that represent the greatest public risk.

California's risk assessment/risk management program is (by law) separate and distinct. First, substances are evaluated in an "identification" phase where it is demonstrated that there is public exposure to a substance which results in an increased public risk. The second step is the "control" phase where risk reduction measures are developed for the identified toxic air contaminants (TACs). California law (California Health and Safety Code Section 39655) requires the ARB to identify all HAPs as TACs."

During the control phase (similar to the US EPA), individual and multi-compound risks are assessed, and corresponding cost-effective reduction options are considered.

Comment 7: As the ARB enters the risk management phase of its consideration of 1,3-butadiene and other airborne toxics, GM recommends that decisions be made based on improved risk assessment methodologies rather than on theoretical upper limits for unrealistic exposure scenarios.

Response: Staffs of the ARB and the OEHHA use the risk assessment methodology outlined in the Guidelines for Chemical Carcinogens: Risk Assessment and Their Scientific Rationale, 1985. While staff endeavor to improve the risk assessment methodologies, the California Health and Safety Code (Section 39650e) states that "while absolute and undisputed scientific evidence may not be available to determine the exact nature and extent of risk from toxic air contaminants, it is necessary to take action to protect public health."

The focus of the risk management phase is directed toward control of emissions from particular types of sources. Exposure scenarios are based

on measured variables such as flow and usage rates, stack parameters, emission factors, and impacted population.

Comment 8: GM urges the ARB to participate in and coordinate its efforts with the federal efforts to improve, harmonize, and reduce the uncertainty in risk assessment. For example, the US EPA is carrying out a study and workshop on the need to control mobile-source related toxics, including 1,3-butadiene. Also, the Office of Science and Technology Policy's 1985 Document on Carcinogenicity will be reviewed by a number of federal agencies as part of an effort to coordinate risk assessment practices.

Response: The risk assessment/risk management process used by the staffs of the ARB, the local air pollution control districts, and the OEHHA is the result of Californian state law (AB 1807 Assemblywoman Tanner, 1983). This California process is presently under review to assess options for dealing with the uncertainties. Public workshops will be held to develop future guidelines and policy. Federal guidelines and policies are included in any state review of California's risk policies. Furthermore, the staff of the ARB and OEHHA regularly work with the US EPA during the development of risk control strategies and risk assessments, and will continue to do so.

Comment 9: In previous comments GM recommended that data from the Auto/Oil Air Quality Improvement Program (AQIRP) be used to provide a better basis for statistically robust mobile source inventories. Instead, ARB staff used unpublished data to estimate that 1,3-butadiene emissions from catalyst equipped vehicles are 0.59 weight percent of total organic gas (TOG) emissions. The AQIRP data demonstrates that 1,3-butadiene is a smaller percentage of TOG (0.38 for 1989 catalyst equipped vehicles and 0.34 for 1983-1985 catalyst equipped vehicles) than the ARB data indicate. The massive additional data on reformulated gasoline also suggest that 1,3-butadiene is between 0.3 and 0.4 percent of TOG, adding substantial credence to the use of the AQIRP data.

Response: The data (available to the public) used by the ARB staff to develop 1,3-butadiene emission estimates for catalyst-equipped light-duty passenger vehicles was derived from ARB emissions testing of 62 in-use passenger vehicles taken (with the exception of 1 vehicle) from the Light-duty Vehicle Surveillance Program. The vehicles were emissions tested in "as-received" condition (no tune-ups, tune-downs, or fuel changes), and were selected to represent, as near as possible, the on-road vehicle population. The emission factor is a composite of the mean value of all of the tested vehicles. Although the AQIRP data and emission estimates are based on fewer vehicles and "industry average" gasoline, the results of the analysis are interesting and useful for comparison with the ARB data. The AQIRP information has been used by ARB staff in the development of the Phase 2 reformulated gasoline regulatory package to estimate reductions in the per-vehicle emissions of 1,3-butadiene when Phase 2 gasoline is sold statewide in 1996.

Comment 10: The staff response to GM's earlier recommendation to use the AQIRP data neglects to mention the industry average gasoline data and

indicates that the gasolines used in AQIRP are prototype. The ARB's recent Phase 2 gasoline regulatory package used the AQIRP data to estimate that a 26 to 29 percent reduction in 1,3-butadiene emissions will be associated with Phase 2 gasoline. Thus, Phase 2 gasoline (because it will be introduced throughout the state and used throughout the vehicle fleet) will substantially reduce statewide 1,3-butadiene emissions.

Response: The AQIRP emission factors are based on "industry average" fuel (a fuel that contains the average fuel components, in average concentrations, for gasoline sold in the United States) and "reformulated" fuel. The ARB's Phase 2 gasoline regulatory package used AQIRP data to estimate a 26 to 29 percent reduction in 1,3-butadiene emissions for vehicles using Phase 2 gasoline rather than "California average" gasoline. Refineries are not required to sell Phase 2 gasoline to gas stations until March 1996. One of the goals of the Phase 2 gasoline program is the reduction of 1,3-butadiene emissions from motor vehicles.

Comment 11: GM continues to believe that the individual organic species data from the South Coast Air Quality Study (SCAQS) should be analyzed in terms of spatial and temporal variability to provide input for more refined exposure analysis. In order to properly account for the population exposure to 1,3-butadiene, spatial and temporal differences in ambient concentrations as well as indoor concentrations will need to be taken into account.

Response: The ARB staff are analyzing the individual organic species data from the SCAQS to learn more about the pollution dynamics of the South Coast Air Basin. While much of the pollution dynamics information from the SCAQS can be applied to other areas of California, the concentrations data derived from the SCAQS is South Coast-specific and cannot be used to represent conditions in California's other 13 air basins.

The 1,3-butadiene concentrations data derived from the ARB's toxic monitoring network have been used to develop air basin and statewide exposure averages for outdoor air. The use of "average" outdoor concentrations reduces the numbers of opportunities to over- or under-estimate the actual exposure (and associated risk). Indoor exposures are considered separately. The ARB is concerned about indoor air pollution and regularly sponsors indoor air exposure research, however, the ARB does not presently have control authority over indoor air.

Comment 12: GM recommends that early-morning SCAQS 1,3-butadiene/TOG ratios be analyzed to shed light on the ARB's estimated inventory.

Response: The on-going analysis of the SCAQS data includes the analysis of 1,3-butadiene/TOG ratios. The SCAQS data represents "episode" conditions in the South Coast Air Basin, while the ARB's emissions inventory seeks to estimate average emissions.

The ARB's motor vehicle inventory has recently (1991) been updated as a result of 1,3-butadiene emissions testing for on-road motor vehicles by the ARB's Mobile Source Division. The revised emission estimates for motor vehicles have been reported in the SRP version Part A.

Comment 13: GM is concerned that ambient exposures from urban monitoring is used to characterize the total population of California. A portion of the population resides in more rural locations where the 1,3-butadiene concentrations are expected to be below typical urban levels. If this is taken into account, the statewide average ambient exposure and accompanying risk would be reduced somewhat.

Response: The toxic monitoring network primarily represents urban exposures, which is appropriate since California is a heavily urbanized state. Analysis of concentrations data from the ARB's toxic monitoring network indicate that 1,3-butadiene concentrations and emissions are higher in urban areas. Rural populations included in the statewide averaging may experience a lower exposure to 1,3-butadiene than the statewide population-weighted average. Conversely, people in the heavily-populated South Coast Air Basin are experiencing a higher exposure than the statewide population-weighted average. The population-weighted exposure average represents the "average" Californian's exposure, and is only an estimate of the average exposure in the State.

Comment 14: GM is concerned that the upper limit risk calculations in Part B do not provide an assessment of the actual risks that Californians experience as they are exposed to 1,3-butadiene in their daily lives. A knowledge of the relative contributions of individual sources is important for better estimates of public exposure.

Response: Staffs of the ARB and the OEHHA agree that information about the relative contributions of individual sources is important for better estimates of public exposure. It is anticipated that individual source data will become available through the AB 2588 Air Toxics "Hot Spots" Program. During the risk management phase, should 1,3-butadiene be identified as a toxic air contaminant, these data will be further assessed.

Comment 15: A National Research Council review of human exposure assessment for airborne pollutants stated that "risk reduction strategies that address only outdoor air are only partially effective. Such strategies need to be modified to better address the importance of indoor exposures." They also indicated that all media and routes of exposure should be assessed.

Response: Staff has previously responded to this comment in Part C, page 569, paragraph 7. The response: "The ARB has been given authority to identify and control outdoor TACs. The ARB staff recognize that indoor air exposures can pose a significant risk, and staff agree that consideration of indoor air and other routes of exposure are important for a complete risk assessment. Available information on indoor air, food, and water exposure has been reported in the document. Because

indoor exposures can be significant, the ARB is continuing to sponsor research to develop data on indoor air exposures to TACs."

Comment 16: The US EPA's comparison of benzene emissions versus human exposure (the TEAM study) indicates that, although motor vehicles emit 82 percent of the benzene, they are only responsible for 18 percent of the individual exposures (based on personal monitoring). The TEAM study showed how significant individual sources of exposure are because the general public spends most of its time indoors.

Response: Staff agree that the average Californian spends the majority of their time indoors, and that indoor exposures can be the most significant exposure that they receive from certain pollutants. In the case of 1,3-butadiene, indoor exposure to 1,3-butadiene from environmental tobacco smoke will probably be higher than the local outdoor concentration (in the absence of "hot spot" outdoor emission sources). As has been discussed in other responses, the ARB is concerned with indoor air pollution.

Comment 17: The report does not provide the proper perspective on the risk from motor vehicles relative to the much larger risks from indoor sources, particularly to the substantial 1,3-butadiene exposure experienced by both smokers and non-smokers from cigarette smoke and environmental tobacco smoke. In response to the Executive Summary question "what about indoor exposure to 1,3-butadiene?", the response indicates that indoor air may be the major route of exposure to individuals exposed to a heavy smoking environment. Based on available data it is clear that indoor air <u>is</u> the major route of exposure for smokers and those exposed to a heavy smoking environment, and that exposure to environmental tobacco smoke <u>is</u> a major route of exposure to 1,3-butadiene for Californians. The response to this question should also include comparisons of the average daily intake of 1,3-butadiene from smoking, typical environmental tobacco smoke exposures, heavy exposures, and exposures to ambient air.

Response: The Executive Summary has been changed to state that indoor air is almost certainly the major route of exposure to 1,3-butadiene for individuals exposed to a heavy smoking environment. Available data do not lead to the unequivocal conclusion that indoor air is the major route of exposure for all Californians in a smoking environment. In the Northern California study, 34 percent of the homes were reported to have smokers present, yet only 8 percent of the total had measurable levels of 1,3-butadiene. The remaining 26 percent of the homes where smoking occurred had 1,3-butadiene concentrations below the limit of detection. The Executive Summary gives estimated inhaled doses of 1,3-butadiene for heavy smoking environments (the tavern and bar). However, broad quantitative comparisons of exposures with different levels of ETS simply cannot be made due to a lack of data.

Comment 18: Regarding indoor air concentrations of 1,3-butadiene, the statement that "it appears reasonable to assume that residential exposures ... may typically be close to ambient levels" is not supported

by the data provided. The detection limit used in the Woodland study (0.54 ppb) is significantly above the statewide average ambient exposure, so no comparison of residential and ambient exposures can be made.

Response: Further research is needed using a greater number of homes sampled over different seasons with an improved detection limit before conclusions can be drawn regarding typical indoor concentrations. The referenced sentence will be changed in the Board version of the report.

Comment 19: The statement that non-smoking residential exposures may typically be close to the statewide average ambient exposure is not supported by the data provided because the limit of detection was too high. The statement should be replaced with one that acknowledges that there is not enough information to make a quantitative comparison between ambient exposures and non-smoking residential exposures.

Response: Please see the response to comment 18.

Comment 20: GM encourages ARB to carry out a study of residences, offices, and commercial spaces using a technique that has a detection limit similar to that of the Woodland pilot study (0.05 ppb). Such measurements, in conjunction with estimates of smoking, would go a long way toward establishing the exposures and health effects of environmental tobacco smoke.

Response: We acknowledge the need for studies that would further monitor a wide variety of indoor environments for TACs. Such studies would need to obtain activity data and ambient concentrations data as well.

Comment 21: Using the NRC reported value for the average emission rate of respirable suspended particulate matter per cigarette in sidestream smoke (26 mg), the US EPA calculated a daily intake for passive exposure of 3 mg. Using the same methodology for 1,3-butadiene (with an emission rate of 400 ug per cigarette), one can calculate the average daily intake of a passive smoker to be 46 ug. This can be compared to an average daily intake of 16.4 ug for an individual exposed for 24 hours to the average ambient concentration of 0.37 ppbv. Thus, the typical exposure from smoking exceeds that of typical outdoor concentrations. GM recommends that calculations of 1,3-butadiene exposure from smoking and from passive exposure to ETS be included in Part A and in the Executive Summary to provide perspective for the reader.

Response: Exposure calculations are based on actual measured indoor concentration estimates, not on extrapolated emissions data. Emission rates are only one of many factors that determine indoor exposures. Concentrations in an indoor environment can be decreased by ventilation (outdoor air exchange) and by reaction with other ETS constituents. As discussed in an earlier response, data from the Northern California study were inconclusive regarding the degree to which the presence of smoking affected butadiene concentrations. In that study, many of the ETS homes with butadiene concentrations below the limit of detection had as many cigarettes smoked in them as ETS homes with measurable butadiene concentrations.

III.

Office of Environmental Health Hazard Assessment Staff Responses to Summarized Comments on the SRP Version Draft Part B and the Executive Summary

OFFICE OF ENVIRONMENTAL HEALTH HAZARD ASSESSMENT (OEHHA)
STAFF RESPONSES TO PUBLIC COMMENTS
ON THE SCIENTIFIC REVIEW PANEL (SRP) VERSION DRAFT
RELEASED SEPTEMBER 1991
OF THE TECHNICAL SUPPORT DOCUMENT (TSD) FOR THE
PROPOSED IDENTIFICATION OF 1,3-BUTADIENE
AS A TOXIC AIR CONTAMINANT

Comments from the Chemical Manufacturers Association (CMA)

1. Comment: OEHHA's "best estimate" of the human cancer potency of butadiene (BD), 0.37/ppm, based on lung tumors in female mice, is inconsistent with the results of epidemiologic studies. It should be noted that every BD epidemiology study conducted thus far has observed a deficit of lung tumors. OEHHA has significantly overstated the human cancer risks arising from butadiene exposure. Thomas B. Starr has found the observed cancer rates in studies of BD-exposed workers to be inconsistent ($p = 6.9 \times 10^{-44}$) with the cancer rate predicted using the 0.37/ppm potency slope (despite methodological flaws in one epidemiologic study that would tend to bias the results toward consistency). John F. Acquavella has obtained similar results in a consistency check of a similar risk assessment. OEHHA's consistency check of its risk assessment is based, without justification, upon potency estimates only up to 0.089/ppm. It would have been far more appropriate to perform a consistency check using OEHHA's separate rat-based and mouse-based "best estimates" of potency. In contrast to the mouse-based estimate, OEHHA's rat-based potency estimate yields predicted numbers of cancer deaths that are not demonstrably inconsistent with those observed. (CMA, comments dated December 6, 1991, pp. i, 2-3, and Appendices II and IV).

Response: The TSD's risk assessment is based upon the newest bioassay (Melnick et al. 1990, or "NTP II"). OEHHA staff did not update the document's consistency check (Part B, Section 5.3) when the new data were incorporated into the risk assessment. However, Staff believe that Dr. Starr's analysis is flawed for the following reasons:

1) It compares upper bound rodent estimates to central tendency human estimates which should overpredict by definition.
2) Concordance of site-to-site extrapolation is not expected across species.
3) Overpredictions may be high for lung-to-lung comparisons for the one study analyzed, but not for total cancer comparisons across species or for other valid comparisons.
4) The predominantly male worker studies would be expected to underpredict population estimates.
5) The epidemiologic studies are generally conceded to be lacking sufficient exposure detail to be quantitatively useful.
6) In response to this comment, staff have prepared an additional "consistency check" analysis for inclusion in the document.

Dr. Starr's analysis compares predictions from a mouse-based upper bound potency estimate with actual observations in one epidemiological

study of humans. Upper bound estimates are designed to predict a value which is not likely to be exceeded, not the median or mean cancer rate. Since the observed cancer rate was not exceeded, the upper bound estimate may be consistent. Dr. Starr concluded that when compared with observed human lung cancers, the upper bound estimate for the mouse was inconsistent while the upper bound estimate for the rat was consistent. However, OEHHA staff believe that based on the comparison made, the upper bound estimate for the rat would be an underprediction of a human population risk since the Starr analysis compares the rodent upper bound to the central tendency in one worker study. In the previous OEHHA analysis (Table 5-1), the maximum likelihood estimate (MLE) from the mouse I study is compared to measures of central tendency from two worker studies. In OEHHA's additional analysis the mouse upper bound estimate is compared to upper bound estimates for two human studies.

Butadiene has been shown to be a multisite carcinogen in animal studies. It produces cancer of the heart, lung, liver, ovaries, forestomach, hematopoietic system and mammary gland. Staff believe that it may act at many sites in humans as well. The female mouse lung was the most sensitive site and sex in the "mouse II" study. It was chosen for the best value since the response at this site appears scientifically valid and consistent with genotoxicity and cancer information on butadiene. The female lung also appears to be the best site to choose for risk assessment. This site exhibited a fairly low background rate (8%) in the controls. It was the only site that was significantly elevated in the lowest dose tested in the study (6.25 ppm). Thus, there are fewer competing risks which confound the lung potency estimate in mice. Due to the compound's lack of site specificity, we do not expect lung cancer produced in mice to be directly correlated with lung cancer rates in humans. OEHHA staff believe that the best comparisons are based on total cancers produced in human studies or significant excesses found in human studies. In OEHHA's most recent analysis, comparisons are made with the best upper bound value to overall human cancers and significantly increased rates of hematopoietic cancers for two epidemiologic studies.

Dr. Starr's analysis shows a 1.3- to 2.8-fold overprediction of all cancers in a worker study. OEHHA staff believe that this indicates that the mouse upper bound estimate is not very inconsistent with the human estimates. Although elevated lung tumor rates have not been observed in exposed humans, there is epidemiologic evidence that butadiene may cause lymphatic cancers in humans (and such cancers have been seen in mice). Butadiene may have caused tumors at other sites (including the lung) in humans that have gone undetected due to the limited power of epidemiologic studies. OEHHA staff believe that comparison of the best value in animal studies to observed excess cancer rates in humans is also a valid consistency check, and such a comparison was made in the additional OEHHA analysis.

There are several reasons why a worker cohort may underpredict the risk for the general population. First, the well-known "healthy worker effect" may have muted the apparent cancer potency of BD in these studies. Second, the age of exposure may affect the potency estimate and the site of tumor development. While the animals were exposed from youth, humans in the epidemiologic studies were exposed as adults. Young animals have a

better likelihood of developing cancer due to the latency for cancer. Furthermore it is possible that younger animals exhibit a different sensitivity to butadiene exposure and that different sites may exhibit cancer due to the development process. Third, rat and mouse studies indicate that females tend to be more sensitive than males to butadiene exposure. One must be concerned about the susceptibility of the young and of women, since they are generally not represented in worker studies. Other factors may also have muted the potency apparent from the epidemiologic studies (see Sections 3.6 and 5.3 of Part B of the TSD).

Data from epidemiologic studies are sometimes used for quantitative cancer risk assessment. The epidemiologic data regarding persons exposed to butadiene are not adequate for this purpose. Few exposure data are available (for BD and other workplace chemicals), and most studies have very limited power to measure a dose-response association. Nevertheless, when epidemiologic studies are used for risk assessment, the reported "best" potency estimates are usually upper bounds (partly to account for the potential non-representativeness of the sample). Thus, comparisons such as those in Dr. Starr's analysis should consider upper bounds on the tumor incidences observed in epidemiologic studies. OEHHA staff considered such upper bounds in preparing the extension to Section 5.3 of the TSD.

The OEHHA reanalysis is summarized in Table 5-1b. In this instance comparisons are made between the OEHHA best value for the upper bound to an upper bound on the observed value in two epidemiologic studies. In the reanalysis all cancers were considered as well as elevated rates of lymphopoietic cancer. An adjustment was made for time and age of exposure (CC Brown and KC Chu (1983) A new method for the analysis of cohort studies: implications of the multistage theory of carcinogenesis applied to occupational arsenic exposure. Environmental Health Perspectives 50:293-308). The analysis compares upper bound of predicted plus background with upper bound observed. The analysis indicates reasonably good consistency between animal-based estimates and human observations.

2. Comment: The epidemiology review in the SRP version of the TSD selectively highlights positive findings, neglects important negative findings, and relies on a number of unproven assumptions. These assumptions include a uniform reduction in industrial BD exposure after 1945, and a lack of correlation between employment duration and cumulative exposure. The reduction in exposure assumption is based only on process changes in polymerization areas of styrene-butadiene rubber (SBR) plants (rather than measurements, which only began in the 1970s), and there were no corresponding process changes in the studied BD monomer plant. The review fails to note that findings of a base cohort study conflict with a nested (lymphopoietic cancer) case-control study (CMA, Appendix IV, pp. 1-3).

Response: First, it should be noted that the document's risk assessment does not rely on epidemiology. The document's epidemiology review is designed to highlight positive findings, and gives consideration to important negative findings. The review mentions, rather than "relies on," the reduction in BD exposure after 1945. If a desire to reduce workplace BD exposure was a driving force behind changes in production processes, it is likely that exposure of all workers, including non-polymerization

workers, was reduced. The TSD does not rely upon a uniform reduction in exposure after World War II. The review reports both the negative findings of the base cohort study and the positive finding of the nested case-control study mentioned in the comment. The review also describes a job classification-based analysis of the cohort data, which had positive findings and prompted the nested case-control study. Many caveats regarding interpretation of these findings are communicated in the review. One must also keep in mind that negative epidemiologic studies do not necessarily contradict positive human or animal studies. Instead, the negative nature of a study may be the result of confounding factors or lack of power of the epidemiologic study.

3. Comment: The conclusion of a carcinogenic effect among workers employed prior to 1946 is not consistent with the available epidemiologic data (CMA, Appendix IV, pp. 3-6).

Response: The comment makes good points regarding the separate analyses of workers employed prior to 1946 (during World War II). Many of these workers had only short term exposure, albeit at high levels. The authors of one analysis note that their mortality rate comparisons are *a posteriori* and only find significant differences with a one-tail test (Lemen et al. 1990, p. 105). Since the World War II worker analyses are limited, OEHHA staff have prepared a suggested modification of the conclusion in the TSD regarding those workers. Nevertheless, the high levels of butadiene in World War II plants certainly imposed cancer risks upon the exposed workers. Scientists agree that BD is a carcinogen, that metabolites of BD may act directly on DNA, and that there is no known dose threshold for BD carcinogenesis. Epidemiological studies have not clearly detected increased cancer rates among World War II butadiene and SBR workers. Given the general limitations of occupational cancer epidemiology, this is not unexpected.

4. Comment: TSD's authors mention that the lymphopoietic cancer findings from the Matanoski et al. cohort study are of limited usefulness because of the omission of early (World War II) workers. However, data from a subset of the plants considered in that study, where early workers were completely enumerated, show no excesses (and borderline significant deficits) of lymphopoietic cancers, lymphosarcoma and leukemia (CMA, Appendix IV, pp. 6-7).

Response: The TSD mentions the omission of some World War II workers as a factor that should be considered in interpreting Matanoski et al.'s results. Although the data presented in the comment do bear on the issue, the effects of this omission remain unknown. The fact that potentially useful person-years were not included in the study remains a valid concern.

5. Comment: Another unproven assumption relied upon by the TSD is biological consistency of varied lymphopoietic cancer findings across studies. The document dismisses the criticism that variation in health endpoints across studies detracts from finding a causal relationship between BD and lymphopoietic cancer. In this regard, the TSD's authors should consider and respond to certain testimony before the U.S. Occupational Safety and Health Administration by P. Cole. The document suggests that individual lymphopoietic cancers are related tumors. Yet, if

this were so, one would expect to see general lymphopoietic cancer excesses (for all sites) rather than the variable findings for specific lymphopoietic cancers actually observed. The document cites diagnostic overlap and changing nomenclature over time in disregarding the heterogeneity of the lymphopoietic cancer findings. This is not a credible explanation because diagnostic overlap would also have occurred in the comparison populations for the cohort studies. Also, diagnostic variability differs by type of lymphopoietic cancer, and will have little effect on cancers with a high percent confirmation. Diagnostic specificity has improved with time, and most of the deaths occurred in the last 10-15 years of the studies (CMA, Appendix IV, pp. 7-9).

Response: The comment enumerates several reasons for not relying on human studies. Clearly, uncertainties exist in using human studies. This requires the risk assessor to consider those uncertainties and weigh them against the uncertainties in using animal studies. Part B of the TSD does so, and applies the epidemiologic data in a supportive manner. The TSD's epidemiology review does consider the possibility that the various blood and lymph cell cancers may be related. The TSD's animal data-based risk assessment does not rely on an assumption of biological consistency among these tumors, however. There are rational bases for considering the cancers to be related. Some of these are cited in Section 3.6.3 of the TSD's Part B. That general increases in lymphopoietic cancers were not seen may be a reflection of the insensitivity of the epidemiologic studies. These studies considered occupationally exposed populations of which only small subsets had high-level exposure to butadiene. If the blood and lymph cancers are indeed related (and related to BD), chaotic variations within the "grouped cancer" incidences might cause different specific cancers to appear as significantly elevated in different studies, resulting in the observed heterogeneity of findings. Finally, if diagnostic overlap or misclassification occurred in both an exposed group and a comparison population, the results of studies would be biased towards the null hypotheses (no effect of exposure). Thus any changes over time in diagnostic specificity should be a matter of concern.

OEHHA staff have considered the referenced testimony of Dr. Cole. It highlights evidence for heterogeneous etiology of heterogeneous lymphohematopoietic cancers ("LHC" in the testimony, or "lymphopoietic cancers" here). Although such cancers are morphologically (and thus etiologically) distinct, it does not follow that butadiene will necessarily be associated with either (1) specific lymphopoietic cancers or (2) all lymphopoietic cancers in studies of worker cohorts. First, one must remember the limited nature of such studies and the uncertainties involved with relying on them. Second, one should note that all cancers, including lymphopoietic cancers, are generally though to have multiple causes. Environmental carcinogens may act upon or in concert with varying genomes to produce varying tumors. Dr. Cole gives examples of varying genetic susceptibilities to leukemias. Environmental carcinogens may act upon or in concert with different environmental (or lifestyle-associated) chemicals or stresses (such as viruses or other disease organisms, or immune-suppressing behaviors) to produce different tumors. It is possible that butadiene may facilitate the development of different blood or lymph system tumors in persons of different genomes or in cohorts of different experience. Clusters of differently-susceptible individuals may thus be found in different

occupational cohort studies. In the epidemiologic studies discussed in the TSD, butadiene may have acted synergistically with different confounding genetic susceptibilities, workplace exposures, or viruses. Thus one cannot rule out a causal role for butadiene.

All lymphopoietic cancers derive from the same embryonic tissue germ layer (mesoderm) and in such cancers similar types of genes may be active. These genes may be similarly susceptible to carcinogens such as butadiene, with BD insult leading to morphologically different lymphopoietic cancers under different circumstances. However, as noted above, the data regarding chronic exposure of humans to BD are limited and the TSD does not rely on these data for the risk assessment. It is possible that confounding factors largely explain the increased cancer rates found in the epidemiologic studies discussed in Part B's review. These were studies of occupational cohorts, however, and more-susceptible subgroups of humans are likely to exist in the general population, with exposure (albeit lower-level exposure) throughout life.

6. Comment: The findings from an SBR workers lymphopoietic cancer case-control study are irreconcilable with findings for the cohort study of the same population. These two studies should be evaluated together, and emphasis should be placed on finding an interpretation for the case-control results that is consistent with the lack of a leukemia excess for the SBR worker cohort overall. The case-control study in particular should be examined critically; there appears to be a large random error component there, making its results sensitive to varying exposure classification schemes (CMA, Appendix IV, pp. 9-15).

Response: The two study reports are not irreconcilable because they are based on the same data. The TSD evaluates them in sequence. It is not surprising for a case-control study to show a significant effect where a cohort study does not, because case-control studies are ordinarily more powerful than cohort studies. The TSD mentions the 1989 observation by Acquavella (who prepared this part of CMA's comments) that an abnormally low leukemia rate among unexposed workers may have caused the case-control study's high odds ratio. In response to this comment, OEHHA staff have prepared additions to the TSD's examination of the case-control study, to indicate that the study's results were sensitive to the choice of exposure classification scheme and that a log transformation was used. The commenter, although critical of the classification scheme, notes that the log transformation decreased the skewness of the exposure data. Although it may not have produced a normal distribution, such a transformation is an accepted technique in statistical analysis.

7. Comment: Although the TSD mentions that Checkoway and Williams (1982) attributed hematological abnormalities to butadiene exposure in a cohort of SBR workers, this statement is not true since the values for the highest exposed group were all within the normal range. The investigators concluded that there was no significant difference between the two exposure groups in their study. Thus the TSD's citation of this study is misleading (CMA, Appendix IV, pp. 1, 15-16).

Response: Checkoway and Williams (1982) did find that changes in blood parameters were associated with butadiene exposure. However, the comment

points out that the word "abnormalities" can rightly be considered inappropriate for describing those changes; the investigators themselves noted that there was no striking indication of bone marrow toxicity related to exposure (1982, p. 168). Checkoway and Williams also noted that causality could not be inferred directly from their study (*Ibid.*). Thus, OEHHA staff have prepared a modification of the TSD, to remove the words "abnormalities" and "attributed" from the summary sentence near the beginning of Section 3.6 of Part B. A more complete description of the Checkoway and Williams study may be found in Section 3.6.3, as released.

8. Comment: The TSD's epidemiology review neglected findings for white production workers and for white and black mechanical workers in its review of Matanoski et al. (1990). These findings are important because process and mechanical workers have frequent opportunity for butadiene exposure. The findings for mechanical workers are particularly important because of these workers' opportunity for intermittent peak exposures, and because the TSD's authors have drawn an analogy between certain peak exposure mouse studies and findings from epidemiology. The omitted findings show no elevation of lymphopoietic or all cancers and are thus inconsistent with the analogy to the high exposure/short-time mouse studies. Also, a more appropriate analysis of the Matanoski et al. (1990) data would have shown that there was not an overall lymphoma excess for all production workers (CMA, Appendix IV, pp. 16-18).

Response: OEHHA staff have noted that the stop exposure studies of Melnick et al. (1990) in mice indicate that short-term higher exposures to BD may result in greater tumor incidences than longer-term lower exposures (see Part C of the TSD, pp. OEHHA C-18 to OEHHA C-19, International Institute of Synthetic Rubber Producers, Inc. [IISRP] comment 5 [and response]). Nevertheless, we would not expect to see elevated incidence rates in every "peak exposed" subcohort of every epidemiologic study.

The "mechanical workers" described in the comment are considered maintenance workers by Matanoski et al (1990). The comment cites a report by Fajen et al. (JM Fajen, DR Roberts, LJ Ungers and ER Krishnan (1990) Occupational exposure of workers to 1,3-butadiene. Environmental Health Perspectives 86:11-18) as indicating that these workers have had opportunity for intermittent peak exposures. However, these exposures may have been few and far between. Based on full-shift personal samples, Fajen et al. found that maintenance workers had the second-lowest geometric mean butadiene exposure (0.122 ppm) among the six job categories with some samples showing exposure in excess of 10 ppm. Although some workers had some days of peak exposure, continuous high exposure for several weeks or months was not documented, and these workers' exposure was not closely analogous to the mouse exposures. In addition, it is not clear that all maintenance workers were similarly assigned to peak exposure tasks.

The TSD's analysis of the Matanoski et al. (1990) data clearly notes that no cancer SMR was significant when the total cohort was analyzed (see Table 3-4, p. 3-27).

9. Comment: The TSD's epidemiology review gives undue attention to studies of tire manufacturing populations in which solvents have previously been associated with elevated rates of lymphatic leukemia. The TSD's

authors have chosen to disregard the earlier comment that studies of tire manufacturing populations are essentially irrelevant for butadiene since butadiene is not liberated during tire manufacturing. Only one of these studies had a subcohort employed in an SBR "synthetic plant," and for these workers it is unclear whether exposures resembled SBR production plants. Further studies by the research group there attributed the elevated leukemia findings to solvent, rather than BD, exposure. The TSD should not confuse the evaluation of BD by discussing tire manufacturing studies in detail (CMA, Appendix IV, pp. 1, 18-19).

Response: As OEHHA staff have previously noted in responding to the earlier comment regarding tire manufacturing populations (see Part C, pp. OEHHA C-17 to OEHHA C-19, IISRP comments 3 and 6), the TSD only presents results from groups of workers involved with rubber synthesis. Whether or not the exposures in the synthetic plant in the tire manufacturing facility resembled those in SBR production plants, the workers there were almost certainly exposed to butadiene. The report of "further studies" referenced by the comment (H Checkoway, T Wilcosky, P Wolf and H Tyroler (1984) An evaluation of the associations of leukemia and rubber industry solvent exposures. Am J Industrial Medicine 5:239-234) is limited. This report uses "data from a small case-control study of lymphocytic leukemia" (p. 239): it does not separately address the synthetic plant, and does not address butadiene.

10. Comment: The TSD's interpretation of Ott et al. (1980)'s findings in SB latex workers reflects an unwillingness to accept negative findings from any study. There were no leukemias among 391 such workers. The contention that this finding is not reliable because "OEHHA staff were not able to confirm that this OTG was the only one in which butadiene exposure occurred" indicates a biased perspective regarding this study. Even if there were other BD-exposed workers in this study, they would not affect the negative findings in SB latex workers (CMA, Appendix IV, pp. 19-20).

Response: The epidemiology review in the TSD clearly reports the negative findings (zero cases of leukemia) in the SB latex group. No biased perspective is reflected in this review. Only 391 workers were in the SB latex group, but elevated SMRs for leukemia and for lymphatic and hematopoietic neoplasms were seen for the whole 2,904-worker cohort. It is thus logical to inquire whether some of the non-SB latex workers were exposed to butadiene (see Part C, pp. OEHHA C-17 to OEHHA C-20, IISRP comments 3, 6 and 8).

11. Comment: OEHHA should use more data for its best estimate of likely human cancer risk. The "best estimate" presented in the TSD is an upper bound based on several "worst case" assumptions that are implicit in generic cancer risk assessment guidelines. The collective use of these assumptions is not realistic and does not provide a reasonable basis for estimating likely excess human cancer deaths from exposure to BD in ambient air (CMA, p. 1).

Response: OEHHA staff believe that the methods used in the TSD are reasonable, and represent prudent public health practice. The assumptions and choices made in the TSD are indeed made in accordance with guidelines (see State of California (1985) Guidelines for Chemical Carcinogen Risk

Assessments and Their Scientific Rationale. Issued by: Health and Welfare Agency, Department of Health Services). The assumptions suggested by these guidelines and used in the TSD reflect health protection and plausible (rather than "worst case") scenarios.

12. Comment: OEHHA's reasons for basing its best estimate of human cancer risks on the B6C3F1 mouse data are not valid. The assertions that the mouse study was repeated at lower doses with consistent results and that the mouse data are available in greater detail do not provide a valid basis for preferring the mouse data. Stuart Z. Cagen has addressed similar arguments advanced by the National Institute for Occupational Safety and Health.

Contrary to OEHHA's assertion, the epidemiologic data do not provide any basis for preferring the mouse over the rat data. The increased incidence of lymphocytic and hematopoietic cancers in B6C3F1 mice has been observed only at relatively high doses and may reflect the presence of an endogenous retrovirus not present in humans. It is extremely doubtful that these tumors are relevant to an assessment of potential human cancer risks from exposure to ppb levels of BD in ambient air.

OEHHA would be more objective to acknowledge that all three cancer bioassays are adequate for risk assessment, and that the risk assessor has the task of choosing the species which provides the best model. The available data on butadiene metabolism and mechanism of action indicate that the B6C3F1 mouse is uniquely susceptible to the carcinogenic effects of butadiene and not an appropriate model for human risk assessment. Most notable is the mouse's relative inability to detoxify putative mutagenic/carcinogenic metabolites via epoxide hydrolase. The rat provides a better model, because of greater similarities in butadiene metabolism. If OEHHA continues to rely on the mouse data, it should acknowledge that this choice is not based on the data's quality or reliability. Rather, it is based on generic quantitative risk assessment guidelines that dictate a preference for the most sensitive species. In the case of butadiene, the weight of the available evidence demonstrates that the most sensitive species is not the best model for human risk assessment (CMA, pp. i, 3-5).

