M E M O R A N D U M

TO: Winston H. Hickox
Agency Secretary

FROM: Joan E. Denton, Ph.D.
Director

DATE: April 25, 2000

SUBJECT: ADOPTION OF CHRONIC REFERENCE EXPOSURE LEVELS FOR AIRBORNE TOXICANTS

In accordance to Health and Safety Code, Section 44300 *et seq*. (The Air Toxics Hot Spots Information and Assessment Act, AB 2588, Connelly as amended by SB 1731, Calderon), the Office of Environmental Health Hazard Assessment (OEHHA) hereby adopts Chronic Reference Exposure Levels (RELs) for 16 chemicals (attachment).

OEHHA is mandated to develop risk assessment guidelines to be used by state and local agencies in implementing the Air Toxics Hot Spots program. Development of these guidelines is proceeding in stages. There are four technical support documents, three of which have been adopted, and the fourth is currently being reviewed by the Scientific Review Panel (SRP) on Toxic Air Contaminants. These describe the scientific basis for (respectively) acute RELs, cancer potency factors, chronic RELs, and exposure assessments. The fifth document is a guidance manual based on the four technical support documents.

The third technical support document, *Air Toxics Hot Spots Program Risk Assessment Guidelines. Part III. The Determination of Chronic Reference Exposure Levels for Airborne Toxicants,* was adopted on February 23, 2000. A chronic REL is an airborne level that would pose no significant health risk to individuals indefinitely exposed to that level. RELs are based solely on health considerations, and are developed from the best available data in the scientific literature. This technical support document provided chronic RELs for 22 chemicals, with a summary for each describing its chemical and physical properties, its chronic health effects, and the data used to calculate the REL.

The SRP had initially reviewed a number of other proposed chronic RELs at previous meetings, beginning in September 1999. At its April 13, 2000 meeting, the SRP endorsed 16 additional RELs, bringing the total number of chemicals for which chronic RELs are provided

to 38. The expanded list and supporting summaries will be available on our Web site. Additional RELs are currently undergoing review by the public and the SRP, and revision by OEHHA; these will be presented in due course.

Should you have any questions, please contact me at (916) 322-6325.

Attachment

Attachment

Chronic Reference Exposure Levels Adopted by OEHHA – April 2000

	Substance (CAS #)	*Chronic Inhalation REL ($\mu g/m^3$)*	*Hazard Index Target(s)*
1	Chloroform (67-66-3)	300	Alimentary system; kidney; development
2	Dioxane (1,4-) (123-91-1)	3,000	Alimentary system; kidney; cardiovascular system
3	Ethyl chloride (75-00-3)	30,000	Development; alimentary system
4	Ethylene glycol (107-21-1)	400	Respiratory system; kidney; development
5	Hexane (n-) (110-54-3)	7000	Nervous system
6	Hydrogen cyanide (74-90-8)	9	Nervous system; endocrine system; cardiovascular system
7	Hydrogen sulfide (7783-06-4)	10	Respiratory system
8	Manganese & manganese compounds	0.2	Nervous system
9	Methanol (67-56-1)	4,000	Development
10	Naphthalene (91-20-3)	9	Respiratory system
11	Phenol (108-95-2)	200	Alimentary system; cardiovascular system; kidney; nervous system
12	Propylene (115-07-1)	3,000	Respiratory system
13	Styrene (100-42-5)	900	Nervous system
14	Toluene (108-88-3)	300	Nervous system; respiratory system; development
15	Trichloroethylene (79-01-6)	600	Nervous system; eyes
16	Xylenes (m-, o-, p-)	700	Nervous system; respiratory system

OEHHA
4/25/2000

CHRONIC TOXICITY SUMMARY

CHLOROFORM

(trichloromethane; formyl trichloride; methenyl trichloride; methyl trichloride)

CAS Registry Number: 67-66-3

I. Chronic Toxicity Summary

Inhalation reference exposure level	**300 µg/m^3** (50 ppb)
Critical effect(s)	Liver toxicity (degenerative, foamy vacuolization, and necrosis) in rats; increased liver weights in male rats Kidney toxicity (cloudy swelling and nephritis) in rats Developmental toxicity
Hazard index target(s)	Alimentary system; kidney; teratogenicity

II. Chemical Property Summary (HSDB, 1995; 1999; CRC, 1994)

Description	Colorless liquid
Molecular formula	$CHCl_3$
Molecular weight	119.49 g/mol
Boiling point	61.1^{o}C
Melting point	–63.6 ^{o}C
Vapor pressure	197-200 torr @ 25 ^{o}C
Solubility	Soluble in water (8220 mg/L); miscible in carbon tetrachloride, carbon disulfide, alcohols, benzene, ethers and oils
Conversion factor	4.9 µg/m^3 per ppb at 25ºC

III. Major Uses and Sources

Chloroform ($CHCl_3$) is used in industry and laboratory settings as a solvent for adhesives, pesticides, fats, oils and rubbers. It is also used as a chemical intermediate in the synthesis of fluorocarbon 22, dyes, pesticides, and tribromomethane. Chloroform is produced as a byproduct of water, sewage, and wood pulp chlorination (HSDB, 1995). In 1996, the latest year tabulated, the statewide mean outdoor monitored concentration of chloroform was approximately 0.037 ppb (CARB 1999a). The annual statewide industrial emissions from facilities reporting under the Air Toxics Hot Spots Act in California based on the most recent inventory were estimated to be 79,949 pounds of chloroform (CARB 1999b).

IV. Effects of Human Exposure

Limited information is available regarding possible adverse health effects in humans following chronic inhalation of chloroform. However, historical clinical reports from patients who underwent chloroform anesthesia indicate that acute inhalation exposure affects the central nervous system, cardiovascular system, stomach, liver, and kidneys (Schroeder, 1965; Smith *et al.*, 1973; Whitaker and Jones, 1965). Acute chloroform toxicity included impaired liver function (Smith *et al.*, 1973), toxic hepatitis (Lunt, 1953; Schroeder, 1965), cardiac arrhythmia (Payne, 1981; Schroeder, 1965; Whitaker and Jones, 1965), and nausea (Schroeder, 1965; Smith *et al.*, 1973; Whitaker and Jones, 1965), and caused central nervous system symptoms (Schroeder, 1965; Whitaker and Jones, 1965). Chronic inhalation studies are limited to a few occupational studies identifying the liver and the central nervous system as target organs (Challen *et al.*, 1958; Li *et al.*, 1993; Phoon *et al.*, 1983; Bomski *et al.*, 1967).

Challen *et al.* (1958) investigated workers manufacturing throat lozenges with exposure to chloroform vapors estimated in the range 77 to 237 ppm with episodes of >1100 ppm. Workers reported symptoms of fatigue, dull-wittedness, depression, gastrointestinal distress, and frequent and burning micturition. No evidence of liver dysfunction was found based on thymol turbidity, serum bilirubin, and urine urobilinogen levels.

Bomski *et al.* (1967) reported 17 cases of hepatomegaly in a group of 68 chloroform-exposed workers. Chloroform concentrations ranged from 2 to 205 ppm (duration 1 to 4 years). Three of the 17 workers with hepatomegaly had toxic hepatitis based on elevated serum enzymes. Additionally, 10 workers had splenomegaly. Workers exposed to chloroform had a 10-fold increased risk of contracting viral hepatitis compared to the general population. The study authors considered the chloroform induced liver toxicity as a predisposing factor for viral hepatitis, but the incidence of viral hepatitis in the workers is in itself a confounding factor.

Phoon *et al.* (1983) described two outbreaks of toxic jaundice in workers manufacturing electronics equipment in Singapore. One plant had 13 cases of jaundice, initially diagnosed as viral hepatitis, in a work area with >400 ppm chloroform. Blood samples from workers (five with jaundice, four without symptoms) contained between 0.10 and 0.29 mg chloroform/100 mL. A second factory reported 18 cases of hepatitis, all from a work area utilizing chloroform as an adhesive. Two samplings indicated air levels of 14.4 to 50.4 ppm chloroform. Due to a lack of fever and hepatitis B surface antigen in the patients, the authors attributed the jaundice to chloroform exposure rather than viral hepatitis.

More recently, Li *et al.* (1993) reported on 61 chloroform-exposed workers from a variety of production factories. Exposure levels at 3 representative worksites varied widely, from 4.27 to 147.91 mg/m^3 (0.9 to 30 ppm) (119 samples), with 45% of the samples below 20 mg/m^3. The exposed workers were subclassified for some studies according to exposure levels into group 1 (mean level = 13.49 mg/m^3 or 2.8 ppm) and group 2 (mean level = 29.51 mg/m^3 or 6 ppm). Workers exposed to chloroform had slight liver damage indicated by higher (abnormal) levels of serum prealbumin (in group 2) and transferrin (in both groups) than those of control workers.

Neurobehavioral functions were also affected, manifested as increases in scores of passive mood states and dose-related, negative changes in neurobehavioral testing.

These cross sectional studies are limited in their ability to establish chronic NOAEL/LOAEL values due to limited exposures, concurrent exposure to other chemicals, inadequate control groups and potential confounders. However, these studies indicate the potential for liver and central nervous system toxicity in humans exposed to chloroform via inhalation.

V. Effects of Animal Exposure

Exposure of experimental animals to chloroform for acute, subchronic or chronic durations results in toxicity to the liver and kidney, as well as to the respiratory and central nervous systems (USDHHS, 1993). The majority of chronic animal studies have used oral routes of chloroform administration (USDHHS, 1993), while only limited data are available on inhalation specific exposures. Both routes of exposure, however, appear to primarily affect the liver and kidney (Chu *et al.*, 1982; Heywood *et al.*, 1979; Jorgenson *et al.*, 1985; Miklashevshii *et al.*, 1966; Munson *et al.*, 1982; Roe *et al.*, 1979; Larson *et al*., 1996; Templin *et al*., 1996; Torkelson *et al.*, 1976).

Larson *et al*. (1996) exposed female and male B6C3F1 mice to atmospheric concentrations of 0, 0.3, 2, 10, 30, and 90 ppm chloroform 6 hr/day, 7 days/week for exposure periods of 4 days or of 3, 6, or 13 consecutive weeks. Additional exposure groups were exposed for 5 days/week for 13 weeks or for 5 days/week for 6 weeks and then examined at 13 weeks. Complete necropsy and microscopic evaluation revealed that chloroform treatment induced dose- and time-dependent lesions only in the livers and nasal passage of the female and male mice and in the kidneys of the male mice. Large increases in the liver cell labeling index were seen in the 90-ppm groups at all time points. The female mice were most sensitive. The no-observed-adverse-effect level (NOAEL) for induced hepatic cell proliferation was 10 ppm. The hepatic labeling indices in the 5 days/week groups were about half of those seen in the 7 days/week groups and returned to the normal baseline in the 6-week recovery groups. The NOAEL for increased liver weight (normalized to body weight) was 10 ppm in male mice. Histologic changes and regenerative cell proliferation were induced in the kidneys of male mice at 30 and 90 ppm with 7 days/week exposures and also at 10 ppm with the 5 days/week regimen. Nasal lesions were transient and occurred only in mice exposed to 10, 30, or 90 ppm for 4 days.

Templin *et al*. (1996) exposed male and female F-344 rats to airborne concentrations of 0, 2, 10, 30, 90, or 300 ppm chloroform 6 hr/day, 7 days/week for 4 days or 3, 6, or 13 weeks. Additional groups were exposed 5 days/week for 13 weeks, or 5 days/week for 6 weeks and held until Week 13. A “full-screen” necropsy identified the kidney, liver, and nasal passages as the only target organs. The primary target in the kidney was the epithelial cells of the proximal tubules of the cortex; significantly elevated increases in the cell labeling index were observed at concentrations of 30 ppm chloroform and above. However, only a marginal increase in the renal cell labeling index in the males was seen after exposures of 90 ppm, 5 days/week. Chloroform induced hepatic lesions in the midzonal and centrilobular regions with increases in the labeling index throughout the liver, but only at 300 ppm, an extremely toxic level. An additional liver lesion seen only at 300 ppm was numerous intestinal crypt-like ducts surrounded by dense connective

tissue. Enhanced bone growth and hypercellularity in the lamina propria of the ethmoid turbinates of the nose occurred at the early time points at concentrations of 10 ppm and above. At 90 days there was a generalized atrophy of the ethmoid turbinates at concentrations of 2 ppm (the lowest concentration tested) and above.