Response: Although some evidence may favor the rat model over the mouse model, the total mass of available evidence is limited. Public health protection requires consideration of all valid models, and use of health protective models unless data clearly and convincingly indicate they are not valid predictors of human toxicity. Such data are not currently available. The available BD-related data from mice, rats and primates do not clearly indicate that the rat data should be used instead of the mouse data for human cancer risk assessment. Indeed, the commenter's own demonstration of consistency between upper-bound rat-based risk projections and observations in a worker cohort provides some evidence that rat-based risk assessment may underestimate human risks and thus might not be prudent (see CMA, Appendix II, pp. 2, 5). The comment is correct in noting that in this case OEHHA staff have not moved away from the practice recommended by guidelines of using the most sensitive site, sex, and species for human health risk assessment. Use of a species other than the mouse might be indicated if there were strong evidence showing that mice are not like humans in their response to BD. However, the available data regarding

human response to BD are limited. Human metabolic data are insufficient for use in addressing this issue, and the mechanism of BD-associated carcinogenesis has not been clearly established in any species. Epidemiologic studies of BD-exposed workers are available, but these studies do not provide evidence that mice and humans differ. Rather, tumor incidence rates seen in these studies are consistent with potency estimates based on mouse bioassay data.

OEHHA staff believe that the mouse data are clearly superior to the rat data. The fact that the findings in mice have been replicated, whereas the findings in rats have not, is relevant. The fact that the mouse data are more detailed than the rat data is also relevant. It is more prudent to rely on detailed, replicated findings than to rely on less detailed, unreplicated findings. The availability of new data consistent with earlier findings reduces the probability that risk estimates are too low due to chance or extraneous factors.

13. Comment: The use of body surface area scaling is not appropriate for butadiene risk assessment. Such scaling implies that the rat would be more sensitive to BD on a mg/kg basis than the mouse. The mouse and rat bioassays, and OEHHA's mouse- and rat-based potency slopes, clearly show that this is not the case (and that body surface scaling is not valid). Such scaling also implies that man would be more sensitive to BD on a mg/kg basis than the mouse. The clear evidence, including both metabolism and direct tumor data, is that the mouse is the most sensitive species. "Routine" scaling is inappropriate when compound-specific information is available; several technical papers have concluded that the use of compound-specific data is preferred. For some chemicals, scaling based on mg intake/kg/day is more accurate. Surface area scaling is inconsistent with the substantial body of data on BD, including metabolism data from mice, rats, monkeys and humans. Scaling based on the 3/4 power of body weight might be used after adjusting for species differences in formation and deactivation of the "direct acting" toxicants, epoxide metabolites of BD (CMA, pp. i, 5-6).

Response: Guidelines for chemical carcinogen risk assessment (such as those cited in the response to CMA comment 11, above) generally suggest the use of a "surface area" scaling factor, in the absence of substance-specific data, largely due to empirical observations. Data from the most sensitive species are generally scaled using such a factor (usually body weight [bw] raised to the 2/3 or 3/4 power; actual surface area is commonly assumed to be proportional to bw raised to the 2/3 power). These factors are thought to represent pharmacodynamic differences among species, as well as differences in metabolic rates (for which body surface area has been thought to be a proxy -- due to heat loss). Pharmacodynamic differences involve differing responses to similar concentrations of a toxicant in tissue of different species or subgroups within species. The number of cells in a tissue, the rapidity of a tissue's growth or maturation, and the passage of a cell line through divisions towards senescence all can affect a tissue's response to a toxicant and add uncertainty to risk assessment. Thus, "surface area" (bw to the 2/3 or 3/4 power) scaling factors are used largely as uncertainty factors. Here, the surface area scaling increases the potency estimate by approximately 2-fold, so it has little impact on the overall risk estimates.

It is not certain that the B6C3F1 mouse is more sensitive than all humans (or all rats). The report of human metabolism data cited by the commenter is limited: it refers to microsomes from only 12 liver samples and 6 lung samples (the latter from surgery patients). Although the report presents Vmax values for activating (BD to BMO) and presumed detoxicating (from BMO to 1,2-dihydroxy-but-3-ene rather than DEB) transformations, it does not present the corresponding Km values which might tell us more about the rate of these steps at low doses of BD. Moreover, the relevance of these data to carcinogenesis is unclear because we do not know with certainty which metabolites are responsible for tumor induction or promotion. Even if we knew that specific epoxides were responsible, there are insufficient data on concentrations of epoxides in target tissues to predict these concentrations in humans exposed to ambient levels of BD. Even within species, different strains or subgroups may have markedly different metabolisms. The varying susceptibility of different groups of humans to different lymphopoietic cancers (see CMA comment 5, above) may be due to differing rates of metabolism of substances like BD. Thus OEHHA staff do not believe that the data should be adjusted as suggested by the comment prior to applying a scaling factor (bw to the 2/3 or 3/4 power) in the risk assessment.

Although quite a bit of data on BD metabolism is available, it does not provide sufficient pharmacokinetic and pharmacodynamic information for OEHHA staff to adjust the ("routine") surface area scaling. The fact that BD appeared less potent in the rat study than in mice does not invalidate the surface area scaling: as noted above, this approach is generally used with data from the most sensitive species, which is not always the largest. Although the mouse data, used with a scaling factor, would poorly predict responses in rats, this does not mean that the mouse necessarily poorly predicts the human response to BD. OEHHA staff have analyzed datasets used in the references cited by the commenter as supporting scaling based on bw to the 3/4 power, and found them to be consistent with 2/3 power as well as 3/4 power scaling. The data used in the report prepared by Clement Associates that suggests mg intake/kg bw/day scaling are also consistent with bw to the 2/3 power scaling. In view of the uncertainties involved with interspecies scaling, and the consistency of empirical data with 2/3 power scaling, OEHHA staff have chosen to use 2/3 power scaling for the butadiene risk assessment.

It is also important to note that since surface area scaling reflects our Office's standard approach, other risk assessments developed from animal estimates for the Toxic Air Contaminants Program have used this procedure. To compare the risk from butadiene to the risk from other compounds, it is best to use similar approaches.

14. <u>Comment</u>: OEHHA should use the internal concentration of butadiene monoepoxide (BMO) as the measure of dose. In all butadiene risk assessment documents, BD's reactive epoxide metabolites are the putative mutagenic and carcinogenic species. BMO is the first metabolic product of BD. Thus, any pharmacokinetic model using BMO would be superior to one based on external air or absorbed levels of BD. The fact that a full physiologic model that includes diepoxybutane (DEB) or other epoxide metabolites has not yet been developed does not justify OEHHA's preference for BD over BMO. Evidence suggests that the mouse is unusual in its ability to produce DEB.

OEHHA's concerns regarding the adequacy of the available metabolism data reflect unreasonable expectations. The lack of perfect data should not be used as a justification for disregarding the enormous body of evidence which weighs in favor of using BMO as the measure of dose. OEHHA's point concerning alleged similarities between the mouse and monkey data of Dahl et al. (1990) is unfounded because it is based on inappropriate scaling techniques. OEHHA should recognize that when adjustments for metabolism are made, the risk estimates based on the mouse and rat tumor data become reasonably consistent. This suggests that use of the metabolized dose reduces uncertainties in the risk estimates (CMA, pp. i-ii, 6-8).

Response: As OEHHA staff have noted previously (see Part C, pp. OEHHA C-4 and OEHHA C-7 to OEHHA C-8, CMA comments 6 and 15), a number of possible dose measures were considered and the continuous internal dose, based on the uptake data of Bond et al. (1986), was judged the most reliable. The available metabolic data show little or no correlation between tissue concentrations of epoxide metabolites and observed incidence of carcinogenic lesions. The production of tumors in multiple organs and lack of correlation with tissue metabolite residues suggests the use of a dose measure that is applicable to the whole animal. The relative distribution of metabolites does not explain either the lower sensitivity of rats to butadiene or the different sites of carcinogenicity in the two species. For example, the estimates and measurements for mammary tissue are not much different between the two species and the difference in response between the two species is not easily explained on the basis of metabolite levels. In view of these facts, a whole body (continuous internal) dose is the most appropriate for low dose extrapolation.

OEHHA staff are aware of the unreasonableness of expecting perfect data. Nevertheless, the lack of human metabolic data on butadiene prevents the application of the metabolic information in a reliable manner. In view of the current state of knowledge of tumor incidences and BD metabolite concentrations, OEHHA staff believe that continuous internal dose is the most reliable measure. OEHHA staff have used metabolic data in risk assessments for many compounds including methylene chloride, vinyl chloride, chloroform, trichloroethylene, perchloroethlyene and formaldehyde.

OEHHA staff found similarity between the mouse (rather than the rat) and the monkey using a common "surface area" (body weight raised to the 2/3 power) adjustment of BD uptake data. Staff do not believe this is an inappropriate technique.

OEHHA staff have addressed the adjustments for metabolism suggested by the commenter in Part C (see pp. OEHHA C-2 to OEHHA C-12, esp. CMA comments 5, 6, 17 and 21). These adjustments are based on data regarding blood epoxide levels. The epoxides referred to are not necessarily the only metabolites associated with carcinogenicity. There is no apparent correlation between tissue epoxide concentrations and tumorigenic response. Given the available data, the whole body continuous internal dose measure used in the TSD is still the most appropriate measure for low dose extrapolation. OEHHA staff declined to use the suggested adjustments in the TSD's ultimate risk assessment, and need not acknowledge properties of

risk estimates based on those adjustments. OEHHA staff do recognize, however, that adjustments to the data (such as those suggested by the commenter) can allow similar risk estimates to be derived from studies in different species. It should be noted, again, that the adjustments suggested by the commenter are not based on human data.

15. Comment: The TSD should at least use the available metabolism data qualitatively. The document should acknowledge that the "best estimate" of likely excess cancer deaths from exposure to BD in ambient air is an upper bound based on conservative assumptions not specific to butadiene. The document should also acknowledge that the data indicate that the B6C3F1 mouse is uniquely susceptible to the carcinogenic effects of butadiene, and that the document's risk assessment may therefore overstate human cancer risks by a substantial margin. This should be stated wherever the document presents its "best estimate" of human cancer risks, and at the end of the fourth paragraph on p. 7 of the Executive Summary. The document should recognize that the estimate presented there is intended to be a conservative upper bound estimate of possible excess human cancer deaths, that the "best estimate" of potency is not necessarily the most plausible and is inconsistent with data from epidemiologic studies, and that the number of excess human cancer deaths from exposure to BD in California's ambient air may actually be zero (CMA, pp. ii, 8-9).

Response: The TSD generally makes clear that the "best estimate" of risk is an upper bound value. The reader can easily discern that many of the assumptions used are health conservative and not specific to butadiene. It is not clear that B6C3F1 mice is uniquely susceptible to the carcinogenic effects of butadiene, although it is apparent that they are more sensitive than Sprague-Dawley rats. Part B's summary makes it clear that the calculations presented there relate to upper bound plausible excess cancer risks and that "the actual risk, which cannot be calculated, may be much lower" (p. 1-4). In response to comments, OEHHA staff have recommended changes to the Executive Summary to note the upper bound nature of the population cancer burden estimate and the fact that actual risk may be significantly lower. However, with 30 million people in California, it is extremely unlikely that the number of excess human cancer deaths from exposure to BD in the ambient air will actually be zero. The consistency of the "best" potency estimate with the epidemiologic data is addressed in responses to several of the comments above, as well as in Section 5.3 of the TSD's Part B. OEHHA staff have compiled additional relevant information for inclusion in the TSD before the document is formally presented to the Air Resources Board.

Comments from the General Motors Corporation (GM)

1. Comment: The document still does not put the risk from butadiene concentrations into the proper perspective. It should better portray the limitations and uncertainty associated with the range of unit risk factors developed for 1,3-butadiene. Although some important caveats are present in the Part B Summary, the Executive Summary still contains an objectionable statement that can be taken out of context (at the end of the fourth paragraph on p. 7). The words "upper limit" should be included in each reference to risk. (GM comments, December 6, 1991, p. 1).

Response: The draft TSD Part B addresses specific limitations and uncertainties of the chosen risk assessment approach, in Section 4. As noted by the commenter, Part B's Summary conveys the uncertainty associated with the range of unit risk factors. OEHHA staff have recommended changes to the TSD's overall Executive Summary to better convey this uncertainty and modify the statement found objectionable by the commenter.

2. Comment: This commenter is concerned that there is such a wide range (nearly two orders of magnitude) in the upper limit risk based on different animal models. This means that there is great uncertainty in judging the need for, and the cost-effectiveness of, any additional measures to reduce the risk. It is, therefore, extremely important to establish which animal model most closely approximates the risk to humans. A better understanding of the mechanism(s) of BD carcinogenicity is thus of paramount importance. The reader of the Executive Summary should be clearly informed of the wide range in upper limit risk, and the reasons for it (GM, pp. 1-2).

Response: As noted in the document, the range in upper limit risk estimates is based on upper confidence limits from various dose modeling approaches as well as different animal models. The TSD's Executive Summary need not detail all the reasons for this range. The Executive Summary notes that carcinogenic effects of butadiene were observed in studies of rodents. The reader can refer to Part B of the TSD for details of these studies and the risk assessment. Reasons for selecting a mouse-based risk estimate as the "best" value are also discussed there (see also the response to CMA comment 12, above). Unfortunately, insufficient data are available to clearly establish with which animal model can be used to most closely approximate the risk to humans.

Comments from Members of the Scientific Review Panel

1. Comment: Please address the dose responses for lung tumors in the two mouse bioassays. Are they consistent? (Dr. Witschi)

Response: In mouse study I the incidences of alveolar and bronchiolar neoplasms for the 0, 625, and 1250 ppm nominal dose groups were 2/50, 4/49 and 5/49 for males, respectively; and 3/49, 12/48 and 23/49 for the females, respectively. The maximum likelihood estimate (MLE) or q_1 values equivalent to the low dose slopes of these dose responses were 0.12 ppm^{-1} and 0.09 ppm^{-1}, respectively. The upper-bound (q_1*) potency values were 0.17 and 0.20 ppm^{-1} respectively. For the mouse II study, the incidences of alveolar and bronchiolar neoplasms for the 0, 6.25, 20, 62.5, 200 and 625 ppm dose groups were 22/48, 23/48, 20/44, 33/46, 42/48, 12/16 for the males, respectively; and 4/50, 15/44, 19/43, 27/44, 32/40 and 25/30 for the females respectively. The MLE (q_1) values were 0.15 ppm^{-1} and 0.28 ppm^{-1} respectively and the q_1* values were 0.22 ppm^{-1} and 0.37 ppm^{-1} respectively. For both studies, the q_1 and q_1* values given here were derived using surface area scaling for interspecies (mouse to human) extrapolation.

As can be seen, the q_1* potency estimates for the 4 data sets are quite close, ranging from 0.17 ppm^{-1} to 0.37 ppm^{-1}. The q_1 values ranged from 0.09 ppm^{-1} to 0.28 ppm^{-1}; the males in the two studies showed the closest

correspondence (0.12 ppm^{-1} vs 0.15 ppm^{-1}). Judging from these results it can be concluded that the extrapolated dose-response "curves" for this tumor are reasonably consistent between the two studies. As we would expect, given the designs for the two studies, the ratios of q_1^*/q_1 values indicate a greater statistical reliability of the mouse II study data.

2. Comment: Please address the varying background lung tumor rates among the males and females in the mouse bioassays. (Dr. Byus)

Response: The male control group in the mouse II study seemed to have a high background incidence of lung tumors (22/48, or 45.8%) compared to the other control groups in both studies. The historical incidence of these tumors in the testing facility was 20.6% (standard deviation = 7.5%, range = 10-30%). There is no explanation at present for this high background incidence. The incidence in the mouse II female controls was 4/50 or 8%. The historical incidence at the testing facility was 8.4% (standard deviation = 3.3%, range = 0-12%). Thus the female background incidence is typical of that seen in other studies at this facility.

3. Comment: Please address the observation by Philip Cole (submitted with earlier public comments) that, when data from the epidemiologic studies are aggregated, employment in occupations with butadiene exposure is not significantly associated with any cancer endpoint. (Dr. Friedman)

Response: This observation is part of testimony presented by Dr. Cole to the U.S. Occupational Safety and Health Administration (OSHA) as part of an OSHA proceeding. Dr. Cole's testimony was submitted as an Appendix to comments of the International Institute of Synthetic Rubber Producers on an earlier draft of the TSD. Nevertheless, it does not directly address the TSD. In addition, it should be noted that Dr. Cole's testimony was not published as peer-reviewed scientific literature.

Dr. Cole's testimony does not make clear the methods used to aggregate data from the epidemiologic studies. His observation is made only in a summary at the beginning of his testimony and repeated in his conclusions. It is not referred to, or discussed, in the body of his testimony.

OEHHA staff caution against mechanically aggregating data from different epidemiologic studies and drawing conclusions. Data from diverse epidemiologic studies are sometimes aggregated in order to examine the hazard posed by similar exposures. An advantage of this is increased statistical power to demonstrate a health effect. Before conducting this type of analysis, stringent conditions should be met. These include comparability of exposure classification, consistent availability of information on potential confounders, and comparability of follow-up time and disease ascertainment. In the case of butadiene the comparability of exposure classification is the most difficult issue to resolve. Failure to satisfy these conditions may unwittingly introduce bias into the analysis, possibly in the direction of the null hypothesis. Thus, for hazard identification, it is often best to examine the data from individual studies. However, each study may contribute to an overall weight-of-evidence judgement.

Epidemiological studies commonly vary in their suitability for use in dose-response assessment, and the risk assessor should consider their strengths and weaknesses, as well as whether their data can be aggregated. If the conditions for aggregating data cannot be met, alternative procedures for examining multiple studies exist. These involve ranking studies by criteria such as statistical power, adequacy of study design, and absence of confounding exposures. The risk assessor may report (as a "best estimate") a measure of association derived from the best study, or, the results from individual studies may be weighted, using the ranking criteria, to generate an overall result. Such weighting may be based on qualitative as well as quantitative factors. Risk assessors generally use similar methods with animal studies; it is common practice to report a geometric mean of potency estimates as an overall estimate, but the raw data from several studies are rarely combined.

Although Dr. Cole's testimony presents no details of his aggregation, several criticisms can be made. In the two styrene-butadiene rubber (SBR) studies included in his analysis (Matanoski et al., 1990; Meinhardt et al., 1982), lymphatic cancers were not significantly elevated in the whole cohorts, but were elevated in cohort subsets defined by job category, race, or time of hire. Job category and time of hire may relate to level of butadiene exposure; therefore, observations of elevated cancer SMRs in such subcohorts are consistent with an association of butadiene and cancer.

Dr. Cole's analysis apparently groups butadiene production workers from the cohort described by Divine (1990) with *all* the SBR workers from the Meinhardt et al. (1982) and Matanoski et al. (1990) studies. This groups workers who were probably exposed to BD with those who may have had little or no exposure, thus diluting any observed associations of butadiene with lymphatic or hematopoietic cancers.

In summary, Dr. Cole's observation is presented without detail or calculations, and the underlying analysis appears to be inappropriate for assessing the potential carcinogenic effects of employment in industries with butadiene exposure.

Table 5-1b. Comparison of Cancer Incidence in Worker Cohorts with Predictions from Mouse-Based Butadiene Risk Assessment.

Study	Cohort	Cancer Type	Number of Cancer Cases In Workers			Number of Cases Predicted from 95% U.C.L. of Mouse-Based Potency Estimate[a]	
			Exp.	Obs.	95% U.C.L. of Obs.	At 10 ppm BD	At 1 ppm BD
Matanoski et al., 1990	Black Production Workers (N=371)	All	16.5	19	30	28	18
		All Lymphopoietic	1.2	6	13	12	2
Divine, 1990	Routinely Exposed Workers (N=705)	All	46.3	42	57	50 - 80[b]	47 - 50[b]
		Lymphosarcoma & reticulosarcoma	0.9	5	12	5 - 40	1 - 5

Abbreviations: Exp., expected. Obs., observed. U.C.L., upper confidence limit. BD, butadiene. ppm, parts per million.

[a]Based on occupational exposure to the indicated concentrations of BD. These concentrations represent a range of plausible estimates (the actual workplace concentrations were not measured). See text for exposure assumptions and description of calculations.

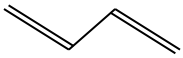
[b]Ranges given here result from using different numbers of stages in multistage model of human cancer incidence. See text for details.

000777

1,3-Butadiene Reference Exposure Levels

(butadiene; buta-1,3-diene; biethylene; bivinyl; divinyl; vinylethylene)

CAS 106-99-0



1 Summary

The Office of Environmental Health Hazard Assessment (OEHHA) is required to develop guidelines for conducting health risk assessments under the Air Toxics Hot Spots Program (Health and Safety Code Section 44360 (b) (2)). In response to this statutory requirement OEHHA developed a Technical Support Document (TSD), adopted in December 2008,that describes acute, 8-hour and chronic RELs. The TSD presents methodology that explicitly includes consideration of possible differential effects on the health of infants, children and other sensitive subpopulations, in accordance with the mandate of the Children's Environmental Health Protection Act (Senate Bill 25, Escutia, chapter 731, statutes of 1999, Health and Safety Code Sections 39669.5 et seq.). These guidelines have been used to develop the following RELs for butadiene; this document will be added to Appendix D of the TSD.

Butadiene is a major commodity product of the petrochemical industry. Workers acutely exposed to butadiene during rubber manufacturing experienced irritation of the eyes, nasal passages, throat, and lung. Some workers also experienced coughing, fatigue, and drowsiness. Inhalation of butadiene is mildly narcotic at low concentrations. Exposure to very high concentrations can result in narcosis, respiratory paralysis, and even death. Repeated exposure can damage human sperm cells and increase ovarian atrophy in mice. The studies reviewed for this document include those published through August 2012.

Although butadiene is a carcinogen (IARC Group 1, IARC, 2012), this document does not discuss issues related to the cancer potency factor. That has been previously derived and is available at www.oehha.ca.gov/air/hot_spots/index.html.

1.1 Butadiene Acute REL

Inhalation reference exposure level	**660 µg/m^3 (297 ppb)**
Critical effect(s)	Lowered male fetal weight
Hazard Index target(s)	Development

1.2 Butadiene 8-Hour REL

Inhalation reference exposure level	**9 µg/m^3 (4 ppb)**
Critical effect(s)	Increased incidence of ovarian atrophy in mice
Hazard Index target	Female reproductive system

1.3 Butadiene Chronic REL

Inhalation reference exposure level	**2 µg/m^3 (1 ppb)**
Critical effect	Increased incidence of ovarian atrophy in mice
Hazard Index target	Female reproductive system

2 Physical & Chemical Properties

Description	Colorless gas
Molecular formula	C_4H_6
Molecular weight	54.09 g/mol
Density	0.6211 g/ml at 20 ºC
Boiling point	-4.4 ºC
Melting point	-108.9 ºC
Vapor pressure	2100 mm Hg at 25 ºC
Flashpoint	- 76 ºC
Flammability limits	Extremely flammable
Solubility	Very slightly soluble in water ; soluble in ethanol, ether, acetone, benzene and organic solvents
Odor threshold	0.0014 mg/l; mildly aromatic with slight petroleum odor
Octanol-water partition coefficient	Log Kow = 1.99
Henry's Law constant	0.074 atm m^3/mole (HSDB, 2011)
Conversion factor	1 ppm = 2.21 mg/m^3 at 20 ºC, 1 bar 1 mg/m^3 = 0.445 ppm at 20 ºC, 1 bar

3 Occurrence and Exposure

1,3-Butadiene (butadiene, BD) is a major commodity product of the petrochemical industry. The largest quantities are used in the production of styrene-butadiene rubber copolymers for car and truck tire manufacturing. Butadiene polymers are also used in a variety of other industrial applications, including the production and synthesis of certain latex products, resins, and nitrile rubbers. Most environmental releases of butadiene (78.8%) are associated with non-point or mobile sources such as motor vehicles. About 1.6% of butadiene releases are fugitive or accidental emissions during manufacture, use, transport, storage, or disposal. The remainder comes from other sources including direct volatilization from gasoline, stack emissions from incinerators, cigarette smoke, and the burning of plastics (U.S. EPA, 2002; HSDB, 2004). Butadiene is a component of gasoline (Kane and Newton, 2010) as well as a product of the combustion of minor gasoline components, olefins and cyclohexane (Zhang et al., 2008). Approximately 26 percent of butadiene emissions in California can be attributed to on-road motor vehicles,

with an additional 27 percent attributed to other mobile sources, such as recreational boats, off-road recreational vehicles, and aircraft. Area-wide combustion sources contribute approximately 21 percent and include agricultural waste burning, open burning associated with forest management, and woodstoves and fireplaces. Stationary sources contribute less than one percent of the statewide 1,3-butadiene emissions. The primary stationary sources with reported 1,3-butadiene emissions are petroleum refining, manufacturing of man-made materials, and oil and gas extraction. The primary natural sources of 1,3-butadiene emissions are wildfires. The total statewide emission inventory for butadiene was estimated at 3,754 tons for 2008 (CARB, 2009).

Butadiene is subject to rapid destruction by reactions in the atmosphere in the presence of O_3, OH radical, and NO_x from which a number of reactive electrophiles are formed, including acrolein, formaldehyde, epoxybutene, and other aldehydes (Tuazon et al., 1999; Baker et al., 2005; Doyle et al., 2007). Baker et al. (2005) investigated the formation of butadiene and hydroxyl radical-initiated reaction products using atmospheric pressure ionization mass spectrometry (API-MS) and solid phase extraction followed by gas chromatography. The products from butadiene were identified as 4-hydroxy-2-butenal ($HOCH_2CH{=}CHCHO$) and its nitro derivative ($HOCH_2CH{=}CHCH_2ONO_2$) and isomers. The rate constant for the reaction of OH radicals (OH) with 1,3-butadiene was 6.66 x 10^{-11} $cm^3molecule^{-1}s^{-1}$ and with 4-hydroxy-2-butenal was (5.7±1.4) x 10^{-11} $cm^3molecule^{-1}s^{-1}$ both at 298±2K. The formation yields of acrolein and 4-hydroxy-2-butenal were 58±10% and 25+15-10%, respectively. Hydroxynitrates from the reaction of hydroxyperoxy radicals with NO account for an additional 7-10% of products from butadiene. Acrolein also reacts with OH radicals to form glycolaldehyde ($HOCH_2CHO$) with yields of 25 to 32 percent. For 1,3-butadiene the reaction with OH radicals is the dominant chemical loss process during daytime, with butadiene lifetimes of 1-2 hr at OH concentrations of 2 x 10^6 molecules/cm^3.

Sexton et al. (2007) investigated the photochemistry of 1,3-butadiene in an outdoor smog chamber. The butadiene atmospheric lifetime and transformation rates were dependent on concentrations of NO, NOx, other organic compounds, sunlight, temperature, and humidity. The results were compared with computer-based simulations of butadiene transformations (Morphecule Mechanism model). The major observed products with daytime BD/NOx were acrolein, formaldehyde, furan, 1,3-butadiene monoxide and an organic nitrate probably peroxyacylnitrate . Other carbonyls found were glyoxal, glycolaldehyde, glycidaldehyde, 3-hydroxy propanaldehyde, hydroxyl acetone, butenedial, and malonaldehyde. The model simulations were representative of the observed results.

Doyle et al. (2004) studied the photochemical degradation of 200 ppb 1,3-butadiene in presence of 50 ppb NO. The initial and major photochemical products were acrolein, acetaldehyde and formaldehyde. They observed that the photoproducts were more toxic to A549 human lung cells in vitro than butadiene. In a similar study Doyle et al. (2007) showed that BD + NOx + 5 hours light was more toxic to A549 cells than a synthetic mixture of acrolein, formaldehyde and ozone photoproducts or ozone alone. The photoproducts of butadiene were primarily acrolein, acetaldehyde, formaldehyde, furan and ozone. Toxicity was assessed by increased lactate dehydrogenase (LDH) release and increased interleukin-8 (IL-8) and interleukin-6 (IL-6) protein release.

3.1 Ambient Exposures

Despite its rapid removal, butadiene is almost always present at very low concentrations in US cities and large suburban areas.. Elevated concentrations have been measured in the vicinity of heavily trafficked areas, refineries, chemical manufacturing plants, and plastic and rubber factories (ATSDR 1992). Statewide levels throughout California have consistently dropped from an annual average high of 0.41 ppb in 1990 to approximately 0.1 ppb in 2007. Sampling of 18 communities in the San Francisco Bay area showed a maximum butadiene concentration of 0.26 ppb (San Jose). The majority of all samples were below the minimum detection limit (MDL) of 0.05 parts per billion by volume (ppbv) (BAAQMD, 2008). Similar sampling of 10 communities in the Los Angeles air basin showed a maximum butadiene concentration of 0.78 ppb (Compton). The majority of all samples were below the MDL of 0.2 ppbv (SCAQMD, 2008). In general, mean values in southern California are about twice those in the San Francisco Bay area e.g., San Jose, 0.069 ppb vs. Compton, 0.14 ppb.

One recent study measured butadiene concentrations prior to and after the Ireland Public Health Tobacco Act of 2002 ban on smoking in pubs (McNabola et al., 2006). The average concentration of butadiene measured inside pubs prior to the ban was 4.15 μg/m^3 (± 1.61 μg/m^3). This is compared to average ambient outside measurement of 0.12 μg/m^3 (0.05 ppm) and an average indoor measurement of 0.22 μg/m^3 (0.1 ppm) following the smoking ban. Similar results were obtained in a survey of 10 Finnish restaurants with 4.3 ± 3.2 SD μg/m^3 (1.9 ppm) butadiene (geometric mean = 2.7, range = 0.26-10.1, N = 20 measurements) in smoking areas compared with 1.1 ± 1.3 SD (0.5 ppm) butadiene (geometric mean = 0.52, range = 0.11-3.9, N =20) in non-smoking areas (Vainiotalo et al., 2008).

The TEACH (Toxic Exposure Assessment, Columbia/Harvard) study characterized personal exposures to urban air toxics among high school students living in inner city neighborhoods of New York City (Kinney et al., 2002). Exposure to air toxics was analyzed in 46 high school students using 48-hr personal monitoring, outdoor ambient monitoring, and in-home ambient monitoring. The students were mainly African American and Hispanic, and were required to be non-smokers from non-smoking families. The subjects ranged in age from 12.5 to 19.6 years old, with 42% male and 58% female. Investigators measured levels of particulates and concentrations of 17 volatile organic compounds (VOCs). Butadiene levels tended to be higher in the home and from personal exposures than what was measured in ambient outdoor air. The mean outdoor concentrations of butadiene were 0.13 μg/m^3 (winter) and 0.14 μg/m^3 (summer), versus in-home ambient concentrations of 1.18 μg/m^3 in winter and 1.01 μg/m^3 in summer. Mean concentrations obtained from personal air monitors were 0.87 μg/m^3 (0.39 ppm) in winter and 1.16 μg/m^3 (0.52 ppm) in summer (Kinney et al., 2002).

Marshall et al. (2006) estimated the intake of butadiene from ambient air pollution in California's South Coast Air Basin. Butadiene concentrations were based on air dispersion modeling for the period April, 1998 to March, 1999. Surveys from 25,064 respondents were used in the study. The 28,746 person-days in the data set are from 11,749 households. Travel survey data were collected for 15-24 week periods in 2000-

2002. The method used four main inputs: temporal information about people's position (latitude and longitude); microenvironment, and activity level; temporally and spatially explicit model estimates of ambient butadiene concentrations; and stochastically determined microenvironmental adjustment factors relating the exposure concentration to the ambient concentration, age-, gender-, and activity-specific breathing rates. The calculated average breathing rate (m^3/day-person) for the study was 13.1. The mean intake rate for 1,3-butadiene was 7.3 µg/day ±7.6 SD. This corresponds to an average concentration of 0.56 µg/m^3 (0.24 ppb)(7.3/13.1) or approximately 8% of the chronic REL for butadiene derived in this document.

A qualitatively similar difference between personal/indoor air and outdoor air concentrations was observed in metropolitan Mexico City (Serrano-Trespalacios et al., 2004). Personal exposures to 34 VOCs were measured for adolescents and their families living close to one of five central monitoring stations over the period of one year. Simultaneous 24-hr indoor, outdoor and central site measurements were also taken. Indoor butadiene concentrations (mean = 2.5 µg/m^3, 1.1 ppb) were 2 to 6 times higher than outdoors measurements (mean 0.9 µg/m^3, 0.40 ppb). Personal exposures for all participants including adolescents (mean = 2.9 µg/m^3, 1.3 ppb) were also significantly higher than ambient outdoor concentrations (Serrano-Trespalacios et al., 2004). Logue et al. (2011) identified 1,3-butadiene as one of nine indoor air pollutants categorized as priority hazards based on available acute and chronic health criteria. Two hundred and sixty-seven chemicals were evaluated with health criteria for 97. Butadiene was categorized based on its cancer risk, however the upper bound estimate of exposure (their Fig.2) is close to the U.S. EPA RfC of 0.9 ppb and the chronic REL derived in this document (1.0 ppb).

Gustafson et al. (2007) measured personal exposures and indoor air concentrations of butadiene, benzene, formaldehyde, and acetaldehyde due to wood burning in a small Swedish town. Ambient air concentrations of butadiene for 1 day sampling (N = 9) had a mean of 0.12µg/m^3(0.05 ppb) (95%CI, 0.04-0.16). Indoor personal monitoring in the wood burning group (N = 14) gave mean butadiene concentrations of 0.31 to 0.38 µg/m^3 (0.14 to 0.17 ppb) for 1 to 7 days sampling. The referent group (N=10) had mean values of 0.11 to 0.14 µg/m^3 (0.049 to 0.06 ppb) ($P < 0.015$-0.03). The authors concluded that domestic wood burning increased personal exposure to ambient butadiene as well as indoor exposures to butadiene.

Additional exposure analyses were conducted by Nazaroff and Singer (2004), who studied hazardous air pollutants including butadiene within US residences. The authors found that the population inhalation intake of butadiene was dominated by residential environmental tobacco smoke (ETS). Mean individual intake of butadiene was also dominated by residential ETS. Data analyses indicated that some 16 million U.S. juveniles (2 months to 16 years old) are exposed to ETS in the home. Assuming between 14-20 cigarettes smoked per day in each residence, with an average of 515 µg butadiene/cigarette, the resulting daily intake of butadiene for juveniles was 16 – 37 µg. The total mean individual inhalation intake for juveniles was estimated to be approximately 10 mg butadiene/year from residential ETS, far exceeding the exposure

to butadiene from ambient sources, estimated at approximately 0.3 mg/year (Nazaroff and Singer 2004).

3.2 Worker Exposures

Investigators in the US, Europe, and China report similar average exposures to butadiene in the workplace. However, approaches to exposure assessment vary. Because butadiene monomer production and extraction are generally operated currently as closed processes, exposures tend to be low. With certain tasks, or with accidents and engineering failures, there can be transiently high exposures that are brief and intense. Air measurement may not accurately reflect these peaks. Historical levels of butadiene exposure in the workplace are thought to have been much higher than present values (Lynch 2001). Few data are available for butadiene concentrations prior to the 1970s. Exposure modeling suggests that levels in the 1940s – 1950s were considerably higher, and estimated to be approximately 20 ppm (Lynch et al., 2001). There has been an annual trend of an approximate 6% drop in butadiene concentrations over time, with large decreases in the late 1950s and the early 1980s (Lynch 2001). More current measurements of butadiene in the workplace were provided by Chan et al. (2006), who measured worker exposure to air toxics in an 11-plant petrochemical complex in Taiwan. For the years 1997-1999, only 15.2% of the measurements were above the limit of detection (LOD), with a mean low concentration of 7.7 ppb, a mean high concentration of 10.5 ppb, and a maximum concentration of 3080 ppb butadiene. Specific LODs were not given for the 39 chemicals studied but the mean low concentrations were calculated using 0 for values below the LOD and the mean high concentrations were estimated using LOD/√2 for values below the LOD.

Sapkota and colleagues evaluated butadiene exposure of tollbooth workers in the Baltimore Harbor Tunnel during the summer of 2001. Mean ambient butadiene concentrations outside the tollbooths varied by shift, with the morning levels (19.8 $\mu g/m^3$) exceeding afternoon levels (14.9 $\mu g/m^3$). The lowest concentrations were measured at night, and averaged 4.9 $\mu g/m^3$. Considerable protection was offered by the tollbooth itself, within which ambient concentrations even in the height of traffic only measured 6.7 $\mu g/m^3$ (3 ppb) butadiene (Sapkota et al., 2005).

Ammenheuser et al. (2002) evaluated BD exposure in 49 workers in a styrene-butadiene rubber plant. The concentration of the butadiene metabolite 1,2-dihydroxy-4-(N-acetylcysteinyl-S)-butane (M1) in urine was used as a biomarker of butadiene exposure and the frequency of hypoxanthine-guanine phosphoribosyl transferase (*HPRT)* mutations in peripheral blood lymphocytes was used as a biomarker of effect. The workers were divided into high and low exposure groups according to job description and work area. The average butadiene exposure concentrations (by personal organic vapor badge dosimeters) for the two groups were high, 1.48 ppm and low, 0.15 ppm ($P < 0.002$). Both mean M1 and *HPRT* mutant frequencies were more than three-fold higher in the high group than in the low group ($P < 0.0005$). For the high exposure group (N = 19-22) urine M1 mean was 2,046 ± 348 SE ng/mg creatinine and the *HPRT* frequency was 6.8±1.2 SE x 10^{-6}. The values for the low exposure group (N = 20-24) were 585±98 ng/mg creatinine and 1.8±0.2 x 10^{-6}, respectively. The study

demonstrated correlation between the three measures of butadiene exposure. All workers, even those not exposed to butadiene, excreted some M1 suggesting other exogenous and/or endogenous sources. It is uncertain whether the observed increase in mutant frequency represents an adverse effect.

4 Metabolism

Common routes of exposure to butadiene include inhalation and dermal exposure.The inhalation absorption of butadiene is dependent on its partition coefficient (log Kow = 1.99) and Henry's Law constant (0.074 atm m^3/mole). Butadiene is absorbed very quickly into the lungs and blood. Physiological modeling uses blood/air partition coefficients of about 1 for butadiene (e.g., lung/air = 0.65 in humans, Pery & Bois, 2009; blood/air = 1.22 in humans, Brochot et al., 2007). The dosimetry of butadiene in rodents has been assessed by measuring steady-state concentrations of butadiene epoxides in blood and deposition in tissues following butadiene exposure. The steady-state in vivo concentrations of epoxybutene in the blood of mice exposed to 62.5, 625, and 1250 ppm butadiene for 4 to 6 hours was 4-8 times higher than those measured in rats at these BD concentrations and exposure periods, i.e. 0.4 to 8.6 μM in mice vs. 0.01 to 2.5μM in rats (Bond and Medinsky, 2001). Average tissue epoxides in mice for exposures of 625 ppm or 1250 ppm butadiene for 6 hours ranged from 2 to 3.5 mmol/g for lung epoxybutene, 0.5 to 0.75 mmol/g for liver epoxybutene, and 0.6 to 1.5 mmol/g for lung diepoxybutane, respectively. In rats at exposures of 625, 1250, or 8000 ppm butadiene, lung epoxybutene ranged from 0.2 to 1.1 mmol/g and liver epoxybutene ranged from 0.1 to 1.2 mmol/g (Bond et al., 1996).

Butadiene metabolites play a role in the toxicity of butadiene. It is not always clear which metabolites are responsible for various observed toxicities (e.g., cardiovascular). In some cases, strong evidence implicates specific toxicological endpoints are associated with particular metabolites.

In general, studies using human tissues showed that humans metabolized butadiene more like rats than mice, with lower rates of diepoxybutane formation (Bond et al., 1996). Perez et al. (1997) report the formation of N-(2,3,4-trihydroxybutyl)valine hemoglobin adducts (THBV) from the butadiene metabolite epoxybutanediol in rats and occupationally exposed humans. The adduct levels in rats exposed to 50, 200, or 500 ppm butadiene were 13.4, 5.9, and 1.15 pmol THBV/g globin/ppm butadiene, respectively. In humans exposed to 1 ppm butadiene, hemoglobin adducts were 8.2 and 10.7 pmol THBV/g globin/ppm. The results indicate that butadiene is absorbed into human blood at relatively low concentrations and forms hemoglobin adducts at levels comparable to those seen in rats.

Once in the body, butadiene is activated, creating the toxic metabolite(s) associated with adverse effects. Activation of butadiene typically occurs in liver microsomes via P450-mediated oxidative metabolism in mammals (Bond and Medinsky, 2001). Epoxybutene is the major oxidative metabolite, which is further oxidized to diepoxybutane and hydrolyzed to epoxybutane diol by microsomal epoxide hydrolase (Bond and Medinsky, 2001; Elfarra et al., 2001) (Figure 1).

Available data indicate that metabolism is qualitatively similar among all species studied, although the rates of metabolite formation differ. Mice form the initial oxidative metabolite, epoxybutene, approximately 6 to 8 times faster than rats or humans (Csanady et al., 1992) and produce a greater proportion of active epoxide metabolites than rats (Bond et al., 1986; Himmelstein et al., 1994; Thornton-Manning et al., 1995; Csanady et al., 2011).

This greater formation of epoxy metabolites may be related to differences in the amino acid composition of the binding pocket of CYP2E1 in rodents that facilitate hydrogen bonding and increase the probability of a second epoxidation. Compared to the rat, mouse CYP2E1 has an additional methylene group in the active site that binds epoxybutene more tightly, thus further enhancing formation of the second epoxide (Lewis et al., 1997). This observed difference in the quantity of epoxides formed correlates with the observed sensitivity and higher overall toxic response in mice versus rats. In vitro data from adult liver samples indicate that humans form epoxides of butadiene at rates (Vmax/Km, where Vmax = maximum reaction rate e.g. µmoles/hr and Km = reactant or substrate concentration (S) in µmol/L giving half the Vmax, where the overall reaction rate is more precisely defined by the Michaelis-Menten relation v = Vmax x S/(Km + S)) similar to mice but less than rats (Duescher and Elfarra 1994; Krause and Elfarra 1997), although it is unclear how this relates to in vivo toxicity in humans. Other authors, notably Csanady et al. (1992), reported that the Vmax/Km ratio obtained with mouse liver microsomes was 5.6- and 8.2-fold higher than the ratios obtained with human or rat liver microsomes, respectively. The observed differences in human butadiene oxidation rates between the studies may be attributed to variability in the expression of P450 2E1, 2A6 or other P450 enzymes in the human liver samples (Duescher and Elfarra, 1994). Detoxification of butadiene epoxides also varies across species, with human tissues preferentially detoxifying the derived epoxides through microsomal epoxide hydrolase to form butane diol followed by conjugation with GSH, whereas detoxification in mice occurs through glutathione-*S*-transferase (Boogaard and Bond 1996; Kemper et al., 2001). As a result, mice predominantly excrete 1-hydroxy-2-(N-acetylcysteinyl)-3-butene (MII), while adult humans excrete mainly 1,2-dihydroxy-4-(N-acetylcysteinyl-S)-butane (MI) (Figure 1). Boogard et al. (2001) reported urinary metabolites resulting from 8 hours occupational exposure to 1 ppm butadiene: MI, 2213 µg/L and MII, 39 µg/L.

The reactive metabolites of butadiene, including 1,2-epoxy-3-butene (EB), 3,4-epoxy-1,2-butane diol (EB diol), diepoxybutane (DEB), and hydroxymethylvinyl ketone (HMVK) have been shown to covalently bind to biological macromolecules, including DNA in the testes, lung, and liver, and certain proteins, including hemoglobin (Koivisto et al., 1998; Hayes et al., 2000; Begemann et al., 2001; Boogaard et al., 2004). In vivo experiments have demonstrated species differences in adduct formation. For example, following a nose-only 6 hr inhalation exposure of 200 ppm ^{14}C-1,3-butadiene, measured uptake in mice was almost twice that for rats; the formation of specific DNA adducts was nearly 10 times higher in mice than rats (Boogaard et al., 2004). Quantitatively similar profiles of adducts were observed in an inhalation study using 20 ppm, although the overall level of DNA adducts formed in rats and mice was very low (Booth et al., 2004).

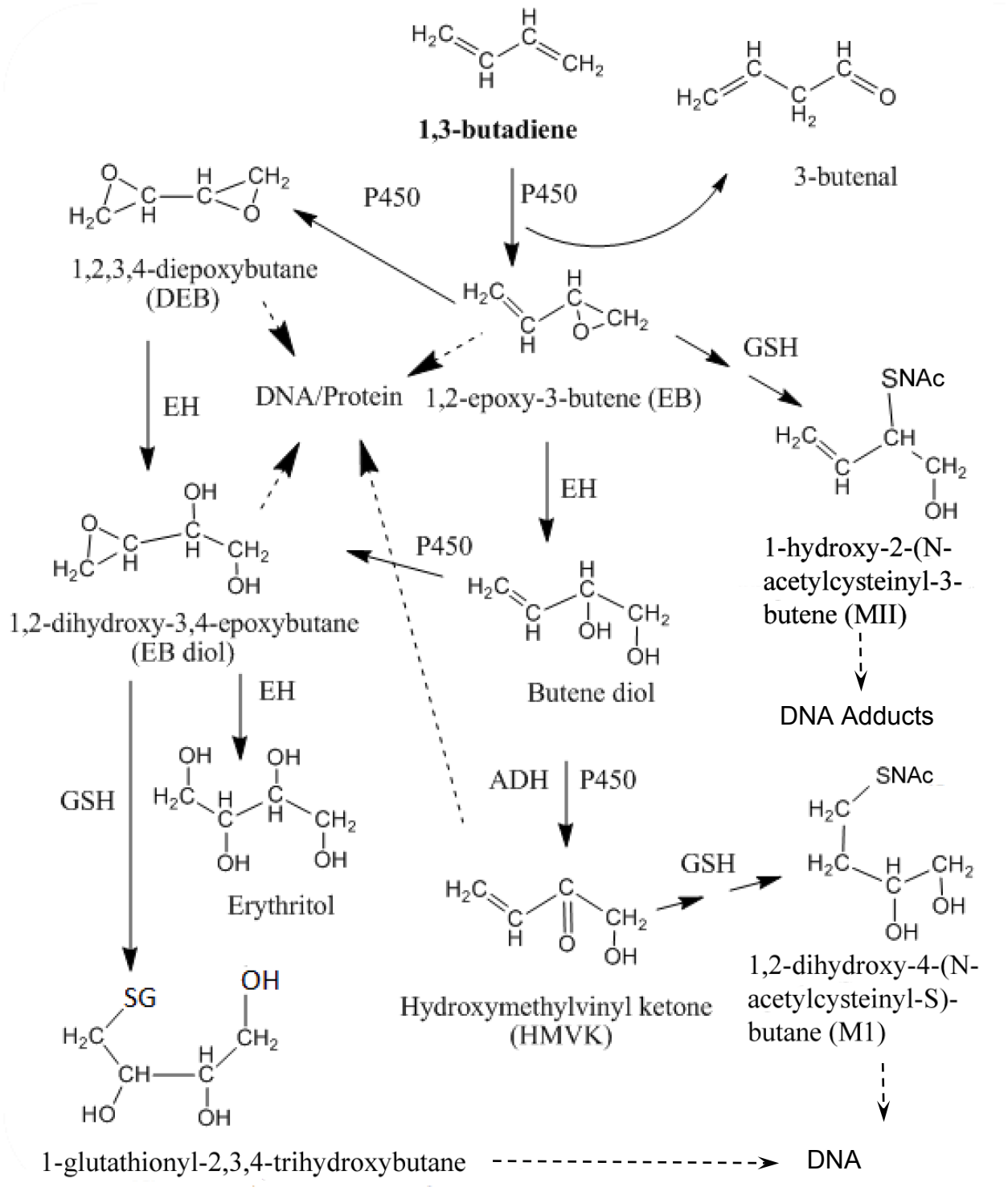


Figure 1 Metabolic scheme for 1,3-butadiene

This scheme presents the major identified reactive metabolites. Dashed arrows emanate from butadiene metabolites reported to form adducts with DNA or proteins. Abbreviations: EH, epoxy hydrolase; GSH, glutathione; ADH, alcohol dehydrogenase; P450, Cytochrome P450; NAc, N acetylcysteinyl; G, glutathionyl.

Cho and Guengerich (2012b) reported the mutagenicity in the *Escherichia* coli *rpoB* gene of the DEB-GSH conjugate *S*-(2-hydroxy-3,4-epoxybutyl)glutathione. Three DNA adducts were identified in the *E.coli* DEB/GST/GSH system: *S*-[4-(N(7)-guanyl)-2,3-dihydroxybutyl]GSH; *S*-[4-(N(3)-adenyl)-2,3-dihydroxybutyl]GSH; and *S*-[4-(N(6)-deoxyadenosinyl)-2,3-dihydroxybutyl]GSH.

Butadiene reactive metabolites also appear to form stable hemoglobin adducts in humans and experimental animals. In one study, Begemann et al. (2001) found that workers in a butadiene monomer production plant exposed to a median concentration of 440 $\mu g/m^3$ (0.2 ppm) had significantly increased levels of epoxybutene hemoglobin adducts versus unexposed controls. Results from another study indicated that levels of hemoglobin adducts of epoxybutane diol were substantially higher than those of epoxybutene in both inhalation-exposed rats and occupationally exposed humans (Perez et al., 1997). Forty-one butadiene polymer production workers in China were exposed to median butadiene concentrations of 2 ppm (6 hr time-weighted average) and had greater levels of hemoglobin adducts when compared with unexposed controls ($p < 0.0001$) (Hayes et al., 2000). These adduct levels were significantly correlated with butadiene concentrations ($p < 0.03$). The exposed workers did not differ from unexposed workers with respect to frequency of diepoxybutane-induced sister chromatid exchanges or aneuploidy, leading the authors to suggest that there were no correlations of biomarkers of butadiene exposure with these specific genotoxic effects (Hayes et al., 2000).

Sangaraju et al. (2012) used a more sensitive liquid chromatography-electrospray ionization tandem mass spectrometry method of measuring DEB-specific adducts in mouse liver DNA (bis-N7G-BD) following 2 weeks exposure (6hr/d, 5d/week) to various concentrations of 1,3-butadiene from 0.5 to 625 ppm. At low concentrations of 0.5, 1.0 and 1.5 ppm butadiene they found bis-N7G-BD (1,4-bis-(guan-7-yl)-2,3-butanediol) adducts at 0.17, 0.14, and $0.14/10^7$ nucleotides/ppm BD, respectively. At higher concentrations of 6.25, 62.5, 200 and 625 ppm BD the adduct levels were significantly lower at 0.051, 0.013, 0.009, and $0.006/10^7$ nucleotides/ppm BD, respectively. A similar pattern of higher efficiency of adduct formation at lower exposures was also seen with DEB-specific hemoglobin adducts by Georgieva et al. (2010). At the same low concentration butadiene exposures (0.5, 1.0 and 1.5 ppm), pyr-Val (N,N-(2,3-dihydroxy-1,4-butadiyl)valine) Hb adducts were formed at 21.0, 20.0 and 26.0 pmol pyr-Val-Hb/g globin/ppm BD. At 6.25, 62.5, 200 and 625 ppm butadiene concentrations, adduct formation was 12.0, 6.7, 3.7, and 2.5 pmol pyr-Val Hb/g globin/ppm BD, respectively.

Overall the results indicate that the efficiency of DEB reaction with cellular macromolecules is higher at lower exposure levels in mice. It is unknown whether a similar increased efficiency at low exposure levels occurs in humans. Boysen et al. (2012) have observed low but clearly detectable levels of pyr-Val-Hb in workers with 8-hr time weighted average butadiene exposures of 0.01 ppm (N = 23, controls), 0.24 ppm (N = 24, monomer workers), or 0.81 ppm (N = 34, polymerization workers). The levels of pyr-Val-Hb adducts found were 0.13, 0.16, and 0.29 ($P<0.05$ vs controls and monomer workers) pmol/g globin, respectively. The authors concluded that humans were less efficient in the formation of DEB than mice or rats at similar butadiene

exposures. HB-Val, indicative of epoxybutene concentrations, was found at 0.22, 0.47, and 2.23 pmol/g globin and THB-Val, indicative of epoxybutane diol concentrations, was found at 95, 179, and 716 pmol/g globin, respectively. The formation of DEB relative to other epoxides was significantly different in the highest exposed workers compared to controls and lower exposures. The pyr-Val concentrations in individual workers were significantly correlated with their HB-Val concentrations when analyzed combined or separately ($P < 0.01$, $P < 0.05$, respectively). In contrast the pyr-Val concentrations were significantly correlated with the THB-Val concentrations when analyzed combined or separately only for the high exposure group ($P < 0.01$).

5 Acute Toxicity of 1,3-Butadiene

5.1 Acute Toxicity to Adult Humans

There is a paucity of data on acute human exposure to butadiene. For eyes and skin, direct contact with liquid butadiene can cause burns similar to frostbite, while vapors or fumes may be irritating to the eyes. Some early occupational health studies indicated that workers exposed to butadiene during rubber manufacturing experienced irritation of the eyes, nasal passages, throat, and lung (Wilson 1944). Some workers also experienced coughing, fatigue and drowsiness, although the symptoms diminished after the exposure ceased. Inhalation of butadiene is mildly narcotic at low concentrations, and may result in a feeling of lethargy and drowsiness (Sandmeyer, 1981). Exposure to very high concentrations of butadiene can result in narcosis, respiratory paralysis, and even death. The first signs of acute exposure to high concentrations are blurred vision, nausea, paresthesia, and dryness of the mouth, throat, and nose. This may be followed by fatigue, headache, vertigo, hypotension, slowed pulse rate, and unconsciousness (ATSDR, 1992).

In more recent occupational health studies, while there were significant differences in short-term inhaled concentrations of butadiene between exposed workers (mean 1.5 $\mu g/m^3$, range 0.2 – 69.0 $\mu g/m^3$) and unexposed workers (mean 0.4 $\mu g/m^3$, range < 0.1 – 3.8 $\mu g/m^3$), biomarkers of effect, such as sister chromatid exchanges, were not different between groups of workers (Lovreglio et al., 2005). A study of 437 full-shift and 14 short-term samples indicated that exposure to butadiene in the polymer industry ranged from < 0.005 – 43.2 ppm for 8 hr samples, and 0.088 – 210 ppm for acute (15 – 120 min) exposures (Fajen et al., 1990). Data on potential adverse effects from acute exposure were not reported. Since the workers in many of these studies were exposed to mixtures of chemicals, the relationship of acute butadiene exposure and adverse effects remains unclear.

In an acute study of human physiological factors, 133 adult subjects (both male and female) were exposed to 2 ppm (4.42 mg/m^3) butadiene for 20 minutes (Lin et al., 2001) followed by purified air for 40 minutes. Five exhaled breath samples collected during exposure were used to measure the respiratory uptake of butadiene. The absorbed dose was defined as µg butadiene uptake/kg body weight. Although the subjects were given identical administered doses, there was a wide range of uptakes, 0.6-4.9 µg/kg.. The total butadiene concentration inhaled was significantly higher in males than in

females; however there was no significant difference in the respiratory uptake fraction of the total concentration inhaled, which ranged from 18-74% for all participants. Females had larger body burdens per kilogram of body weight than males. Additionally, both age and smoking were negatively associated with butadiene uptake. The authors suggested that the wide range of butadiene uptake values indicates that administered dose is a poor estimator of absorbed dose or body burden (Lin et al., 2001).

Khalil et al. (2007) evaluated neurological parameters for a random cohort of 310 subjects who had been exposed to 1,3-butadiene due to accidental leakage and release of BD in an industrial area and 50 matched unexposed controls. A set of clinical neurological tests was utilized including electroencephalograms (EEGs), somatosensory evoked potentials (SSEPs), brainstem auditory evoked potentials (BAEPs), visual evoked potentials (VEPs), and electromyography/nerve conduction tests (EMG/NCT). Exposed individuals were divided into mild (N = 212) and severe (N = 98) subgroups based on preliminary testing. Among the mild subgroup the most common finding was encephalopathy characterized by headaches and light headedness that resolved within hours to six months. The electroneurodiagnostic tests were normal except for abnormal BAEPs in 21/212 (9.91%) subjects. In the severe subgroup encephalopathy was characterized by confusion, irritability, impaired judgment, altered levels of consciousness, disequilibrium, numbness, paresthesia, ataxia, weakness, nausea, vomiting, and memory deficits. The EEGs were mildly abnormal (12/98, 12%) and BAEPs and VEPs strongly so (63/98, 49/98, respectively). Within the 50 controls, one had an abnormal EEG on heavy medication and 2 (4%) had polyneuropathy with diabetes and alcohol abuse, and two had abnormal BAEPs due to deafness or hearing abnormalities. All controls had normal VEPs. The authors concluded that butadiene met the definition of a neurotoxicant. The butadiene-induced neurotoxic effects of most of the subjects were reversible except for 18 (6%) subjects that demonstrated irreversible neurotoxic effects.

5.2 Acute Toxicity to Infants and Children

The exposures most infants and children have to butadiene tend to be chronic and low level. However, by correlating changes in health effects with short-term changes in ongoing measurements of butadiene and other VOC's, it may be possible to discern the potential toxicity associated with acute exposures. This is the approach taken below to determine the effects of air pollutants on asthma exacerbation in children.

Delfino and colleagues studied the relationship between asthma symptoms in children and concentrations of ambient VOCs and criteria air pollutants, including butadiene (Delfino et al., 2003). To be eligible to participate, children were required to have a minimum 1-year history of physician diagnosed asthma and at least two symptomatic days per week that required use of a β-agonist inhaler. Twenty-two Hispanic children, aged 10 – 16, who were living within the Los Angeles air basin and who were nonsmokers from nonsmoking households, were enrolled in the study. The children kept daily symptom diaries from November 1999 through January 2000 and the severity of their symptoms was analyzed for association with ambient concentrations of particulates, ozone, and VOCs. Regression analysis examined air pollution

concentrations on the same day or up to 4 days prior to the reported asthma symptoms, thereby allowing the analysis of the lag between the measured concentrations and the onset of health effects.

Adjusted odds ratios (OR's) for more severe asthma symptoms were as follows: for 1.4 ppb NO_2, 1.27 (95% CI, 1.05-1.54); 1.00 ppb benzene, 1.23 (95% CI, 1.02-1.48); 3.16 ppb formaldehyde, 1.37 (95% CI, 1.04-1.80); 37 μg/m^3 PM_{10}, 1.45 (95% CI, 1.11-1.90); 2.91 μg/m^3 elemental carbon, 1.85 (95% CI, 1.11-3.08); and 4.64 μg/m^3 organic carbon, 1.88 (95% CI, 1.12-3.17).

Butadiene was detected in 74 daily air pollution measurements with a mean concentration of 0.51 ppb (range 0.05 – 1.50 ppb). This study presented an OR of 1.32 (95% CI = 0.97-1.80) for the association between asthma symptoms and ambient butadiene concentrations on the previous day, and an OR of 1.16 (95% CI = 0.90-1.49) for the association between asthma symptoms and same-day butadiene levels. While these estimates are elevated, the confidence intervals included no effect. Thus, the authors interpreted these results as suggestive of an increased risk of asthma symptoms in children with butadiene exposure. However, the study was limited by small numbers, as only seven subjects reported symptom scores >2 and only 16 with scores >1. Also, associations for scores >2 were strongly influenced by one subject with moderate persistent asthma (Delfino et al., 2003). Since many of these pollutant chemicals occur together it will always be difficult to tease out effects due to specific pollutants. However, such studies may be of use in comparison with studies of accidental releases of butadiene where the problem of concurrent exposures is much less.

5.3 Acute Toxicity to Experimental Animals and In Vitro Toxicity

Few studies have reported the acute or short-term effects of butadiene. The lethal concentration resulting in 50% mortality in a population (LC_{50}) was measured in an acute inhalation study using 2 hr exposures for mice and 4 hr exposures for rats (Shugaev 1969). The mouse LC_{50} was determined to be 122,000 ppm, and the rat LC_{50} was 129,000 ppm; no other effects were reported. In a National Toxicology Program study, male and female B6C3F1 mice were exposed to 0, 625, 1,250, 2,500, 5,000, or 8000 ppm butadiene in air for 15 days (NTP, 1984). Survival rates were unaffected by dosing, and no respiratory effects, cardiovascular lesions, or hepatic/gastrointestinal histopathology were noted even at the highest concentration. There was, however, a non-significant decrease in mean body weight at 8000 ppm butadiene when compared with controls (NTP 1984). No severe immunological changes were noted in humoral or cell-mediated immunity in mice exposed to 1250 ppm butadiene for 6 weeks (Thurmond et al., 1986).

The effects of butadiene inhalation (12.5 and 1250 ppm) on birth outcomes following a single acute 6-hr exposure of male mice were reported by Anderson et al. (1996). The mean number of implants per female was reduced at both concentrations compared to controls, but statistically significant only at the higher level ($p < 0.05$). A dominant lethal

effect (see Section 7.2) was not evident with the acute exposure, and there was no significant increase in post-implantation losses or fetal abnormalities.

In contrast to the results of the studies of the parent compound cited above, short-term studies of butadiene metabolites have shown acute effects, some of which were severe. Henderson and colleagues investigated the acute and sub-acute toxicity of the oxidative metabolite butadiene diepoxide in female B6C3F1 mice and in Sprague Dawley rats. Examination of the organs, including the liver, kidney, marrow, heart, and ovaries revealed that a single 6 hr inhalation exposure to 18 ppm butadiene diepoxide (3 to 4/group) induced no clinical signs of toxicity. However, the mice showed a slight increase in the number of alveolar macrophages and focal necrosis of the olfactory epithelium that started to reverse 7 days after exposure (Henderson et al., 1999). In the sub-acute portion of the study, mice (15/group) were exposed to either 2.5 or 5 ppm butadiene diepoxide for 6 hr/day for 5 days. The animals showed no body weight changes and no histopathology of the nose, larynx, or lung. However, in an earlier part of the study there was significant toxicity when mice and rats (56/group) were exposed to either 10 or 20 ppm butadiene diepoxide for 6 hr/day for 7 days. Both mice and rats experienced significant weight loss, corneal opacity, labored breathing, ruffled coats and hunched posture, as well as nasal mucosa degeneration, necrosis, inflammation, and ulceration. Additionally there were individual instances of focal hepatic necrosis and bone marrow atrophy in the mice. All but one of 56 mice from the high dose group were dead by the end of 12 days (98.2 %); by comparison, mortality in the low dose group was only 29% at 10 days of the 21 day study duration.

In a study of the butadiene metabolite 3-butene-1,2-diol, male B6C3F1 mice (4 to 5/group) were given a one-time intraperitoneal (i.p.) dose of 250 mg/kg and male Sprague Dawley rats (4 to 5/group) were given either 125, 200, or 250 mg/kg 3-butene-1,2-diol i.p. (Sprague et al., 2004). Two of the four rats dosed with 250 mg/kg died within 24 hours. Prior to death, the rats were lethargic and experienced seizures. At necropsy, it was evident that rats dosed at the highest level experienced severe hepatic necrosis and hemorrhage. The rats dosed with 200 mg/kg also showed indications of hepatic necrosis, inflammation, and hemorrhage. No other rat organ examined showed consistent dose-related lesions, although dosed rats exhibited significant hematological differences and significantly lower hepatic levels of glutathione when compared with controls (Sprague et al., 2004). Mice necropsied 4 hrs after treatment with 250 mg/kg 3-butene-1,2-diol did not exhibit any lesions, or any significant hematological changes. However, the mice did have significantly lower levels of hepatic glutathione 1 hr after treatment when compared to controls (Sprague et al., 2004). Half-lives for 3-butene-1,2-diol were calculated to be 2.12 hr in the rat and 0.44 hr in the mouse, which may partially account for the observed differences in acute toxicity between the two species (Sprague et al., 2004).

5.3.1 Toxicity In Vitro

Results from a human embryonic lung fibroblast study suggest that in vitro exposure to the butadiene metabolite 1,2:3,4-diepoxybutane (100 μM for 1 hr) is associated with substantial inhibition of cell proliferation and cell cycle arrest at G_1 (Schmiederer et al.,

2005). The authors suggested that cell proliferation was inhibited by a diepoxybutane-mediated alteration to cell division (Schmiederer et al., 2005). Another in vitro study found that products from the incomplete combustion of butadiene were differentially taken up and retained by human bronchial epithelial cells (Penn et al., 2005). Investigators found that the combustion-generated "ultrafine" particles (defined by the authors as <2.5 µm aerodynamic diameter) migrated from culture medium to the cell membrane, but not into the cell interior. The organic chemicals bound to the particles, however, were found to migrate from the particle surface, through the cell membrane into the cytosol, and into cellular vesicles. The authors suggested that toxicants, including butadiene, may transfer into cells directly without the cellular uptake of the carrier particles i.e., direct uptake from the vapor phase (Penn et al., 2005).

6 Developmental and Reproductive Toxicity

6.1 Developmental Effects

Many investigators have studied the potential for butadiene and its metabolites to cause reproductive or developmental toxicity. Earlier studies focused on overall reproductive fitness following short-term exposures, while more recent studies have focused on specific effects and mechanisms of toxicity.

Some of the first studies on the reproductive and developmental toxicity of butadiene were nested within chronic studies (Owen et al., 1987; Melnick et al., 1990). The National Toxicology Program developed the first series of short-term studies to evaluate developmental and reproductive toxicity of inhaled butadiene in mice and rats (Morrissey et al. 1990). In a three-part study, Sprague Dawley rats (24-28/group), and B6C3F1 and Swiss (CD-1) mice (18-22/group) were exposed to 0, 40, 200, or 1000 ppm butadiene on gestation days 6-15 for 6 hr/day and examined for teratological effects. A separate set of male mice was exposed to 0, 200, 1000, or 5000 ppm for 6 hr/day for 5 days for a sperm morphology study. Pregnant rats exhibited toxicity at 1000 ppm in the form of reduced extragestational weight gain and, during the first week of treatment, decreased body weight gain; there were no other differences in fertility or developmental parameters between exposed and control groups. In mice, however, there were increased numbers of fetal variations (supernumerary ribs, reduced ossification) in litters from dams exposed to 200 and 1000 ppm butadiene. These teratogenic effects occurred in the presence of maternal toxicity and fetal weight gain reductions. Male mice treated with 1000 or 5000 ppm butadiene showed significant increases in abnormal sperm 5 wks after exposure, consistent with an effect of butadiene on mature spermatozoa and spermatids (Morrissey et al., 1990).

Teratological effects at maternal butadiene exposures of 200 ppm and higher were also observed by Hackett et al. (1987a), along with signs of maternal toxicity. However, in their study of reproductive performance, and maternal and fetal toxicity, Hackett et al. included a lower dose of 40 ppm. For this study, 78 pregnant female CD-1 mice received whole-body exposure to 0, 40, 200, or 1000 ppm butadiene for 6 hr/day on gestation days (gd) 6-15, with necropsy on gd 18. Mice were weighed prior to mating and repeatedly during gestation. They were observed for signs of toxicity during

exposure and examined for gross tissue abnormalities at necropsy. Reproductive parameters included numbers of implantations sites, resorptions, and live and dead fetuses. Live fetuses were examined for signs of morphological anomalies and growth retardation. The incidences of fetal variations (supernumerary ribs and reduced ossification of the sternebrae) were significantly elevated in litters from mice exposed to 200 and 1000 ppm ($P < 0.05$). At these exposure levels, there was also evidence of maternal toxicity as shown by significantly lower maternal weight gain ($P \leq 0.05$). There was a significant dose-dependent reduction of fetal body and placental weights at the two higher doses for female fetuses, and at all doses in males ($P \leq 0.05$) (Table 1). The observation that males fetuses appeared to be susceptible to butadiene at levels that were not maternally toxic is the basis of the acute REL.

Green (2003) reanalyzed the data of Hackett et al. (1987) and found inconsistencies associated with the presentation and calculation of mean values for maternal and fetal body weights, sex ratio, and reproductive data. When the data were analyzed in the context of analysis of covariance (ANCOVA) with sex ratio and litter size as covariates, no statistically significant difference was found between fetal weights at the 40 ppm exposure level and the controls. The results of the Green (2003) re-analysis are shown in Table 2B. In addition to the covariance adjusted pairwise tests of means in Table 2, Green also evaluated the ratios of treatment means to control means and calculated a 95% confidence interval (CI) for this ratio using the variance-covariance matrix from the ANCOVA. For male fetal weights the ratios (and 95% CI) were: control, 100 (referent); 40 ppm, 95.7(100.1); 200 ppm, 82.6(87.2); and 1000 ppm, 77.0(81.5). Since the upper 95% CI for the 40 ppm exposure level includes 100 there is no statistically significant difference from the control at this level. The other data sets for combined sexes and female fetal weights gave similar results.

In a companion study, Hackett et al. (1987b) exposed female Sprague-Dawley rats to whole body inhalation of 1,3-butadiene for 6 hr/day at 0, 40, 200, or 1000 ppm. The female rats (24 to 28/dose group) that had been mated to unexposed males were exposed on gestation days 6 thru 15 and sacrificed on gd 20. There were no significant differences among treatment groups in maternal body weights or extragestational weights at 40 or 200 ppm, but at 1000 ppm depressed body weight gains were observed during the first 5 days of exposure and extragestational weight gains were lower than control values. Placental weights, fetal body weights and sex ratios were unaffected by butadiene treatment. There were no significant differences among groups for incidences of fetal malformations.

The dose-response analyses of the Hackett et al. (1987) and the Green (2003) re-analysis data sets are described and discussed in Section 8.1. Those analyses employ the benchmark dose software (BMDS v. 2.3.1) and do not depend on a specific no observed adverse effect level (NOAEL) or lowest observed adverse effect level (LOAEL). Rather, the benchmark model uses the entire dose-response data set. The key value for a non-cancer dose response is usually the benchmark concentration level $BMCL_{05}$, or the 95% lower confidence limit on the concentration causing a 5% response. Considerable experience has shown that the $BMCL_{05}$ is a better choice for a point of departure than a single dose NOAEL.

Table 1. Body Weight and Fetal and Placental Measures after 1,3-Butadiene Exposure (Hackett et al., 1987a)

Observation	1,3-Butadiene Concentration (ppm)			
	0	40	200	1000
Number examined				
Mothers	18	19	21	20
Litters	18	19	21	20
Fetuses	211	237	259	244
Maternal weight gain (g)[a] (11-16 gd)	13.3 ± 0.6[b]	12.7 ± 0.4[b,c]	11.4 ± 0.5[c,d]	10.6 ± 0.4[d]
Pup body weight (g)[a]				
Females	1.30 ± 0.02[b]	1.25 ± 0.01[b]	1.10 ± 0.02[c]	1.02 ± 0.03[d]
Males	1.38 ± 0.03[b]	1.31 ± 0.02[c]	1.13 ± 0.02[d]	1.06 ± 0.02[e]
Sex ratio (% male)	51.6 ± 3.91	49.8 ± 3.06	51.5 ± 3.68	51.8 ± 3.29
Placental weight (mg)[a]				
Females	83.1 ± 3.03[b]	80.9 ± 2.46[b]	74.7 ± 3.52[b,c]	70.1 ± 2.33[c]
Males	89.3 ± 3.05[b]	89.5 ± 2.27[b]	80.1 ± 2.35[c]	74.5 ± 1.81[c]

[a]Mean ± standard error; [b-e] Values that do not share a common superscript letter are significantly different ($p \leq 0.05$) from one another.

Table 2. Body Weight and Fetal and Placental Measures after 1,3-Butadiene Exposure (Green, 2003 Reanalysis of Hackett et al., 1987a data)

Observation	1,3-Butadiene Concentration (ppm)			
	0	40	200	1000
Number examined				
Mothers	18	19	21	20
Litters	18	19	21	20
Fetuses	211	237	259	244
Pup body weight (g)	Mean ± SE	Mean ± SE	Mean ± SE	Mean ± SE
Combined	1.350 ± 0.028	1.283 ± 0.013	1.126 ± 0.021*	1.038 ± 0.025*
Females	1.309 ± 0.021	1.253 ± 0.012	1.100 ± 0.022*	1.015 ± 0.026*
Males	1.382 ± 0.033	1.307 ± 0.016	1.132 ± 0.016*	1.060 ± 0.024*
Sex ratio (% male)	51.55 ± 3.866	49.66 ± 2.947	51.44 ± 3.677	51.80 ± 3.310
Placental weight (mg)				
Combined	86.99 ± 2.960	85.30 ± 2.283	78.65 ± 3.243	73.06 ± 1.826*
Females	83.15 ± 3.023	80.89 ± 2.474	74.33 ± 3.540	70.84 ± 2.284*
Males	89.58 ± 2.995	89.71 ± 2.263	80.27 ± 2.324	74.64 ± 1.785*

Note: * significantly different from controls ($P < 0.05$).