Torkelson and associates (1976) exposed rats (12/sex/group), rabbits (2-3/sex/group), and guinea pigs (8-12/sex/group) for 7 hours/day, 5 days/week over 6 months to 0, 25, 50 or 85 ppm chloroform vapor. Dogs were exposed to 25 ppm chloroform, for 7 hours/day, 5 days/week for 6 months. Dose and species-dependent pathological changes in the liver included mild to severe centrilobular granular degeneration, foamy vacuolization, focal necrosis, and fibrosis in both sexes of all species tested. Guinea pigs were the least sensitive and male rats the most sensitive to chloroform induced hepatotoxicity; the above adverse effects occurred at 25 ppm. Adverse kidney effects observed in all species included cloudy swelling of the renal tubular epithelium and interstitial and tubular nephritis. Pneumonitis was observed in the high (85 ppm) exposure groups of male rats, female guinea pigs, and male rabbits, and in the lower dose group of female rabbits (25 ppm). Clinical and blood parameters were also examined in rats and rabbits, but no alterations were attributable to chloroform exposure.

Effects on average body weight, and relative liver and kidney weights of rats due to chloroform exposure 7 hours/day for 6 months (Torkelson *et al*., 1976)

Sex	Parameter	Unexposed control	Air control	25 ppm	50 ppm	85 ppm
male	survival	11/12	10/12		9/10	6/10
	avg. bw	343	356		305*	316
	liver	2.45	2.52		2.48	2.76*
	kidney	0.69	0.70		0.81*	0.84*
male	survival	8/12	12/12	9/12		
	avg. bw	319	347	335		
	liver	2.67	2.41	2.65		
	kidney	0.75	0.70	0.83*		
female	survival	10/12	9/12		10/10	10/10
	avg. bw	202	223		203	206
	liver	2.92	2.99		3.00	3.12
	kidney	0.82	0.81		0.95	1.06
female	survival	10/12	12/12	12/12		
	avg. bw	211	202	194		
	liver	3.02	2.93	3.08		
	kidney	0.83	0.84	0.94*		

* $p < 0.05$

VI. Derivation of Chronic Reference Exposure Level (REL)

Study	Torkelson *et al.*(1976)
Study population	Rats, unspecified strain (12/sex/group)
Exposure method	Discontinuous whole-body inhalation exposures (0, 25, 50, 85 ppm)
Critical effects	Pathological changes in liver (degenerative), and kidneys (cloudy swelling)
LOAEL	25 ppm
NOAEL	Not observed
Exposure continuity	7 hr/day for 5 days/week for 6 months
Average experimental exposure	5.3 ppm for LOAEL group (25 x 7/24 x 5/7)
Human equivalent concentration	15.9 ppm for LOAEL group (gas with systemic effects, based on RGDR = 3.0 for lambda (a) : lambda (h) (Gargas *et al.*, 1989))
Exposure duration	6 months
LOAEL uncertainty factor	10
Subchronic uncertainty factor	1
Interspecies uncertainty factor	3
Intraspecies uncertainty factor	10
Cumulative uncertainty factor	300
Inhalation reference exposure level	0.05 ppm (50 ppb; 0.30 mg/m^3; 300 µg/m^3)

In the study of Torkelson and associates (1976) rats were the most sensitive species and guinea pigs the least sensitive to chloroform vapors. Though of subchronic duration, this inhalation study still exposed rats discontinuously for 25% of a lifetime (25.8 weeks/104 weeks/lifetime). Pathological changes were observed in both sexes of rat at 50 and 85 ppm (244 or 415 mg/m^3) and in male rats at 25 ppm (122 mg/m^3) chloroform. These hepatic changes included mild to severe centrilobular granular degeneration, foamy vacuolization, focal necrosis, and fibrosis. Adverse effects in the kidney including cloudy swelling and nephritis were seen in all species tested at 25 ppm (122 mg/m^3) chloroform.

An unexpected finding in animals was the generalized atrophy of the ethmoid turbinates of F344 rats after a 90 day exposure at concentrations of 2 ppm chloroform and above (Templin *et al*., 1996). Nasal lesions have also been reported in F344 rats given chloroform by gavage (Larson *et al*., 1995). This severe and extensive chloroform-induced olfactory mucosal degeneration in rats is not associated with detectable olfactory deficit (Dorman *et al*., 1997). As the basis of the REL we have used the more usual chloroform organ targets of liver and kidney. However, confirmation of nasal effects in other rat strains and other species may require reassessing the basis of the REL for chloroform.

The human occupational studies have reported jaundice with or without alterations in liver enzymes at similar ambient concentrations: 2 to 204 ppm chloroform (10 to 995 mg/m^3) after at least 1 year (Bomski *et al.*, 1967) and 14 to 400 ppm chloroform (68 to 1952 mg/m^3) after 6 months or less (Phoon *et al.*, 1983). The presence of jaundice and hepatitis in these 2 reports

made them questionable for use in developing a REL. In the Li *et al*. (1993) study the workers were exposed for an average of 7.8 years (range = 1-15 years) and the air concentrations ranged from 4.27 to 141.25 mg/m^3 with a geometric average of 20.46 mg/m^3. The exposed workers were subdivided into higher (n=46) and lower (n=14) exposures, but the separation was not indicated for all results. If the lower exposure level of 2.8 ppm (13.49 mg/m^3) is classified as a mild LOAEL based on a significant difference from controls in one type of neurobehavioral test, the exposure level can be time adjusted to an equivalent continuous exposure of 1 ppm, then divided by a LOAEL UF of 3 and an intraspecies UF of 10 to yield a REL of 30 ppb, in good agreement with the proposed REL of 50 ppb (300 µg/m^3) based on animals (rats).

Chloroform is metabolized by the cytochrome P-450 dependent mixed function oxidase system, primarily in the liver, the respiratory epithelium, and the kidney. In the rat liver and kidneys, chloroform is metabolized to phosgene (Pohl *et al.*, 1984). The hepatotoxicity and nephrotoxicity of chloroform is thought to be due largely to phosgene (Bailie *et al.*, 1984). Individuals with concurrent exposure to certain chemical inducers of liver cytochrome P450 activity, including barbiturates, may be at potentially greater risk of chloroform toxicity (Cornish *et al.*, 1973). Others with possible higher sensitivity to chloroform include persons with underlying liver, kidney or neurological conditions.

VII. Data Strengths and Limitations for Development of the REL

Strengths of the chronic REL for chloroform derive from the critical effect being found in the liver, a well-established site of chloroform toxicity. Limitations in the data include the lack of a NOAEL in the key study, the less than lifetime duration of the key study, and the limited number of chronic inhalation studies available.

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CHRONIC TOXICITY SUMMARY

1,4-DIOXANE

(Synonym: dihydro-p-dioxin, diethylene dioxide, p-dioxane, glycolethylene ether)

CAS Registry Number: 123-91-1

I. Chronic Toxicity Summary

Inhalation reference exposure level	**3,000 mg/m^3** (800 ppb)
Critical effects	Liver, kidney, hematologic changes in rats
Hazard index target(s)	Alimentary system; kidney; circulatory system

II. Chemical Property Summary (HSDB, 1995; 1999; CRC, 1994)

Description	Colorless liquid with a faint, pleasant odor
Molecular formula	$C_4H_8O_2$
Molecular weight	88.10 g/mol
Boiling point	101.5 ^oC
Melting point	11.8^oC
Vapor pressure	37 torr @ 25^oC
Solubility	Miscible with water, aromatic solvents, and oils
Kow	0.537
Conversion factor	3.60 µg/m^3 per ppb at 25^oC

III. Major Uses and Sources

1,4-Dioxane (dioxane), a cyclic ether, is used as a degreasing agent, as a component of paint and varnish removers, and as a wetting and dispersion agent in the textile industry. Dioxane is used as a solvent in chemical synthesis, as a fluid for scintillation counting, and as a dehydrating agent in the preparation of tissue sections for histology (Grant and Grant, 1987; HSDB, 1995). The annual statewide emissions from facilities reporting under the Air Toxics Hot Spots Act in California based on the most recent inventory were estimated to be 155,549 pounds of 1,4-dioxane (CARB, 1999).

IV. Effects of Human Exposure

Dioxane is absorbed by all routes of administration (HSDB, 1995). In humans, the major metabolite of dioxane is β-hydroxyethoxyacetic acid (HEAA) and the kidney is the major route of excretion (Young *et al.*, 1976). The enzyme(s) responsible for HEAA formation has not been studied, but data from Young *et al.* (1977) indicate saturation does not occur up to an inhalation exposure of 50 ppm for 6 hours. Under these conditions the half-life for dioxane elimination is

59 min (plasma) and 48 min (urine). Although physiologically based pharmacokinetic (PBPK) modeling suggests HEAA is the ultimate toxicant in rodents exposed to dioxane by ingestion, the same modeling procedure does not permit such a distinction for humans exposed by inhalation (Reitz *et al.*, 1990).

Several anecdotal reports have appeared in which adverse health effects due to chronic dioxane exposure are described. Barber (1934) described dioxane exposed factory workers, some of whom exhibited signs of liver changes, increased urinary protein and increased white blood cell counts, and some of whom died from apparent acute exposures. Although the kidney and liver lesions were considered manifestations of acute exposure, the author suggested a chronic component that was manifested by increased white blood cells. A case was reported in which a worker, who died following exposure by inhalation and direct skin contact to high (unspecified) dioxane levels, exhibited lesions in the liver, kidneys, brain and respiratory system. However, the effects could not be easily separated from the effects due to high intake of alcohol (Johnstone, 1959).

In a German study (Thiess *et al.*, 1976 / in German, described in NIOSH, 1977) 74 workers exposed to dioxane in a dioxane-manufacturing plant (average potential exposure duration - 25 years) underwent evaluation for adverse health effects. Air measurements indicated dioxane levels varied from 0.01 to 13 ppm. Clinical evaluations were applied to 24 current and 23 previous workers. Evidence of increased (i.e., abnormal) aspartate transaminase (also known as serum glutamate-oxalacetic transaminase or SGOT), alanine transaminase (serum glutamate pyruvate transaminase or SGPT), alkaline phosphatase, and gamma glutamyltransferase activities (liver function) was noted in these workers, but not in those who had retired. The indicators of liver dysfunction, however, could not be separated from alcohol consumption or exposure to ethylene chlorohydrin and/or dichloroethane.

A follow-up mortality study was conducted on 165 chemical plant manufacturing and processing workers who were exposed to dioxane levels ranging from less than 25 to greater than 75 ppm between 1954 and 1975 (Buffler *et al.*, 1978). Total deaths due to all causes, including cancer, did not differ from the statewide control group, but the data were not reanalyzed after removing the deaths due to malignant neoplasms. The study is limited by the small number of deaths and by the small sample number. The study did not assess hematologic or clinical parameters that could indicate adverse health effects in the absence of mortality.

Yaqoob and Bell (1994) reviewed human studies on the relationship between exposure to hydrocarbon solvents - including dioxane - and renal failure, in particular rare glomerulonephritis. The results of their analysis suggest that such solvents may play a role in renal failure, but dioxane was not specifically discussed. Of interest to the discussion on chronic exposure to dioxane is the suggestion that the mechanism of the disease process involves local autoimmunity with decreased circulating white blood cells (see below).

V. Effects of Animal Exposure

In rats, the major metabolite of dioxane is HEAA, which is excreted through the kidneys (Braun and Young, 1977). Exposure to dioxane by ingestion results in saturation of metabolism above 100 mg/kg given in single dose. Saturation of metabolism was also observed as low as 10 mg/kg if dioxane was administered in multiple doses. Dioxane itself is not cleared through the kidney. A decrease in metabolic clearance with increasing dose (iv) has been interpreted as the saturation of metabolism at the higher doses (Young *et al.*, 1978).

For Sprague-Dawley rats, the metabolic fate of inhaled dioxane (head only exposure) was based on one air concentration (50 ppm). At this level, nearly all the dioxane was metabolized to HEAA since HEAA represented 99 percent of the total dioxane + HEAA measured. The plasma half-life for dioxane under these conditions was 1.1 hours. The absorption of dioxane through the inhalation pathway could not be exactly determined, because of a high inhalation rate (0.24 liters/min), calculated on the basis of complete absorption (Young *et al.*, 1978; U.S. EPA, 1988). Although the high inhalation rate could be dioxane-related, another explanation may be the stress incurred when the jugular veins were cannulated as part of the experiment. Extensive absorption by inhalation is also inferred from the high tissue/air partition coefficients (Reitz *et al.*, 1990).