6.2 Dominant Lethal Effects

The primary purpose of the dominant lethal assay is to identify toxicants that have the ability to enter the gonad and cause a genetic lesion in the gamete, which results in cytogenetic damage in the fertilized zygote without recognized changes in fertilizability. Generally, male animals are either given 1 – 5 doses of the test agent, or are dosed over the entire cycle of spermatogenesis, then mated at weekly intervals with untreated females. The females are necropsied prior to giving birth and examined for uterine contents. The total number of implanted embryos is compared over dose groups and with controls. Dominant lethal assays use conceptus failure as an endpoint for potential male-mediated developmental toxicity.

Nested within the larger National Toxicology Program study discussed above, dominant lethality was studied in litters sired from male mice exposed to 70, 200, 1000, or 5000 ppm butadiene for 6 hr/day for 5 days (Morrissey et al., 1990). There was no mortality noted in the male mice even at the highest butadiene concentrations, and body weights were unaffected. One week following exposure to 1000 ppm, investigators observed a statistically significant increase in dead implants as a percentage of total implants ($p \leq 0.05$). There were smaller non-statistically significant increases in dead implants at 200 and 5000 ppm (p values not given). During the second week following exposure, there were significant increases in dead implants in both the 200 and 1000 ppm groups ($p \leq 0.05$). The investigators noted that the dose-dependency of the effects weakens at higher dose levels, but the effect in week 1 and 2 is consistent with an effect of butadiene on mature spermatids and spermatozoa. The authors suggested that because of the effects noted in sperm morphology and dominant lethality, butadiene might act as a germ cell mutagen in mice (Morrisey et al., 1990).

In 1993, Anderson and colleagues considered dominant lethality in a more focused study of male-mediated reproductive toxicity following acute and short-term butadiene exposure. In the acute study, male CD-1 mice were exposed to 0 (n = 25), 1250 (n = 25), or 6250 (n = 50) ppm butadiene one time for 6 hr, then mated with untreated females. In the short-term study, male CD-1 mice were also exposed to 0, 12.5, or 1250 ppm butadiene for 6 hr/day 5 days/wk for 10 weeks prior to mating, then mated with untreated females. The necropsied dams were examined for the number of live fetuses, the number of early and late post-implantation deaths, and the number and type of fetal gross malformations. A single 6 hr exposure did not elicit a dominant lethal effect, although there was a non-significant reduction in the mean number of implants in the 1250 and 6250 ppm dose groups. Such findings may indicate germ cell toxicity or pre-implantation losses, which are generally not associated with heritable changes (Anderson et al., 1993). Subchronic exposures to 12.5 ppm butadiene were associated with increased congenital malformations and late fetal deaths. In addition, there was a significant incidence of dominant lethality at 1250 ppm butadiene. The authors concluded that butadiene is mutagenic and teratogenic following subchronic exposures (Anderson et al., 1993).

To further elucidate the dominant lethality of butadiene, Adler and Anderson (1994) exposed CD-1 male mice to 1250 ppm for 6 hr/day, 5 day/week for 10 weeks prior to

mating, and then mated the exposed males with unexposed females for 4 successive weeks. There was a total (summed) incidence of 23.1% dominant lethality over 3 weeks of mating. The highest weekly incidence of dominant lethality was 12.4% after week 2 of mating. No other fertility differences were noted between exposed and control animals. From the timing of the dominant lethal effect, the authors suggested that spermatozoa and late spermatids were the most sensitive germ cell types to butadiene inhalation (Adler and Anderson, 1994). Most of these results were duplicated in another report by Anderson and colleagues (Anderson et al., 1996) and are consistent with dominant lethal findings by Morrisey et al. (1990).

In 1994, Adler and colleagues designed a cross-bred (102/E1 x C3H/E1) mouse study to consider not only dominant lethality but teratogenicity in pregnancies allowed to come to term. Male mice (20 per group) were exposed to 0 or 1300 ppm butadiene for 6 hr/day for 5 days, and then mated with unexposed females for 4 consecutive weeks. Groups of pregnant dams were sacrificed at gestation day 14 following successive weeks of mating. The percentage of dead implants and dominant lethals was elevated in litters that were sired during weeks 1, 2, and 3 following butadiene exposure in the males. The effect, however, was highly significant ($p \leq 0.01$) in week 2. All other fertility parameters were similar to controls (Adler et al., 1994). From the timing of effects, the authors concluded that dominant lethal mutations were induced in spermatozoa and late spermatids, suggesting that butadiene may induce heritable translocations in these germ cell stages (Adler et al., 1994), consistent with their previous work in CD-1 mice (Adler and Anderson 1994).

Two other studies have demonstrated a positive association between inhalation of low concentrations of butadiene and dominant lethality, with mice being more susceptible than rats (Anderson et al., 1998; Brinkworth et al., 1998). Brinkworth and colleagues exposed male CD-1 mice to 0, 12.5 or 125 ppm butadiene for 6 hrs/day for 5 days a week for 10 weeks followed by mating with untreated females. A significant increase in dominant lethality was noted, but only in the 125 ppm group. Anderson and colleagues exposed male CD-1 mice to 0, 12.5, 65, or 130 ppm butadiene for 6 hr/day, 5 days/week for four weeks and groups of male Sprague Dawley rats to 0, 65, 400, or 1250 ppm butadiene for 6 hr/day, 5 days/week for 10 weeks. There was a significant increase in dominant lethality in mice in the 65 and 130 ppm dose groups, although the effect was not dose-related. Male-specific effects in the rats were not observed at any exposure level (Anderson et al., 1998). Additionally, one study showed that the butadiene metabolite diepoxybutane is also associated with dominant lethality. Male mice were given either 0 or 54 mg/kg diepoxybutane (DEB), and mated for up to 16 days after treatment with untreated females. A single dose of 54 mg/kg DEB was toxic to mature spermatozoa; therefore, there was a low number of total implants in the first 8 days after treatment. However, there was an increase in dominant lethals throughout all mating intervals. The authors suggested that diepoxybutane is likely a late spermatid mutagen (Adler et al., 1995).

Accumulated data appear to suggest that inhalation exposure of butadiene is associated with an increase in dominant lethal effects even at concentrations below the threshold for acute toxicity. There is evidence of species and strain differences in

susceptibility, with mice being more susceptible than rats, and outbred CD-1 mice appearing to show dominant lethality at lower butadiene concentrations than other strains of mice. Regardless of the length of pre-mating dosing (i.e., a single 6 hr, 5 day, or 4-10 week exposures), dominant lethal effects were associated with butadiene effects in the more mature male germ cells, specifically mature sperm and late spermatids.

6.3 Germ Cell Mutagenicity

Results from dominant lethal experiments suggest that butadiene exposure may be associated with germ cell mutagenicity. As such, several researchers investigated the potential for butadiene to induce heritable mutations. In one study, C3H/E1 male mice were exposed to 1300 ppm butadiene for 6 hr/day for 5 days and mated with untreated females. Half the offspring were examined for dominant lethality while the other half were allowed to mature in order to study heritable effects in subsequent generations (Adler et al., 1995). There was a non-significant increase in dominant lethal mutations in F_1 litters sired from butadiene-exposed males. However, when the F_1 males were allowed to mature and mate, the resulting F_2 litters were approximately half as large as litters sired by control F_1 mice. Additionally, there was a 2.7% translocation frequency in F_2 pups following F_0 paternal butadiene exposure compared with a 0.05% translocation frequency from historical controls (Adler et al., 1995).

In a follow-up study, mice exposed to 0, 130, or 500 ppm butadiene for 6 hr/day for 5 days were mated for 4 consecutive weeks with untreated females. A positive dominant lethal effect was observed for 130 and 500 ppm during week one of mating (a sperm effect) and for 500 ppm during week two of mating (a late spermatid effect). Among 434 F_1 offspring from butadiene-exposed F_0 sires, there was a 1.15% incidence of translocation when compared with 0.05% translocation in historical controls ($p < 0.001$) (Adler et al., 1998). The heritable mutation was identified as a reciprocal translocation with insertion of the central portion of chromosome 9 into chromosome 19 (Adler et al., 1998). The genetic risk from butadiene exposure was calculated using data from these two studies with a linear dose-response model [$Y = 0.05 + 6.9 \times 10^{-5} X$] where Y is the percentage of offspring with heritable translocations and X is butadiene exposure in ppm hr. Results from the calculations suggest that butadiene has the potential to cause heritable mutations in mouse sperm that can be transmitted to subsequent generations with a doubling dose of 725 ppm-h butadiene (Adler et al., 1998).

Pacchierotti et al. (1998) investigated the paternal transmission of butadiene-induced chromosomal aberrations to one cell embryos. Male mice (102/E1 x C3H/E1) were exposed to 0, 130, 500, or 1300 ppm butadiene for 6 hr/day for 5 days then mated 1:2 with untreated B6C3F1 females. The resulting zygotes were arrested at first cleavage and analyzed for chromosomal aberrations in each of the first three weeks of mating following paternal exposure. Zygotes conceived in weeks one and two of mating were produced from oocytes from untreated females fertilized with either mature sperm or late spermatids. Zygotes conceived in week three of mating were the product of oocyte-early spermatid or oocyte-late prophase spermatocyte unions. Investigators found a dose-dependent increase in structural chromosomal aberrations in first-cleavage

embryos conceived in week one of mating, with significance ($p < 0.005$) achieved at 500 ppm butadiene. Embryos conceived during week two also showed dose-dependent increases in chromosomal aberrations, with significance achieved at 1300 ppm butadiene ($p < 0.025$). The authors concluded that the male germ cells associated with the observed embryo mutations were likely the same types as those associated with male-mediated dominant lethality in other studies, and that sperm in the final stages of maturation were most susceptible to the mutagenic effects of butadiene.

Anderson et al. (1997) evaluated the effects of 1,2-epoxybutene (BMO) and three other chemicals (β-estradiol, daidzein, dibromochloropropane) on human sperm using the alkaline Comet assay for DNA damage in vitro. Fresh and frozen samples from two fertile donors each and frozen samples from two infertile donors were analyzed. All the chemicals induced significant effects in all exposed samples ($P < 0.01$). For BMO exposures of 0, 80, 160, and 320 μM, significant dose-response model fits were obtained only with frozen samples. Using the continuous Hill and polynomial models, $BMCL_{05}$ values ranged from 0.6 to 24.2 μM with an arithmetic mean of 12.3 μM and a geometric mean of 4.64 μM. The effects seen with BMO were comparable to those seen with dibromochloropropane, a known male reproductive toxicant, at 20 to 200 μM. Internal exposure estimates obtained with human PBPK models based on Kohn and Melnick (2001) indicate that BMO blood concentrations in the range of $BMCL_{05}$'s might be achieved by acute occupational exposures to butadiene (e.g., 200 ppm butadiene x 2 hr = 6.8 to 15.6 μM BMO Cmax in venous blood). Alternatively, the human PBPK model of Brochot et al. (2007) predicts lower blood and tissue concentrations of BMO for the same exposure scenario (i.e., 0.1 to 0.7 μM).

The majority of acute reproductive toxicity studies of butadiene have focused on male animals. No similar studies were found for female animals. However, one study by Tiveron and colleagues (1997) considered the acute female reproductive toxicity of the butadiene metabolite diepoxybutane. Female B6C3F1 mice received a single intraperitoneal injection of 26, 34, 43, or 52 mg/kg diepoxybutane, and then mated with unexposed males. The embryos were arrested at first-cleavage metaphase and examined for chromosomal aberrations. There were no consistent diepoxybutane-associated effects on mating, fertilization, or cell-cycle progression of the fertilized oocytes, although there was a decrease in the average number of zygotes harvested from each female. There was, however, a significant increase in frequency and number of chromosomal aberrations in exposed zygotes when compared with controls in all dose groups ($p < 0.05$-0.001).

When comparing the sensitivity of male and female gametes to pre-conception exposures, it appears that zygotes produced from exposed sperm-nonexposed oocytes are many times more likely to contain chromosomal aberrations (Adler et al., 1995) than zygotes conceived with exposed oocytes (Tiveron et al., 1997). While chromosomal damage may be transmitted via female germ cells (Adler et al., 1995), sperm appear to be at much higher risk of the mutagenic effects of butadiene and butadiene metabolites.

6.4 Ovarian Atrophy

Reproductive organs appear to be critical targets of chronic butadiene exposure, and ovotoxicity is the basis of the chronic and 8-hour RELs. Effects in the female reproductive tract were identified in an NTP chronic study, where B6C3F1 mice were exposed to 0, 625, or 1250 ppm butadiene for 6 hr/day, 5 days/wk for a planned period up to 103 weeks (NTP, 1984). Significant ovarian atrophy was observed in female mice in both dose groups (40 of 45 females at 625 ppm; 40 of 48 females at 1250 ppm). NTP investigators identified a chronic LOAEL of 625 ppm, based on the observed gonadal effects (NTP, 1984). In an effort to further elucidate the reproductive toxicity, NTP investigators conducted a study in B6C3F1 mice with lower butadiene concentrations (Melnick et al., 1990; NTP 1993). The animals were exposed to 0, 6.25, 20, 62.5, 200, or 625 ppm for 6 hr/day, 5 days/wk for up to 65 weeks. While there was significant mortality, a concentration-related increase in ovarian atrophy was also observed. At 40 weeks, ovarian atrophy was present in females exposed to 200 and 625 ppm butadiene. At 65 weeks, ovarian atrophy was present in all groups exposed to ≥ 20 ppm butadiene, and female mice exposed to the lowest concentrations of butadiene (6.25 ppm) exhibited atrophy at the end of the study at 105 weeks (Melnick et al., 1990; NTP, 1993). Based on these results, NTP investigators identified a chronic LOAEL of 6.25 ppm for reproductive toxicity (NTP, 1993). The 9-, 15- and 24-month ovarian atrophy data from NTP (1993) were subjected to benchmark dose analysis as summarized in Table 3. Only the 9-month interim sacrifice data were adequately fit by the multistage model (X^2 = 2.47, P = 0.78, $BMCL_{05}$ = 19.2 ppm). The 24-month data were adequately fit by the log probit model if the top dose was excluded. If the dose response used the average mixed venous concentration of diepoxybutane (DEB) from the mouse PBPK model an excellent fit to all the 24 months data was obtained (X^2 = 0.1, P = 0.99, $BMCL_{05}$ = 0.58μM DEB equivalent to 0.71 ppm BD). If the data were included in a time-adjusted model, all of the data could be fit (N = 435). Using the log probit model, a $BMCL_{05}$ of 1.01 ppm butadiene was obtained with a X^2 of 1.7, P = 0.64 (BMDS v 2.2). This study forms the basis of the derivation of 8-hour and chronic RELs described in sections 8.2 and 8.3, respectively.

While atrophy may be associated with normal reproductive senescence in rodents, it is noteworthy that butadiene-associated ovarian atrophy was observed in female mice as early as 9 months of age in association with butadiene exposure (NTP 1984). Depletion of ovarian follicles and ovotoxicity were also observed following intraperitoneal administration of butadiene monoepoxide or diepoxide (Doerr et al., 1996). Doerr et al. (1996) administered butadiene monoepoxide (BMO; 0.005-1.43 mmol/kg b.w.) or the diepoxide (DEB; 0.002-0.29 mmol/kg b.w.) to female $B6C3F_1$ mice (n = 10/dose) intraperitoneally daily for 30 days. The investigators analyzed the data on ovarian and uterine weight depression in mice (% body weight ± SD) using a PBPK model to simulate i.p. administrations and extrapolate internal dose metrics to external butadiene inhalation equivalents.

Table 3a. Ovarian Atrophy in Female Mice in 2-Year Inhalation Study of 1, 3-Butadiene (NTP, 1993).

Exposure Period	Model	X^2	P	BMC_{05} ppm	$BMCL_{05}$ ppm	$BMCL_{05}$ continuous, ppm	Comments
9 months	Multistage	2.47	0.78*	35.0	19.25	3.44	Full data set, N = 58
15 months	Log probit	10.74	0.030	11.2	3.66	0.654	Full data set, N = 52
	Log probit	10.64	0.014	11.1	3.45	0.616	Without top dose, N = 50
24 months	Log probit	6.47	0.091	0.056	0.0034	0.00054	Full data set, N = 325
	Log probit	2.80	0.42*	0.254	0.031	0.0055	Without top dose, N = 246
24 months#	Log logistic PBPK mean blood DEB concn	0.1	0.99	1.21 μM	0.56 μM, 0.71 ppm BD equivalent	0.51 ppm BD equivalent	Full data set, N = 325
9-24 mo time adjusted**	Log probit	1.7	0.64*	2.04	1.01	0.18	Full data, N = 435
9-24 mo time adjusted**	Log logistic	1.13	0.89*	2.03	1.58	0.28	Full data, N = 435

Note: * indicates adequate model fit to data, P ≥ 0.1; ** time adjustment by fitting % atrophy data to the hyperbola y (%) = a – b/(1 + c x dose)^d where a, b, c, and d are parameters, the dose was ppm butadiene (6hr/day, 5days/week), and the % atrophy response was weighted for time (PSI-Plot Hyperbola c, www.polysoftware.com). The fitted values were reconverted to quantal equivalents and analyzed by BMDS 2.2 with log probit, log logistic and multistage models (see Tables A8-A11 in appendix for additional details). # PBPK model simulation was for 24 hr and the 24 hr DEB AUC was divided by 24 to give average daily concentration so continuous adjustment is only 5/7 for 5 days/week exposure.

The values from Figure 2 of Doerr et al. (1996) were estimated as shown in Table 4. Internal dose metrics were then analyzed by benchmark dose methods using BMDS (versions 1.4.1c and 2.1.2). The benchmark dose BMD_{05} was the 0.05 relative deviation of a given dose metric typically using the Hill or Polynomial continuous dose response model and the $BMDL_{05}$ was the 95% lower confidence limit on that dose. The 5% response level is typically chosen by OEHHA for noncancer toxicity endpoints. The PBPK model was based on Kohn and Melnick (2001). The formation of hemoglobin adducts in the model was based in part on Csanady et al. (2003) with additional ACSL model code obtained from Prof. J.G. Filser (personal communication).

Table 4. Mean Ovarian and Uterine Weights (% Body Weight) in Mice Estimated from Figure 2 of Doerr et al. (1996).

Compound	Dose i.p., mmol/kg-d	Ovary	SD*	Uterus	SD*
BMO	0.005	0.0425	0.007	0.27	0.126
	0.02	0.0425	0.007	0.30	0.126
	0.09	0.038	0.007	0.28	0.126
	0.36	0.034	0.007	0.27	0.126
	1.43	0.020	0.007	0.10	0.126
DEB	0.002	0.0375	0.007	0.34	0.126
	0.009	0.034	0.007	0.33	0.126
	0.036	0.035	0.007	0.22	0.126
	0.14	0.020	0.007	0.11	0.126
	0.29	0.015	0.007	0.03	0.126

*SD values assumed equal for BMD analysis since they appear similar for low doses and decrease for the high doses.

Following the 30 days of exposure, ovaries and uteri were weighed, fixed, stained, and the follicular populations were assessed microscopically. In mice receiving 1.43 mmol BMO /kg, body weights were slightly decreased (10%) while ovarian and uterine weights were significantly decreased ($p < 0.05$). Administration of DEB caused significant decreases in both ovarian and uterine weights at 0.14 and 0.29 mmol/kg b.w. ($p < 0.05$), along with a 15% decrease in body weights at the higher dose. A dose-dependent decrease in ovarian follicle numbers was observed with both epoxides. To facilitate comparison, the dose required to reduce follicle numbers by 50% relative to controls (ED_{50}) was estimated. For small and growing follicles, the ED_{50} values for BMO were 0.29 and 0.40 mmol/kg, respectively. DEB showed greater potency with ED_{50} values for these same follicle populations of 0.10 and 0.14 mmol/kg, respectively. These authors suggested that butadiene ovotoxicity may be associated with metabolism of the parent compound to reactive diepoxides in situ.

Perez et al. (1997) measured N-(2,3,4-trihydroxybutyl)valine (THBV) adducts of hemoglobin in rats exposed to 1,3-butadiene or butadiene epoxides. Sprague-Dawley rats (2 to 3/group) were treated with single i.p. doses of either epoxybutene (78.3 mg/kg), epoxybutanediol (30 mg/kg), or diepoxybutane (16,7, 33.4, 60 mg/kg). The animals were sacrificed and blood was collected after 48 hr. Rats (3/group) were exposed to 1,3-butadiene by inhalation for 6 hr/d for 5 days at concentrations of 0, 50, 200 or 500 ppm. Two humans occupationally exposed to 1 ppm butadiene were also analyzed. The HBI (hemoglobin binding index, pmol THBV adduct/g/µmol/kg for epoxides, or /ppm-hr for butadiene exposures) for rats was 0.07 for epoxybutene, 3.4 for epoxybutanediol, and 4.0 to 9.3 for diepoxybutane. For butadiene exposures in rats, THBV adducts ranged from 0.04 to 0.5 pmol/g/ppm-hr. For the exposed human subjects, the THBV adduct levels were 0.15 and 0.20 pmol/g. The results indicate that epoxybutanediol is comparable to DEB in it adduct forming ability and considerably more active than BMO.

In the Hazelton Laboratories study, Sprague Dawley rats were exposed to 0, 1000, or 8000 ppm butadiene for 6 hr/day, 5 day/week, for 105 weeks. Although investigators reported tumors in certain reproductive organs, there was no clear statement on the presence or absence of ovarian atrophy, or any other reproductive effect in females (Owen et al., 1987).

Male reproductive effects have also been observed in relation to chronic butadiene exposure, although generally at concentrations greater than those that elicited effects in female animals. In the NTP study, investigators observed a significant concentration-related decrease in testis weight at weeks 40 and 65 for males exposed to ≥ 62.5 ppm butadiene (Melnick et al., 1990). In addition, testicular atrophy (measured at weeks 40 and 60) was observed in males exposed to 625 ppm butadiene (Melnick et al., 1990). In the later NTP study (1993), investigators once again observed testicular atrophy at 625 ppm, and reduced testicular weights in the 200 ppm and 625 ppm exposure groups at the end of the study. However, no effect was seen at the lower butadiene concentrations, as noted in female mice (NTP 1993). In the Hazelton Laboratories study, there was no clear statement of the presence or absence of testicular atrophy in male rats exposed to up to 8000 ppm butadiene. However Leydig cell tumors were observed in 3 males in the 1000 ppm group and 8 males in the 8000 ppm group (Owen et al., 1987).

Kirman and Grant (2012) conducted a meta-analysis with dose-response data from rats and mice that were normalized using an internal dose estimate of diepoxybutane (DEB) in blood that is causally related to ovarian toxicity. A time-to-response (multistage-Weibull) model was used to fit the pooled data sets with exposures from 13 to 105 weeks. Reference concentration (RfC) values were derived for scenarios with average, low and high follicle counts at birth with differing durations of susceptibility to toxic action of DEB. The $BMCL_{01}$ points of departure for these scenarios in ppm continuous butadiene exposure were 17, 1.5, and 240 ppm, respectively. The authors applied a 10-fold uncertainty factor to these values to derive a range of presumably health protective values for human exposures.

7 Chronic Toxicity of 1,3-Butadiene

7.1 Chronic Toxicity to Adult Humans

Studies on the chronic effects of butadiene have been centered in the styrene-butadiene rubber manufacturing industry and the butadiene monomer industry. The Occupational Health Studies Group at the University of North Carolina, Chapel Hill, organized one of the earliest retrospective epidemiological studies conducted in the rubber industry. Investigators observed rubber workers during the period of 1964 – 1972 and reported an increase in overall mortality, emphysema, and cardiovascular diseases (chronic rheumatic and atherosclerotic heart disease) among the subjects (McMichael et al., 1976).

Penn and Snyder (2007) reviewed the evidence supporting an association between butadiene exposure and cardiovascular disease. Two independent longitudinal epidemiological studies have indicated an association. Matanoski et al. (1990) found that butadiene exposure in a styrene-butadiene manufacturing facility was associated with a significant increase in the standardized mortality ratio (SMR: the ratio for mortality in the workers compared to the general population) for arteriosclerotic heart disease in exposed black workers (SMR = 1.48 (95%CI, 1.23-1.76)). The SMRs were adjusted for age, calendar time, and race. Circulatory system disease was also significantly elevated in 214 of 1195 exposed black workers (SMR = 1.18 (95%CI, 1.03-1.35)). Significant differences in these diseases were not seen in 10,915 exposed white workers. In both cases the referent population was U.S. males. Devine (1990) and Devine and Hartman (1996) studied a cohort of nearly 2800 male workers employed at least 6 months at a 1,3-butadiene manufacturing facility. They found significantly elevated SMRs for all causes of death including arteriosclerotic heart disease in nonwhite workers (SMR = 1.28 (95%CI, 1.06-1.53)). For arteriosclerotic heart disease the SMR was 1.42 (95%CI, 0.95-2.04). The referent population was the U.S. nonwhite male mortality rates. Butadiene exposure was also associated with atherogenesis in an avian model (see 7.3 below).

Additional arguments have been made in regard to polymorphisms for xenobiotic metabolizing genes including GSTM1, GSTT1, CYP2E1, and microsomal epoxide hydrolase (EH) and susceptibility to atherosclerosis. Salama et al.(2002) studied 120 atherosclerotic subjects and 90 matched controls for associations between polymorphisms in metabolizing genes and atherosclerotic susceptibility. They found increased atherosclerotic susceptibility associated with the GSTT1 null allele and the fast allele of EH. Also individuals with one of three combined phenotypes (GSTM1 null/CYP2E1 5B, GSTM1 null/mEH YY and GSTT1 null/mEH) exhibited significantly increased atherosclerotic susceptibility (ORs = 3.4-15.42). The importance of EH as a potential cardiovascular disease susceptibility gene in population subgroups and as a key player in butadiene metabolism may influence the AR potential of butadiene in individuals exposed to ETS.

Johns Hopkins University and the National Institute for Occupational Safety and Health initiated two large cohort studies of North American synthetic rubber industry workers (Meinhardt et al., 1982; Matanoski et al., 1990). Investigators studied male workers at eight synthetic rubber plants and two plant complexes in Texas, Louisiana, Kentucky, and Ontario, and included men who began their employment as far back as 1943. Results from these studies were the first to report increased risk of hematologic neoplasms from workplace exposure to butadiene. The University of Alabama designed a series of follow-up epidemiological studies, and has since reported an excess of leukemia among hourly workers with long duration of employment and high butadiene exposure (Delzell et al., 1996), as well as a positive association between cumulative exposure to butadiene and leukemia risk (Macaluso et al., 1996). More recently, University of Alabama investigators evaluated all-cause mortality for the same synthetic rubber industry workers from 1944 – 1998 (Sathiakumar et al., 2005). The subjects had an overall mortality rate 14% lower than expected, including lower rates of cardiovascular, digestive, and genitourinary deaths than the general population.

However, the total group of 17,924 subjects had 16% more leukemia deaths than expected. While this increase was not limited to a single form of leukemia, it was concentrated in long-term hourly workers particularly those in polymerization, coagulation, and maintenance positions (Sathiakumar et al., 2005).

Results from studies of the genotoxic outcomes in workers exposed to butadiene have not been consistent. Begemann et al. (2001) analyzed blood samples from 17 workers in butadiene monomer production and 19 controls from a heat plant in the Czech Republic. Butadiene exposure was assessed by personal monitoring, with a median exposure concentration of 440 μg/m^3 for exposed workers and < 6 μg/m^3 for control subjects. The hemoglobin adduct HBVal, which is formed by a reaction of the *N*-terminal valine with the carbon-1 of epoxybutene, was measured with a limit of detection of 0.2 pmol/g globin. Results showed that the median butadiene-hemoglobin adduct level in monomer production workers was significantly higher than that of controls, with no consistent correlation with any other parameter except smoking (Begemann et al., 2001).

Hayes et al. (2000) also measured a spectrum of genotoxic outcomes, including aneuploidy and sister chromatid exchange, in 41 butadiene polymer production workers and 38 non-exposed controls in China. Full-shift personal exposure varied widely, and short-term breathing zone grab samples showed even greater extremes (median 6.5 ppm, range 0 – 1078 ppm). Hemoglobin adducts were significantly more common in butadiene exposed workers than controls ($p < 0.0001$). In addition, butadiene exposed workers had greater lymphocyte and platelet counts than controls. However, the frequency of total aneuploidy (chromosomes 1, 7, 8, 12) did not differ significantly between exposed and non-exposed workers, nor did structural or numerical chromosomal abnormalities. The authors found no correlation between hemoglobin adducts and any genotoxic markers, nor did they find a positive correlation between butadiene concentrations in air and sister chromatid exchanges (Hayes et al., 2000).

Lovreglio and colleagues (2005) conducted personal monitoring throughout an 8 hr work shift at a petrochemical plant where butadiene was produced and polymerized. Personal monitoring was conducted 3 to 4 times over a 6-week period, with the last session followed by blood sampling of the subjects. Twenty-seven healthy petroleum plant workers were matched with 26 controls not occupationally exposed. Results indicated significantly higher airborne butadiene levels for exposed workers when compared with controls, ranging from 0.2 to 69 μg/m^3. Blood samples were analyzed for sister chromatid exchange and percent chromosomal aberrations in peripheral blood lymphocytes. After correcting for cigarette smoking, the investigators found no genetic marker differences between butadiene exposed and control workers. In their conclusion, the authors stated that there was a lack of genotoxicity in workers exposed to very low concentrations of butadiene similar to ambient levels currently characterized in the general population (Lovreglio et al., 2005).

The Health Effects Institute reported on a genetic epidemiology study of Czech workers exposed to butadiene (Albertini et al., 2003). The study was designed to determine whether biomarkers of exposure could be correlated with in situ levels of butadiene and

personal workplace exposure. Blood and urine samples were collected from males, working in either a butadiene monomer production plant or in a butadiene-styrene polymerization facility. Samples were also collected from male administrative workers who had very limited occupational exposure to butadiene, and served as controls. In addition, full-shift personal monitoring was conducted on each of 10 days over a 60-day period for both groups of workers, including the days on which biological samples were collected. The mean concentrations ranged from 0.026 mg/m^3 for controls to 1.76 mg/m^3 for polymer workers, with maximum concentrations of 0.125 mg/m^3 and 39 mg/m^3, respectively (Albertini et al., 2003). Cytogenetic assays included chromosomal aberrations, breaks, translocations, and sister chromatid exchanges. No significant relations could be identified between group mean chromosomal aberrations, determined by the conventional method or by fluorescent in situ hybridization, and butadiene exposure levels after adjusting for age and smoking. Since tobacco smoke contains butadiene it is possible that this latter adjustment affected the results. In addition, there was no relationship found between butadiene exposure and aberrations associated with glutathione-S-transferase genotypes. Regression analyses showed no significant effects of butadiene exposure on sister chromatid exchanges, although the effect of smoking on sister chromatid exchanges was significant after adjusting for age and occupational butadiene exposure. Hemoglobin adducts of butadiene (to the valine residue) were analyzed as biomarkers for butadiene exposure. There was a significant correlation between the individual subject's exposure levels and two types of hemoglobin adducts. However, the authors cautioned that one adduct, trihydroxybutyl valine (THBVal), also appeared to be formed by an endogenous material other than butadiene and its metabolites, indicating relatively high natural background levels. Overall conclusions from the authors indicate that the cytogenetic endpoints did not prove to be sensitive indicators of butadiene exposure at the levels encountered in these facilities (Albertini et al., 2003).

Since butadiene exposures rarely occur in the absence of other air toxic contaminants it is usually difficult to isolate the effects due to butadiene from those due to other chemicals or combinations. It is OEHHA policy to use human data in preference to animal data when they are of suitable quality. However, in the case of butadiene there are no human dose-response data that can be used to derive a chronic REL with confidence.

7.2 Chronic Toxicity to Infants and Children

Infants and children are rarely exposed to butadiene alone but rather experience long-term exposure to butadiene as one of the combustion products in vehicle exhaust, environmental tobacco smoke (ETS), and other combustion processes. As can be expected with many toxic air contaminants, it is difficult to elucidate the effects of one particular pollutant when it exists in the presence of multiple co-pollutants. Studies of the chronic effects of butadiene in children primarily focus on carcinogenesis as the endpoint. Butadiene has been associated with asthma exacerbation and increased respiratory tract infections, but only in the presence of other pollutants. While some recent studies have aimed to quantify children's exposure to butadiene, ascribing

chronic respiratory effects to butadiene alone remains problematic (see discussion of TEACH study in section 3 above).

7.3 Chronic Toxicity to Experimental Animals

Butadiene is considered a multi-site carcinogen in all identified long-term studies in mice and rats, although there appear to be strain and species differences in sensitivity and severity of effect (NTP 1984; Owen et al., 1987; Melnick et al., 1990; NTP 1993). Instances of tumors include malignant lymphomas, histiocytic sarcomas, cardiac hemangiosarcomas, as well as neoplasms of the liver, lung, mammary gland, ovary, and forestomach (NTP, 1993).

Far fewer data are available for the non-cancerous effects of long-term exposure to butadiene. However, based on these limited data, sensitivity to non-neoplastic events appears to be consistent with species variations in metabolism of butadiene and quantitative differences in the formation of butadiene metabolites (Hughes et al., 2001). For mice, National Toxicology Program (NTP) investigators exposed B6C3F1 males and females to 0, 625, or 1250 ppm butadiene for 6 hrs/day, 5 days/week, for up to 61 weeks (NTP 1984). Liver necrosis was observed in male mice at both doses and in female mice at 1250 ppm. At the highest dose, adverse changes were observed in the nasal cavity, including non-neoplastic lesions, chronic inflammation, fibrosis, cartilaginous metaplasia, osseous metaplasia, and atrophy of the sensory epithelium. In a later study, investigators studied the effects of lower concentrations of butadiene in mice exposed for 6 hrs/day, 5 days/week, for up to 2 years (Melnick et al., 1990; NTP 1993). Two-year survival was significantly decreased in mice exposed to ≥ 20 ppm, which the investigators attributed to butadiene-induced malignant neoplasms. Beyond the neoplastic changes, there were increased incidences of angiectasis, alveolar epithelial hyperplasia, forestomach epithelial hyperplasia, and cardiac endothelial hyperplasia.

Chronic effects in rats were studied by Hazelton Laboratories and reported later by Owen and colleagues (1987; 1990). Groups of 110 male and female Sprague Dawley rats were exposed to 0, 1000, or 8000 ppm butadiene for 6 hrs/day, 5 days/week, for up to 2 years. After the first 52 weeks, investigators euthanized multiple female rats in both dose groups because of the high incidence of large subcutaneous masses. Increased mortality in males was observed in the highest dose group and associated with increased nephropathy. Body weight decreases were observed in the first twelve weeks in the high dose males and females, along with liver and kidney organ weight changes. Although there were minor clinical effects, including slight ataxia, no major changes in neuromuscular function could be definitively associated with butadiene exposure (Owen et al., 1987). Further reporting on this 2-year chronic investigation indicated that there were decreased survival rates at 105 weeks for females and 111 weeks for males (Owen and Glaister 1990). There were no effects on hematology, blood chemistry, and urinalysis, although changes in body and organs weights continued until study termination. Although somewhat equivocal, the rats displayed both common and uncommon tumor types, leading the investigators to suggest that butadiene might be a rat oncogen (Owen and Glaister 1990).

Other studies have identified hematological effects following chronic exposure to butadiene at concentrations at or below those that were associated with systemic toxicity (Irons et al., 1986a; Irons et al., 1986b; NTP 1993). Such hematotoxicity may be associated with the observed changes in organ weights (NTP, 1993). Butadiene exposure has also been associated with bone marrow changes, including bone marrow atrophy, bone marrow toxicity (regenerative anemia), and decreased cellularity as observed in mice exposed to ≥ 62.5 ppm (Melnick et al., 1990; NTP, 1993). Owen and colleagues (1987), however, found no hematological effects in rats exposed to as much as 8000 ppm butadiene in a 2-yr bioassay.