Although the PBPK modeling suggests that in rat the parent dioxane is a better dose surrogate than HEAA for exposure by ingestion, the inhalation modeling did not use more than one inhalation dose. No studies were located on the biological or biochemical properties of HEAA or the properties of the enzyme(s) that are responsible for the transformation of dioxane into HEAA.

Rats (Wistar) were exposed by inhalation to dioxane (111 ppm; 7 hours/day, 5 days/week) for 2 years (Torkelson *et al.*, 1974). Increased mortality and decreased body weight gains, compared to unexposed control rats, were not observed. Among the male rats, decreased blood urea nitrogen (kidney function), decreased alkaline phosphatase (cholestatic liver function), increased red blood cells, and decreased white blood cells were observed. According to the authors, exposure-related, non-cancerous tissue lesions were not observed during the 2-year period.

In another inhalation study, rats were exposed to dioxane at levels of 0.15, 1.3, and 5.7 ppm (Pilipyuk *et al.*, 1978). Frequency was not specified, but the duration is given as “90 successive days”. At the end of the 3-month exposure, increased SGOT activity at the two highest doses and increased SGPT activity at all doses were measured in the sera of the exposed rats. Rats exposed to the highest dose also exhibited increased urinary protein and chloride levels, each of which returned to control levels during an unspecified recovery period. Pilipyuk *et al.* (1978) also report changes in the minimum time (ms) required for an electric stimulus to result in excitation of extensor and flexor muscles. Although Pilipyuk *et al.* (1978) consider the changes to be a reflection of adverse effects due to exposure to dioxane, Torkelson *et al.* (1974) do not consider the hematologic and clinical changes of toxicologic importance. In particular, toxic manifestations are usually associated with increased blood urea nitrogen and alkaline phosphatase levels, whereas these levels decreased in the Torkelson *et al.* (1974) investigation. The reason for the discrepancies between the two studies, in particular the extremely low dioxane exposure levels in the Pilipyuk *et al.* (1978) study, is unknown. One explanation could

be the purity of the dioxane used, which was not described in the latter study, although such contamination would be unlikely to account for the large difference in exposure levels.

Kociba *et al.* (1974) exposed rats (Sherman) to dioxane by ingestion of drinking water for up to 2-years. The drinking water levels were 0, 0.01, 0.1, and 1.0 percent, which were converted to daily intake according to measured rates of water consumption during exposure. Exposure to the highest level resulted in decreased body weight gain and increased deaths. According to the authors, exposure related hematologic changes did not occur. Histopathologic examination revealed evidence of regeneration of hepatic and kidney tissues in rats exposed to 1.0 or 0.1 percent, but not in rats exposed to 0.01 percent dioxane. On the assumption of total absorption of dioxane from the gastrointestinal tract, the exposure levels in female and male rats is as follows: 0.01%-18 ppm/F, 9.3 ppm/M; 0.1% -144 ppm/F, 91 ppm/M.

The teratogenic potential of dioxane was studied in rats (Giavini *et al.*, 1985). Dioxane was administered by gavage at doses of 0, 0.25, 0.5, and 1.0 ml/kg-day, on gestation days 6-15, and observations continued through day 21. Dams exposed to the highest dose exhibited nonsignificant weight loss and a significant decrease in food consumption during the first 16 days. During the remaining 5 days, food consumption increased, but the weight gain reduction in the presence of dioxane continued. At the 1.0 ml/kg-day dose, mean fetal weight and ossified sternebrae were also reduced. The inability to separate the developmental toxicity from maternal or embryotoxicity renders these data inconclusive as to the developmental toxicity of dioxane. If toxicity to the dam and/or embryo exists, the NOAEL for dioxane (based on density = 1.03 gm/ml) is 517 mg/kg-day.

VI. Derivation of Chronic Reference Exposure Level (REL)

Study	Torkelson *et al.* (1974)
Study populations	Rats
Exposure method	Discontinuous inhalation
Critical effects	No effects on liver, kidney, or hematologic function were noted in this study. Such dysfunctions, however, were observed in rats exposed to dioxane by ingestion (Kociba *et al.* 1974) and humans (Thiess, *et al.*, 1976, described by NIOSH, 1977).
LOAEL	Not observed in inhalation studies
NOAEL	111 ppm
Exposure continuity	7 h/d x 5 days/wk
Average experimental exposure	23 ppm (111 x 7/24 x 5/7)
Human equivalent concentration	23 ppm (gas with systemic effects, based on RGDR = 1.0 using default assumption that lambda (a) = lambda (h))
Exposure duration	2 years
LOAEL uncertainty factor	1
Subchronic exposure	1
Interspecies uncertainty factor	3
Intraspecies uncertainty factor	10
Cumulative uncertainty factor	30
Inhalation reference exposure level	0.8 ppm (800 ppb; 2.8 mg/m^3; 3000 µg/m^3)

The lifetime rat inhalation study of Torkelson *et al.* (1974) is the only detailed inhalation study available in the literature. The Pilipyuk *et al.* (1977) study contains useful and consistent data, but the absence of necessary details prevents the use of these results for the determination of a chronic reference exposure level (REL). Although the ingestion study (Kociba *et al.*, 1974) shows unequivocal toxic responses (liver and kidney) of the rat to dioxane by ingestion, exposure to 111 ppm by inhalation leads to equivocal results (Torkelson *et al.*, 1974). In particular, serum markers for liver and kidney dysfunction decrease in value, whereas toxic responses are associated with increased levels. The lack of toxic hematologic endpoints observed in the ingestion study suggests that toxicity of dioxane may be route-of-exposure specific. Hematologic changes were also observed in the early worker study wherein changes in white blood cell count occurred (Barber, 1934), but the directions are different. The studies on humans and rodents therefore suggest inhalation of dioxane may lead to adverse biologic effects, but good dose-response data are not available. A partial explanation may lie in the dose-response characteristic of the metabolism of dioxane, wherein toxicity may be a function of the saturation of metabolism. For inhalation, neither the point of saturation nor the mechanism has been established. Importantly, the end-point for dioxane chronic exposure may not be established.

VII. Data Strengths and Limitations for Development of the REL

Although a free-standing NOAEL is not a desirable parameter to use for the development of a chronic REL, other studies support the conclusion that exposure to dioxane leads to adverse health effects. These observations have been documented among experimental animals (Kociba *et al.*, 1974; Pilipyuk *et al.*, 1977) and humans (Thiess *et al.*, 1976, described in NIOSH, 1977). Until additional data from inhalation dose-response studies become available, a chronic REL based on the free-standing NOAEL is considered the best available.

The strength of the REL for 1,4-dioxane is that it is based on a full lifetime study, with a large number of toxic endpoints and a good sample size. The weaknesses include use of a free standing NOAEL, the limited human data, and the lack of developmental studies.

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CHRONIC TOXICITY SUMMARY

ETHYL CHLORIDE

(Chloroethane; monochloroethane; ether hydrochloric)

CAS Registry Number: 75-00-3

I. Chronic Toxicity Summary

Inhalation reference exposure level	**30,000 µg/m^3** (10,000 ppb)
Critical effect(s)	Delayed fetal ossification in mice
Hazard index target(s)	Teratogenicity; alimentary system

II. Physical and Chemical Properties (HSDB, 1995; 1999)

Description	Colorless gas
Molecular formula	C_2H_5Cl
Molecular weight	64.52
Density	0.9214 g/cm^3 @ 0°C
Boiling point	12.3 °C
Melting point	–138.7 °C
Vapor pressure	1000 torr @ 20 °C
Conversion factor	1 ppm = 2.64 mg/m^3 @ 25°C

III. Major Uses or Sources

Ethyl chloride has been used as a starting point in the production of tetraethyl lead and as a refrigerant, solvent and alkylating agent (HSDB, 1995). It is also used as a topical anesthetic (Clayton and Clayton, 1994). The annual statewide industrial emissions from facilities reporting under the Air Toxics Hot Spots Act in California based on the most recent inventory were estimated to be 291,300 pounds of ethyl chloride (CARB, 1999).

IV. Effects of Human Exposure

Neurological symptoms have been observed in human case studies in instances of ethyl chloride abuse. Cerebellar-related symptoms including ataxia, tremors, speech difficulties, and hallucinations were observed in a 28-year old female who had sniffed 200-300 ml ethyl chloride off her sleeve daily for 4 months (Hes *et al.* 1979). The patient's liver was enlarged and tender. Four weeks following cessation of exposure, all symptoms were absent.

V. Effects of Animal Exposure

Pregnant mice were exposed to 1300, 4000, or 13000 mg/m^3 ethyl chloride in air for 6 hours per day on days 6-15 of gestation (Scortichini *et al.*, 1986). No effects on fetal resorption rates, litter size, body weight or maternal health were observed. A statistically significant increase in the incidence of delayed ossification of the skull bones was observed in fetuses from the 13,000 mg/m^3 (4900 ppm) ethyl chloride exposed group. This skull effect was accompanied by a non-significant increased incidence of cervical ribs (a supernumerary rib is considered to be a malformation). No significant adverse effects were observed in fetuses from the 4000 mg/m^3 (1500 ppm) exposure group.

No significant adverse effects were observed in rats and mice exposed to 0 or 15,000 ppm ethyl chloride for 6 hours per day, 5 days per week for 102 weeks (rats) or 100 weeks (mice) (NTP, 1989). At necropsy, a complete histopathologic examination (approximately 35 tissues) failed to identify evidence of non-cancer toxicity. The same study also exposed rats and mice to 2500, 5000, 10,000 or 19,000 ppm ethyl chloride 6 hours per day, 5 days per week for 13 weeks. No exposure-related clinical signs of toxicity or histological changes were observed in exposed animals. Thus the subchronic NOAEL for mice and rats is 19,000 ppm, which is equivalent to a continuous exposure of 3400 ppm, and a free-standing chronic NOAEL is 15,000 ppm, which is equivalent to a continuous exposure of 2700 ppm (7100 mg/m^3).

Increased relative liver weights and a slight increase in hepatocellular vacuolation were observed in mice exposed to 5000 ppm ethyl chloride 23 hours per day for 11 days (Landry *et al.*, 1989). No effects were observed in mice exposed to 0, 250, or 1250 ppm ethyl chloride for the same period.

Following acclimatization to an inhalation chamber, two groups of 10 female mice were exposed to 0 or 15,000 ppm (40,000 mg/m^3) ethyl chloride 6 hours per day for 2 weeks (Breslin *et al.*, 1988). Groups of five male mice were housed in each inhalation chamber to synchronize and promote regular cyclicity. The mean length of the estrous cycle in control mice remained constant at 4.5 days during both pre-exposure and exposure periods. Mice in the 15,000 ppm exposure group showed a 0.6 day increase in the mean cycle length during exposure (5.6 days) when compared to the pre-exposure period (5.0 days). The authors attribute this increase in estrous cycle length to a general stress response although they note that it does not preclude direct effects on neuroendocrine function.

VI. Derivation of Reference Exposure Level

Study	Scortichini *et al.*, 1986
Study population	Mice
Exposure method	Discontinuous whole-body inhalation (on days 6-15 of gestation)
Critical effects	Delayed ossification of skull foramina
LOAEL	13,000 mg/m^3
NOAEL	4,000 mg/m^3
Exposure continuity	6 hours per day
Exposure duration	Days 6-15 of gestation
Average experimental exposure	1,000 mg/m^3 for NOAEL group (4000 x 6/24)
Human equivalent concentration	1,000 mg/m^3 for NOAEL group (gas with systemic effects, based on RGDR = 1.0 using default assumption that lambda (a) = lambda (h))
LOAEL uncertainty factor	1
Subchronic uncertainty factor	1
Interspecies uncertainty factor	3
Intraspecies uncertainty factor	10
Cumulative uncertainty factor	30
Inhalation reference exposure level	30 mg/m^3 (30,000 µg/m^3; 10 ppm; 10,000 ppb)

To develop the chronic REL OEHHA used the same study on which U.S. EPA based its RfC of 10,000 µg/m^3. The REL is based on a developmental toxicity study. In accordance with U.S. EPA methodology, a time-weighted average concentration for the discontinuous exposure experiment is not used by U.S. EPA when the key effect is developmental toxicity. However, OEHHA prefers to make a time adjustment to equivalent continuous exposure because the chronic REL assumes continuous exposure. U.S. EPA also used a Modifying Factor (MF). The database deficiencies leading U.S. EPA to employ a modifying factor include the lack of a multigenerational reproductive study. The criteria for use of such modifying factors are not well described. Such MFs were not used by OEHHA.