Penn and Snyder (2007) have identified vapor phase butadiene as the principal component in environmental tobacco smoke (ETS) promoting plaque development in an avian model of accelerated atherosclerosis (20 ppm butadiene x 16 weeks). The total butadiene doses that produced atherogenic effects were only 1/80th and 1/1000th the minimal doses causing cancer in mice and rats, respectively. The authors note that side-stream smoke, which represents about 90 % of ETS, contains butadiene at 205 to 400 µg/cigarette versus 16 to 77 µg/cigarette in main-stream tobacco smoke. Furthermore, Penn and Snyder (1996, 2007) found that the atherogenic effect of butadiene exposure was apparently not associated with either of the two principal epoxy metabolites of BD; 1,3-epoxybutene and 1,2:3,4-diepoxybutane. Neither of these metabolites was increased by butadiene exposure in avian liver in contrast to mouse liver.

7.4 Mode of Action

There is currently no accepted mode of action for the acute or chronic effects of butadiene exposure noted in this document. The metabolism of butadiene in all species studied produces epoxide, diepoxide and epoxydiol metabolites in addition to glutathione conjugates and 1,2-butene-3,4-diol. The epoxides all exhibit mutagenic activity in various test systems and react with DNA and proteins to form adducts. Butene diol has exhibited the ability to cause single strand breaks in DNA (Zhang et al., 2012). Adduct forming activity has also been observed for the glutathione conjugate of the butadiene metabolite DEB (Cho and Guengerich, 2012a). One of the DEB-conjugates (*S*-(2-Hydroxy-3,4-epoxybutyl)glutathione) was observed to be more mutagenic than DEB or other BD-epoxides in *Salmonella typhimurium* TA1535 (Cho and Guengerich, 2012b). The ability of DEB-GSH conjugates to form DNA adducts and cause mutations indicate a level of reactivity that may extend to protein binding. While mutagenicity of epoxide metabolites and other genetic toxicity is usually associated with oncogenic effects of butadiene and related chemicals, these genotoxic effects may also be associated with non-cancer effects such as cardiovascular disease, neurodegenerative disease, diabetes mellitus, rheumatoid arthritis, and aging (Burnet, 1974, Cooke et al., 2006). Recent reports indicate a specific mutation (*TREM2*) associated with increased risk of Alzheimer's disease in humans (Guerreiro et al., Jonsson et al., 2012).

In addition to the formation of pyr-Val-Hb and related hemoglobin adducts and their use as biomarkers of internal exposure to specific butadiene epoxy metabolites in

butadiene exposed human subjects, such adducts are also indicative of more general reaction of these metabolites with cellular proteins. The generalized organ and body adverse effects induced by butadiene in mice: fetal body weight decreases, relative organ weight decreases (lung, liver and kidney), and tissue atrophy (ovary, testes, nasal olfactory epithelium) are indicative of, or at least could be plausibly associated with, epoxy adduct formation of key cellular proteins and/or nucleic acids. Such adducts may lead to impaired cellular function and observed organ/tissue effects. The observed neurotoxic effects of butadiene exposure may also be due to reactive epoxide metabolites binding to key proteins and nucleic acids of the central nervous system.

Oxidative stress induced by BD metabolism may also play an important role in the observed tissue/organ toxicity and possible chronic effects in humans exposed to butadiene (Primavera et al., 2008; Yadavilli et al., 2007). Products of butadiene combustion have been observed to inhibit catalase activity and induce oxidative DNA damage repair enzymes in human bronchial epithelial cells in vitro (Kennedy et al., 2009). While the atherogenic effect of butadiene seen in an avian model does not have a clear link to the principal epoxide metabolites of butadiene, it may be related to ETS-induced oxidative stress as seen in mouse heart tissue (Yang et al., 2004). Alternatively, previously unidentified metabolites like the GSH-conjugates of Cho and Guengerich (2012a,b) may be involved in atherogenesis.

8 Derivation of Reference Exposure Levels

8.1 1.3-Butadiene Acute Reference Exposure Level (aREL)

Study	Hackett et al. (1987)
Study population	78 Pregnant CD-1 mice and offspring
Exposure method	Whole body inhalation of 0, 40, 200 or 1000 ppm on GD 6 through 15
Critical effects	Lowered male fetal weight at GD 18
LOAEL	200 ppm
NOAEL	40 ppm (based on Green re-analysis)
$BMCL_{05}$	17.7 ppm (Hill Model)
Exposure duration	6 hr
Time-adjusted exposure	n/a
Human Equivalent Concentration	29.7 ppm (17.7 ppm * 1.68 DAF)
Interspecies uncertainty factor	
Toxicokinetic (UF_{A-k})	1
Toxicodynamic (UF_{A-d})	√10
Intraspecies uncertainty factor	
Toxicokinetic (UF_{H-k})	10
Toxicodynamic (UF_{H-d})	√10
Cumulative uncertainty factor	100
Inhalation reference exposure level	297 ppb (660 µg/m^3)

Acute Reference Exposure Levels are levels at which intermittent one-hour exposures are not expected to result in noncancer adverse health effects (see Section 5 of the Technical Support Document).

The study by Hackett et al. (1987) was selected as it addressed the most sensitive noncancer endpoint associated with butadiene inhalation: developmental effects. A continuous benchmark dose analysis of the male mouse fetal weight data from Table 2 was conducted and the results are presented in Table 5. Both applied and PBPK doses were used but the latter showed little improvement in model fit or values derived. The best value based on the applied dose and the Hill model using the values from the Green (2003) re-analysis of the Hackett data was 17.7 ppm for the mouse and 29.7 ppm for the human equivalent concentration. This analysis in BMDS (v 2.3.1) was based on the male pup data and the homogenous variance assumption, due to the exact model fit and little evidence of differing variance in the exposed groups. We analyzed all the data (male and female) as well as the males only and the males consistently gave better model fits and lower $BMCL_{05}$ values (Table 12A). The nonhomogeneous variance assumption based BMC/BMCL values were about 50% lower than the homogeneous variance values and the model fit, while acceptable, was not exact (see Table A12). In the end we chose the lowest BMCL value giving the best model fit (male pups, Green, 17.7 ppm).

A second mouse PBPK model based on rat and human pregnancy (Gentry et al., 2002; Poet et al. 2010) allowed estimates of fetal exposure to BMO (mean fetal AUC GD 9-18). This metric also gave an exact fit to the fetal body weight data (Table 5). In the summary acute REL calculation above, we employed the standard intraspecies toxicokinetic uncertainty subfactor (UF_{H-k}) of 10: this is specifically justified to account for observed human variability due to polymorphisms in the microsomal epoxide hydrolase (mEH) gene reported by Abdel-Rahman, Wickliffe, Ward and co-workers (Abdel-Rahman et al., 2003, 2005; Wickliffe et al. 2003). When the interaction between BD exposure and inheritance of a variant ATT allele for mEH was evaluated in 49 exposed workers, the increase in HPRT variant mutant frequency was increased to $10.89 \pm 2.16 \times 10^{-6}$ (95% C.I. = 6.56-15.20 ($\times 10^{-6}$), $p = 0.0027$) over the background rate of $4.02 \pm 1.32 \times 10^{-6}$ (Abdel-Rahman et al. 2005). In this case, the background is not an unexposed general population but rather that portion of the worker study population (N= 33) with exposures < 150 (18.4 ± 5.5) ppb BD. Since mEH is a key enzyme in the detoxification of butadiene epoxide metabolites, low activities in this gene/enzyme translate to increased internal concentrations of these metabolites and risk of related toxic effects. The dosimetric adjustment factor (DAF) is related to the ratio of human and animal blood/air partition coefficients for butadiene and was estimated using mouse and human PBPK models. For example, the butadiene blood/air partition coefficient for rodents used by Kohn and Melnick (2001) was 1.95 and the butadiene blood/air partition coefficient for humans used by Brochot et al. (2007) was 1.22 ± 0.30. This would indicate a DAF based on these values of 1.60. OEHHA's estimates using rodent and human PBPK models was 1.68. That is:

DAF = PBPK predicted human blood concentration/PBPK predicted animal blood concentration and;
Human equivalent concentration (HEC) = Animal experimental concentration x DAF.

Table 5. Benchmark Dose Analysis of Male Mouse Fetal Weight Data of Hackett et al. (1987).

Dose Metric	N, male fetuses	Model	BMC_{05}	$BMCL_{05}$	$BMCL_{05}$ ppm BD equivalent	Human ppm equivalent 1.68 DAF
Applied BD ppm 6 hr/d						
0	109	Hill (Green reanalysis)	37.2**	17.7**	17.7**	29.7
40	118	Hill (Hackett et al. original values)	28.5**	13.4**	13.4**	22.5
200	133	Polynomial	48.8	41.1	41.1	69.0
1000	126	Power	261 n.s.	225 n.s	225 n.s.	378 n.s.
PBPK AUC Maternal BMO µMhr/d						
0	109	Hill	134**	70.1**	16.5**	27.8
166.4	118	Polynomial	100	69.2	14.9	25.0
371.9	133	Power	106	90.7	19.8	33.2
493.8	126					
PBPK AUC Fetal BMO µMhr/d[a]						
0	109	Hill	10.9**	5.1**	13.4**	22.5
15.2	118					
74.3	133					
356.7	126					

Note ** indicates exact model fit by graph and tabular output, P values were not applicable for exact fits of the Hill model to the continuous data sets or given as P <0.0001 for the other models despite obvious high degrees of fit visually and by tabular output of observed and predicted values; n.s. indicates insignificant model fit (P < 0.1)

[a] based on average fetal BMO AUC during gestation days 9-18.

8.2 1,3-Butadiene 8-Hour Reference Exposure Level

Study	NTP (1993) supported by Doerr et al. (1996)
Study population	B6C3F1 mice
Exposure method	Daily inhalation
Exposure continuity	6 hr/d, 5d/wk
Exposure duration	9-24 months
Critical effects	Increased incidence of ovarian atrophy
$BMCL_{05}$	1.01 ppm
Time-adjusted exposure	758 ppb (1.01 ppm x 6/8 hr/d)
Human Equivalent Concentration	1.27 ppm (0.758 ppm x1.68 DAF)
Subchronic uncertainty factor (UFs)	1
Interspecies uncertainty factor	
Toxicokinetic (UF_{A-k})	1
Toxicodynamic (UF_{A-d})	10
Intraspecies uncertainty factor	
Toxicokinetic (UF_{H-k})	10
Toxicodynamic (UF_{H-d})	√10
Cumulative uncertainty factor	300
Reference Exposure Level	4.2 ppb (9 µg/m^3)

The 8-hour Reference Exposure Level is a concentration at or below which adverse non-cancer health effects would not be anticipated for repeated 8-hour exposures (see Section 6 in the TSD). The study of NTP (1993) and the endpoint of ovarian atrophy was selected as the basis of both the 8 hr and chronic RELs. The benchmark concentration analysis of this study is summarized in Table 3 above. We selected the value of 1.01 ppm for the $BMCL_{05}$ based on the full data sets of 9, 15 and 24 months exposures as the best basis for the derivations. This study is supported by that of Doerr et al. (1996). The Doerr study involves PBPK modeling (Table 6) to estimate internal dose metrics for ovarian effects resulting from i.p. doses of mono- or diepoxide metabolites of BD. Dose-response modeling (BMDS v.2.1.2) was used to estimate $BMDL_{05}$'s for the various dose metrics evaluated.

The selected best point of departure, 1.27 ppm, is the $BMCL_{05}$ adjusted for time and human equivalent exposure. The interspecies uncertainty subfactor for toxicokinetics (UF_{A-k}) was reduced from √10 to 1 because we think that the mouse is more active in metabolizing butadiene to reactive epoxide metabolites than either the rat or human, thus reducing the uncertainty in the interspecies toxicokinetic extrapolation.

The other departure from defaults which we have made in this derivation is that we have increased the toxicodynamic subfactor (UF_{A-d}) from the default value of √10 to 10, based on the uncertainty arising from potentially greater human response to the ovotoxic effects of butadiene epoxide metabolites, particularly diepoxybutane (DEB), as compared to the mouse model from which the primary data for risk calculations were derived. Humans differ substantially from mice in lifespan and in the time available for

chronic exposure to effect ovotoxicity which is far longer in humans, and the generally greater robustness of the mouse reproductive system relative to the human (e.g., see He et al., 2007). Chemicals structurally similar to butadiene, specifically the butadiene dimer 4-vinylcyclohexene (VCH) and its diepoxide (VCD) are established rodent ovotoxicants. These related chemical species produce selective destruction of primordial and primary follicles, premature ovarian failure and increased risk of ovarian tumors (Smith et al., 1990; Ito et al., 2009; Mark-Kappeler et al., 2011). Mechanistic studies of the ovotoxic effects of VCD indicate that activation of proapoptotic signaling events in the Bcl-2 and mitogen-activated protein kinases play a role (Hu et al., 2001a, b, 2002). VCD causes a physiological form of ovotoxicity in which follicle loss is "silent" and mimics normal follicular atresia. Thus the damage caused by VCD, were this to be paralleled in humans, could go unnoticed in exposed and affected individuals leading one investigator to surmise that "As a result, because VCD only targets ovarian preantral follicles, chronic exposure in women to low levels of this chemical may represent a risk for early menopause without prior evidence of disrupted menstrual cycles" (Mark-Kappeler et al., 2011) (also see Hoyer and Sipes, 2007).

Table 6. Modeled Butadiene Inhalation Resulting in Ovarian and Uterine Atrophy Following I.P. Butadiene Mono- and Diepoxide Exposures

Data Set	Metric	Model	P for fit	BMD_{05}	$BMDL_{05}$	6 hr ppm mouse equivalent
Ovary						
BMO i.p.	AUC BMO blood µMhr/d	Power	0.76	2688	1765	N.A.
	AUC DEB blood µMhr/d	Poly	0.92	57.4	20.5	1.80
BMO i.p.	Hb adducts nmol/g/d	Poly	0.92	34.8	15.6	N.A.
DEB i.p.	Hb adducts nmol/g/d	Hill	0.43	183.3	85.1	N.A.
Uterus						
BMO i.p.	AUC BMO blood µMhr/d	Power	0.36	2688	1765	N.A.
	AUC DEB blood µMhr/d	Poly	0.84	103.1	37.9	3.47
BMO i.p.	Hb adducts nmol/g/d	Poly	0.85	799	117	N.A.
DEB i.p.	Hb adducts nmol/g/d	Poly	0.85	799	116.8	N.A.

Notes: BMO = butadiene mono epoxide; DEB = diepoxybutane; EBD = epoxybutenediol; AUC = area under the blood concentration x time curve; Hb = hemoglobin,
table BMD/L values are the average of 30 consecutive daily i.p. administrations,
ppm equivalent is inhalation exposure of butadiene (BD) giving the same internal BMDL dose,
N.A. indicates values unachievable by BD inhalation,
Poly is the polynomial model for continuous dose response in BMDS software,
BMD_{05} indicates 5% relative risk and BMDL the 95% lower confidence limit on the BMD value.

VCH is an industrial chemical released during the manufacture of rubber tires, plasticisers and pesticides, and human exposures to that chemical are likely to be limited to occupational settings. The similarity of the ovotoxcity of VCD, as a model system, however, indicates that of DEB may pose risk derived from mechanistic similarities. Should such mechanistic similarities exist, human females exposed chronically to DEB via butadiene may be at risk of silent ovotoxic effects that would not be manifest for many years. Because of these reproductive uncertainties, OEHHA believes it is adviseable to reject the lower and less conservative default value in favor of a larger UF_{A-d} until further relevant information is available.

The PBPK modeling used two related models. PBPK Model 1 simulates butadiene plus three metabolites BMO, DEB, and butadiene diol (BDD) and estimates EBD as a metabolized dose (AMET). PBPK Model 2 added EBD as a circulating metabolite and erythritol as a metabolized dose (AMET). Both models use parameters and model structure from Kohn & Melnick, 2001. The ovary and uterus data sets (% of body weight vs. i.p. dose) are from Doerr et al. (1996) Fig 2. The general metabolic scheme is: BD →BMO→DEB→EBD→Erythritol and BMO→BDD→EBD→Erythritol. All metabolic steps have Michaelis-Menten kinetics for P450 oxidation, epoxide hydrolases (EH) or the ordered bi bi rate law for glutathione sulfotransferases (GST). Further details of the GST kinetics can be found in Kohn and Melnick (2000). Essentially, the GST reaction rate (moles/hr) is the product of the Vmax, substrate (Ci) and glutathione concentrations (CGSH) divided by the sum of the cross products of the Km's (Kmi, Kmgsh) and tissue concentrations of the substrate and glutathione:

$$V = Vmax*Ci*CGSH/(Kmi*Kmgsh + Kmi*CGSH + Kmgsh*Ci + Ci*CGSH)$$

All model simulations were conducted with Berkeley Madonna software. Additional details on the PBPK modeling including parameters, graphical and tabular model output, benchmark dose analysis, and sample model code are given in the Appendix.

8.3 1,3-Butadiene Chronic Reference Exposure Level (cREL)

Study	NTP (1993) supported by Doerr et al. (1996),
Study population	B6C3F1 mice
Exposure method	Daily inhalation
Exposure continuity	6 hr/d, 5d/wk
Exposure duration	9-24 months
Critical effects	Increased incidence of ovarian atrophy
$BMCL_{05}$	1.01 ppm
Time-adjusted exposure	180 ppb (1.01 ppm x 6/24h x 5/7d)
Human Equivalent Concentration	302 ppb (180 ppb x 1.68 DAF)
Subchronic uncertainty factor (UFs)	1
Interspecies uncertainty factor	
Toxicokinetic (UF_{A-k})	1
Toxicodynamic (UF_{A-d})	10
Intraspecies uncertainty factor	
Toxicokinetic (UF_{H-k})	10
Toxicodynamic (UF_{H-d})	√10
Cumulative uncertainty factor	300
Reference Exposure Level	1.0 ppb (2.2 μg/m^3)

The chronic Reference Exposure Level is a concentration at which adverse noncancer health effects would not be expected from continuous chronic exposures (see Section 8 in the Technical Support Document). In the derivation of the chronic REL above, the $BMCL_{05}$ is the same as the 8-hour REL but the time adjustment is for potentially continuous exposure rather than 8 hours/day. The uncertainty factors applied are the same in both cases (i.e., 8-hour and chronic) and the proposed RELs are similar to those derived from the supporting study of Doerr et al. (1996), namely 23 ppb and 8 ppb for 8-hour and chronic RELs , respectively. This latter study uses the same toxicity endpoint but with parenteral administration of the butadiene epoxide metabolites BMO and DEB.

Butadiene causes non-cancer effects following chronic exposure, the most important of which appears to be reproductive toxicity. Inhalation exposure to relatively low concentrations of butadiene over a lifetime is associated with adverse effects in the reproductive organs of female and male mice (NTP, 1984; Melnick et al., 1990; NTP, 1993; Doerr et al., 1996). A PBPK model was developed to model the disposition of butadiene and its metabolites based on a rodent model described by Kohn and Melnick (2001), and data on the ovarian toxicity of 1,3-butadiene epoxide in rats and mice (Doerr et al.,1996).

As reported earlier, ovarian atrophy was observed at all concentrations in the NTP chronic bioassays (NTP, 1984; Melnick et al., 1990; NTP, 1993), and showed a dose-dependent effect associated with increased inhalation exposure. In all exposure groups, ovarian atrophy was associated with what might be considered premature

reproductive senescence. It is noteworthy that such senescence was observed as early as nine months in female mice exposed to 200 or 625 ppm, and after 15 months in female mice exposed to ≥20 ppm butadiene (NTP, 1984; NTP, 1993). It is currently unknown how ovarian atrophy in rodents relates to human reproductive senescence. Reproductive senescence is a general term referring to the loss of reproductive function with age. In the female, this involves a loss of normal ovarian, and hypothalamic or pituitary function (Valdez and Petroff, 2004). The correlation between the loss of oocytes and declining fertility with age is well characterized, and it is generally accepted that depletion of follicles results in the final loss of fertility. The actual end of ovarian activity is preceded by a period of irregular reproductive cycles and subfertility in both women and rodents (Valdez and Petroff, 2004). Age-related changes in the rodent hypothalamus and pituitary appear to bring about the final cessation of ovarian cyclicity before follicular depletion occurs (Wise et al., 2002). However, exhaustion of ovarian follicular reserves appears to drive menopause in women (vom Saal et al., 1994). Despite these apparent differences in final reproductive senescence, the biological mechanisms leading to prolonged cycles and aberrant endocrinology appear similar in primates and rodents (Wise et al., 2002; Bellino and Wise 2003).

Currently, there are few data detailing the potential role of butadiene on the etiology of ovotoxicity and late-stage infertility (Hughes et al., 2001). Potential mechanisms might involve the in situ formation of butadiene reactive intermediates, which may have a direct effect on ovarian function (Doerr et al., 1995; Hughes et al., 2001), or a more general role on the hypothalamic-pituitary-gonadal axis. Available data seem to suggest that humans potentially form qualitatively similar metabolites to experimental rodent species, although the rates of formation and the quantities of specific metabolites differ (Bond et al., 1986;Csanady, 1992). Mice appear to produce a greater proportion of active epoxide metabolites than rats (Bond et al., 1986; Himmelstein et al., 1994; Thornton-Manning et al., 1995). However there are conflicting in vitro data that suggest humans may form epoxides of butadiene at comparable rates to mice or at rates several fold less than mice (Csanady et al., 1992; Duescher and Elfarra 1994; Krause and Elfarra 1997). Dahl et al. (1991) reported that following equivalent inhalation exposures, the concentrations of total butadiene metabolites in blood were 5-50 times lower in monkeys than in mice, and 4-14 times lower than in rats. Humans may more closely resemble the monkey than the mouse or the rat in their formation of epoxides from butadiene, and several models adjust for species differences in butadiene pharmacokinetics (Sweeney et al., 1997). In view of the fact that we have used a pharmacokinetic model to assess relevant dosimetry and the weight of published evidence that mice are more active butadiene metabolizers than other species studied, we used a UF of 1 to account for interspecies kinetic differences. However, an interspecies toxicodynamic adjustment for ovarian atrophy endpoint with butadiene is still needed to reflect the expected substantial toxicodynamic differences between mice and women (see the discussion in the derivation of the 8-hour REL). Therefore OEHHA used an interspecies toxicodynamic uncertainty factor of 10. We applied an intraspecies uncertainty factor of 30, 10 for toxicokinetics and $\sqrt{10}$ for toxicodynamics. The overall uncertainty factor was 300, and the cREL is 1.0 ppb.

For comparison, the U.S. EPA's RfC is 0.9 ppb. This value is based on a benchmark concentration (BMC) and a Weibull time-to response model for the ovarian atrophy endpoint in mice in the NTP 2-year inhalation study (NTP, 1993; U.S.EPA, 2002). U.S.EPA (2002) calculated a BMC_{10} of 1.05 ppm and a $BMCL_{10}$ of 0.88 ppm (continuous) without the top dose. They applied an overall uncertainty factor (UF) of 1000 to derive a final value of 0.88 ppb or 0.9 ppb rounded. The composite UFs included √10 for interspecies, 10 for effect level, 10 for intraspecies, and √10 for data deficiencies.

The Texas Commission on Environmental Quality (TCEQ) used a similar analysis employing the top dose in the same data set to calculate a $BMCL_{05}$ of 462 ppb. Their chronic reference value of 15 ppb employs an overall UF of 30, which includes 10 for intraspecies differences and √10 for data deficiencies (Grant et al., 2010).

Kirman and Grant (2012) conducted a meta-analysis of pooled rodent data on ovarian atrophy resulting from butadiene exposure. They used data in mice from NTP (1993), NTP (1984) and Bevan et al. (1996) together with negative data in rats from Owen et al. (1987) and Bevan et al. (1996). Instead of using a PBPK model to estimate internal dosimetry they used an empirical equation relating average blood concentration of diepoxybutane (DEB) to DEB-specific pyr-Val hemoglobin adducts with separate rate constants and erythrocyte lifespans for mice and rats (4.7×10^{-5}, 40.3; 5.5×10^{-5}, 64.4, L/g hr., days, respectively). This resulted in estimated DEB blood concentrations in mice of 11 to 823 nM for butadiene exposures of 6.25 to 1250 ppm. For rats, estimated blood concentrations of 2.9 to 8.1 nM DEB were obtained for exposure concentrations of 1000 and 8000 ppm butadiene, respectively. Dose response analysis was conducted using the Multistage-Weibull (MSW) time and dose model for incidental effects (ovarian atrophy, extra risk, non-fatal analysis). Analyses were conducted for the mouse data at 823, 686, 471, 186, 73, 29, and 11 nM DEB, for rat data at 2.9 nM DEB, and for pooled mouse and rat data at 0 nM DEB (controls).

The advantage of this approach was the prediction of effects at specific exposure durations that could be compared with human ovarian reserves at times prior to menopause. Three human scenarios for duration of susceptibility to ovarian effects were defined by human menopause at 60 yr (low susceptibility), 49.6 yr. (average susceptibility) and 38.7 yr. (high susceptibility). For these scenarios points of departure (PODs) were determined at the 10% and 1% effect levels with 95% confidence limits i.e., $BMCL_{10}$, $BMCL_{01}$. The $BMCL_{01}$ PODs for high, average and low susceptibility were 1.3, 0.88, and 0.67 nM DEB in blood, respectively. The human equivalent concentrations of butadiene (continuous exposure) $BMCL_{01}$s were 10, 17, and 39 ppm butadiene, respectively. Comparable rodent durations for the human scenario durations were 53.7, 68.8, and 83.2 weeks, respectively. The MSW predicted dose response curve for low follicle count at birth (high susceptibility scenario) was shifted by factors of 3 and 8.5 (25.5 total) to the left to reflect a sensitive individual due to variation in toxicokinetics and toxicodynamics. Similarly, the curve for high follicle count at birth (low susceptibility) was shifted to the right by the same factors. The dose-response curve was not shifted for the average scenario. For the POD $BMCL_{01}$s of 17 ppm BD (average), 1.5 ppm BD (high, 25.5 TK+TD shift UF) , and 240 ppm BD (low,1/25.5

TK+TD shift UF) the derived reference values including an additional net uncertainty factor of 10 were 2, 0.2, and 20 ppm butadiene rounded, respectively. The lowest value of 0.2 ppm or 200 ppb butadiene is over an order of magnitude higher than that proposed by Grant et al. (2010) for the Texas chronic reference value of 15 ppb butadiene and 67 times higher than the cREL proposed in this document.

This is a novel approach to butadiene non-cancer risk assessment. It rests on a number of assumptions which may not be justified. First of all, it assumes that the effects of butadiene in humans will be exactly as seen in mice, i.e. ovarian atrophy. Ovarian atrophy was the most sensitive non-neoplastic effect noted among several organ effects in mice (decreased lung, liver, and kidney weights) and uterine, testicular and nasal olfactory epithelial atrophies. Next it assumes that all the ovarian effects are due to DEB and that DEB acts the same in rats as in mice. An examination of their Table 3 shows some problems with this latter assumption since at a comparable estimated blood DEB concentration of 11 nM in mice 19/49 showed atrophy, whereas at 8.1 nM in rats no effect was seen (0/110). It appears that mice are much more sensitive to blood DEB than rats. There is also a concern that the effect of including the negative rat data (350 animals) may have reduced the overall response. The choice of a 1% response rate may also be problematic. Usually for non-cancer data the lower bound on the 5% response rate is chosen as the POD i.e., $BMCL_{05}$. While the authors note that several hundred animals were included in the analysis it is questionable if the analysis had the power to detect a 1% increase ($BMCL_{01}$) since the largest denominator for any specific dose level causing an effect was 79 mice. The $BMCL_{01}$ is an extrapolated response well beyond the bulk of observed values. There appears to be a discrepancy in Table 8 which lists a POD of 240 ppm and a reference level of 20 ppm (rounded) where the text gives a value of 255 ppm (10 x 25.5) which would give 26 ppm rounded.

In order to compare their method with ours we used the same TWA DEB internal dose estimates of Kirman & Grant (2012) and conducted a Multistage Weibull non-fatal time to effect analysis (MSW) using U.S.EPA software (U.S.EPA, 2010a) to estimate the benchmark concentration level time adjusted $BMCLt_{05}$ values for the largest and most important data set in their meta-analysis and our cREL derivation, namely the NTP (1993) 24 months data (N= 325). Using individual animal data (dose, time, incident, censored, number) we obtained a value of $BMCLt_{05}$ = 0.502 ppm butadiene equivalent (Table 7). This value is half the 1.01 ppm value based on our time-weighted analysis of 9, 15 and 24 months quantal data (dose, time, number with ovarian atrophy/total number exposed) and the $BMCL_{05}$ for a log probit model (N = 435). While there is no chi-square goodness of fit statistic for the $BMCLt_{05}$, graphical comparisons of the MSW parametric and nonparametric models in probability-time (pr), dose-response (dr), quantile-quantile (qq), and probability-probability (pp) plots indicate excellent fit of the MSW model to the 24 months ovarian atrophy data (Appendix B, U.S.EPA, 2010b). Since the $BMCLt_{05}$ is similar to the $BMCL_{05}$, but is based on fewer animals and conducted with new software with which we have limited experience, the MSW analysis is considered a supporting analysis for the cREL.

Table 7. Multistage Weibull Analysis of Ovarian Atrophy in Female Mice in 2-Year Inhalation Study of 1, 3-Butadiene (NTP, 1993).

Exposure Period	Model	AIC	$BMCt_{05}$ nM DEB	$BMCLt_{05}$ nM DEB	$BMCLt_{05}$ ppm BD equiv.	$BMCLt_{05}$ continuous ppm BD HEC	Comment
24 months	Multi-stage Weibull[1]	300.781[2]	1.56	1.28[3]	0.502[4]	0.151[5]	N = 325; good fit by pr, dr, qq, & pp plots[6]

Note: (1) P(response) = 1-exp{-(t-t0)^c * (beta_0 + beta_1*Dose^1 + beta_2*Dose^2 + beta_3*Dose^3)} run as nonfatal analysis, t0 = 0; (2) Akaike's Information Criterion; (3) $BMCLt_{05}$ 95% lower bound on the 5% response level by MSW with internal DEB dose metric of Kirman and Grant (2012); (4) external equivalent of 0.50 nM DEB in 6hr/d BD; (5) continuous human equivalent concentration of BD (6/24h x 5/7d x 1.68 dosimetric adjustment factor; (6) goodness of fit graphical tests: probability v. time (pr), probability v. dose (dr), time v time (qq), probability v. probability (pp) for parametric and nonparametric msw models (U.S.EPA, 2010b).

It is important to note that in deriving the cREL for butadiene, OEHHA makes no assumption concerning target site concordance in experimental animals and humans. We assume only that the most sensitive site and species with appropriate uncertainty factors will protect against any adverse effect in exposed humans including children.

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Appendix A

This appendix includes information on genotoxicity of butadiene, except discussion of dominant lethal mutations and germ cell mutagenicity, which are covered in the developmental and reproductive toxicity section of the main document. An overview of the genotoxicity of butadiene and its metabolites is presented in Table A1. Also included are data on PBPK modeling of butadiene employed in the derivation of the $BMCL_{05}$ and the 8-hr and chronic RELs. Table A2 gives a historical overview of the PBPK and related models that were more or less available for use.

While genetic toxicology generally provides key supporting documentation for cancer risk assessment rather than the present noncancer assessment, we believe that mutagenicity and other genetox effects, particularly oxidative DNA damage, may contribute to chronic diseases such as heart disease, neurodegenerative diseases, diabetes mellitus, rheumatoid arthritis, and aging, irrespective of their role in initiation and promotion of tumors (F.M.Burnet,1974; Cooke et al., 2006).

A.1 Genetic Toxicity: Introduction

This section includes information on genetic toxicity. Albertini et al. (2010) have provided a comprehensive review of 1, 3-butadiene genotoxicity. As noted above, the metabolism of butadiene produces the major electrophilic metabolites EB, EBD, and DEB. These epoxide metabolites are responsible both for the mutagenicity and carcinogenicity of 1, 3-butadiene. They are DNA reactive forming a variety of adducts, and all are genotoxic in vitro and in vivo. Also butadiene epoxide metabolites form adducts with proteins notably hemoglobin and may exert or influence noncancer endpoints. In general genotoxicity, specifically oxidative DNA damage, may influence a number of chronic diseases and aging in addition to cancer (Cooke et al., 2006; Evans & Cooke, 2006).

A.2 Microbial test systems

Butadiene has induced mutagenic responses in all microbial systems that were provided with exogenous microsomal activation (S-9) that allowed the formation of oxidative metabolites. The purified metabolites epoxybutene (EB) and diepoxybutane (DEB) have also been shown to be mutagenic in all microbial systems without exogenous activation (Albertini, 1999). Carmical et al. (2000) found evidence of stereospecific mutagenicity of diolepoxide derived adducts in an *E.coli* system. The BDE R,R isomer induced a mutantion frequency of 0.14% (A→G mutations exclusively), whereas the S,S isomer induced 0.25% (A→C mutations exclusively). The mutation frequencies induced by BMO isomers were not significantly different from controls (<0.05%). Curiously, a recent report by Cho et al. (2010) demonstrated the mutagenicity in *S. typhimurium* TA1535 of the glutathione conjugate of DEB, *S*-(2-hydroxy-3,4-epoxybutyl)GSH. In general GSH conjugates are regarded as detoxification products but in this case the conjugate was reported to have higher mutagenic potency than DEB.

A.3 Higher organism test systems

Butadiene and its diepoxide have been tested in the fruit fly *Drosophila melanogaster*. BD was negative in sex-linked recessive lethal mutations and the spot wing test whereas DEB was positive in these tests. Studies in mammalian cell systems parallel those in microbial systems with BD, EB, DEB and epoxybutane diol (EBD) showing positive results in systems provided with exogenous activation. In these systems the rank order of mutagenic potency was generally DEB> EB (BMO)>EBD>BD. DEB was generally 40 to 100-fold more potent than EDB. DEB is more likely to cause deletions, whereas all the epoxides induce base substitutions with a preference for A:T base pairs (Albertini, 1999). Both EB and DEB occur in different stereochemical configurations, which have been evaluated in the TK6 human lymphoblastoid cell line for increases in hypoxanthine-guanine phosphoribosyltransferase (HPRT) and thymidine kinase (TK) mutant frequencies. Meng et al. (2007) found no differences in cytotoxicity or mutagenicity among three isomers of DEB or the two isomers of EB. Thus, stereochemistry seems likely to play little role in the mutagenicity of BD. These findings were consistent with in vivo results, which showed little difference in the mutagenic efficiencies of racemic-DEB or meso-DEB in rodents. However, the results seem somewhat at odds with those of Carmical et al. (2000) above, where *S*,*S*-EBD derived adducts preferentially induced mutagenicity in *E.coli*.

Mutagenic studies in whole mammals have generally given positive responses when the animals (mice or rats) were exposed to butadiene by inhalation (Albertini et al., 2010). The tests included the mouse spot test, which involves in utero exposure of a developing embryo, which results in mutations in hair coloring in the offspring. Most of the assays were for mutations in the *hprt* locus in lymphocytes of exposed rodents.

A.4 Genotoxicity in humans

Genotoxicity in humans is usually assessed by the molecular epidemiology of exposed populations via the monitoring of genetic endpoints, including mutations. Several specific biomarkers of BD exposure are available which allow measurement of internal dosimetry of different BD metabolites. Two BD urinary metabolites: M1 (1, 2-dihydroxy-4-[*N*-acetylcysteinyl]-butane) and M2 (an isomeric mixture of 1-hydroxy-2-[*N*-acetylcisteinyl]-3-butene and 2-hydroxy-1-[*N*-acetylcisteinyl]-3-butene have frequently been used as measures of BD exposure (Albertini et al., 2010). N-terminal valine adducts of hemoglobin have also been used as in vivo BD dosimeters (Perez et al., 1997; Boogaard et al., 2001).

There have been several small studies involving one or more biomarkers of exposure and nine populations that have been subjected to comprehensive studies with biomarkers of BD exposure and specific genotoxicity endpoints including somatic gene mutations and chromosome aberrations (Albertini et.al., 2010). Overall the results of these investigations have been mixed. With respect to mutational endpoints, only studies of BD-exposed workers in Texas have reported significantly increased frequencies of *hprt* gene mutations in peripheral blood lymphocytes determined by autoradiography (Albertini et al., 2010; Wickliffe et al., 2009).

Albertini et al. (2007) conducted a molecular epidemiological study of 53 BD-exposed Czech workers and 51 controls. The mean 8-hr time weighted average (TWA) concentrations of BD were 0.008 mg/m^3 (3.5 ppb) and 0.397 mg/m^3 (180 ppb) for female controls and exposed, respectively, with individual 8-hr TWA values up to 9.8 mg/m^3 (4.45 ppm). For male workers, mean TWA values for control and exposed workers were 3.2 and 370 ppb, respectively with single individual values up to 5.72 ppm. For both sexes, the urinary BD metabolites M1 and M2 were elevated in exposed vs. control subjects, reaching statistical significance in males. *HPRT* mutations, via cloning assays, and multiple measures of chromosome level changes (SCEs) were not associated with BD exposures.

Wickliffe et al. (2009) evaluated the frequencies of HPRT mutant lymphocytes in BD polymer workers in a Southeast Texas facility. Thirty workers were exposed to BD concentrations of 93.5 ppb (mean and 2.5 ppb (median) with only one individual's exposure estimate (1684 ppb) exceeding the OSHA PEL of 1.0 ppm. The minimum detection level was 2.5 ppb and 50% of exposures were below this level. *HPRT* mutant frequencies by autoradiographic lymphocyte assay were not significantly associated with current exposures or age (N = 29). However *HPRT* mutant frequencies were significantly associated with occupational longevity (≥ 30 yr, N = 29, R^2 = 0.107, P < 0.046). The authors speculate that chronic or past high-level BD exposures may have produced retention of mutant long-term memory T-cells.

Table A1. Genotoxicity of 1, 3-Butadiene and Metabolites
(Adapted from Albertini 1999 and Albertini et al., 2010).

Compound	Test System	Results	Comments
Microbial, Procaryotic			
BD	*Salmonella typhimurium* TA 1530, TA1535	+	Rat and human S-9, multiple tests
BD	*Salmonella typhimurium* TA 1530, TA1535	-	Without S-9, usually negative, positive results attributed to volatile metabolites
EB (BMO)	*Salmonella typhimurium* TA 1530, TA1535,TA100	+	Without S-9, LEC = 2431 µM
EB (BMO)	*Escherichia coli*	+	Without S-9
EB N^6 adenine adducts	*E.coli* transfected with adducted oligonucleotides	-	Neither isomer adduct was significantly mutagenic, Carmical et a. 2000
EB (BMO)	*Klebsiella pneumoniae*	+	Without S-9
DEB	*Salmonella typhimurium*	+	Without S-9, more potent than EB, LEC = 302µM
DEB	*E.coli*	+	Without S-9
DEB	*Bacillus megaterium*	+	Without S-9
DEB	*Pseudomonas pyocyanea*	+	Without S-9
DEB	*K. pneumonia*	+	Without S-9
DEB-GSH	*Salmonella typhimurium* TA1535	+	*S*-(2-hydroxy-3,4-epoxybutyl)GSH reported more mutagenic than DEB (Cho et al., 2010)
EBD	*Salmonella typhimurium*	+	With or without S-9, LEC = 980 µM
EBD N^6 adenine adducts	*E.coli* transfected with adducted oligonucleotides	+	S,S- stereoisomer of EBD gave higher mutagic frequency than the R,R isomer, Carmical et al., 2000

Table A1. Genotoxicity of 1, 3-Butadiene and Metabolites
(Adapted from Albertini 1999 and Albertini et al., 2010).

Compound	Test System	Results	Comments
Microbial, Eucaryotic			
DEB	*Neurospora crassa*	+	Without S-9
DEB	*Schizosaccharomyces pombe*	+	Without S-9
DEB	*Saccharomyces cerevisiae*	+	Without S-9
DEB	*Aspergillus nidulans mitotic crossive over and recombinant selection, gene mutations*	+	20μM, 1 min; LEC = 50 mM, 13 min
DEB	*Penicillium multicolor*	+	Both stereoisomers
Insect			
BD	*Drosophila melanogaster*	-	Sex-linked recessive lethal mutations, spot wing test somatic mutations
ED (BMO)	*Drosophila melanogaster* adult males exposed to EB by inhalation for 24 hr. Gene mutations scored	+	Sex-linked recessive lethal mutations induced highest exposure level on 3800 ppm EB (91,200 ppm-hr) w/ DNA repair or at 2000 ppm (24,000 ppm-hr) w/o DNA repair.
DEB	*Drosophila melanogaster,* gene mutations, deletions, CAs	+	Sex-linked recessive lethal mutations, semi lethal and visible mutations, LEDs 100-800 μg/kg by injection.
DEB	*Drosophila melanogaster,* heritable gene deletions, point mutations	+	500 μM (single dose), high frequency of deletions vs. point mutations. Males fed DEB progeny phenotypes scored, or adults from treated larvae.

Table A1. Genotoxicity of 1, 3-Butadiene and Metabolites
(Adapted from Albertini 1999 and Albertini et al., 2010).

Compound	Test System	Results	Comments
Mammalian cells in vitro			
BD	Mouse Lymphoma	+	S-9
DEB	Human TK6 (*hprt* and *tk* loci)	+	Mutations induced preferentially at A:T base pairs, 40-100x more potent than BDE, LECs = 3.5, 2.3 µM, respectively.
EB (BMO)	Human TK6 (*hprt* and *tk* loci)	+	Mutations induced preferentially at A:T base pairs, 3.5x more potent than BDE, LECs = 122, 243 µM, respectively.
BDE	Human TK6 (*hprt* and *tk* loci)	+	Mutations induced preferentially at A:T base pairs, LECs = 200, 300 µM, respectively.
DEB	Human TK6 (*hprt* locus)	+	Mutations induced preferentially at A:T base pairs
EB (BMO)	Human TK6 (*hprt* locus)	+	Mutations induced preferentially at A:T base pairs
Mammalian, whole animal			
BD inhalation	Mouse melanocyte mutations, mouse spot test	+	500 ppm, 6hr/d on gestation days 8-12, offspring inspected for color spots at 2-3 weeks of age.
BD inhalation	Mouse B6C3F1 *hprt* splenic lymphocytes	+	Mutagenic potency mouse/rat = 5
BD inhalation	Mouse B6C3F1 *hprt* thymic lymphocytes	+	
BD inhalation	Rat Fischer 344 *hprt* splenic lymphocytes	+	
BD inhalation	Rat Fischer 344 *hprt* thymic lymphocytes	+	
BD inhalation	Mouse 102XC3h *hprt* splenic lymphocytes	+	
BD inhalation	Mouse CD1 *hprt* splenic lymphocytes	-	
BD inhalation	Mouse MM *lac I*	+, -	Lung positive, bone marrow and liver negative
BD inhalation	Mouse BB *lac I*	+	

Table A1. Genotoxicity of 1, 3-Butadiene and Metabolites
(Adapted from Albertini 1999 and Albertini et al., 2010).

Compound	Test System	Results	Comments
BD inhalation	Mouse 2-year Bioassay K-ras mutation	+	Lung and liver tumors, lymphoma
BD inhalation	Mouse 2-year Bioassay H-ras mutation	+	Liver tumors
BD inhalation	Mouse 2-year Bioassay p53, Rb, Chr.4 gene, allele loss	+	Lung and mammary tumors
EB (BMO)	Mouse B6C3F1 *hprt* splenic lymphocytes	+	
EB (BMO)	Mouse 102XC3h *hprt* splenic lymphocytes	±	
EB (BMO)	Rat Fischer 344 *hprt* splenic lymphocytes	±	
EB (BMO)	Rat Lewis *hprt* splenic lymphocytes	-	
DEB	Mouse B6C3F1 *hprt* splenic lymphocytes	+	
DEB	Mouse 102XC3h *hprt* splenic lymphocytes	-	
DEB	Mouse C57B1 *hprt* splenic lymphocytes	-	
DEB	Rat Fischer 344 *hprt* splenic lymphocytes	+	
DEB	Rat Lewis *hprt* splenic lymphocytes	-	
Mammalian cytogenetics in vitro			
BD	Chinese hamster ovary cells, sister chromatid exchanges (SCEs)	+	S-9
BD	Human lymphocytes, SCEs	±	± S-9, S-9 not necessary
EB (BMO)	Mouse, rat and human lymphocytes, Cas	-	EB negative for all species tested

Table A1. Genotoxicity of 1, 3-Butadiene and Metabolites
(Adapted from Albertini 1999 and Albertini et al., 2010).

Compound	Test System	Results	Comments
EB (BMO)	Mouse and rat splenocytes, Cas	-	EB negative in both species, HIC = 931 μM EB
EB (BMO)	Human peripheral blood lymphocytes, MN induction	+	LEC = 1.0 μM
EB (BMO)	Rat spermatids, MN. Treatment of late pachytene-diakinetic spermatocytes for 4 days, score MN in meiosis.	-	Negative at 1000 μM
DEB	Rat spermatids, MN. Treatment of late pachytene-diakinetic spermatocytes for 4 days, score MN in meiosis.	+	LEC = 5 μM
DEB	Human peripheral blood lymphocytes, MN induction	+	LEC = 0.5 μM
DEB	Mouse and rat splenocytes, Cas	+	DEB positive in both species, 0.47 and 0.36 aberrant metaphases/100 metaphases/μM, respectively. LEC mouse = 40μM, LEC rat = 80μM.
DEB	Mouse, rat and human lymphocytes, Cas	+	DEB positive for all species tested, aberrant metaphases = 0.36/100/μM DEB for mice and rats.
DEB	Rat embryo fibroblasts, chromosome aberrations	+	Induction of Cas and high degree of tetraploidy, LEC = 93 μM
DEB	Rat liver cells, cytological scoring	+	Concentration not stated.
DEB	Human skin fibroblasts	+	Cas positive in Fanconi's anemia heterozygotes, LEC = 0.1 μM.

Table A1. Genotoxicity of 1, 3-Butadiene and Metabolites
(Adapted from Albertini 1999 and Albertini et al., 2010).

Compound	Test System	Results	Comments
EBD	Rat spermatid, MN induction. Treatment of late pachytene-diakinetic spermatocytes for 4 days, score MN in meiosis.	-	HIC = 100 µM
Mammalian cytogenetics in vivo			
BD inhalation	Mouse Chromosome aberrations (Cas)	+	Lymphocytes and bone marrow multiple tests
BD inhalation	Mouse induced micronuclei (MN)	+	Lymphocytes and bone marrow multiple tests
BD inhalation	Mouse, induced MN in lung fibroblasts, 500 ppm (males) and 1300 ppm, 6hr/d x 5d	+	Significant increase in kinetochore (+) MN in males at 500 ppm, and kinetochore (+) and (-) MN in females at 1300 ppm.
BD inhalation	Mouse SCEs	+	Lymphocytes and bone marrow multiple tests
BD inhalation	Rat Cas	-	
BD inhalation	Rat MN	-	
BD inhalation	Rat SCEs	±	
EB (BMO), i.p. injection	Mouse, Cas in bone marrow cells	+	Positive dose response 25-150 mg/kg
EB (BMO), i.p. injection	Mouse and Rat, MN in splenic lymphocytes	+	Positive in both species, LED mice = 40 mg/kg, LED rats = 80 mg/kg
DEB, s.c. injection	Rat, peripheral blood lymphocytes Cas	+	Stickiness and clumping of chromosomes at 1 mg/kg, chromosome fragmentation at 3 mg/kg
DEB, i.p. injection	Mouse and Rat, MN in splenic lymphocytes by cytokinesis-block method; characterization of induced MN by FISH.	+	Positive in both species, LED mice = 15 mg/kg, LED rats = 30 mg/kg
DEB, i.p. injection	Mouse, Cas in spermatogenic cells	+	Significant increase in chromosome breaks.

Table A1. Genotoxicity of 1, 3-Butadiene and Metabolites
(Adapted from Albertini 1999 and Albertini et al., 2010).

Compound	Test System	Results	Comments
EBD, i.p. injection	Rat, MN in bone marrow cells at 30 and 60 mg.kg	±	Weak positive at 30 mg/kg only.
EBD, i.p. injection	Rat, MN in spermatids at 30 amd 60 mg/kg	+	Significant induction of MN on days 2 and 3 post-exposure (diploteme and late pachyteme)
Mammalian germ cell genotoxicity in vivo			
BD inhalation	Mouse dominant lethals	+	Multiple tests
BD inhalation	Mouse heritable translocation	+	2 studies
BD inhalation	Mouse sperm head abnormalities	+	Increases at 200 ppm, statistically significant at 1,000 and 5,000 ppm, 6hr/d x 5 d
BD inhalation	Mouse spermatid micronuclei	+	
BD inhalation	Mouse testicular cells Comet Assay	+	DNA fragmentation
BD inhalation	Rat dominant lethal	-	
EB (BMO), i.p. injection	Mouse splenic lymphocytes, SCEs	+	Statistically significant increases at 48.8 and 73.2 mg/kg. HID = 24.4 mg/kg
DEB, i.p., i.v. injection	Mouse SCEs in bone marrow cells, alveolar macrophages and liver cells	+	Positive SCE induction in all cell types.
DEB, 1 hr aerosol inhalation, i.p. injection.	Mouse and Chinese hamsters, SCEs in bone marrow cells	+	Positive in mice, LED = 20-30 mg/kg (based on blood concentration). Hamster less sensitive.
EBD, i.p. injection	Mouse, dominant lethal mutations	-	Single EBD dose of 120 mg/kg

Table A1. Genotoxicity of 1, 3-Butadiene and Metabolites
(Adapted from Albertini 1999 and Albertini et al., 2010).

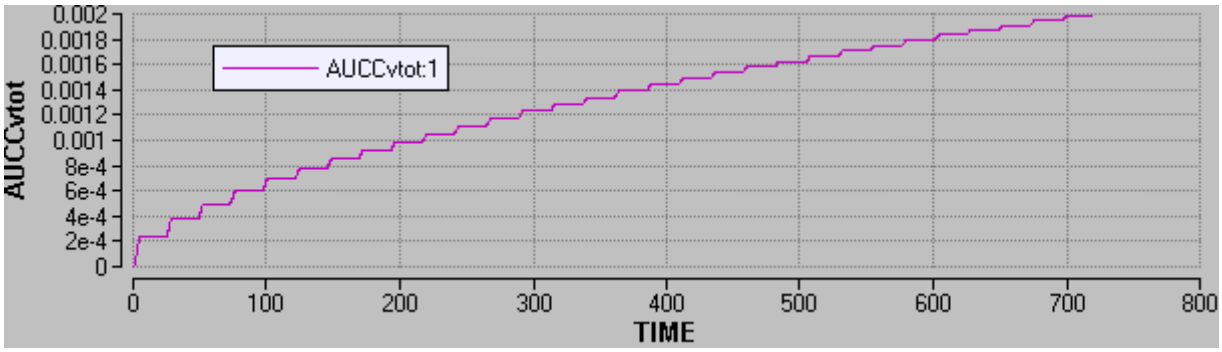
Compound	Test System	Results	Comments
Human cytogenetics in vivo			
BD occupational exposure	Cas -	±	+ for $GST^{-/-}$ genotype
BD occupational exposure	SCEs	±	
BD occupational exposure	MN	-	
BD occupational exposure	Comet Assay	-	
BD occupational exposure	*hprt* T-cell (Autoradiography)	+	Variant frequencies significantly associated with occupational longevity (N = 29, R^2 = 0.107, P < 0.046), Wickliffe et al., 2009.
BD occupational exposure	*hprt* T-cell (Cloning Assay)	-	
BD occupational exposure	*ras* oncoprotein	-	

A.5 PBPK Modeling Data

A general summary of the PBPK models and related reports in the literature is given in Table A2. Of the available models, the one that seemed the most developed in view of relevant metabolites covered in mice and rats was that of Kohn and Melnick (2001). Unfortunately, due to the untimely death of the senior author we were unable to secure the actual model code for this model and were limited to the published report. The values given for the physiological parameters for mice and rats are reproduced in Table A3. Average reported values for alveolar ventilation rates of 20.2 and 20.0 L/hr/$kg^{0.7}$ for mice and rats, respectively were used in the model. For cardiac output the values used were 15.3 and 14.7 L/hr/$kg^{0.7}$, for mice and rats respectively. Typical body weights were 0.025 to 0.030 kg for mice and 0.215 to 0.360 kg for rats. Model partition coefficients are shown in Table A4. The biochemical parameters are listed in Table A5. The activities of P450 and epoxide hydrolase (EH) in mice and rats were multiplied by 30, 9, and 9 mg of microsomal protein per gram of tissue for liver, lung, and kidney, respectively. The glutathione *S*-transferase activities were multiplied by 82.8 or 108 mg of cytosolic protein per gram of tissue for mice and rats, respectively. Michaelis-Menten kinetics were used for P450 and EH and the ordered bi-bi rate law was used for GST.

The results of PBPK simulations of Doerr et al (1996) are shown in Table A6. Daily i.p. doses of BMO are administered to a young mouse over 30 days (720 hr). The

cumulative AUC for DEB in the mixed venous blood (Cvtot) is divided by 30 to give the dose metric of μMhr/d, which is then analyzed by the benchmark dose method (Table A7) to obtain a $BMDL_{05}$. A Berkeley Madonna graph of the 30 days simulation of the 0.09 mmol/kg mid dose is shown in the Figure below. The units are AUC_{DEB} in mixed venous blood Mhr versus time in hours. The terminal value (0.002Mhr) corresponds to the 1985 μMhr value in Table A5 obtained from the tabular output of the same simulation.



An example of code for the OEHHA implementation of the Kohn and Melnick (2001) butadiene model is shown after the tables.

Table A2. Summary of Butadiene PBPK and Related Modeling Literature.

Study	Species	Chemicals modeled	Key parameters	Comments or other factors
Hattis & Wasson, 1987	Mice, Rats	BD	Pb = 0.35 Pf = 118.2 Plvrg = 5.4 Pm = 5.26 P_{450} Vmax = 400µmol/kg-hr (mice), 200µmol/kg-hr (rats) Km = 5.0 µM	3 compartment model liver combined with vessel rich group. Model runs in moles, minutes, liters and Vmax = 1.46×10^{-6} mol/min (rat)
Csanady et al., 1992	Mice, Rats, Humans	BD, BMO	P_{450} Vmax/Km = 157-1295; EH Vmax/Km = 3.6-35; GST Vmax/Km = 4.3-17 nmol/mg protein/min/mM, all values for liver	2-compartment kinetic model for BD and BMO metabolism in vitro: BD gas and liquid, BMO gas and liquid.
Johanson & Filser, 1993	Mice, Rats	BD, BMO, GSH (liver)	BD Pb = 3.03; Pf = 7.2; Pmusc = 0.24; Pl = 0.31; Pkid = 0.30; Pbrain = 0.14; BMO Pb = 83.4; Pf = 1.85; Pmusc = 0.72; Pl = 0.65; Pkid = 0.84; Pbrain = 0.62.	Closed chamber experiments simulated.
Evelo et al., 1993	Mice, Rats	BD	Pb = 1.184, 0.603 Pf = 32.36; Pl = 2.675; Pmusc = 1.871; Pkidney = 1.69; Plung = 1.272; Pbrain = 2.355; Pvrg = 2.02	2-compartment lung with gas exchange in alveoli, metabolism in bronchi and alveoli; Mice Vmax liver = 318, pulmonary = 70, bronchi = 77 µmol/hr-kg, Km = 8 µM

Table A2. Summary of Butadiene PBPK and Related Modeling Literature.

Study	Species	Chemicals modeled	Key parameters	Comments or other factors
Kohn & Melnick, 1993	Mice, Rats, Humans	BD, BMO	BD Pb = 1.5; BD Pf = 118.2; BD Pl = 5.49; BD Pmusc = 5.26; BD Pviscera = 5.34; BMO Pb = 60; BMO Pf = 1.81; BMO Pl = 0.6545; BMO Pmusc = 0.6533; BMO Pviscera = 0.6348	Sensitivity Analysis; Vmax BD P_{450} = 155.4 (mouse), 35.4 (rat), 70.8 (human) nmol/hr-mg prot., liver microsomal protein = 11.6, 16.8, 14.5 g/L, Km = 2, 3.75, 5.14 μM, respectively.
Medinsky et al., 1994	Mice, Rats	BD, BMO	BD Pb = 1.49, 1.34; BD Pf = 14.9, 14.3; BD Pl = 0.799, 1.01 BD Pm = 0.99, 2.99 BMO Pb = 50.4, 36.6; BMO Pf = 2.74, 2.49, BMO Pl = 1.43, 1.15 BMO Pm = 0.39, 0.64 (rats and mice, respectively.)	BD liver and lung oxidation; BMO liver oxidation, hydrolysis, and conjugation. Liver oxidation Vmax/Km = 16.5, 169 μmol/hr/kg/μM, rats and mice, respectively.
Johanson & Filser,1996	Mice, Rats, Humans	BD,BMO,GSH (liver)	BD Km = 83 μM GST Km(BMO) = 100 mM	Intrahepatic oxidation of BD and hydrolysis of BMO
Csanady et al., 1996	Mice, Rats, Humans	BD,BMO,DEB	Rats BD Vmax = 220 μmol/kg-hr, Km = 36 μM; Mice BD Vmax = 400 μmol/kg-hr, Km = 26 μM; BMO k_{P450} = 0.00126 L/hr; DEB k_{elim} = 0.0046 L/hr	Hemoglobin adducts from BMO reaction with N-terminal valine:2.9E-5 L/hr/g Hb

Table A2. Summary of Butadiene PBPK and Related Modeling Literature.

Study	Species	Chemicals modeled	Key parameters	Comments or other factors
Sweeney et al., 1997	Mice, Rats	BD, BMO, DEB in Liver and Lung;	DEB P_{liver} = 1.53, P_{kidney} = 1.41, P_{muscle} = 1.82, P_{fat} = 2.20; Mice BD Liver Vmax = 338 µmol/kg-hr, Km = 2.0µM, Lung Vmax = 21.6 µmol/kg-hr, Km = 5.01 µM; Liver DEB EH Vmax = 4193µmol/kg-hr, Km = 8100 µM; GST Vmax = 5.03 x 10^4 µmol/kg-d, Km = 6400 µM	Mice BMO Liver P_{450} Vmax = 176.6 µmol/kg-hr, Km = 145 µM, EH Vmax= 754 µmol/kg-hr, Km = 1590 µM, GST = 1.54 x 10^5 µmol/kg-hr, Km = 3.53 x 10^4 µM
Kohn & Melnick, 2000	Mice, Rats	BD, BMO, DEB	BD Pb = 1.95 BD Pf = 10.8 BMO Pb = 56.8 BMO Pf = 2.25 DEB Pf = 2.19 BD liver Vmax/Km = 1292, 578 nmol/min/mg protein/mM for mice and rats, respectively	Diffusion-limited model, liver, lung and kidney metabolism P_{450}, EH, GST; P_{450}-EH complex ER bound.
Kohn & Melnick, 2001	Mice, Rats	BD, BMO, DEB, BDD, EBD	BD Pb = 1.95 BD Pf = 10.8 BMO Pb = 56.8 BMO Pf = 2.25 DEB Pf = 2.19 BDD Pf = 0.573 EDB Pf = 0.496	Diffusion-limited model, liver, lung and kidney metabolism P_{450}, EH, GST; P_{450}-EH complex ER bound.

Table A2. Summary of Butadiene PBPK and Related Modeling Literature.

Study	Species	Chemicals modeled	Key parameters	Comments or other factors
Sweeney et al., 2001	Human	BD,BMO, DEB,BDD,EBD	Vmax/Km: BD→BMO, 0.0132/0.7; BD→DEB, 0.031/880; BMO→BDD, 1.4/540; BMO→$GS_{conj,}$ 2.7/10,400; DEB→GS_{conj}, 0.4/3390; BDD→EBD, 0.031/880; DEB→EBD, 9.2/4605; EBD→Erythritol, 4.6/4605; EBD→GS_{conj}, 0.2/339, µmol/mg prot.-hr/µM.	Tissue/blood partition coefficients for BDD and EBD assumed to be 1.0. Sensitivity analysis (SA) for exhaled BD (2hr human exposure to 5 ppm BD): BD blood/air = 0.36; alveolar ventilation = -0.36; cardiac output = 0.28; Qliver = -0.40; Qfat = -0.23. SA for DEB AUC: Vmax EB→DEB = -0.98;Vliver = -0.96; Km DEB→EBD = 0.94; Vmax DEB→EBD = -0.93; Vmax BMO→BDD = -0.87, microsomal protein liver = -0.80; BD blood/air = 0.78.
Smith et al. 2001	Humans	BD 3 compartment model fit to data from human subjects, N = 133	BD uptake µg/kg; kmet /min, CYP2E1 genotype, phenotype	BD uptake and rate of metabolism not related in this analysis. No significant differences in uptake or kmet with genetic makeup.

Table A2. Summary of Butadiene PBPK and Related Modeling Literature.

Study	Species	Chemicals modeled	Key parameters	Comments or other factors
Mezzetti et al., 2003	Humans	BD 3-compartment model fit to data from human subjects, N= 130 8 parameters analyzed	1120 parameters sampled, the most sensitive: W_{wp_i} the weight fraction of the well perfused tissues; P_{fat}, the fat/blood partition coeff; P_{wp} = wp/blood partition coeff.	Bayesian Analysis Model, MCMC analysis. Subgroups analyzed: males, females; age<30yr; age ≥30 yr; African-American; Hispanic; Asian, Caucasian
Brochot et al., 2007	Humans	BD, BMO, DEB, BDD, EBD	BD Pb = 1.22 BD Pf = 18.4 BD P_{450} Kmet = 0.19 ± 0.06 /min	Global sensitivity analysis
Pery & Bois, 2009	Human	BD	Pb = 0.653 Pf = 22.0 Kmet = 0.3 L/min	23 tissue compartments PBPK model coupled to PD model of carcinogenicity
Beaudouin et al., 2010	Humans, N = 133	BD	BD P_{450} Vmax = 2.28 x 10^{-5} mmol/min/mg microsomal protein, Km = 0.39 mM	Acute and chronic (lifetime) exposures modeled. Human inter-individual variability in exhaled BD well described, within 95% CI: median x 0.3 to 1.6.

Table A3. Physiological Parameter Values for Kohn & Melnick (2001) PBPK Model for Butadiene and Metabolites

Tissue compartment volumes, % body weight	Mouse	Rat
Liver	5.5	3.7
Lung (bronchi)	0.6	0.52
Alveolar	0.5	0.515
Kidney	1.67	1.48
GI Tract	7.5	7.5
Viscera	3.93	14.3
Fat	6.0	5.4
Muscle and skin	64.5	54.2
Blood	6.0	5.4
Capillary blood volume, % tissue volume		
Liver	11.0	13.8
Lung	11.0	18.0
Kidney	10.2	16.0
GI tract	2.9	2.65
Viscera	7.1	7.1
Fat	3.0	2.0
Muscle and skin	1.3	2.0
Blood flow rate, % cardiac output		
Liver (hepatic artery only)	4.4	3.9
Kidney	16.3	13.3
GI tract	18.1	18.1
Viscera	22.4	24.8
Fat	5.0	6.5
Muscle and skin	33.8	33.4

Table A4. Partition Coefficients for Kohn & Melnick (2001) PBPK Model for Butadiene and Metabolites

Partition Coefficient	Butadiene	Epoxybutene	Butenediol	Epoxybutane diol	Diepoxybutane
Blood:air	1.95	56.8	-	-	-
Liver:blood	0.595	0.984	1.04	0.903	1.41
Lung:blood	0.615	0.977	1.107	0.958	1.41
Kidney:blood	0.472	0.842	0.962	0.833	1.54
GI tract:blood	0.446	0.908	1.22	1.06	1.41
Viscera:blood	0.446	0.908	1.22	1.06	1.41
Fat	10.8	2.25	0.573	0.496	2.19
Muscle and Skin:blood	0.564	0.736	1.139	0.986	1.82

Table A5. Biochemical Parameters for Kohn & Melnick (2001) PBPK Model for Butadiene and Metabolites

	Mouse Liver	Rat Liver	Mouse Lung	Rat Lung	Mouse Kidney	Rat Kidney
Butadiene						
P450 Vmax	155	130	139	9.6	1430	30
P450 Km	0.002	0.00375	0.00501	0.00775	0.00501	0.00216
Epoxybutene						
P450 Vmax	45.1	24.3	10.2	9.84	48.6	12.6
P450 Km	0.0156	0.145	0.0156	0.145	0.0156	0.145
EH Vmax	347	584	34.8	42.8	113	14.7
EH Km	1.59	0.26	1.59	0.7	1.59	0.7
GST Vmax	6420	4260	720	196	960	494
GST Kmx, adjusted	3.59	2.59	3.59	4.94	3.59	4.39
Butenediol						
P450 Vmax	16.3	67.1	1.0	31.5	1.0	85.0
P450 Km	0.0156	0.145	0.0156	0.145	0.0156	0.145
GST Vmax	3280	1230	491	276	1070	658
GST Kmx	34	34	34	34	34	34
Epoxybutanediol						
EH Vmax	363	1150	69.5	169	10.0	152
EH Km	8.1	2.76	7.5	7.1	7.5	7.1
GST Vmax	2260	271	50.0	100	50.0	138
GST Kmx	6.40	4.17	6.40	4.17	6.40	4.17
Diepoxybutane						
EH Vmax	1920	3170	10.0	1160	35.2	1000
EH Km	8.1	2.76	7.5	7.1	7.5	7.1
GST Vmax	9720	1940	100	100	100	100
GST Kmx, adjusted	6.40	4.17	6.40	4.17	6.40	4.17
Cysteine						
Tissue cysteine	0.193	0.195	0.171	0.127	0.280	0.326
γ-GCS Vmax	420	396	54	50	7920	6080

Table Vmax values in nmol/hr/mg protein, Km values in mM.

Table A6. PBPK Modeling ot the Doerr et al. (1996) Data on Ovarian Atrophy in Mice (Model based on Kohn and Melnick, 2001)

BMO i.p. dose mmol/kg-d x 30 d	AUC DEB in blood µM hr (30 d)	AUC DEB in blood µM hr/day	Hb adducts nmol THBV/g globin-day
0.005	449	15.0	2.43
0.02	1046	34.9	21.8
0.09	1985	66.2	41.4
0.36	2717	90.6	56.7
1.43	3971	132.4	82.9

Table A7. Benchmark Dose Analysis of AUC DEB and Hb Adduct Dose Metrics with the Doerr et al. (1996) Data Set on Ovarian and Uterine Atrophy in Mice Induced by Parenteral Administration of Epoxybutene (BMO).

Model, continuous, site	Fit Statistic, P	BMD_{05} µMhr/d	$BMDL_{05}$ µMhr/d
Polynomial, ovary	0.919	57.4	20.5
Power, ovary	0.0284 n.s.	2688	1765
Polynomial, uterus	0.84	103.1	37.9
		BMD_{05} nmol Hb/g-d	$BMDL_{05}$ nmol Hb/g-d
Hill, ovary	0.661	31.75	18.3
Polynomial, ovary	0.9225	34.81	15.60
Polynomial, uterus	0.80	58.2	23.9

Table A8 Ovarian Quantal Toxicity Data (NTP, 1993)

Dose group	9 months	15 months	24 months	N
0	0/10	0/10	4/49	69
6.25	0/10	0/10	19/49	69
20.0	0/10	0/10	32/48	68
62.5	0/10	9/10	42/50	70
200.0	9/10	7/10	43/50	70
625.0	8/8	2/2	69/79	89
$BMCL_{05}$	3.44	0.65	0.00054	435

Table A9 Ovarian Toxicity Continuous Data for Weighted Analysis (Time Adjustment)

Dose group, Xi, ppm	Yi, %	Wi, weight time in months*
0	0	9
0	0	15
0	8.46	24
6.25	0	9
6.25	0	15
6.25	38.78	24
20	0	9
20	0	15
20	66.67	24
62.5	0	9
62.5	90.0	15
62.5	84.0	24
200	90.0	9
200	70.0	15
200	86.0	24
625	100.0	9
625	100.0	15
625	87.3	24

Note:* weighting by $X^2 = \sum_i W_{i(Yi - Y(Xi, P1 \ldots Pm))}^2$, $Y = a - b/(1 + c(X))^d$, fitted parameters a = 93.52 ±0.236, b = 89.23±0.2933, c = 0.01558±0.000632, d = 1.7097±0.05887 (SD).

Table A10 Ovarian Continuous to Quantal Conversion

Dose group	Y predicted ,% with parameters a, b, c, d	Adjusted quantal response for BMC analysis	Observed at 24 months, %	Observed average 9-24 months, %
0	4.29	3/69	8.16	2.8
6.25	17.4	12/69	38.78	12.5
20.0	37.4	25/68	66.67	22.2
62.5	65.6	46/70	84.0	58.0
200.0	85.6	60/70	86.0	82.0
625.0	91.98	82/89	87.3	95.8

Table A11. Continuous Benchmark Analysis of Adjusted Quantal Response from Time Weighted Regression*

Model	X^2, P	BMC_{05} ppm	$BMCL_{05}$ ppm	$BMLC_{05}$ ppm continuous
Log Logistic	1.13, 0.8896	2.05	1.58	0.28
Log Probit	**1.70, 0.6364**	**2.04**	**1.009**	**0.18**
Multistage	58.35, -	7.54	6.16	n.s.
Multistage	10.19, 0.017	4.10	3.37	Without top dose, n.s.

Note:* BMDS version 2.2

Table A 12. Benchmark Dose Analysis of Fetal Weight Data from Hackett et al. (1987) and Green (2003) Re-Analysis.

Data Set	BMC_{05}	$BMCL_{05}$	Variance assumption	Fit*	Model	Comment
Hackett et al. (1987), total males and females	44.4 183.9	20.0 111.2	Equal Unequal	Exact No	Hill Hill	Best fit
	57.6 89.6	45.9 62.2	Equal Unequal	Exact No	Polynomial Polynomial	Nonmonotonic
Male fetuses	39.5 28.5	19.3 13.4	Equal Unequal	Exact Yes	Hill Hill	Best fit
Green (2003), total males and females	40.3 172.5	19.2 48.0	Equal Unequal	Exact No	Hill Hill	Best fit
	58.2 54.4	46.7 44.4	Equal Unequal	Exact No	Polynomial Polynomial	Nonmonotonic
Male fetuses	37.2 22.6	**17.7** 9.6	Equal Unequal	Exact Yes	Hill Hill	**Best fit overall**

Note * Exact = predicted values equal observed by table and plot, all absolute value residuals <3E-7, Yes = test 4 for fit, A3 vs. fitted, all absolute value residuals <2 . All values obtained with BMDS version 2.3.1.