As a comparison to the proposed REL of 10 ppm, NTP (1989) found a free-standing NOAEL of 15,000 ppm in rats and mice exposed to ethyl chloride for 6 hours per day, 5 days per week for 2 years. Time adjusting to continuous exposure results in an adjusted NOAEL of 2679 ppm. Applying an RGDR of 1, a UF_A of 3 and a UF_H of 10 results in an estimated REL of 90 ppm.

VII. Data Strengths and Limitations for Development of the REL

The strengths of the inhalation REL for ethyl chloride include the availability of controlled exposure inhalation studies in multiple species at multiple exposure concentrations and with adequate histopathogical analysis, and the observation of a NOAEL. Major areas of uncertainty are the lack of adequate human exposure data, and the lack of a multigenerational reproductive study.

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CHRONIC TOXICOLOGY SUMMARY

ETHYLENE GLYCOL

(1,2-dihydroxyethane; 1,2-ethanediol)

CAS Registry Number: 107-21-1

I. Chronic Toxicity Summary

Chronic reference exposure level	**400** mg/**m**^{3} (200 ppb)
Critical effects	Respiratory irritation in human volunteers
Hazard index target(s)	Respiratory system; kidney; teratogenicity

II. Physical and Chemical Properties (HSDB, 1996; 1999)

Description	Clear, colorless, odorless liquid
Molecular formula	$C_2H_6O_2$
Molecular weight	62.07 g/mol
Density	1.1088-1.1135 g/cm^3 @ 20° C
Boiling point	197.6° C
Melting point	–13° C (CRC, 1994)
Vapor pressure	0.06 torr @ 20°C; 0.092 torr @ 25°C
Solubility	Soluble in water and ethanol; slightly soluble in ether. Insoluble in benzene and petroleum ether.
Conversion factor	1 ppm = 2.5 mg/m^3 @ 25° C

III. Major Uses and Sources

Ethylene glycol is used as an antifreeze agent in cooling and heating systems (HSDB, 1996). It is used in hydraulic brake systems; as an ingredient in electrolytic condensers; as a solvent in the paint and plastics industries; and in inks for ball-point pens and printer's inks. It is used in the manufacture of some synthetic fibers (Terylene and Dacron), and in synthetic waxes. It is used in some skin lotions and flavoring essences. Also, it is used in asphalt emulsion plants, in wood stains and adhesives, and in leather dyeing. It has been used as a de-icing fluid for airport runways. The annual statewide emissions from facilities reporting under the Air Toxics Hot Spots Act in California based on the most recent inventory were estimated to be 66,636 pounds of ethylene glycol (CARB, 1999).

IV. Effects of Human Exposure

Laitinen *et al.* (1995) found that 10 motor servicing workers had significantly higher urinary levels of ethylene glycol and ammonia, and decreased urinary glycosaminoglycan levels, compared with 10 controls. The ethylene glycol levels in air were undetectable in the workers' breathing zones (i.e. below 1.9 ppm), therefore dermal absorption appeared to be the primary route of exposure. Because the dermal absorption rate is high, airborne ethylene glycol concentrations in workplaces likely underestimate the total exposure.

In a study of 20 volunteer male prisoners in Alabama, 20 hour/day exposure to aerosolized ethylene glycol concentrations varying up to a mean of 20 ppm (49 mg/m^3) for 30 days was without effect (Wills *et al.*, 1974). The actual concentrations measured in the exposure chamber were:

Concentration of ethylene glycol in air (mg/m^3)

Days	Low	High[a]	Mean
1-7	3.6	75.0	37
8-14	18.8	44.8	29
15-21	0.8	41.6	17
22-28	3.5	49.2	23
29-35	20.6	66.8	49
36-37	14.4	39.0	31

[a] does not include the very high concentrations maintained for comparatively brief periods.

Respiratory irritation was noted after 15 minutes at an exposure concentration of 75 ppm (188 mg/m^3), and became quickly intolerable at 123 ppm (308 mg/m^3). No effects were observed in normal clinical chemistry, clinical serum enzyme levels for liver and kidney toxicity (including SGOT and serum alkaline phosphatase), hematotoxicity (including % hematocrit and gm hemogloin per 100 ml blood), or psychological responses (including simple reaction time, weight discrimination, and depth perception). The respiratory irritation at 75 ppm resolved soon after exposure with no long term effects noted after a 6-week follow-up period.

V. Effects of Animal Exposure

A chronic feeding study in rats and mice was conducted by DePass *et al.* (1986a). In this study, rats (130 per sex per group) and mice (80 per sex per group) were exposed to 0, 0.04, 0.2, or 1 g/kg/day for up to 2 years. All male rats in the high dose group died by 475 days. A large number of effects were observed in this group, including: reduced body weight, increased water intake, increased blood urea nitrogen and creatinine, reduced erythrocyte counts, reduced hematocrit and hemoglobin, increased neutrophil count, and increased urine volume. Heart, kidney, lung, parathyroid, stomach, and other vascular mineralization and hyperplasia were observed histologically in the high dose group of the male rats. Female rats exhibited fatty changes and granulomas in the liver at the high dose. Liver effects were not reported for the males. The NOAEL in rats for chronic oral ethylene glycol toxicity was 200 mg/kg/day. No effects were observed in mice. Therefore, the NOAEL for mice was 40 mg/kg/day.

Coon *et al*. (1970) exposed groups of rats (as well as guinea pigs, rabbits, dogs, and monkeys) to ethylene glycol intermittently 8 hours/day, 5 days per week for 6 weeks (30 exposures) to 10 or 57 mg/m^3 or continuously to 12 mg/m^3 for 90 days. At 10 mg/m^3 2 rabbits had conjunctivitis and liver changes were noted in a few animals of the other species. At 57 mg/m^3 no signs of toxicity were seen during the exposure. Nonspecific inflammatory changes were noted in some lungs and hearts of all species. A few livers also showed necrotic areas. Continuous exposure to 12 mg/m^3 led to moderate to severe eye irritation in rats and rabbits. Edema in the rabbits led to eye closure. Two rats developed corneal opacities. All hematologic parameters and various enzymes assayed were within normal limits. At necropsy organs appeared normal. Histopathological analysis revealed inflammatory changes in the lungs of all species, but the controls also showed a lesser degree of inflammation. Several guinea pigs showed foci of inflammatory cells in the kidney.

Mortality in Coon *et al*. (1970)

			<----------Number died/number exposed---------->				
Ethylene glycol (mg/m^3)	Exposure duration	Equivalent continuous concentration	Rat	Guinea pig	Rabbit	Dog	Monkey
0 (control)	90 days	0	4/123	0/73	0/12	0/12	0/8
10±1	6 wk	2.4	0/15	0/15	0/3	0/2	0/2
57±14	6 wk	13.6	0/15	0/15	0/3	0/2	0/2
12±2	90 days	12.0	1/15	3/15	1/3	0/2	0/3

Studies on the effects of inhaled ethylene glycol on reproduction and development of rats and mice were conducted by Tyl *et al.* (1995a, 1995b). In a study using whole-body exposure of rats and mice to ethylene glycol at analyzed concentrations of 0, 119, 888, or 2090 mg/m^3 for 6 hours/day on days 6-15 of gestation, mice were found to be the more sensitive species. Maternal toxicity in rats included a significant increase in absolute and relative liver weight at 2090 mg/m^3. No effects on weight gain, organ weights other than liver, fecundity, live fetuses per litter, or pre- or post-implantation loss were observed in rats. In addition, terata were not observed at any concentration. Reduced ossification in the humerus, zygomatic arch, and the metatarsals and proximal phalanges of the hindlimb was present in fetuses exposed to 888 or 2090 mg/m^3. The NOAEL for maternal toxicity in rats was 888 mg/m^3, while the NOAEL for fetotoxicity was 119 mg/m^3.

In mice, reduced body weight and gravid uterine weight during and after the exposure were observed at the 888 and 2090 mg/m^3 concentrations. Increased nonviable implants per litter and reduced fetal body weights were also observed in groups exposed to 888 or 2090 mg/m^3. External, visceral, skeletal, and total malformations were increased in the 888 and 2090 mg/m^3 groups. The NOAEL for these effects in mice was 119 mg/m^3.

A similar experiment in mice using nose-only exposures was conducted by these researchers (Tyl *et al.*, 1995a) to determine the role of dermal absorption and/or ingestion on the effects observed with the whole-body exposure. Nose-only exposures to ethylene glycol were for 6

hours/day, on gestational days 6 through 15 at concentrations of 0, 500, 1000, and 2000 mg/m^3. The NOAEL for maternal effects (increased kidney weight) was 500 mg/m^3, and the NOAEL for fetal toxicity (skeletal variations and fused ribs) was 1000 mg/m^3. Thus, secondary dermal and/or oral exposures appear to have contributed significantly to the developmental and maternal toxicity in mice exposed to ethylene glycol aerosol. The nose-only inhalation exposure study by Tyl *et al.* (1995a) was conducted in addition to the whole-body inhalation study since extensive adsorption of ethylene glycol onto the fur of the animals was demonstrated in the whole-body experiment. Normal grooming behavior would have resulted in significantly larger doses of ethylene glycol than that expected by inhalation only.

A 3-generation study on the effects of ethylene glycol on reproductive performance and gross health of offspring in rats was conducted by DePass *et al.* (1986b). Rats were exposed orally to 40, 200, or 1000 mg/kg/day ad libitum in the feed through 3 generations. No effects on pup survivability or pup body weight were observed. Total and viable implants were also not affected. Teratogenic effects were not examined in this study.

Tyl *et al.* (1993) studied the reproductive and developmental effects of ethylene glycol in rabbits exposed by gavage on days 6 to 19 of gestation. Dams were exposed to 0, 100, 500, 1000, or 2000 mg/kg/day. Exposure to 2000 mg/kg/day resulted in 42% mortality, and abortion or early delivery in 4 does. No evidence of embryotoxicity or teratogenicity was observed in the groups exposed to 1000 mg/kg/day or less. The NOAEL for maternal toxicity was determined to be 1000 mg/kg/day.

VI. Derivation of Chronic Reference Exposure Level

Study	Wills *et al.* (1974)
Study population	Human volunteer prisoners
Exposure method	Discontinuous whole-body inhalation
Critical effects	Respiratory tract irritation
LOAEL	75 ppm
NOAEL	20 ppm
Exposure continuity	20 hours/day
Exposure duration	30 days
Average exposure	16.7 ppm for NOAEL group (20 x 20/24)
Human equivalent concentration	16.7 ppm
LOAEL uncertainty factor	1
Subchronic uncertainty factor	10
Interspecies factor	1
Intraspecies factor	10
Cumulative uncertainty factor	100
Inhalation reference exposure level	0.2 ppm (200 ppb; 0.4 mg/m^3; 400 μg/m^3)

The subchronic study by Wills *et al.* (1974) represents the only human inhalation data for ethylene glycol toxicity. The experiment showed a concentration-response relationship, with onset of irritation occurring at 188 mg/m^3 and intense and intolerable irritation occurring at 308 mg/m^3. The volunteers were followed for 6 weeks without any apparent long-term effects from

the exposures. Although the irritation experienced in the human subjects appears to be an acute phenomenon and not a cumulative lasting effect, the subchronic uncertainty factor of 10 was retained to protect against other systemic effects associated with ethylene glycol such as kidney damage which may occur over a long-term exposure.