The following model code is an example of OEHHA implementation of the Kohn and Melnick (2001) butadiene model in Berkeley Madonna v. 8.3.9. In Berkeley Madonna lines preceded by semi colons or enclosed in curved brackets are not executed.

```
METHOD Stiff

STARTTIME = 0
STOPTIME = 720
DT = 0.0005
DTOUT = 0.25
;Butadiene multimetabolite mouse model based on Kohn & Melnick (Chemico-Biol. Interact. 135-136:285-301(2001)). Symbols: A = mass of BD in moles, AB = BMO, AC = DEB, AD = BDD; AE = BDE; f = fat, l = liver, m = muscle, vrg = vessel rich group, br = bronchi, pu = alveoli, uo = uterus-ovary, kid, k = kidney, cap = tissue capillary bed; exh = exhaled, perit = peritoneum,C = concentration, Cv = concentration leaving tissue, Cvtot = mixed venous concentration, AUC = area under the time x concentration curve, V = volume, Q = flow, P = partition coeff.,Vmax = metabolic rate, Km = affinity constant, P450 = oxidase; EH = epoxide hydrolase, GST = glutathione sulfotransferase, BW = body weight for adult or young mouse for 30 day simulations, Amet = amount metabolized
{butadiene, moles}
init Af = 0
Limit Af >= 0
init Al = 0
Limit Al >= 0
init Am = 0
Limit Am >= 0
init Avrg = 0
Limit Avrg >= 0
init Abr = 0
Limit Abr >= 0
init Apu = 0
Limit Apu >= 0
init Auo = 0
Limit Auo >= 0
init AUCvtot = 0
Limit AUCvtot >= 0
init Aexh = 0
Limit Aexh >= 0
init Akid = 0
Limit Akid >= 0

init Afcap = 0
Limit Afcap >= 0
init Alcap = 0
Limit Alcap >= 0
init Amcap = 0
Limit Amcap >= 0
init Avrgcap = 0
Limit Avrgcap >= 0
;init Abrcap = 0
;init Apucap = 0
init Auocap = 0
```

```
Limit Auocap >= 0
init Akidcap = 0
Limit Akidcap >= 0

{butadienemonoxide moles}
init ABperit = 0
init ABf = 0
Limit ABf >= 0
init ABl = 0
Limit ABl >= 0
init ABlfree = 0
init ABm = 0
Limit ABm >= 0
init ABvrg = 0
Limit ABvrg >= 0
init ABbr = 0
Limit ABbr >= 0
init ABbrfree = 0
init ABpu = 0
Limit ABpu >= 0
init ABpufree = 0
init ABuo = 0
Limit ABuo >= 0
init AUCBuo = 0
Limit AUCBuo >= 0
init AUCBvtot = 0
Limit AUCBvtot >= 0
init ABkid = 0
Limit ABkid >= 0
init ABkidfree = 0

init ABfcap = 0
Limit ABfcap >= 0
init ABlcap = 0
Limit ABlcap >= 0
init ABmcap = 0
Limit ABmcap >= 0
init ABvrgcap = 0
Limit ABvrgcap >= 0
;init ABbrcap = 0
;init ABpucap = 0
init ABuocap = 0
Limit ABuocap >= 0
init ABkidcap = 0
Limit ABkidcap >= 0

{diepoxybutane moles}
init ACperit = 0
init ACf = 0
Limit ACf >= 0
init ACl = 0
Limit ACl >= 0
init AClfree = 0
init ACm = 0
Limit ACm >= 0
init ACvrg = 0
```

```
Limit ACvrg >= 0
init ACbr = 0
Limit ACbr >= 0
init ACbrfree = 0
init ACpu = 0
Limit ACpu >= 0
init ACpufree = 0
init ACuo = 0
Limit ACuo >= 0
init AUCCuo = 0
Limit AUCCuo >= 0
init AUCCvtot = 0
Limit AUCCvtot >= 0
init ACkid = 0
Limit ACkid >= 0
init ACkidfree = 0

init ACfcap = 0
init AClcap = 0
init ACmcap = 0
init ACvrgcap = 0
;init ACbrcap = 0
;init ACpucap = 0
init ACuocap = 0
init ACkidcap = 0

{dihydroxybutene}
init ADf = 0
Limit ADf >= 0
init ADl = 0
Limit ADl >= 0
init ADm = 0
Limit ADm >= 0
init ADvrg = 0
Limit ADvrg >= 0
init ADbr = 0
Limit ADvrg >= 0
init ADpu = 0
Limit ADpu >= 0
init ADuo = 0
Limit ADuo >= 0
init AUCDvtot = 0
init ADkid = 0
Limit ADkid >= 0

init ADfcap = 0
init ADlcap = 0
init ADmcap = 0
init ADvrgcap = 0
;init ADbrcap = 0
;init ADpucap = 0
init ADuocap = 0
init ADkidcap = 0

{epoxybutanediol}
init AEf = 0
```

```
Limit AEf >= 0
init AEl = 0
Limit AEl >= 0
init AElfree = 0
init AEm = 0
Limit AEm >= 0
init AEvrg = 0
Limit AEvrg >= 0
init AEbr = 0
init AEbrfree = 0
Limit AEvrg >= 0
init AEpu = 0
Limit AEpu >= 0
init AEpufree = 0
init AEuo = 0
Limit AEuo >= 0
init AUCEvtot = 0
init AEkid = 0
Limit AEkid >= 0
init AEkidfree = 0

init AEfcap = 0
init AElcap = 0
init AEmcap = 0
init AEvrgcap = 0
;init AEbrcap = 0
;init AEpucap = 0
init AEuocap = 0
init AEkidcap = 0

{moles of butadiene metabolized}
init Ametl = 0
init Ametpu = 0
init Ametbr = 0
init Ametk = 0

{moles of butadienemonoxide metabolized}
init ABmetl1 = 0
Limit ABmetl1 >= 0
init ABmetl2 = 0
Limit ABmetl2 >= 0
init ABmetpu1 = 0
Limit ABmetpu1 >= 0
init ABmetpu2 = 0
Limit ABmetpu2 >= 0
init ABmetbr1 = 0
Limit ABmetbr1 >= 0
init ABmetbr2 = 0
Limit ABmetbr2 >= 0
init ABmetl3 = 0
Limit ABmetl3 >= 0
init ABmetpu3 = 0
Limit ABmetpu3 >= 0
init ABmetbr3 = 0
Limit ABmetbr3 >= 0
```

```
{moles of dihydroxybutene metabolized}
init ADmetpu6 = 0
init ADmetbr6 = 0
init ADmetl6 = 0

{moles of diepoxybutane metabolized}
init ACmetpu4 = 0
init ACmetbr4 = 0
init ACmetl4 = 0

{Cysteine metabolism, moles, mol/L}
init AFl = Ccysl*Vl
init AFlu = Ccyslu*Vlu
init AFkid = Ccyskid*Vkid
Ccysl = 1.93E-4
Ccyslu = 1.71E-4
Ccyskid = 2.8E-4
Vmax10lC = 420E-9
Vmax10l = Vmax10lC*CPL*Vl
Vmax10luC = 54E-9
Vmax10lu = Vmax10luC*CPLu*Vlu
Vmax10kidC = 7920E-9
Vmax10kid = Vmax10kidC*CPLu*Vkid
Kmcys = 3.5E-4
Kl = 2.3E-3
Kl0 = 1.5*Vl
Klu0 = 1.5*Vlu
Kkid0 = 1.5*Vkid

{Glutathione metabolism, moles, mol/L}
init AGl = CGSHl*Vl
Limit AGl >= 0
init AGlu = CGSHlu*Vlu
Limit AGlu >= 0
init AGkid = CGSHkid*Vkid
Limit AGkid >= 0
CGSHl = 5E-3
CGSHlu = 1E-3
CGSHkid = 2E-3
CGl = AGl/Vl
Limit CGl >= 0
CGlu = AGlu/Vlu
Limit CGlu >= 0
CGkid = AGkid/Vkid
Limit CGkid >= 0
Ka = 3E-3

{Adduct formation}
init Adduct = 0

{tissue flows L/hr}
Qtot = 15.3*BW^0.7
Qalv = 20.2*BW^0.7
Qf = 0.05*Qtot
Ql = 0.044*Qtot
Qkid = 0.163*Qtot
```

```
Qm = Qtot - (Qf + Ql + Qvrg + Quo + Qkid)
Qvrg = 0.389*Qtot
Qpu = 0.928*Qtot
Qbr = 0.072*Qtot
Quo = 0.016*Qtot

{tissue volumes, L}
Vf = 0.06*BW
Vl = 0.055*BW
Vkid = 0.0167*BW
Vm = BW - (Vf + Vl + Vvrg + Vlu + Vuo + Vkid)
Vvrg = 0.1143*BW
Vlu = 0.011*BW
Vpu = 0.454*Vlu
Vbr = 0.545*Vlu
Vuo = 0.001*BW
;BW = 0.030
BW = 0.01263 + 3.69E-4*(T/24) - 5.59E-6*(T/24)^2

{capillary blood volumes, L}
Vfcap = 0.03*Vf
Vlcap = 0.11*Vl
;Vbrcap = 0.11*Vbr
;Vpucap = 0.11*Vpu
Vkidcap = 0.102*Vkid
Vvrgcap = 0.071*Vvrg
Vuocap = 0.071*Vuo
Vmcap = 0.013*Vm

{blood/air and tissue/blood partition coefficients, butadiene}
Pb = 1.95
Pl = 0.595
Pf = 10.8
Pkid = 0.472
Pm = 0.564
Pvrg = 0.472
Ppu = 0.615
Pbr = 0.615
Puo = 0.446
Pibd = 1.183

{blood/air and tissue/blood partition coefficients, butadienemonoxide}
PBb = 56.8
PBl = 0.984
PBkid = 0.842
PBf = 2.25
PBm = 0.736
PBvrg = 0.842
PBpu = 0.977
PBbr = 0.977
PBuo = 0.908
Pibmo = 2.125

{blood/air and tissue/blood partition coefficients, diepoxybutane}
PCl = 4.41
PCkid = 1.54
```

```
PCf = 2.19
PCm = 1.82
PCvrg = 1.54
PCpu = 1.41
PCbr = 1.41
PCuo = 1.41
Pideb = 1.174

{blood/air and tissue/blood partition coefficients, dihydroxybutene}
PDl = 1.04
PDkid = 0.833
PDf = 0.573
PDm = 1.139
PDvrg = 0.962
PDpu = 1.107
PDbr = 1.107
PDuo = 1.22
Pidhb = 1.198

{blood/air and tissue/blood partition coefficients, epoxybutanediol}
PEl = 0.903
PEkid = 1.06
PEf = 0.496
PEm = 0.986
PEvrg = 1.06
PEpu = 0.833
PEbr = 0.833
PEuo = 1.06
Piebd = 1.237

{butadiene oxidation metabolic parameters, mol/hr, mol/L}
Vmaxlu = 139E-9*9.0E3*Vlu
Vmaxbr = 0.50*Vmaxlu
Vmaxpu = 0.50*Vmaxlu
Kmlu = 5.01E-6
Vmaxl = 155.0E-9*30.0E3*Vl
Km = 2.0E-6
VmaxkC = 1430E-9
Vmaxk = VmaxkC*Vkid*30.0E3
Kmk = Kmlu
MPL = 3.0E4
MPLu = 9.0E3

{butadienemonoxide metabolic parameters, mol/hr, mol/L, /hr; 1 = hydrolysis EH, 2 = conjugation GST, 3
= oxidation P450}
;EH
Vmaxl1 = 347.0E-9*30.0E3*Vl
Km1 = 1.59E-3
Vmaxlu1C = 34.8E-9
Vmaxbr1 = Vmaxlu1C*Vbr*9.0E3
Vmaxpu1 = Vmaxlu1C*Vpu*9.0E3
Kmlu1 = 1.59E-3
Vmaxk1C = 113E-9
Vmaxk1 = Vmaxk1C*Vkid*9.0E3
Kmk1 = Kmlu1
krel = 3.76
```

```
R = 0.052

;GST
Vmaxl2C = 6420E-9
Vmaxl2 = Vmaxl2C*Vl*8.28E4
Km2 =3.59E-3
Vmaxlu2C = 720.0E-9
Vmaxbr2 = Vmaxlu2C*Vbr*8.28E4
Vmaxpu2 = Vmaxlu2C*Vpu*8.28E4
Kmlu2 = 3.59E-3
Vmaxk2C = 960.0E-9
Vmaxk2 = Vmaxk2C*Vkid*8.28E4
Kmk2 = 3.59E-3
CPL = 8.28E4
CPLu = 8.28E4
Kmgsh = 1.0E-3

;P450
Vmaxl3 = 45.1E-9*30.0E3*Vl
Vmaxlu3C = 10.2E-9
Vmaxpu3 = Vmaxlu3C*Vpu*9.0E3
Vmaxbr3 = Vmaxlu3C*Vbr*9.0E3
Km3 = 1.56E-5
Kmlu3 = Km3
Kmk3 = Km3
Vmaxk3C = 48.6E-9
Vmaxk3 = Vmaxk3C*Vkid*9.0E3

{Diepoxybutane (DEB) metabolic parameters, 4 = EH, 5 = GST, mol/hr, mol/L}
Vmaxl4C = 1920E-9
Vmaxl4 = Vmaxl4C*Vl*30E3
Km4 = 8.1E-3
Vmaxlu4C = 10E-9
Vmaxbr4 = Vmaxlu4C*Vbr*9.0E3
Vmaxpu4 = Vmaxlu4C*Vpu*9.0E3
Kmlu4 = 7.5E-3
Vmaxk4C = 35.2E-9
Vmaxk4 = Vmaxk4C*Vkid*9.0E3
Kmk4 = Kmlu4
k4rel = 9.75

Vmaxl5C = 9720E-9
Vmaxl5 = Vmaxl5C*Vl*CPL
Km5 = 6.4E-3
Vmaxlu5C = 100E-9
Vmaxbr5 = Vmaxlu5C*Vbr*CPlu
Vmaxpu5 = Vmaxlu5C*Vpu*CPlu
Vmaxk5C = 100E-9
Vmaxk5 = Vmaxk5C*Vkid*CPlu

{Dihydroxybutene (DHB) metabolic parameters, 6 = P450, 7 = GST, mnol/hr, mol/L}
Vmaxl6C = 16.3E-9
Vmaxl6 = Vmaxl6C*Vl*30E3
Km6 = 1.56E-5
Vmaxlu6C = 1.0E-9
Vmaxbr6 = Vmaxlu6C*Vbr*9.0E3
```

```
Vmaxpu6 = Vmaxlu6C*Vpu*9.0E3
Vmaxk6C = 1.0E-9
Vmaxk6 = Vmaxk6C*Vkid*9.0E3

Vmaxl7C = 3480E-9
Vmaxl7 = Vmaxl7C*Vl*CPL
Km7 = 34E-3
Vmaxlu7C = 491E-9
Vmaxbr7 = Vmaxlu7C*Vbr*CPlu
Vmaxpu7 = Vmaxlu7C*Vpu*CPlu
Vmaxk7C = 1070E-9
Vmaxk7 = Vmaxk7C*Vkid*CPlu

{Epoxybutanediol metabolic parameters 8 = EH, 9 = GST}
Vmaxl8C = 363E-9
Vmaxl8 = Vmaxl8C*Vl*30E3
Km8 = 8.1E-3
Vmaxlu8C = 69.5E-9
Vmaxbr8 = Vmaxlu8C*Vbr*9.0E3
Vmaxpu8 = Vmaxlu8C*Vpu*9.0E3
Kmlu8 = 7.5E-3
Vmaxk8C = 10E-9
Vmaxk8 = Vmaxk8C*Vkid*9.0E3
Kmk8 = Kmlu8
k8rel = 50.6

Vmaxl9C = 2260E-9
Vmaxl9 = Vmaxl9C*Vl*CPL
Km9 = 6.4E-3
Vmaxlu9C = 50E-9
Vmaxbr9 = Vmaxlu9C*Vbr*CPlu
Vmaxpu9 = Vmaxlu9C*Vpu*CPlu
Vmaxk9C = 50E-9
Vmaxk9 = Vmaxk9C*Vkid*CPlu

Kadduct = 1E-4

Kip = 1.25
T = TIME
{exposure in ppm converted to moles}
Cair = IF TIME <= 6 THEN 0*(1E-6/25.45) ELSE 0

{calculated concentrations of butadiene}
Cart = (Qpu*Cvpu + Qbr*Cvbr)/Qtot
Cvf = Af/(Vf*Pf)
Cvl = Al/(Vl*Pl)
Cvkid = Akid/(Vkid*Pkid)
Cvm = Am/(Vm*Pm)
Cvvrg = Avrg/(Vvrg*Pvrg)
Cvpu = Apu/(Vpu*Ppu)
Cvbr = Abr/(Vbr*Pbr)
Cvuo = Auo/(Vuo*Puo)
Cvtot = (Ql*Cvl + Qf*Cvf + Qm*Cvm + Qvrg*Cvvrg + Quo*Cvuo + Qkid*Cvkid)/Qpu
Cvtotg = Cvtot*MWBD*1000
Cvipu = (Qalv*Cair + Qpu*Cvtot)/((Qalv/Pb) + Qpu)
Cexh = Cvipu/Pb
```

```
MWBD = 54.1


{calculated concentrations of butadienemonoxide}
CBart = (Qpu*CBvpu + Qbr*CBvbr)/Qtot
CBvf = ABf/(Vf*PBf)
CBvl = ABl/(Vl*PBl)
CBvkid = ABkid/(Vkid*Pkid)
CBvm = ABm/(Vm*PBm)
CBvvrg = ABvrg/(Vvrg*PBvrg)
CBvpu = ABpu/(Vpu*PBpu)
CBvbr = ABbr/(Vbr*PBbr)
CBvuo = ABuo/(Vuo*PBuo)
CBvtot = (Ql*CBvl + Qf*CBvf + Qm*CBvm + Qvrg*CBvvrg + Quo*CBvuo + Qkid*CBvkid)/Qtot
CBvtotg = CBvtot*MWEB*1000
CBair = CBvtot/PBb
CBvipu = (Qalv*CBair + Qpu*CBvtot)/((Qalv/PBb) + Qpu)
CBexh = CBvipu/PBb
MWEB = 70.1

{calculated concentrations of diepoxybutane}
CCart = (Qpu*CCvpu + Qbr*CCvbr)/Qtot
CCvf = ACf/(Vf*PCf)
CCvl = ACl/(Vl*PCl)
CCvkid = ACkid/(Vkid*PCkid)
CCvm = ACm/(Vm*PCm)
CCvvrg = ACvrg/(Vvrg*PCvrg)
CCvpu = ACpu/(Vpu*PCpu)
CCvbr = ACbr/(Vbr*PCbr)
CCvuo = ACuo/(Vuo*PCuo)
CCvtot = (Ql*CCvl +Qf*CCvf + Qm*CCvm + Qvrg*CCvvrg + Quo*CCvuo + Qkid*CCvkid)/Qtot
CCvtotg = CCvtot*MWDEB*1000
CCvipu = (Qalv + Qpu*CCvtot)/(Qalv + Qpu)
MWDEB = 86.0

{calculated concentrations of dihydroxybutene}
CDart = (Qpu*CDvpu + Qbr*CDvbr)/Qtot
CDvf = ADf/(Vf*PDf)
CDvl = ADl/(Vl*PDl)
CDvkid = ADkid/(Vkid*PDkid)
CDvm = ADm/(Vm*PDm)
CDvvrg = ADvrg/(Vvrg*PDvrg)
CDvpu = ADpu/(Vpu*PDpu)
CDvbr = ADbr/(Vbr*PDbr)
CDvuo = ADuo/(Vuo*PDuo)
CDvtot = (Ql*CDvl + Qf*CDvf + Qm*CDvm + Qvrg*CDvvrg + Quo*CDvuo + Qkid*CDvkid)/Qtot
CDvtotg = CDvtot*MWDHB*1000
CDvipu = (Qalv + Qpu*CDvtot)/(Qalv +Qpu)
MWDHB = 72.0

{calculated concentrations of epoxybutanediol}
CEart = (Qpu*CEvpu + Qbr*CEvbr)/Qtot
CEvf = AEf/(Vf*PEf)
CEvl = AEl/(Vl*PEl)
CEvkid = AEkid/(Vkid*PEkid)
CEvm = AEm/(Vm*PEm)
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CEvvrg = AEvrg/(Vvrg*PEvrg)
CEvpu = AEpu/(Vpu*PEpu)
CEvbr = AEbr/(Vbr*PEbr)
CEvuo = AEuo/(Vuo*PEuo)
CEvtot = (Ql*CEvl + Qf*CEvf + Qm*CEvm + Qvrg*CEvvrg + Quo*CEvuo + Qkid*CEvkid)/Qtot
CEvtotg = CEvtot*MWEBD*1000
CEvipu = (Qalv + Qpu*CEvtot)/(Qalv +Qpu)
MWEBD = 102.1

{differential equations for butadiene uptake and metabolism}
d/dt(Apu) = Qpu*(Cvipu - Cvpu) - Vmaxpu*Cvpu/(Kmlu + Cvpu)
d/dt(Abr) = Qbr*(Cart - Cvbr) - Vmaxbr * Cvbr/(Kmlu + Cvbr)
d/dt(Alcap) = Ql*(Cart - Alcap/Vlcap) - Ql*Pibd*(Cart - Cvl)
d/dt(Al) = Ql*Pibd*(Alcap/Vlcap - Cvl) - Vmaxl*Cvl/(Km + Cvl)
d/dt(Akidcap) = Qkid*(Cart - Akidcap/Vkidcap) - Qkid*Pibd*(Cart - Cvkid)
d/dt(Akid) = Qkid*Pibd*(Cart - Cvkid) - Vmaxk*Cvkid/(Kmk + Cvkid)
d/dt(Afcap) = Qf*(Cart - Afcap/Vfcap) - Qf*Pibd*(Cart - Cvf)
d/dt(Af) = Qf*Pibd*(Cart - Cvf)
d/dt(Amcap) = Qm*(Cart - Amcap/Vmcap) - Qm*Pibd*(Cart - Cvm)
d/dt(Am) = Qm*Pibd*(Cart - Cvm)
d/dt(Avrgcap) = Qvrg*(Cart - Avrgcap/Vvrgcap) - Qvrg*Pibd*(Cart - Cvvrg)
d/dt(Avrg) = Qvrg*Pibd*(Cart - Cvvrg)
d/dt(Auocap) = Quo*(Cart - Auocap/Vuocap) - Quo*Pibd*(Cart - Cvuo)
d/dt(Auo) = Quo*Pibd*(Cart - Cvuo)
d/dt(AUCvtot) = Cvtot
d/dt(Aexh) = Cexh*0.15

{amount of butadiene metabolized in liver and lung}
d/dt(Ametl)  = Vmaxl*Cvl/(Km + Cvl)
d/dt(Ametpu) = Vmaxpu*Cvpu/(Kmlu + Cvpu)
d/dt(Ametbr) = Vmaxbr*Cvbr/(Kmlu + Cvbr)
d/dt(Ametk) = Vmaxk*Cvkid/(Kmk + Cvkid)

{differential equations for BMO metabolism}
d/dt(ABperit) = PULSE(1.31*9.0E-5*BW,0,24) -ABperit*Kip
init AUCABperit = 0
d/dt(AUCABperit) = ABperit

d/dt(ABpufree) = ABpu*krel

d/dt(ABpu) = Qpu*(CBart - CBvpu) + Vmaxpu*Cvpu/(Kmlu + Cvpu) - Vmaxpu1*ABpufree/(Km1*Vpu +
ABpufree) - Vmaxpu1*ABpu/(R*Km1*Vpu + ABpu) - Vmaxpu2*CBvpu*CGSHlu/(Kmlu2*Kmgsh +
CBvpu*Kmgsh + CGSHlu*Kmlu2 + CBvpu*CGSHlu) - Vmaxpu3*CBvpu/(Kmlu3 + CBvpu)

d/dt(ABbrfree) = ABbr*krel

d/dt(ABbr) = Qbr*(CBart - CBvbr) + Vmaxbr * Cvbr/(Kmlu + Cvbr) - Vmaxbr1*ABbrfree/(Km1*Vbr +
ABbrfree) - Vmaxbr1*ABbr/(R*Km1*Vbr + ABbr) -Vmaxbr2*CBvbr*CGSHlu/(Kmlu2*Kmgsh +
CBvbr*Kmgsh + CGSHlu*Kmlu2 + CGSHlu*CBvbr) - Vmaxbr3*CBvbr/(Kmlu3 + CBvbr) + ABperit*Kip

d/dt(ABlcap) = Ql*(CBart - ABlcap/Vlcap) - Ql*Pibmo*(CBart - CBvl)

d/dt(ABlfree) = ABl*krel
```

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d/dt(ABl) = Ql*Pibmo*(CBart - CBvl) + Vmaxl*Cvl/(Km + Cvl) - Vmaxl1*ABlfree/(Km1*Vl + ABlfree) -
Vmaxl1*ABl/(R*Km1*Vl + ABl) - Vmaxl2*CBvl*CGSHl/(Km2*Kmgsh + CBvl*Kmgsh + CGSHl*Km2 +
CGSHl*CBvl) - Vmaxl3*Cbvl/(Km3 + CBvl)

d/dt(ABkidcap) = Qkid*(CBart - ABkidcap/Vkidcap) - Qkid*Pibmo*(CBart - CBvkid)

d/dt(ABkidfree) = ABkid*krel

d/dt(ABkid) = Qkid*Pibmo*(CBart - CBvkid) + Vmaxk*Cvkid/(Kmk + Cvkid) -
Vmaxk1*ABkidfree/(Km1*Vkid + ABkidfree) - Vmaxk1*ABkid/(R*Km1*Vkid + ABkid) -
Vmaxk2*CBvkid*CGSHkid/(Kmk2*Kmgsh + CBvkid*Kmgsh + CGSHkid*Kmk2 + CGSHkid*CBvkid) -
Vmaxk3*CBvkid/(Kmk3 + CBvkid)

d/dt(ABfcap) = Qf*(CBart - ABfcap/Vfcap) - Qf*Pibmo*(CBart - CBvf)
d/dt(ABf) = Qf*Pibmo*(CBart - CBvf)

d/dt(ABmcap) = Qm*(CBart - ABmcap/Vmcap) - Qm*Pibmo*(CBart - CBvm)
d/dt(ABm) = Qm*Pibmo*(CBart - CBvm)

d/dt(ABvrgcap) = Qvrg*(CBart - ABvrgcap/Vvrgcap) - Qvrg*Pibmo*(CBart - CBvvrg)
d/dt(ABvrg) = Qvrg*pibmo*(CBart - CBvvrg)

d/dt(ABuocap) = Quo*(CBart - ABuocap/Vuocap) - Quo*Pibmo*(CBart - CBvuo)
d/dt(ABuo) = Quo*Pibmo*(CBart - CBvuo)

{Hb and DNA adducts based on styrene oxide induced formation rates}
init HB1 = 0
d/dt(HB1) = CCart*kher ; mole /hr/g Hb
kher = 3.2E-5 ; L/hr/g Hb
Ter = 960 ; lifetime of RBC in hr
HB = (1E9)*HB1*(1 - (T/(2*Ter))) ; nmol/g Hb
init DNA1 = 0
d/dt(DNA1) = CCart*kfdna - DNA*keldna/1E9
DNA = DNA1*1E9 ; nmol/gDNA
kfdna = 9.6E-5 ; N7 DNA adducts/gDNA/hr
keldna = 0.0077 ; elimination of DNA adducts /hr

{AUCs for butadienemonoxide}
d/dt(AUCBuo) = CBvuo
d/dt(AUCBvtot) = CBvtot

{amounts of butadienemonoxide metabolized}
d/dt(ABmetl1) = Vmaxl1*ABlfree/(Km1*Vl + ABlfree) + Vmaxl1*ABl/(R*Km1*Vl + ABl)
d/dt(ABmetl2) = Vmaxl2*CBvl*CGSHl/(Km2*Kmgsh + CBvl*Kmgsh + CGSHl*Km2 + CGSHl*CBvl)

d/dt(ABmetpu1) = Vmaxpu1*ABpufree/(Km1*Vpu + ABpufree) + Vmaxpu1*ABpu/(R*Km1*Vpu + ABpu)
d/dt(ABmetpu2) = Vmaxpu2*CBvpu*CGSHlu/(Km2*Kmgsh + CBvpu*Kmgsh + CGSHlu*Km2 +
CGSHlu*CBvpu)

d/dt(ABmetbr1) = Vmaxbr1*ABbrfree/(Km1*Vbr + ABbrfree) + Vmaxbr1*ABbr/(R*Km1*Vbr + ABbr)
d/dt(ABmetbr2) = Vmaxbr2*CBvbr*CGSHlu/(Km2*Kmgsh + CBvbr*Kmgsh + CGSHlu*Km2 +
CGSHlu*CBvbr)

d/dt(ABmetl3) = Vmaxl3*CBvl/(Km3 + CBvl)
d/dt(ABmetpu3) = Vmaxpu3*CBvpu/(Km3 + CBVpu)
d/dt(ABmetbr3) = Vmaxbr3*CBvbr/(Km3 + CBvbr)
```

```
init ABmetk1 = 0
init ABmetk2 = 0
init ABmetk3 = 0
d/dt(ABmetk1) = Vmaxk1*ABkidfree/(Km1*Vkid + ABkidfree) + Vmaxk1*ABkid/(R*Km1*Vkid + ABkid)
d/dt(ABmetk2) = Vmaxk2*CBvkid*CGSHkid/(Km2*Kmgsh + CBvkid*Kmgsh + CGSHkid*Km2 +
CGSHkid*CBvkid)
d/dt(ABmetk3) = Vmaxk3*CBvkid/(Km3 + CBvkid)


{differential equations for diepoxybutane}
d/dt(ACperit) = PULSE(0*BW,0,24) -ACperit*Kip

d/dt(ACpufree) = ACpu*k4rel

d/dt(ACpu) = Qpu*(CCart - CCvpu) + Vmaxpu3*CBvpu/(Km3 + CBvpu) - Vmaxpu4*ACpufree/(Kmlu4*Vpu
+ ACpufree) - Vmaxpu4*ACpu/(R*Kmlu4*Vpu + ACpu) - Vmaxpu5*CCvpu*CGSHlu/(Km5*Kmgsh +
CCvpu*Kmgsh + CGSHlu*Km5 + CGSHlu*CCvpu)

d/dt(ACbrfree) = ACbr*k4rel

d/dt(ACbr) = Qbr*(CCart - CCvbr) + Vmaxbr3*CBvbr/(Km3 + CBvbr)  - Vmaxbr4*ACbrfree/(Kmlu4*Vbr +
ACbrfree) - Vmaxbr4*ACbr/(R*Kmlu4*Vbr + ACbr) - Vmaxbr5*CCvbr*CGSHlu/(Km5*Kmgsh +
CCvbr*Kmgsh + CGSHlu*Km5 + CGSHlu*CCvbr) + ACperit*Kip

d/dt(AClfree) = ACl*k4rel

d/dt(AClcap) = Ql*(CCart - AClcap/Vlcap) - Ql*Pideb*(CCart - CCvl)
d/dt(ACl) = Ql*Pideb*(CCart - CCvl) + Vmaxl3*CBvl/(Km3 + CBvl) - Vmaxl4*AClfree/(Km4*Vl + AClfree) -
Vmaxl4*ACl/(R*Km4*Vl + ACl) - Vmaxl5*CCvl*CGSHl/(Km5*Kmgsh + CCvl*Kmgsh + CGSHl*Km5 +
CGSHl*CCvl)

d/dt(ACkidfree) = ACkid*k4rel

d/dt(ACkidcap) = Qkid*(CCart - ACkidcap/Vlcap) - Ql*Pideb*(CCart - CCvkid)
d/dt(ACkid) = Qkid*Pideb*(CCart - CCvkid) + Vmaxk3*CBvkid/(Km3 + CBvkid) -
Vmaxk4*ACkidfree/(Kmlu4*Vkid + ACkidfree) - Vmaxk4*ACkid/(R*Kmlu4*Vkid + ACkid) -
Vmaxk5*CCvkid*CGSHkid/(Km5*Kmgsh + CCvkid*Kmgsh + CGSHkid*Km5 + CGSHkid*CCvkid)

d/dt(ACfcap) = Qf*(CCart - ACfcap/Vfcap) - Qf*Pideb*(CCart - CCvf)
d/dt(ACf) = Qf*Pideb*(CCart - CCvf)

d/dt(ACmcap) = Qm*(CCart - ACmcap/Vmcap) - Qm*Pideb*(CCart - CCvm)
d/dt(ACm) = Qm*Pideb*(CCart - CCvm)

d/dt(ACvrgcap) = Qvrg*(CCart - ACvrgcap/Vvrgcap) - Qvrg*Pideb*(CCart - CCvvrg)
d/dt(ACvrg) = Qvrg*Pideb*(CCart - CCvvrg)

d/dt(ACuocap) = Quo*(CCart - ACuocap/Vuocap) - Quo*Pideb*(CCart - CCvuo)
d/dt(ACuo) = Quo*Pideb*(CCart - CCvuo)

{metabolism of diepoxybutane to epoxybutanediol}
d/dt(ACmetpu4) = Vmaxpu4*ACpufree/(Kmlu4*Vpu + ACpufree) + Vmaxpu4*ACpu/(R*Kmlu4*Vpu +
ACpu)

d/dt(ACmetbr4) = Vmaxbr4*ACbrfree/(Kmlu4*Vbr + ACbrfree) + Vmaxbr4*ACbr/(R*Kmlu4*Vbr + ACbr)
```

```
d/dt(ACmetl4) =  Vmaxl4*AClfree/(Km4*Vl + AClfree) + Vmaxl4*ACl/(R*Km4*Vl + ACl)
init ACmetk4 = 0
d/dt(ACmetk4) = Vmaxk4*ACkidfree/(Kmlu4*Vkid + ACkidfree) + Vmaxk4*ACkid/(R*Kmlu4*Vkid + ACkid)

{AUCs for diepoxybutane}
d/dt(AUCCuo) = CCvuo
d/dt(AUCCvtot) = CCvtot

{metabolism of diepoxybutane to DEB-SG}
init ACmetpu5 = 0
init ACmetbr5 = 0
init ACmetl5 = 0
Limit ACmetl5 >= 0
init ACmetk5 = 0

d/dt(ACmetpu5) = Vmaxpu5*CCvpu*CGSHlu/(Km5*Kmgsh + CCvpu*Kmgsh + CGSHlu*Km5 +
CGSHlu*CCvpu)
d/dt(ACmetbr5) = Vmaxbr5*CCvbr*CGSHlu/(Km5*Kmgsh + CCvbr*Kmgsh + CGSHlu*Km5 +
CGSHlu*CCvbr)
d/dt(ACmetl5) = Vmaxl5*CCvl*CGSHl/(Km5*Kmgsh + CCvl*Kmgsh + CGSHl*Km5 + CGSHl*CCvl)
d/dt(ACmetk5) = Vmaxk5*CCvkid*CGSHkid/(Km5*Kmgsh + CCvkid*Kmgsh + CGSHkid*Km5 +
CGSHkid*CCvkid)


{differential equations for dihydroxybutene}
d/dt(ADpu) = Qpu*(CDart - CDvpu) + Vmaxpu1*ABpufree/(Km1*Vpu + ABpufree) +
Vmaxpu1*ABpu/(R*Km1*Vpu + ABpu)  - Vmaxpu6*CDvpu/(Km6 + CDvpu) -
Vmaxpu7*CDvpu*CGSHlu/(Km7*Kmgsh + CDvpu*Kmgsh + CGSHlu*Km7 + CGSHlu*CDvpu)

d/dt(ADbr) = Qbr*(CDart - CDvbr) + Vmaxbr1*ABbrfree/(Km1*Vbr + ABbrfree) +
Vmaxbr1*ABbr/(R*Km1*Vbr + ABbr) - Vmaxbr6*CDvbr/(Km6 + CDvbr) -
Vmaxbr7*CDvbr*CGSHlu/(Km7*Kmgsh + CDvbr*Kmgsh + CGSHlu*Km7 + CGSHlu*CDvbr)

d/dt(ADlcap) = Ql*(CDart - ADlcap/Vlcap) - Ql*Pidhb*(CDart - CDvl)
d/dt(ADl) = Ql*Pidhb*(CDart - CDvl) + Vmaxl1*ABlfree/(Km1*Vl + ABlfree) + Vmaxl1*ABl/(R*Km1*Vl +
ABl)  - Vmaxl6*CDvl/(Km6 + CDvl) - Vmaxl7*CDvl*CGSHl/(Km7*Kmgsh + CDvl*Kmgsh + CGSHl*Km7 +
CGSHl*CDvl)

d/dt(ADkidcap) = Qkid*(CDart - ADkidcap/Vkidcap) - Qkid*Pidhb*(CDart - CDvkid)
d/dt(ADkid) = Qkid*Pidhb*(CDart - CDvkid) +  Vmaxk1*ABkidfree/(Km1*Vkid + ABkidfree) +
Vmaxk1*ABkid/(R*Km1*Vkid + ABkid)  - Vmaxl6*CDvkid/(Km6 + CDvkid) -
Vmaxl7*CDvkid*CGSHkid/(Km7*Kmgsh + CDvkid*Kmgsh + CGSHkid*Km7 + CGSHkid*CDvkid)

d/dt(ADfcap) = Qf*(CDart - ADfcap/Vfcap) - Qf*Pidhb*(CDart - CDvf)
d/dt(ADf) = Qf*Pidhb*(CDart-CDvf)

d/dt(ADmcap) = Qm*(CDart - ADmcap/Vmcap) - Qm*Pidhb*(CDart - CDvm)
d/dt(ADm) = Qm*Pidhb*(CDart - CDvm)

d/dt(ADvrgcap) = Qvrg*(CDart - ADvrgcap/Vvrgcap) - Qvrg*Pidhb*(CDart - CDvvrg)
d/dt(ADvrg) = Qvrg*Pidhb*(CDart - CDvvrg)

d/dt(ADuocap) = Quo*(CDart - ADuocap/Vuocap) - Quo*Pidhb*(CDart - CDvuo)
d/dt(ADuo) = Quo*Pidhb*(CDart - CDvuo)
```

```
d/dt(AUCDvtot) = CDvtot
init (AUCDvuo) = 0
d/dt(AUCDvuo) = CDvuo

{metabolism of DHB to EBD }
d/dt(ADmetpu6) = Vmaxpu6*CDvpu/(Km6 + CDvpu)
d/dt(ADmetbr6) = Vmaxbr6*CDvbr/(Km6 + CDvbr)
d/dt(ADmetl6) = Vmaxl6*CDvl/(Km6 + CDvl)
init ADmetk6 = 0
d/dt(ADmetk6) = Vmaxk6*CDvkid/(Km6 + CDvkid)

{metabolism of DHB to DHB-SG conjugate}
init ADmetpu7 = 0
init ADmetbr7 = 0
init ADmetl7 = 0
init ADmetk7 = 0

d/dt(ADmetpu7) = Vmaxpu7*CDvpu*CGSHlu/(Km7*Kmgsh + CDvpu*Kmgsh + CGSHlu*Km7 +
CGSHlu*CDvpu)
d/dt(ADmetbr7) = Vmaxbr7*CDvbr*CGSHlu/(Km7*Kmgsh + CDvbr*Kmgsh + CGSHlu*Km7 +
CGSHlu*CDvbr)
d/dt(ADmetl7) = Vmaxl7*CDvl*CGSHl/(Km7*Kmgsh + CDvl*Kmgsh + CGSHl*Km7 + CGSHl*CDvl)
d/dt(ADmetk7) = Vmaxl7*CDvkid*CGSHkid/(Km7*Kmgsh + CDvkid*Kmgsh + CGSHkid*Km7 +
CGSHkid*CDvkid)


{differential equations for epoxybutanediol}
d/dt(AEpufree) = AEpu*k8rel

d/dt(AEpu) = Qpu*(CEart - CEvpu) +  Vmaxpu4*ACpufree/(Kmlu4*Vpu + ACpufree) +
Vmaxpu4*ACpu/(R*Kmlu4*Vpu + ACpu) + Vmaxpu6*CDvpu/(Km6 + CDvpu) -
Vmaxpu8*AEpufree/(Km8*Vpu + AEpufree) - Vmaxpu8*AEpu/(R*Km8*Vpu + AEpu) -
Vmaxpu9*CEvpu*CGSHlu/(Km9*Kmgsh + CEvpu*Kmgsh + CGSHlu*Km9 + CGSHlu*CEvpu)

d/dt(AEbrfree) = AEbr*k8rel

d/dt(AEbr) = Qbr*(CEart - CEvbr) + Vmaxbr4*ACbrfree/(Kmlu4*Vbr + ACbrfree) +
Vmaxbr4*ACbr/(R*Kmlu4*Vbr + ACbr) +  Vmaxbr6*CDvbr/(Km6 + CDvbr)  - Vmaxbr8*AEbrfree/(Km8*Vbr
+ AEbrfree) - Vmaxbr8*AEbr/(R*Km8*Vbr + AEbr) - Vmaxbr9*CEvbr*CGSHlu/(Km9*Kmgsh +
CEvbr*Kmgsh + CGSHlu*Km9 + CGSHlu*CEvbr)

d/dt(AElfree) = AEl*k8rel

d/dt(AElcap) = Ql*(CEart - AElcap/Vlcap) - Ql*Piebd*(CEart - CEvl)
d/dt(AEl) = Ql*Piebd*(CEart - CEvl) + Vmaxl4*AClfree/(Km4*Vl + AClfree) + Vmaxl4*ACl/(R*Km4*Vl +
ACl) + Vmaxl6*CDvl/(Km6 + CDvl) - Vmaxl8*AElfree/(Km8*Vl + AElfree) - Vmaxl8*AEl/(R*Km8*Vl + AEl) -
Vmaxl9*CEvl*CGSHl/(Km9*Kmgsh + CEvl*Kmgsh + CGSHl*Km9 + CGSHl*CEvl)

d/dt(AEkidfree) = AEkid*k8rel

d/dt(AEkidcap) = Qkid*(CEart - AEkidcap/Vkidcap) - Qkid*Piebd*(CEart - CEvkid)
d/dt(AEkid) = Qkid*Pidhb*(CEart - CEvkid) +  Vmaxk4*ACkidfree/(Kmlu4*Vkid + ACkidfree) +
Vmaxk4*ACkid/(R*Kmlu4*Vkid + ACkid)  + Vmaxl6*CDvkid/(Km6 + CDvkid) -
Vmaxk8*AEkidfree/(Km8*Vkid + AEkidfree) - Vmaxk8*AEkid/(R*Km8*Vkid + AEkid) -
Vmaxl9*CEvkid*CGSHkid/(Km9*Kmgsh + CEvkid*Kmgsh + CGSHkid*Km9 + CGSHkid*CEvkid)
```

```
d/dt(AEfcap) = Qf*(CEart - AEfcap/Vfcap) - Qf*Piebd*(CEart - CEvf)
d/dt(AEf) = Qf*Piebd*(CEart-CEvf)

d/dt(AEmcap) = Qm*(CEart - AEmcap/Vmcap) - Qm*Piebd*(CEart - CEvm)
d/dt(AEm) = Qm*Piebd*(CEart - CEvm)

d/dt(AEvrgcap) = Qvrg*(CEart - AEvrgcap/Vvrgcap) - Qvrg*Piebd*(CEart - CEvvrg)
d/dt(AEvrg) = Qvrg*Piebd*(CEart - CEvvrg)

d/dt(AEuocap) = Quo*(CEart - AEuocap/Vuocap) - Quo*Piebd*(CEart - CEvuo)
d/dt(AEuo) = Quo*Piebd*(CEart - CEvuo)

d/dt(AUCEvtot) = CEvtot
init (AUCEvuo) = 0
d/dt(AUCEvuo) = CEvuo

{metabolism of  epoxybutanediol to erythitol}
init AEmetpu8 = 0
init AEmetbr8 = 0
init AEmetl8 = 0
init AEmetk8 = 0
d/dt(AEmetpu8) =  Vmaxpu8*AEpufree/(Km8*Vpu + AEpufree) + Vmaxpu8*AEpu/(R*Km8*Vpu + AEpu)
d/dt(AEmetbr8) = Vmaxbr8*AEbrfree/(Km8*Vbr + AEbrfree) + Vmaxbr8*AEbr/(R*Km8*Vbr + AEbr)
d/dt(AEmetl8) = Vmaxl8*AElfree/(Km8*Vl + AElfree) + Vmaxl8*AEl/(R*Km8*Vl + AEl)
init AEmetk8 = 0
d/dt(AEmetk8) = Vmaxk8*AEkidfree/(Km8*Vkid + AEkidfree) + Vmaxk8*AEkid/(R*Km8*Vkid + AEkid)

{metabolism of EBD to EBD-SG}
init AEmetpu9 = 0
init AEmetbr9 = 0
init AEmetl9 = 0
init AEmetk9 = 0

d/dt(AEmetpu9) = Vmaxpu9*CEvpu*CGSHlu/(Km9*Kmgsh + CEvpu*Kmgsh + CGSHlu*Km9 +
CGSHlu*CEvpu)
d/dt(AEmetbr9) = Vmaxbr9*CEvbr*CGSHlu/(Km9*Kmgsh + CEvbr*Kmgsh + CGSHlu*Km9 +
CGSHlu*CEvbr)
d/dt(AEmetl9) = Vmaxl9*CEvl*CGSHl/(Km9*Kmgsh + CEvl*Kmgsh + CGSHl*Km9 + CGSHl*CEvl)
d/dt(AEmetk9) = Vmaxl9*CEvkid*CGSHkid/(Km9*Kmgsh + CEvkid*Kmgsh + CGSHkid*Km9 +
CGSHkid*CEvkid)

{differential equations for cysteine metabolism}
d/dt(AFl) = Kl0 - Vmax10l*AFl/((Kmcys*Vl + AFl)*(1 + AGl/(Kl*Vl)))
d/dt(AFlu) = Klu0 - Vmax10lu*AFlu/((Kmcys*Vlu + AFlu)*(1 + AGlu/(Kl*Vlu)))
d/dt(AFkid) = Kkid0 - Vmax10kid*AFkid/((Kmcys*Vkid + AFkid)*(1 + AGkid/(Kl*Vkid)))

{differential equations for glutathione metabolism}
d/dt(AGl) = Vmax10l*AFl/((Kmcys*Vl + AFl)*(1 + AGl/(Kl*Vl))) - ABmetl2 - ACmetl5 - ADmetl7- AEmetl9
d/dt(AGlu) = Vmax10lu*AFlu/((Kmcys*Vlu + AFlu)*(1 + AGlu/(Kl*Vlu))) - ABmetpu2 - ABmetbr2 -
ACmetpu5 - ACmetbr5 - ADmetpu7 - ADmetbr7 - AEmetpu9 - AEmetbr9
d/dt(AGkid) = Vmax10kid*AFkid/((Kmcys*Vkid + AFkid)*(1 + AGkid/(Kl*Vkid))) - ABmetk2 - ACmetk5 -
ADmetk7 - AEmetk9

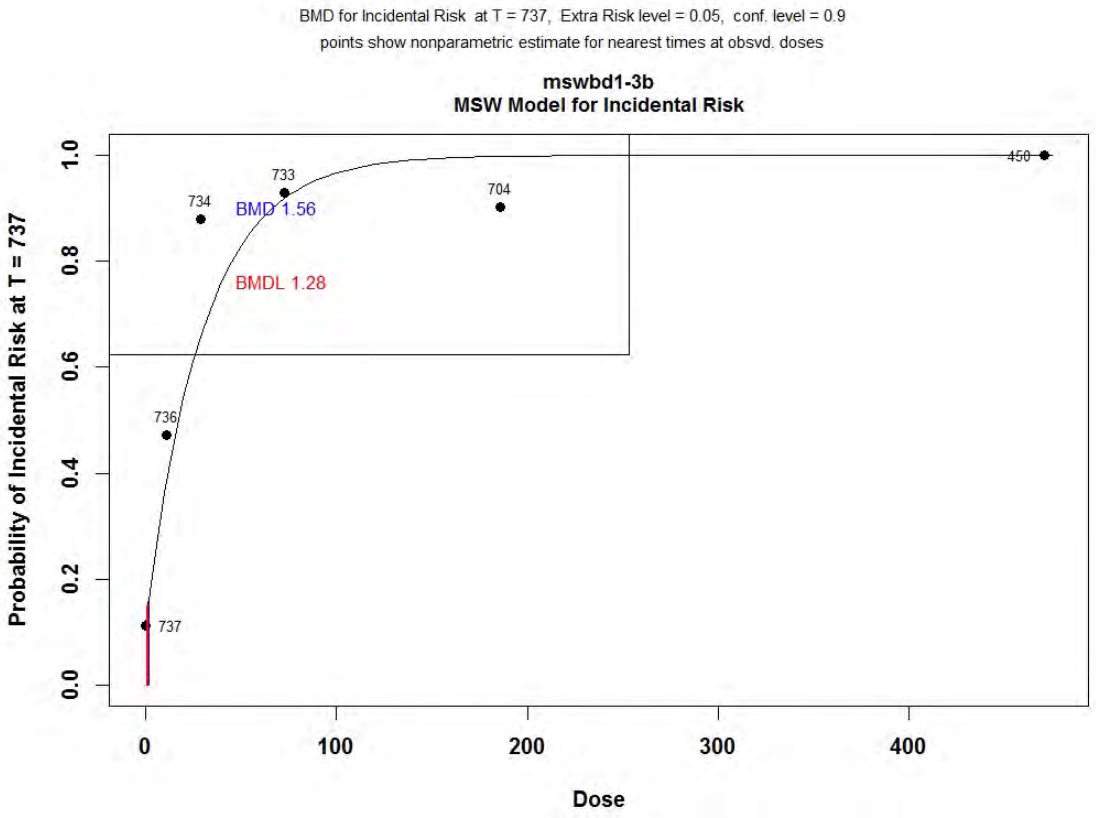
{differential equation for  Hb adduct formation, pmol/g Hb-ppm-hr}
d/dt(Adduct) = CEvtot*Kadduct*1E12/(476.7*8000)
; Mass balance
```

```
MassA = Af + Al + Am + Avrg + Abr + Apu + Auo + Aexh + Akid
MassB = ABf + ABl + ABm + ABvrg + ABbr + ABpu + ABuo + ABkid
MassBfree = ABlfree + ABpufree + ABbrfree + ABkidfree
MassC = ACf + ACl + ACm + ACvrg + ACbr + ACpu + ACuo + ACkid
MassCfree = AClfree + ACpufree + ACbrfree + ACkidfree
MassD = ADf + ADl + ADm + ADvrg + ADbr + ADpu + ADuo + ADkid
MassE = AEf + AEl + AEm + AEvrg + AEbr + AEpu + AEuo + AEkid
MassEfree = AElfree + AEpufree + AEbrfree + AEkidfree
Massfree = MassBfree + MassCfree + MassEfree
MassG2 = ABmetl2 + ABmetpu2 + ABmetbr2 + ABmetk2
MassG5 = ACmetl5 + ACmetpu5 + ACmetbr5 + ACmetk5
MassG7 = ADmetl7 + ADmetpu7 + ADmetbr7 + ADmetk7
MassG9 = AEmetl9 + AEmetpu9 + AEmetbr9 + AEmetk9
MassG = MassG2 + MassG5 + MassG7 + MassG9
MassE8 = AEmetl8 + AEmetpu8 + AEmetbr8 + AEmetk8
TotMass = MassA + MassB + MassC + MassD + MassE + MassG + MassE8 + Massfree
init Inh = 0
d/dt(Inh) = Cair*Qalv
```

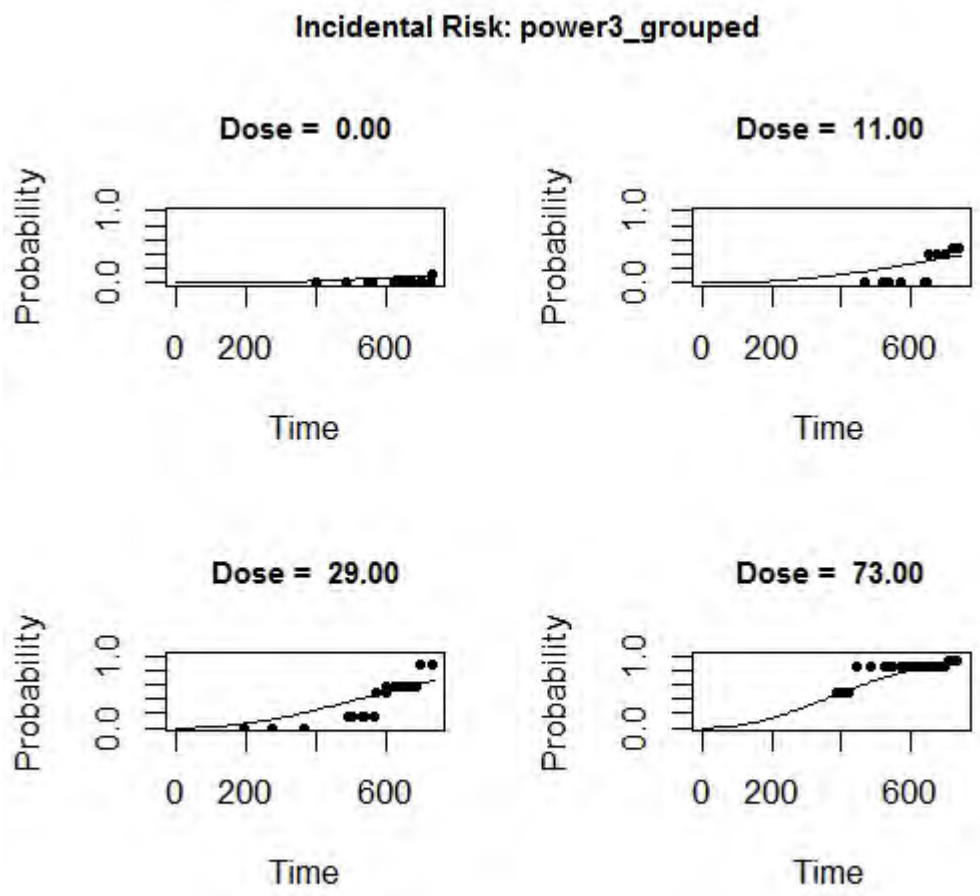
APPENDIX B

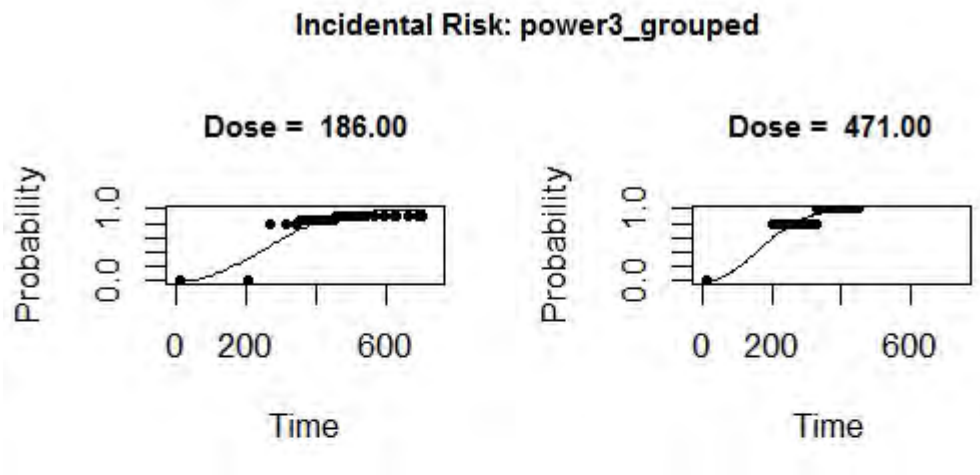
This appendix contains the goodness of fit (gof) plots for the Multistage Weibull model (MSW) fits to the 24 month mouse ovarian atrophy data in NTP (1993). The plots are generated in the statistical program R using slightly modified input files that were used in the main MSW analysis.

Appendix B1. GOFPLOT: DR Plot for Incidental Risk of Ovarian Atrophy in Mice Versus Butadiene Exposure ppm for 24 months. Solid line is Multistage Weibull Model Prediction, Point are Nonparametric Model Estimates. BMD and BMDL are for 95% Lower Bounds on 5% Response Level. Points Closer to Line Indicate Better Model Fit.

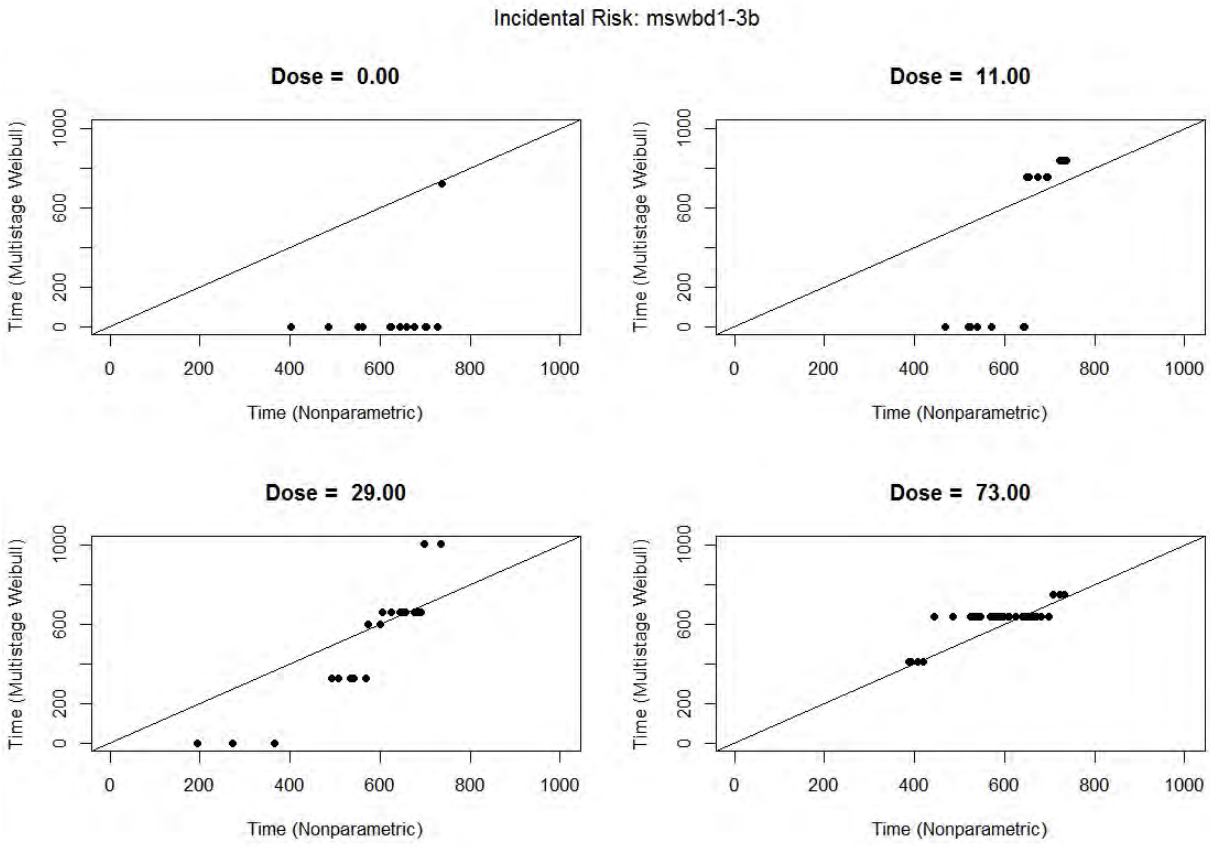

BMD for Incidental Risk at T = 737, Extra Risk level = 0.05, conf. level = 0.9
points show nonparametric estimate for nearest times at obsvd. doses
mswbd1-3b
MSW Model for Incidental Risk
Probability of Incidental Risk at T = 737
Dose
BMD 1.56
BMDL 1.28
734
733
704
450
736
737
0.0
0.2
0.4
0.6
0.8
1.0
0
100
200
300
400

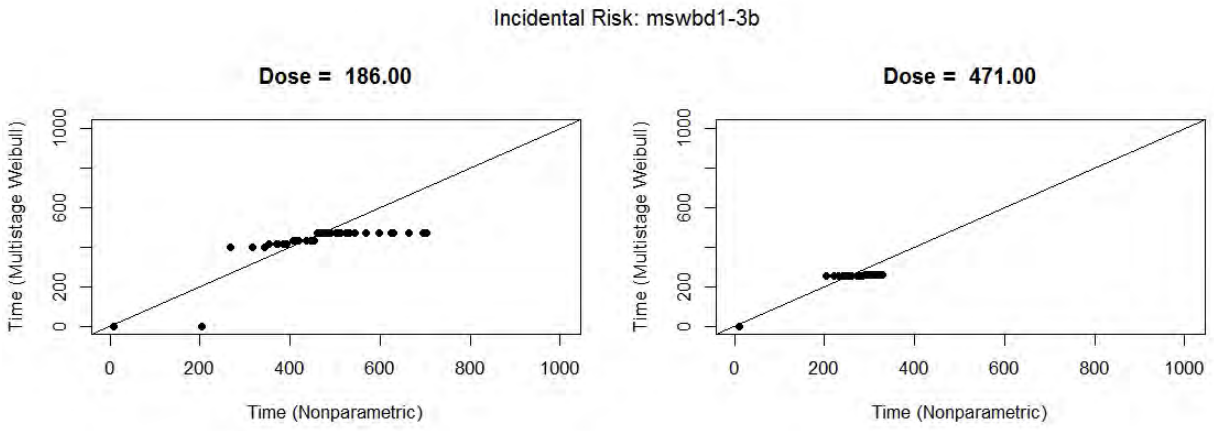
Appendix B2. GOFPLOT: PR Plot. Probability of Ovarian Atrophy in Butadiene Exposed Mice by Multistage Weibull Model (Solid Line) versus Time for Butadiene Internal Doses nM Diepoxybutane in Blood. Points Represent Nonparametric Model Estimates. More Points Closer to the Line Indicates Better Model Fit.



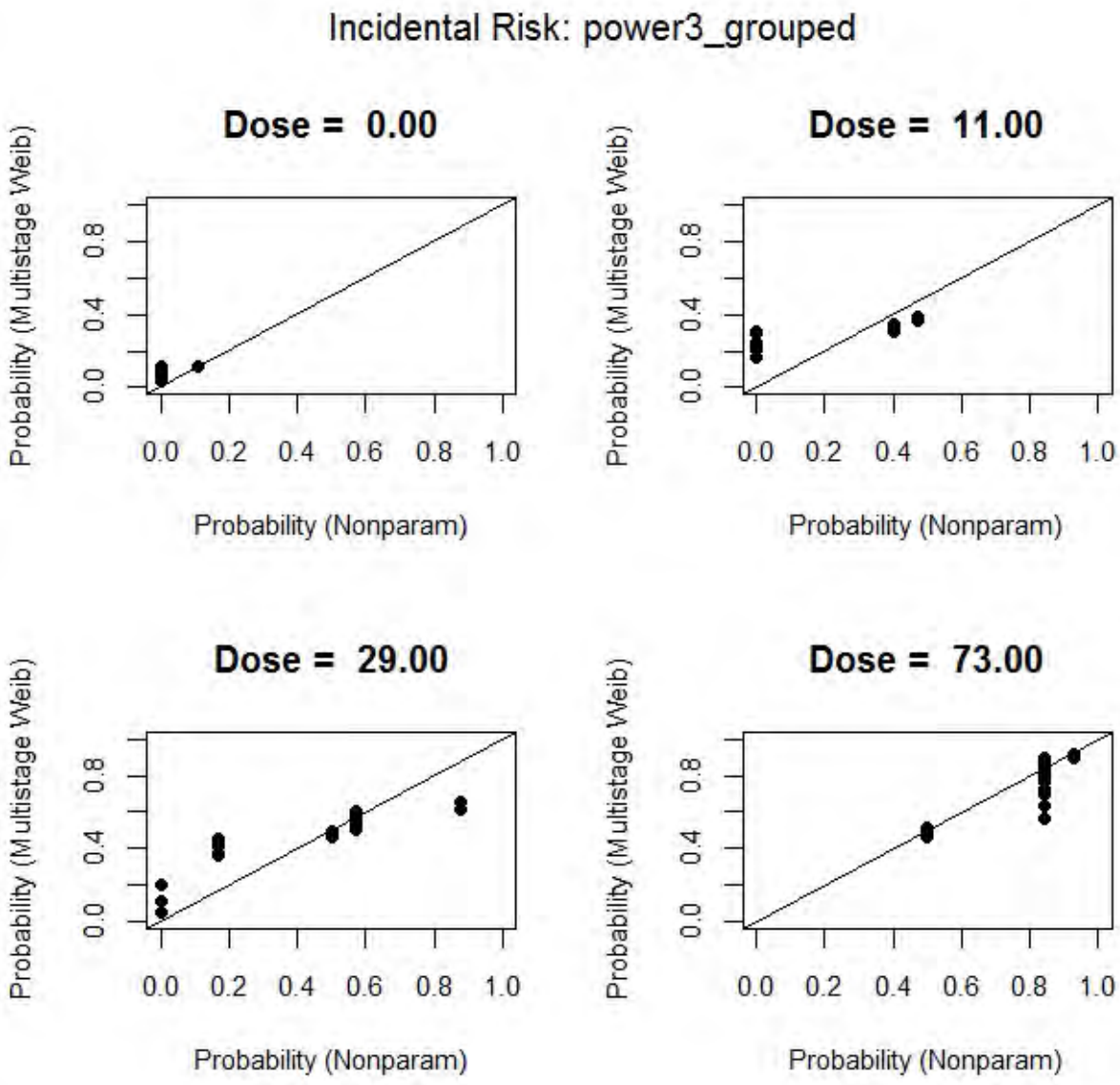

Incidental Risk: power3_grouped
Dose = 186.00
Dose = 471.00
Probability
0.0
1.0
0
200
600
Time

Appendix B3. GOFPLOT: QQ Plot. Incidental Risk of Ovarian Atrophy in Butadiene Exposed Mice. Time-Multistage Weibull Model versus Time-Nonparametric Model Estimates for Internal Blood Diepoxybutane nM. Solid line is for Equivalence of Model Predictions. More Points Closer to Line Indicates Better Model Fit.




Incidental Risk: mswbd1-3b
Dose = 186.00
Dose = 471.00
Time (Multistage Weibull)
Time (Nonparametric)
0
200
400
600
800
1000

Appendix B4. GOFPLOT: PP Plot. . Incidental Risk of Ovarian Atrophy in Butadiene Exposed Mice. Probability-Multistage Weibull Model versus Probability-Nonparametric Model Estimates for Internal Blood Diepoxybutane nM. Solid line is for Equivalence of Model Predictions. More Points Closer to Line Indicates Better Model Fit.



Responses to Comments on the Draft Technical Support Document for Proposed Reference Exposure Levels (RELs) for 1, 3-Butadiene:

1. Comments from the American Chemistry Council (7/16/12)

Comment 1. Occurrence and Exposure p.2.

The OEHHA document states "Most environmental releases of butadiene are associated with fugitive or accidental emission during manufacture, use, transport, storage, or disposal." This sentence is misleading because the primary source of butadiene environment releases is via emissions from gasoline and diesel-powered vehicles and equipment, not via industrial manufacture. According to the U.S. Environmental Protection Agency, 1.6% of environmental emissions of butadiene are from industrial production and use, 78.8% from mobile sources, and 19.9% from other miscellaneous combustion sources (EPA, 2002[4]). The document should be revised to accurately state the major source contributors for butadiene exposure.

Response to Comment 1.

The text refers to "point sources" which are the primary focus of the hot spots program. The sentence will be revised to clearly distinguish contributions of point and non-point or mobile sources of butadiene emissions.

Comment 2. p.10 and 11, Asthma.

This section notes the study conducted by Delfino et al., 2003, in which the authors interpreted the results as suggestive of an increased risk of asthma symptoms associated with butadiene exposure. As the document clearly states, this study had several limitations including study size and the results were strongly influenced by one subject. The document would be improved to also include further detail provided by Delfino et al. on the low reporting of positive symptoms by the study participants. The study size, low reporting and influence of one subject could have skewed the results. Additionally, the sentence on page 11 that states "Misclassification of VOC exposures may have occurred for some chemicals such as formaldehyde with important indoor sources but data from other studies support the view that motor vehicle emissions strongly influence the exposures to other VOCs such as benzene, ethylbenzene, toluene, xylenes and ***probably butadiene*** *(bolded for emphasis)." There is nothing in the text that warrants the inclusion of butadiene in this sentence and thus the reference to butadiene should be removed.*

Response to Comment 2.

OEHHA believes the sentence in question is a reasonable extension to related volatile compounds included in the study. In their discussion (p.652), the authors clearly state,

"Although CIs were wider, ORs were positive for symptom scores >1 in relation to lag 1 concentrations of the same VOCs **as well as 1,3-butadiene"** (bolded for emphasis).

Comment 3. DART Rat Study (Section 6).

"This section discusses several studies that were conducted in mice that have shown some toxicity but it fails to include a discussion of a rat study which was previously provided to OEHHA by the Panel. On October 1, 2003…"

Response to Comment 3.

We have added a description of the Hackett et al. (1987b) rat developmental toxicity inhalation study. This study is largely negative and appears to be the companion to the mouse developmental study we have used in the derivation of the acute REL.

Comment 4. Hackett et al. (1987) Study and Green (2003) Re-analysis.

"As well, this section discusses at length the findings from the Hackett et al., 1987[6] study and notes toxicity effects on male fetal body weight as a critical effect which was ultimately used as the basis for the REL. However, previously the Panel provided extensive comments to OEHHA in 2003 that dispute the apparent finding of reduced fetal body weights in male mice from the Hackett et al. study. The Panel noted in those comments that this finding was based on an inappropriate statistical analysis and provided additional documentation in support of this point. The Panel's comments also consisted of the Green statistical re-analysis[7] of the mouse data reported by Hackett et al. which confirmed that a reduction in male fetal body weight is not seen at 40 ppm when proper statistical methods are applied. The Panel has included an additional copy of this statistical re-analysis in the attachment."

Response to Comment 4.

We have reviewed the Green 2003 re-analysis of the Hackett et al. (1987) data and agree that the low dose in the study now appears to be a NOAEL rather than a LOAEL. However, as reported in our draft document, our proposed acute REL is not based on a NOAEL/LOAEL approach but rather on a benchmark dose method which uses the entire dose response to derive an alternative to the NOAEL, namely the $BMCL_{05}$, or the 95% lower bound on the 5% response level. In our draft analysis this value was 13.4 ppm. We have now repeated this analysis using the Green revised values (Table 1, p.15 in the Green report) and find a $BMCL_{05}$ of 17 ppm based on this latter analysis (Hill model, exact fit). This new analysis and a revised acute REL are included in the revised draft.

Comment 5. Derivation of the REL Values.

As well, OEHHA selected ovarian atrophy in mice as the key non-cancer health effect for butadiene to derive the 8-hr and chronic REL. While the Owen et al., 1987

publication indicated only that gonads were examined, the original study report shows ovarian atrophy was observed in 2 of 46 control rats and 1 of 24 rats in the 8000 ppm exposure group (Table 24, Page B55 of the report). Thus, it appears that ovarian atrophy is an effect specific to the mouse and likely a consequence of the mouse's high rate of butadiene metabolism compared to other species. Given available knowledge of interspecies differences in metabolism, the selected endpoint is of questionable human relevance.

Response to Comment 5.

The ovarian atrophy in female mice in the NTP study was the most sensitive non-neoplastic effect noted among several organ weight effects (lung, liver, and kidney) and uterine, testicular and nasal olfactory epithelial atrophies. It is difficult to extrapolate toxic effects between rodent species, let alone between rodents and humans. OEHHA does not accept the notion that studies in mice are not relevant to human risk assessment or that rats are necessarily "more human" than mice. Duescher and Elfarra (1994) reported that: "Butadiene monoxide formation rates in human liver microsomes were similar, or higher, than the rate obtained in mouse liver microsomes, whereas 1,3-butadiene oxidation rates in human and mouse liver microsomes were higher than the rate obtained in rat liver microsomes. These results provide direct evidence that 1,3-butadiene is a substrate for multiple P450 enzymes and suggest that humans may be at higher risk of expressing 1,3-butadiene toxicity compared to mice or rats. In addition, these results suggest that the mouse may be the more appropriate animal model to assess human risk."

Comment 6. Intraspecies Uncertainty Factor.

Finally, the REL for chronic exposure to butadiene includes an intraspecies uncertainty factor of 30, which included an uncertainty factor of 10 for toxicokinetics however OEHHA provides minimal justification for the selection of this value. The document should be updated to include greater justification for the selection of this uncertainty factor based on the available database.

Response to Comment 6.

The use of a UF of 10 for intraspecies uncertainty in toxicokinetics is based on OEHHA's non-cancer risk assessment guidance (OEHHA, 2008), which was developed in response to the California Children's Environmental Health Act of 1999. Unless we have adequate information on all segments of the exposed population we must acknowledge that uncertainty and apply a larger UF_{Hk}. As noted in the draft, the human metabolism of butadiene is based on studies in relatively few (deceased) adults (e.g., Duescher and Elfarra, 1994) and in our view is insufficient to encompass the possible range of metabolism and toxicokinetics, particularly in young children.

Comment 7. Interspecies Uncertainty Factor.

OEHHA is also encouraged to refer to the recent analysis described in the TCEQ DSD for Butadiene regarding the chronic REL and the reliance on the ovarian atrophy effect

in mice and only mice. The TCEQ assessment and the assessment conducted by the US EPA (Health Assessment of 1, 3 Butadiene, EPA/600/P-98/001F October 2002) both provide several references in the literature regarding the essential role of the diepoxide metabolite of butadiene as the metabolite critical to the occurrence of ovarian atrophy in mice (including references pertaining to observations of ovarian atrophy in rats when exposed directly to the diepoxide metabolite). Critically, significant evidence is provided that this diepoxide metabolite is produced in the mouse in far greater quantities than any other species, including and especially humans, with limited conclusive evidence that humans can produce this metabolite at all. This information should inform OEHHA regarding the magnitude of specific uncertainty factor related to interspecies differences pertaining to ovarian atrophy and argues strongly that this value should be ***less*** *than 1.*

Response to Comment 7.

We have cited a published summary of the TCEQ butadiene assessment in the draft (Grant et al., 2010) and have reduced our usual interspecies uncertainty subfactor for toxicokinetics from √10 to 1 based on the published evidence of greater metabolism of butadiene to epoxide metabolites in the mouse compared to results with other species. As noted in the responses above, human data on this point are relatively limited and at this time OEHHA does not favor the use of fractional UFs. As also noted above the ovarian atrophy endpoint was the most sensitive observed in the experimental animals. Our assessment does not assume that this is the exact effect that will occur in exposed humans. Butadiene exposure caused many other toxic effects that may be more relevant to humans. This is part of the uncertainty.