The chronic feeding study in rats by DePass *et al.* (1986a) showed significant chronic effects. These included reduced body weight, increased water intake, increased blood urea nitrogen and creatinine, reduced erythrocyte counts, reduced hematocrit and hemoglobin, increased neutrophil counts, increased urine volume, and reduced urine specific gravity and pH in rats exposed to a concentration of 1000 mg/kg/day. However, no effects were reported in mice. In contrast, reproductive and developmental toxicity studies in mice, rats, and rabbits have shown the mouse to be the most sensitive species for both terata and maternal toxicity endpoints (Tyl *et al.*, 1995a; Tyl *et al.*, 1993; Neeper-Bradley *et al.*, 1995). In addition, the 3-generation reproductive toxicity study by DePass *et al.* (1986b) showed no significant effects on rat pup survival or body weight at concentrations up to 1000 mg/kg/day. However, developmental endpoints were not reported in this study. From the available data, the toxicity of ethylene glycol is apparently greatest in the maternal mouse. The estimated equivalent air concentrations (assuming a 70 kg human inhales 20 m^3/day) from the feed in the 3-generation study by DePass *et al.* (1986b) are 700 mg/m^3 and 3500 mg/m^3 for the NOAEL and LOAEL, respectively.

For comparison with the proposed REL of 400 μg/m^3 based on a one month human study, the inhalation NOAEL of 48 ppm, obtained by Tyl *et al*. (1995) in mice discontinuously exposed for 10 days on gestation days 6-15, was used to estimate a REL based on animal data. Use of a time adjustment from 6 to 24 hours/day, an RGDR of 1, an interspecies UF of 3, and an intraspecies UF of 10 resulted in an estimated REL of 0.4 ppm (1000 μg/m^3) for ethylene glycol.

VII. Data Strengths and Limitations for Development of the REL

The strengths of the inhalation REL for ethylene glycol include the use of human exposure data, the use of controlled, nearly continuous inhalation exposures, the observation of a NOAEL, and the similar REL value estimated from an animal study. Major areas of uncertainty are the short length of the key study and the lack of chronic inhalation exposure studies in both animals and man (LaKind *et al*., 1999).

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CHRONIC TOXICITY SUMMARY

N-HEXANE

(normal hexane)

CAS Registry Number: 110-54-3

I. Chronic Toxicity Summary

Inhalation reference exposure level	**7000 mg/m^3** (2000 ppb)
Critical effect(s)	Neurotoxicity; electrophysiological alterations in humans
Hazard index target(s)	Nervous system

II. Physical and Chemical Properties (HSDB, 1999)

Description	Colorless liquid, gas
Molecular formula	C_6H_{14}
Molecular weight	86.10
Density	0.660 g/cm^3 @ 20° C
Boiling point	68.95°C
Melting point	–95.3°C
Vapor pressure	150 torr @ 25° C
Solubility	Insoluble in water; soluble in most organic solvents; very soluble in alcohol
Conversion factor	1 ppm = 3.52 mg/m^3 @ 25° C

III. Major Uses or Sources

n-Hexane is used in the extraction of vegetable oil from seeds such as safflower, soybean, cotton, and flax (HSDB, 1995). It is also used as a alcohol denaturant and as a paint diluent. The textile, furniture and leather industries use n-hexane as a cleaning agent. Many petroleum and gasoline products contain n-hexane. The annual statewide industrial emissions from facilities reporting under the Air Toxics Hot Spots Act in California based on the most recent inventory were estimated to be 999,225 pounds of hexane (CARB, 1999).

IV. Effects of Human Exposure

In an offset printing factory with 56 workers, symptomatic peripheral neuropathy was noted in 20 of 56 (36%) workers, while another 26 (46%) had evidence of subclinical neuropathy (Chang

et al., 1993). Reduced sensory action potentials; reduced motor action potentials; decreased motor nerve conduction velocity; and increased distal latency were found in most workers. Giant axonal swellings with accumulation of 10 nm neurofilaments, myelin sheath attenuation, and widening of nodal gaps were noted upon sural nerve biopsy of a severe case. Optic neuropathy and CNS impairment were not usually found. Personal air samples had 80 to 210 ppm hexane (mean = 132 ppm), 20 to 680 ppm isopropanol (mean = 235 ppm), and 20 to 84 ppm (mean = 50 ppm) toluene. The workers worked 12 hours per day for 6 days per week. The mean duration of employment was 2.6 years, with a range of 1 month to 30 years.

An epidemiologic study was performed on workers employed in a factory producing tungsten carbide alloys and exposed for an average of 6.2 years to solvent vapors consisting of an 8-hour time weighted average of 58 ppm (±41 ppm) n-hexane and 39 ppm (±30 ppm) acetone (Sanagi *et al.*, 1980). Neurological examinations performed on both control and exposed workers examined cranial nerves, motor and sensory nerves, reflexes, coordination and gait. Neurophysiological and nerve stimulation studies were also performed. While no overt neurological abnormalities were noted, the mean motor nerve conduction velocity and residual latency of the exposed group were significantly decreased as compared to unexposed workers. The effects observed are consistent with other reports of n-hexane-induced peripheral neuropathy. The study reports a LOAEL of 58 ppm n-hexane.

Polyneuropathy with subsequent development of muscular atrophy and paresthesia in the distal extremities was observed in workers exposed to between 500 and 1000 ppm n-hexane in a pharmaceutical plant (Yamada, 1967).

A group of 15 industrial workers exposed to n-hexane in vegetable oil extracting and adhesive bandage manufacturing processes was examined for signs of neurotoxicity and ophthalmological changes (Raitta *et al*., 1978; Seppalainen *et al*., 1979). The workers (11 males and 4 females) had been exposed to hexane for 5 to 21 years (mean of 12 years). Ten healthy workers served as controls. Exposures were found to be variable; concentrations as high as 3000 ppm were found on some occasions, although exposure concentrations were usually well below 500 ppm. The authors concluded that the high short-term exposures, occurring occasionally for 1 to 2 hours at a time, could have been major factors in the effects observed. Visual evoked potentials (VEPs) were generally reduced among the exposed subjects and latencies tended to be increased (Seppalainen *et al*., 1979). Visual acuity, visual fields, intraocular pressure, and biomicroscopical findings were normal. Macular changes were noted in 11 and impaired color discrimination was found in 12 of the 15 subjects, largely in the blue-yellow spectrum (Raitta *et al*., 1978).

Fifteen (25%) of 59 press proofing workers had polyneuropathy (Wang *et al*., 1986). All of the patients with polyneuropathy were regularly exposed to n-hexane, and there was a significant association between n-hexane concentration and prevalence of polyneuropathy. The ambient concentration of n-hexane of 190 ppm was found in one factory in which all six workers developed polyneuropathy. Workers exposed to less than 100 ppm n-hexane who frequently worked overtime demonstrated significant decreases in motor nerve conduction velocities in median, ulnar, and peroneal nerves. Twelve of 13 workers who regularly slept in the factory had polyneuropathy compared to three (7%) of 46 employees who did not sleep in the factory.

Ninety-three of 1662 Japanese workers were found to have polyneuropathy (Yamamura, 1969; Sobue *et al*., 1978). All of the workers developing polyneuropathy were employed in pasting with rubber cement containing 70% or more hexane and small amounts of toluene. The worksites were poorly ventilated and concentrations in workrooms were measured at between 500 and 2500 ppm hexane. One patient developed numbness and weakness of the legs after 6 months of exposure to hexane-based solvents. This patient was hospitalized for over a year until the muscle weakness and atophy improved enough to discharge the patient.

Urinary 2,5-hexanedione concentrations were significantly higher in 35 male workers exposed to n-hexane than in an unexposed group (Karakaya *et al*., 1996). Significant decreases in serum IgG, IgM and IgA levels were also found, and a significant correlation was noted between urinary 2,5-hexanedione concentrations and serum Ig level of the exposed group.

An association between n-hexane and parkinsonism has been proposed based on two case reports (Pezzoli *et al*., 1989; 1995). Regional striatal abnormalities of the nigrostriatal dopaminergic system and of glucose metabolism, observed with positron emission tomography studies, were considered distinct from those seen in idiopathic Parkinson's disease.

Co-exposure to acetone increased the urinary concentrations of free and total 2,5-hexanedione (2,5-HD) in a study of 87 hexane-exposed workers (Cardona *et al*., 1996). Increased urinary 2,5-HD is noted also with coexposure to hexane and methyl ethyl ketone (Ichihara *et al*., 1998).

V. Effects of Animal Exposure

Groups of 12 Sprague-Dawley (SD) rats inhaled n-hexane (0, 6, 26, or 129 ppm) for 6 hours/day, 5 days/week for 26 weeks (Bio/dynamics, 1978). A second experiment from the same report involved inhalation exposures of SD rats for 26 weeks to 0, 5, 27, or 126 ppm hexane for 21 hours/day, 7 days/week. There were no consistent dose-related differences between exposed and control animals, although small numbers of animals were involved and examinations were limited to physical observation, body weight, hematological parameters, clinical chemistry, and necropsy of spontaneous deaths. The highest concentration (126 ppm for 21 hours/day, 7 days/week) was a NOAEL and represents a time-weighted average exposure of 110.2 ppm over the duration of the experiment.

F-344 rats and B6C3F1 mice (50/sex/concentration/species) inhaled commercial hexane solvent (0, 900, 3000, or 9000 ppm) for 6 h/day, 5 days/week over 2 years (Daughtrey *et al*., 1999). No significant differences in mortality were noted between hexane-exposed and control groups. Small statistically significant reductions in body weight gain were noted in male and female rats inhaling 3000 ppm or more and in female mice inhaling 9000 ppm. Epithelial cell hyperplasia was increased in the nasoturbinates and larynx of exposed rats.

Fischer 344 rats (5/sex/dose) inhaled >99.5% pure n-hexane (0, 3000, 6500, or 10,000 ppm) for 6 hours/day, 5 days/week over 13 weeks (Cavender *et al*., 1984). No statistically significant differences were notes in food consumption, ophthalmologic examination, neurological function,

or hematological or serum chemistry parameters in either males or females. Female body weights and clinical observations were unaltered by hexane treatment. The mean body weight gain of male rats in the 10,000-ppm group was significantly decreased compared with controls at 4 weeks of exposure and thereafter. Axonopathy was noted in the tibial nerve of four of five male rats exposed to 10,000 ppm and in one of five male rats exposed to 6500 ppm. Axonopathy in the medulla was noted in one male rat exposed to 10,000-ppm. Males inhaling 10,000 ppm had slightly but significantly lower brain weights. No other adverse histopathological effects were reported. This study identifies a NOAEL for neurotoxicity of 3000 ppm, with an average experimental exposure of 540 ppm.

B6C3F$_1$ mice were exposed to 500, 1000, 4000, or 10,000 ppm n-hexane 6 hours per day, 5 days per week for 13 weeks or to 1000 ppm n-hexane for 22 hours per day, 5 days per week for 13 weeks (Dunnick *et al.*, 1989). Mild inflammatory, erosive and regenerative lesions in the olfactory and respiratory epithelium were observed in the nasal cavity of mice exposed to 1000 ppm n-hexane and higher. "Minimal lesions" were noted in those mice exposed to 500 or 1000 ppm n-hexane. Paranodal axonal swelling in the tibial nerve was observed in 6/8 mice exposed to 1000 ppm for 22 hours per day and in 6/8 mice exposed to 10,000 ppm for 6 hours per day. No such swelling was noted in neurohistological examination of the control animals; neurohistological examination was not performed in those animals exposed to 500 and 1000 ppm for 6 hours per day. A NOAEL for histological lesions of the nasal turbinates of 500 ppm n-hexane was identified. Because neurohistological examinations were not performed in animals exposed to 500 or 1000 ppm (the NOAEL and LOAEL, respectively), the interpretation of the results from this study are seriously limited.

Male SM-A strain mice (10/group) were exposed continuously to 0, 100, 250, 500, 1000, or 2000 ppm commercial grade hexane (65 to 70% n-hexane with the remainder being other hexane isomers) for 6 days/week for 1 year (Miyagaki, 1967). Electromyography, strength-duration curves, electrical reaction time, and flexor/extensor chronaxy ratio, gait posture and muscular atrophy were studied. Increased complexity of NMU (neuromuscular unit) voltages during electromyographic analysis was noted in 0/6 controls, 1/6 in the 100 ppm group, 3/6 in the 250 ppm group, 5/6 in the 500 ppm group, 3/3 in the 1000 ppm group, and 4/4 in the 2000 ppm group. A dose-related increase in incidence and severity of reduced interference voltages from muscles was noted in mice exposed to 250 ppm or more, but not in controls (0/6 examined) or in the 100 ppm group (0/6). Dose-related abnormal posture and muscle atrophy were noted at 250 ppm or more. This study identifies a NOAEL of 100 ppm for neurotoxicity (68 ppm when adjusted for 67.5% n-hexane).

Rats inhaling 400-600 ppm n-hexane developed peripheral neuropathy after forty-five days of exposure (Schaumburg and Spencer, 1976). Giant axonal swellings and fiber degeneration were observed in the central and peripheral nervous systems. The changes were most notable in tibial nerves and in the cerebellum, medulla and spinal cord.

A dose-dependent decrease in motor nerve conduction velocity and body weight gain was observed in rats exposed to 500, 1200, or 3000 ppm n-hexane for 12 hours per day, 7 days per week for 16 weeks (Huang *et al.*, 1989). The neurotoxicity was significant in the two highest

exposure groups; peripheral nerve degeneration, characterized by paranodal swellings and demyelination and remyelination in the myelinated nerve fibers, was observed and was more advanced in the highest exposure group.

Available studies indicate that the neurotoxicity of n-hexane is potentiated by concurrent exposure to methyl ethyl ketone (Altenkirch *et al.*, 1982).

Acetone has also been shown to potentiate the neurotoxicity of hexane and 2,5-HD. Male rabbits administered acetone and 2,5-HD intravenously had decreased body clearance of 2,5-HD (Lagefoged and Perbellini, 1986). Male rats were treated for 6 weeks with 0.5% w/v 2,5-hexanedione alone or in combination with 0.50% w/v acetone in the drinking water (Ladefoged *et al*., 1994). Acetone potentiated effects on open field ambulation, or rearing and on the rotarod test. Giant axonal swelling was greater in acetone administered animals. During a dose-free 10-week recovery period, the acetone-supplemented group had less improvement in neurological parameters. Male Wistar rats were administered 0.5% w/v 2,5-hexanedione alone or in combination with 0.50% w/v acetone in the drinking water for 7 weeks (Lam *et al*., 1991). Effects on radial arm maze behavior, a "brain-swelling" reaction, and synaptosomal functions were noted with 2,5-HD and exacerbated with acetone coexposure. In another study of male rats using the same doses for 6 weeks, testis weight, testis tubuli diameter and fertility were reduced with 2,5-HD exposure and potentiated with acetone coexposure (Larsen *et al*., 1991).

Pregnant rats were exposed to 200, 1000, or 5000 ppm n-hexane 20 hours per day on days 9-19 of gestation (Mast *et al.*, 1987). A statistically significant decrease in fetal body weight compared to controls was observed in male offspring following maternal exposure to 1000 and 5000 ppm n-hexane. Maternal toxicity, indicated by decreased body weight gain, was observed in all exposure groups.

Pregnants rats were exposed to hexane (0, 93.4, or 408.7 ppm) on days 6 through 15 of gestation (Litton Bionetics, 1979). There were no adverse effects noted in dams, and no hexane-induced teratogenicity, changes in sex ratio, embryotoxicity, or impaired fetal growth or development.

Male New Zealand rabbits exposed to 3000 ppm n-hexane for 8 hours per day, 5 days per week for 24 weeks developed exposure-related lesions of the respiratory tract with the terminal bronchioles exhibiting the most characteristic damage (Lungarella *et al.*, 1984). These changes were noted even after a 120-day recovery period. Clinical signs of ocular and upper respiratory tract irritation and respiratory difficulties (such as gasping, lung rales, mouth breathing) were observed throughout the study in exposed rabbits.

Derivation of Chronic Reference Exposure Level

Key study	Miyagaki (1967)
Study population	Male mice
Exposure method	Discontinuous inhalation
Critical effects	Peripheral neuropathy (electromyographic alterations; dose-related abnormal posture and muscle atrophy)
LOAEL	250 ppm
NOAEL	100 ppm
Exposure continuity	24 hours/day, 6 days/week
Exposure duration	1 year
Average experimental exposure	57.9 ppm for LOAEL group (100 ppm x 0.675 x 6/7)
Human equivalent concentration	57.9 ppm (gas with systemic effects, based on RGDR = 1 using default assumption that lambda (a) = lambda (h))
LOAEL uncertainty factor	1
Subchronic uncertainty factor	1
Interspecies uncertainty factor	3
Intraspecies uncertainty factor	10
Cumulative uncertainty factor	30
Inhalation reference exposure level	2 ppm (2000 ppb; 7 mg/m^3; 7000 µg/m^3)

Three studies, an experimental study with mice (Miyagaki, 1967) and two occupational studies (Sanagi *et al*., 1980; Chang *et al*., 1993), were considered by OEHHA to be most informative and relevant to the derivation of a chronic REL. This was because these studies (1) evaluated the most sensitive endpoint (peripheral neuropathy) and (2) involved exposures over a significant fraction of a lifetime. While significant limitations may be noted for each of these studies individually, viewed collectively they provide a consistent view of the chronic inhalation toxicity of hexane and yield a stronger basis for deriving a chronic inhalation REL.

While the animal study has the disadvantage of introducing the uncertainty of interspecies differences, the limitations of the human studies were considered to be more significant. Specifically, both human studies were considered likely to overestimate effects of inhalation exposures to hexane.

The Sanagi study, which U.S. EPA used as the basis of its RfC, may overestimate hexane effect because of a confounding coexposure to acetone, which is known to potentiate hexane neuropathy. The minimum effective acetone inhalation concentration for potentiating hexane neuropathy is unclear, as studies (Ladefoged *et al*., 1994; Lam *et al*., 1991; Larsen *et al*., 1991) have used orally administered acetone. The minimum effective acetone inhalation dose for potentiation of carbon tetrachloride hepatotoxicity in male Sprague-Dawley rats was 2500 ppm over 4 hours (Charbonneau *et al*., 1986). A dose of 0.5% acetone in human drinking water is comparable, assuming equal absorption, to an inhalation concentration of approximately 1400

ppm (0.5% w/v x 2 L/day ÷ 2 m^3/day = 5 g/m^3; 5 g/m^3 x 1000 mg/g ÷ 3.52 mg/m^3 per ppm = 1400 ppm). As the acetone potentiating effects were all noted at higher exposures than are being considered in occupational studies and are at much higher concentrations than the REL itself, the significance of these findings is uncertain.

In the Chang study, the workers were probably intermittently exposed to higher inhalation exposures than were estimated from ambient air sampling, and significant dermal exposures were also likely. Furthermore, coexposure to high levels of isopropanol and toluene, may have confounded the results, although CNS effects were not noted and these substances are not known to induce or potentiate peripheral neuropathy.

As shown in Table 1, the human studies by Sanagi *et al*. (1980) and Chang *et al*. (1993) yield 7 to 10-fold lower RELs than the Miyagaki study. In view of the likely overprediction of hexane risks from these studies, due to co-exposure to other materials, which may potentiate the effects of hexane, these calculations may be viewed as generally supporting the 7000 µg/m^3 REL.

Table 1: Reference Exposure Levels (RELs) from Selected Human Studies

Study	Duration	Effect	LOAEL (ppm)	LOAEL (ppm) (TWA)	NOAEL (ppm)	NOAEL (ppm) (TWA)	total UF	REL (ppb)	REL (µg/m^3)
Sanagi *et al.*, 1980	6.2 years	decreased motor nerve conduction velocity; increased residual latency	58	20.7	Not observed		100[a]	200	700
Chang *et al.*, 1993	mean 2.6 years: range 1 month to 12 years	Symptomatic peripheral neuropathy; decreased motor nerve conduction velocity; increased residual latency; axonal swelling of sural nerve	mean 132: range 80 - 210	83	Not observed		300[b]	300	1000

[a] LOAEL uncertainty factor, 10; Intraspecies uncertainty factor, 10
[b] LOAEL uncertainty factor, 10; Subchronic uncertainty factor, 3; Intraspecies uncertainty factor, 10

The hexane exposure estimate was reduced for the Miyagaki data as the solvent used contained 67.5% n-hexane.

The average occupational exposure for the Chang study, which involved an unusual 72-hour work week, was calculated by assuming that 12 hours of occupational exposures at an inhalation rate of 20 L/min was followed by 4 hours of light work at 20 L/min and 8 hours of rest at 7.5 L/min. With these assumptions an estimated 63% of daily inhaled air occurred at the workplace.

The Chang study found that the severity of effects was not correlated with the length of exposure, suggesting that (1) susceptibility may differ markedly between individuals and/or (2) shorter exceedances of the time-weighted average concentration might be significant. Thus the subchronic uncertainty factor was reduced to 3-fold.

VII. Data Strengths and Limitations for Development of the REL

There is a substantial database on the health effects of n-hexane in both humans and animals from which to derive a chronic reference exposure level. Some relevant studies are summarized in the table below.

Study	***Species***	***Exposure concentration***	***Exposure regimen***	***TWA from NOAEL[a]***	***TWA from LOAEL[a]***
Sanagi *et al*. (1980)	Humans	58 ppm (mean)	10 m^3/d, 5 d/wk, 6.2 yr (mean)	None	20.7 ppm
Chang *et al*. (1993)	Humans	130 ppm (mean)	12 hr/d, 6 d/wk, 2.6 yr (mean)	None	83 ppm
Miyagaki (1967)	Male mice	0, 100, 250, 500, 1000, 2000 ppm	Continuous, 6 d/wk, 1 yr	57.9 ppm	121 ppm
Daughtrey *et al*. (1999)	F344 rats	0, 900, 3000, 9000 ppm	6 hr/d, 5 d/wk, 2 yr	None	161 ppm
Daughtrey *et al*. (1999)	B6C3F1 mice	0, 900, 3000, 9000 ppm	6 hr/d, 5 d/wk, 2 yr	None	161 ppm
Dunnick *et al*. (1989)	B6C3F1 mice	0, 500, 1000, 4000, 10,000 ppm	6 hr/d, 5 d/wk, 13 wk	89 ppm	179 ppm
Huang *et al*. (1989)	Wistar rats	0, 500, 1200, 3000 ppm	12 hr/d, 7 d/wk, 16 wk	None	250 ppm
Bio/dynamics (1978)	SD rats	0, 5, 27, 126 ppm	21 hr/d, 7 d/wk, 26 weeks	110 ppm	None
Cavender *et al*. (1984)	F344 rats	0, 3000, 6500, 10,000 ppm	6 hr/d, 5 d/wk, 13 wk	540 ppm	1160 ppm

[a] The experimental exposure was extrapolated to an equivalent (time-weighted average or TWA) continuous exposure.

The major strengths of the REL for hexane include: (1) the primary use of an animal study (Miyagaki, 1967) with controlled, nearly continuous chronic hexane exposures not confounded by coexposure to other solvents, which observed both a NOAEL and LOAEL; and (2) the results obtained from two different human studies (Sanagi, 1980; Chang *et al*., 1993) which were viewed as being generally consistent with the animal study based REL.
There is uncertainty about interspecies as well as intraindividual differences in susceptibility to n-hexane peripheral neuropathy. In one study, controlled TWA exposures of 540 ppm (Cavender *et al*., 1984) were not found to cause neuropathy in rats. Also human studies (especially that of Chang *et al*., 1993) have shown that some individuals develop peripheral neuropathy within months, whereas others remain symptom-free despite years of employment at the same occupation at the same workplace.

OEHHA staff also estimated RELs from two other animal studies for comparison. In Bio/Dynamics (1978), 126 ppm for 21 hours/day, 7 days/week for 26 months was a NOAEL and represents a time-weighted average exposure of 110.2 ppm. Using an RGDR of 1 and a cumulative 30-fold uncertainty factor (3 for interspecies differences not accounted for by the RGDR method and 10-fold for intraspecies differences), a REL of 4 ppm (10,000 $\mu g/m^3$) was derived. Cavender *et al*. (1984) identified a NOAEL for neurotoxicity of 3000 ppm, with an average experimental exposure of 540 ppm. A REL based on this study, using an RGDR of 1 and a 100-fold uncertainty factor (3 for subchronic (13 weeks) to chronic, 3 for interspecies, and 10 for intraspecies) would be 5.4 ppm (19,000 $\mu g/m^3$).

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CHRONIC TOXICITY SUMMARY

HYDROGEN CYANIDE

(*Formonitrile; hydrocyanic acid; prussic acid)*

CAS Registry Number: 74-90-8

I. Chronic Toxicity Summary

Inhalation reference exposure level	**9 µg/m^3** (8 ppb)
Critical effect(s)	CNS effects, thyroid enlargement, and hematological disorders in workers
Hazard index target(s)	Nervous system; endocrine system; cardiovascular system

II. Physical and Chemical Properties (HSDB, 1999)

Description	Colorless liquid/gas
Molecular formula	HCN
Molecular weight	27.03
Boiling point	25.6 ^{o}C
Melting point	–13.4 ^{o}C
Vapor pressure	630 torr @ 20°C
Solubility	Miscible in water, alcohol; slightly soluble in ether
Conversion factor	1 ppm = 1.10 mg/m^3 @ 25 ^{o}C

III. Major Uses or Sources

Hydrogen cyanide is used in a variety of syntheses including the production of adiponitrile (for nylon), methyl methacrylate, sodium cyanide, cyanuric chloride, chelating agents, pharmaceuticals, and other specialty chemicals. Manufacturing activities releasing hydrogen cyanide include electroplating, metal mining, metallurgy and metal cleaning processes. Additionally, hydrogen cyanide has some insecticide and fungicide applications (ATSDR, 1993). Fires involving some nitrogen-containing polymers, often found in fibers used in fabrics, upholstery covers, and padding, also produce hydrogen cyanide (Tsuchiya and Sumi, 1977).

Another common source of hydrogen cyanide is cigarette smoke. Levels in inhaled mainstream cigarette smoke range from 10 to 400 µg per cigarette (U.S. brands); 0.6% to 27% (w/w) of these mainstream levels are found in secondary or sidestream smoke (Fiskel *et al.*, 1981). The annual statewide industrial emissions from facilities reporting under the Air Toxics Hot Spots Act in California based on the most recent inventory were estimated to be 188,665 pounds of hydrogen cyanide (CARB, 1999b).

IV. Effects of Human Exposure

Occupational epidemiological studies of hydrogen cyanide exposure are complicated by the mixed chemical environments, which are created by synthetic and metallurgic processes. However, several reports indicate that chronic low exposure to hydrogen cyanide can cause neurological, respiratory, cardiovascular, and thyroid effects (Blanc *et al.*, 1985; Chandra *et al.*, 1980; El Ghawabi *et al.*, 1975). Although these studies have limitations, especially with incomplete exposure data, they also indicate that long-term exposure to inhaled cyanide produces CNS and thyroid effects.

El Ghawabi *et al.* (1975) studied 36 male electroplating workers in three Egyptian factories exposed to plating bath containing 3% copper cyanide, 3% sodium cyanide, and 1% sodium carbonate. Breathing zone cyanide concentrations ranged from 4.2 to 12.4 ppm (4.6 to 13.7 mg/m^3), with means from 6.4 to 10.4 ppm (7.1 to 11.5 mg/m^3), in the three factories at the time of this cross-sectional study. The men were exposed for a duration of 5 to 10 years, except for one man with 15 years exposure. Twenty non-exposed male volunteers were used as controls. None of the subjects, controls or workers, currently smoked cigarettes. Complete medical histories were taken, and medical exams were performed. Urinary levels of thiocyanate (a metabolite of cyanide) were utilized as a biological index of exposure. Thyroid function was measured as the uptake of radiolabeled iodine, since thiocyanate may block the uptake of iodine by the thyroid leading to iodine-deficiency goiters. Frequently reported symptoms in the exposed workers included headache, weakness, and altered sense of taste or smell. Lacrimation, abdominal colic, and lower stomach pain, salivation, and nervous instability occurred less frequently. Increased blood hemoglobin and lymphocyte counts were present in the exposed workers. Additionally, punctate basophilia were found in 78% (28/36) of the exposed subjects. Twenty of the thirty six exposed workers had thyroid enlargements, although there was no correlation between the duration of exposure with either the incidence or the degree of enlargement. Thyroid function test indicated significant differences in uptake between controls and exposed individuals after 4 and 24 hours. Urinary excretion of thiocyanates correlated with the breathing zone concentrations of cyanides. Symptoms persisted in 50% of the dyspneic workers in a 10-month nonexposure follow up period. This study reported a LOAEL of 6.4 ppm (7.1 mg/m^3) for the CNS symptoms and thyroid effects.

Another retrospective study (Blanc *et al.*, 1985) examined 36 former silver-reclaiming workers with long-term exposure to hydrogen cyanide fumes. The authors found significant trends between the incidence of self-reported CNS symptoms during active employment (headache, dizziness, nausea, and bitter almond taste), the symptoms reported post-exposure, and a qualitative index of exposure retroactively defined by the investigators as low-, moderate-, or high-exposure through work histories. Some symptoms persisted for 7 months or more after exposure. None of the workers had palpable thyroid gland abnormalities, but clinical tests revealed decreases in vitamin B12 absorption and folate levels and statistically significant increases in thyroid-stimulating hormone levels, which in combination with the CNS effects, suggest long-term adverse effects associated with cyanide exposure.

Due to the systemic nature of the lesions produced by cyanide, orally ingested cyanide will likely result in injuries similar to that seen by inhalation exposure. Cassava root, a dietary staple in many tropical regions, contains cyanogenic glycosides, such as linamarin, which release cyanide (CN^-) when metabolized endogenously (Sharma, 1993; Kamalu, 1995). Consumption of insufficiently processed cassava roots over a period of time in combination with a protein deficient diet has been implicated in neurotoxic effects. One such neuropathy known as konzo results in nerve cell degeneration leading to a permanent but non-progressive spastic weakness of the legs and degeneration of corresponding corticospinal pathways (Tylleskar *et al*., 1992; Tor-Agbidye *et al*., 1999). The development of this sydrome is hypothesized to depend on (a) the amount and duration of exposure to dietary cyanide, and (b) the ability of the body to detoxify cyanide, a function that may vary with nutritional status. The endogenous conversion of cyanide to cyanate (OCN^-) is thought to be a contributor to the neurotoxic symptoms, but other substances found in cassava flour have been implicated (Obidoa and Obasi, 1991; Tor-Agbidye *et al*., 1999; Kamalu, 1995). Tylleskar *et al*. (1992) determined daily cassava flour consumption at above 0.5 kg per adult in a konzo-affected, albeit malnourished, African population. Thus, the potential daily cyanide exposure was estimated to be 0.5-1 mmol (13-26 mg), which correlated well with urinary concentrations of the metabolite, thiocyanate. A similar daily cyanide intake via cassava ingestion was estimated at 15-31.5 mg (approximately 0.2-0.45 mg/kg) following a major outbreak of konzo in Mozambique (Casadei *et al*.,1984; Cliff *et al*., 1984).

Other effects associated with cassava consumption include pancreatic diabetes, vitamin B_{12} deficiency and decreased iodine uptake (Sharma, 1993; Jansz and Uluwaduge, 1997). Cretinism in children, associated with a deficiency of dietary iodine, is worsened by eating cassava (Miller, 1974). Excess thiocyanate due to cyanide metabolism results in a depressed uptake of iodine by the thyroid gland that may lead to symptoms of iodine deficiency, including goiter. A comparison of three villages in Ethiopia observed increased total goiter rate with increasing rate of cassava consumption (Abuye *et al*., 1998). Goiter was also more prevalent in females and in individuals under 20 years of age. In one village, the incidence of goiter increased following the introduction of cassava, indicating that cassava exacerbated pre-existing iodine deficiency. Urinary iodine levels of school children revealed marginal dietary consumption of iodine, but were within the normal range. However, low T4 and high TSH levels indicated insufficient iodine uptake by the thyroid gland due to cassava consumption.

V. Effects of Animal Exposures

There is little animal data for chronic inhalation exposure to hydrogen cyanide; only two subchronic studies were noted by U.S. EPA, one in rabbits (Hugod, 1979, 1981) and the other in dogs (Valade, 1952). Continuous exposure of rabbits to 0.5 ppm HCN (0.55 mg/m^3) for either 1 or 4 weeks produced no microscopically detectable morphological changes of the lungs, pulmonary arteries, coronary arteries or aorta. This study observed a subacute inhalation NOAEL for HCN in rabbits of 0.5 ppm (Hugod, 1979, 1981). Four dogs exposed to 50 mg/m^3 (45 ppm) hydrogen cyanide in a series of 30-minute inhalation periods conducted at 2-day intervals demonstrated extensive CNS toxicity, including dyspnea and vomiting, with vascular and cellular CNS lesions identified post-mortem (Valade, 1952).

Male Sprague-Dawley rats were administered potassium cyanide (0, 40, 80, or 160 mg KCN/kg bw-day) in the drinking water for 13 weeks (Leuschner *et al*., 1991). At the highest dose, blood cyanide concentrations were between 16 and 26 mmol CN^-/ml blood and thiocyanate ranged between 341 and 877 mmmol SCN^-/ml plasma. The high dose group exposure was reduced to 140 mg/kg-day after 12 weeks because of decreased body weight gain, reduced drinking water consumption, and mortality in this group.

Male New Zealand white rabbits (6 per group) were administered potassium cyanide in the diet over a 40 week experiment (Okolie and Osagie, 1999). The average cyanide intake was 36.5 mg/day. Based on the growth data presented in the report, cyanide intake was estimated at approximately 20 mg/kg-day. The cyanide-exposed group had higher feed consumption with reduced weight gain, and focal necrosis was noted in the liver and kidney.

Male weanling rats (strain not identified, 10 animals per group) were administered potassium cyanide (1500 ppm) in the diet for 11.5 months (Philbrick *et al*., 1979). There were no deaths or overt signs of toxicity. There was a reduction in body weight gain in the exposed group. Myelin degeneration was noted in the spinal cord white matter of cyanide exposed animals.

Kamalu (1993) fed groups of dogs (6/group; strain not specified) either a control diet containing rice as the carbohydrate source, a diet with cassava as a carbohydrate source, or a control diet containing NaCN, for 14 weeks. Both the cassava and NaCN diets were adjusted to release 10.8 mg HCN/kg cooked food. Growth was depressed only in the dogs fed rice + NaCN. Plasma thiocyanate was significantly lower in dogs fed cassava compared to dogs fed rice + NaCN. These effects indicate that all the intact cyanogenic glycosides absorbed from cassava, primarily linamarin, was not hydrolyzed to HCN. However, evidence of liver inflammation and hemorrhage were observed only in the cassava fed dogs. Kidney, adrenal, myocardial, and testicular lesions were noted in both treated groups, but were considered more severe in the cassava fed dogs. It was concluded that the lesions, observed in the cassava fed dogs, were not entirely due to cyanide.

No information was found regarding developmental and reproductive effects in humans for any route of hydrogen cyanide exposure. No animal studies utilizing dermal exposure have been reported for either hydrogen cyanide or cyanide salts. Dietary studies of the high cyanogenic glycoside cassava diet have shown adverse effects, increased runting and decreased ossification in hamsters (Frakes *et al.*, 1986), but not in rats fed cassava alone, or supplemented with potassium cyanide (Tewe and Maner, 1981). Hamsters with gestational cassava exposure did not display reproductive effects (Frakes *et al.*, 1986).

VI. Derivation of Chronic Reference Exposure Level

Study	El Ghawabi *et al.* (1975); U.S. EPA (1994)
Study population	36 male electroplating workers
Exposure method	Discontinuous occupational inhalation exposures
Critical effects	CNS effects, thyroid enlargement, and hematological disorders
LOAEL	7.1 mg/m^3
NOAEL	Not observed
Exposure continuity	8 hr/day (10 m^3/day/20 m^3/day), 5 days/week
Average occupational exposure	2.5 mg/m^3 for LOAEL group
Human equivalent concentration	2.5 mg/m^3 for LOAEL group
Exposure duration	5 to 10 years (except one man for 15 years)
LOAEL uncertainty factor	10
Subchronic uncertainty factor	3
Interspecies uncertainty factor	1
Intraspecies uncertainty factor	10
Cumulative uncertainty factor	300
Inhalation reference exposure level	0.008 ppm (8 ppb, 0.009 mg/m^3, 9 µg/m^3)

The USEPA based its RfC of 3 µg/m^3 on the same study but included a Modifying Factor (MF) of 3 for lack of chronic and multigenerational reproduction studies. The criteria for use of modifying factors are not well specified by U.S. EPA. Such modifying factors were not used by OEHHA. OEHHA used a 3-fold subchronic uncertainty factor because most workers were exposed for less than ten years (78%) and many were exposed for less than 5 years (39%)..

An alternative analysis was conducted using data from an animal ingestion study reporting effects at low cyanide concentrations:

Study	Jackson (1988)
Study population	Miniature swine
Exposure method	Daily oral administration of aqueous potassium cyanide
Critical effects	Behavioral effects; decreased blood T_3 and T_4
LOAEL	0.4 mg/kg-day
NOAEL	Not observed
Exposure continuity	Apparently 7 days per week
Average exposure	0.4 mg/kg-day (1.4 mg/m^3 for LOAEL group assuming 20 m^3/day inhalation by a 70 kg person)
Human equivalent concentration	Not derived due to lack of species-specific data
Exposure duration	24 weeks
LOAEL uncertainty factor	3 (minimal effects at lowest dose)
Subchronic uncertainty factor	10 (based on assumed 27 year lifespan)
Interspecies uncertainty factor	10
Intraspecies uncertainty factor	10

Cumulative uncertainty factor	3,000
Inhalation reference exposure level	0.0005 mg/m^3 (0.5 µg/m^3; 0.0004 ppm; 0.4 ppb)

This study reported neurobehavioural and thyroid effects at cyanide exposure levels (equivalent to 1.4 to 4.2 mg/m^3) similar to that reported by El Ghawabi (2.5 mg/m^3). However, as greater uncertainty factors are required for use of the animal study, a lower REL was derived. Use of a cross-route extrapolation also introduces uncertainty. Therefore the REL derived from the human data is more appropriate.

VII. Data Strengths and Limitations for Development of the REL

The major strength of the RfC for hydrogen cyanide is the use of human health effects data. The major uncertainties are the lack of a NOAEL observation in the key study, the difficulty in estimating exposures, and the discontinuous and variable nature of the exposures.

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CHRONIC TOXICITY SUMMARY

HYDROGEN SULFIDE

(*hydrogen sulphide; dihydrogen sulfide; dihydrogen monosulfide; sulfur hydride; sulfureted hydrogen; hydrosulfuric acid*)

CAS registry number: 7783-06-4

I. Chronic Toxicity Summary

Inhalation reference exposure level	**10 mg/m^3** (8 ppb)
Critical effect(s)	Nasal histological changes in B6C3F1 mice
Hazard index target(s)	Respiratory system

II. Physical and Chemical Properties (HSDB, 1999)

Description	Colorless gas
Molecular formula	H_2S
Molecular weight	34.08
Density	1.4 g/L @ 25° C (air = 1) (AIHA, 1991)
Boiling point	–60.7° C (CRC, 1994)
Melting point	–85.5ºC (CRC, 1994)
Vapor pressure	15,600 torr @ 25ºC
Solubility	Soluble in water, hydrocarbon solvents, ether, and ethanol
Odor threshold	8.1 ppb (11 µg/m^3) (Amoore and Hautala, 1983)
Odor description	Resembles rotten eggs
Conversion factor	1 ppm = 1.4 mg/m^3 @ 25° C

III. Major Uses or Sources

Hydrogen sulfide (H_2S) is used as a reagent and an intermediate in the preparation of other reduced sulfur compounds (HSDB, 1999). It is also a by-product of desulfurization processes in the oil and gas industries and rayon production, sewage treatment, and leather tanning (Ammann, 1986). The annual statewide industrial emissions from point sources at facilities reporting under the Air Toxics Hot Spots Act in California based on the most recent inventory were estimated to be 5,688,172 pounds of hydrogen sulfide (CARB, 1999).

IV. Effects of Human Exposure

Although numerous case studies of acutely toxic effects of H_2S exist, there is inadequate occupational or epidemiological information for specific chronic effects in humans exposed to H_2S.

Bhambhani and Singh (1991) showed that 16 healthy subjects exposed for short durations to 5 ppm (7 mg/m^3) H_2S under conditions of moderate exercise exhibited impaired lactate and oxygen uptake in the blood. Bhambhani and Singh (1985) reported that exposure of 42 individuals to 2.5 to 5 ppm (3.5 to 7 mg/m^3) H_2S caused coughing and throat irritation after 15 minutes.

In another study, ten asthmatic volunteer subjects were exposed to 2 ppm H_2S for 30 minutes and pulmonary function was tested (Jappinen *et al.*, 1990). All subjects reported detecting “very unpleasant” odor but “rapidly became accustomed to it.” Three subjects reported headache following exposure. No significant changes in mean FVC or FEV_1 were reported. Although individual values for specific airway resistance (SR_{aw}) were not reported, the difference following exposure ranged from –5.95% to +137.78%. The decrease in specific airway conductance, SG_{aw}, ranged from -57.7% to +28.9%. The increase in mean SR_{aw} and decrease in mean SG_{aw} were not statistically significant.

Kilburn and Warshaw (1995) investigated whether people exposed to sulfide gases, including H_2S, as a result of working at or living downwind from the processing of "sour" crude oil demonstrated persistent neurobehavioral dysfunction. They studied thirteen former workers and 22 neighbors (of a California coastal oil refinery) who complained of headaches, nausea, vomiting, depression, personality changes, nosebleeds, and breathing difficulties. Their neurobehavioral functions and a profile of mood states were compared to 32 controls (matched for age and educational level). The exposed subjects' mean values were statistically significantly different (abnormal) compared to controls for several tests (two-choice reaction time; balance (as speed of sway); color discrimination; digit symbol; trail-making A and B; immediate recall of a story). Their profile of mood states scores were much higher than those of controls. Visual recall was significantly impaired in neighbors, but not in the former workers. The authors concluded that neurophysiological abnormalities were associated with exposure to reduced sulfur gases, including H_2S from crude oil desulfurization.

Xu *et al*. (1998) conducted a retrospective epidemiological study in a large petrochemical complex in Beijing, China in order to assess the possible association between petrochemical exposure and spontaneous abortion. The facility consisted of 17 major production plants divided into separate workshops, which allow for the assessment of exposure to specific chemicals. Married women (n = 2853), who were 20-44 years of age, had never smoked, and who reported at least one pregnancy during employment at the plant, participated in the study. According to their employment record, about 57% of these workers reported occupational exposure to petrochemicals during the first trimester of their pregnancy. There was a significantly increased risk of spontaneous abortion for women working in all of the production plants with frequent exposure to petrochemicals compared with those working in nonchemical plants. Also, when a comparison was made between exposed and non-exposed groups within each plant, exposure to

petrochemicals was consistently associated with an increased risk of spontaneous abortion (overall odds ratio (OR) = 2.7 (95% confidence interval (CI) = 1.8 to 3.9) after adjusting for potential confounders). When the analysis was performed with the exposure information obtained from interview responses for (self reported) exposures, the estimated OR for spontaneous abortions was 2.9 (95% CI = 2.0 to 4.0). When the analysis was repeated by excluding those 452 women who provided inconsistent reports between recalled exposure and work history, a comparable risk of spontaneous abortion (OR 2.9; 95% CI = 2.0 to 4.4) was found. In analyses for exposure to specific chemicals, an increased risk of spontaneous abortion was found with exposure to most chemicals. There were 106 women (3.7% of the study population) exposed only to hydrogen sulfide, and the results for hydrogen sulphide (OR 2.3; 95% CI = 1.2 to 4.4) were significant. No hydrogen sulfide exposure concentration was reported.

Four workers were exposed for several minutes to concentrations of hydrogen sulfide sufficient to cause unconciousness. Four other workers were exposed chronically to H_2S and developed lacrimation, eye irritation, nausea, vomiting, headache, sore throat, and skin irritation but retained conciousness as the result of a 150-minute release. Both groups were subjected to olfactory testing 2 to 3 years later (Hirsch and Zavala, 1999). Six of eight workers showed deficits in odor detection and identification, with the workers who had experienced unconciousness most severely affected in the followup tests.

Three patients exposed acutely to unknown concentrations of hydrogen sulfide developed persistent cognitive impairment (Wasch *et al*,. 1989). While standard neurological and physical examinations were unremarkable, all three subjects had prolonged P-300 latencies and persistent neurological and neurobehavioral deficits.

V. Effects of Animal Exposure

Rats (Fischer and Sprague-Dawley, 15 per group) were exposed to 0, 10.1, 30.5, or 80 ppm (0, 14.1, 42.7, or 112 mg/m^3, respectively) H_2S for 6 hours/day, 5 days/week for 90 days (CIIT, 1983a,b). Measurements of neurological and hematological function revealed no abnormalities due to H_2S exposure. A histological examination of the nasal turbinates also revealed no significant exposure-related changes. A significant decrease in body weight was observed in both strains of rats exposed to 80 ppm (112 mg/m^3).

In a companion study, the Chemical Industry Institute of Toxicology conducted a 90-day inhalation study in mice (10 or 12 mice per group) exposed to 0, 10.1, 30.5, or 80 ppm (0, 14.1, 42.7, or 112 mg/m^3, respectively) H_2S for 6 hours/day, 5 days/week (CIIT, 1983c). Neurological function was measured by tests for posture, gait, facial muscle tone, and reflexes. Ophthalmological and hematological examinations were also performed, and a detailed necropsy was included at the end of the experiment. The only exposure-related histological lesion was inflammation of the nasal mucosa of the anterior segment of the noses of mice exposed to 80 ppm (112 mg/m^3) H_2S. Weight loss was also observed in the mice exposed to 80 ppm. Neurological and hematological tests revealed no abnormalities. The 30.5 ppm (42.5 mg/m^3) level was considered the NOAEL for histological changes in the nasal mucosa. (Adjustments were made by U. S. EPA to this value to calculate an RfC of 0.9 μg/m^3.)

Fischer F344 rats inhaled 0, 1, 10, or 100 ppm hydrogen sulfide for 8 hours/day for 5 weeks (Hulbert *et al*, 1989). No effects were noted on baseline measurements of airway resistance, dynamic compliance, tidal volume, minute volume, or heart rate. Two findings were noted more frequently in exposed rats: (1) proliferation of ciliated cells in the tracheal and bronchiolar epithelium, and (2) lymphocyte infiltration of the bronchial submucosa. Some exposed animals responded similarly to controls to aerosol methacholine challenge, whereas a subgroup of exposed rats were hyperreactive to concentrations as low as 1 ppm.

Male rats were exposed to 0, 10, 200, or 400 ppm H_2S for 4 hours (Lopez *et al.*, 1987). Samples of bronchoalveolar and nasal lavage fluid contained increased inflammatory cells, protein, and lactate dehydrogenase in rats treated with 400 ppm. Lopez and associates later showed that exposure to 83 ppm (116 mg/m^3) for 4 hours resulted in mild perivascular edema (Lopez *et al.*, 1988).

A study by Saillenfait *et al.* (1989) investigated the developmental toxicity of H_2S in rats. Rats were exposed 6 hours/day on days 6 through 20 of gestation to 100 ppm hydrogen sulfide. No maternal toxicity or developmental defects were observed..

Hayden *et al*. (1990) exposed gravid Sprague-Dawley rat dams continuously to 0, 20, 50, and 75 ppm H_2S from day 6 of gestation until day 21 postpartum. The animals demonstrated normal reproductive parameters until parturition when delivery time was extended in a dose dependent manner (with a maximum increase of 42% at 75 ppm). Pups which were exposed in utero and neonatally to day 21 postpartum developed with a subtle decrease in time of ear detachment and hair development and with no other observed change in growth and development through day 21 postpartum.

VI. Derivation of Chronic REL

Study	CIIT, 1983c
Study population	B6C3F1 mice (10-12 per group)
Exposure method	Discontinuous inhalation
Critical effects	Histopathological inflammatory changes in the nasal mucosa
LOAEL	80 ppm (112 mg/m^3)
NOAEL	30.5 ppm (42.5 mg/m^3)
Exposure continuity	6 hours/day, 5 days/week
Exposure duration	90 days
Average experimental exposure	5.4 ppm for NOAEL group (30.5 x 6/24 x 5/7)
Human equivalent concentration	0.85 ppm (gas with extrathoracic respiratory effects, RGDR = 0.16, based on mouse MV_a = 0.033 L/min; MV_h = 13.8 L/min; $SA_a(ET)$ = 3.0 cm^2; $Sa_h(ET)$ = 200 cm^3) (U.S. EPA, 1994)
LOAEL uncertainty factor	1
Subchronic uncertainty factor	3
Interspecies uncertainty factor	3
Intraspecies uncertainty factor	10
Cumulative uncertainty factor	100
Inhalation reference exposure level	8 ppb (10 μg/m^3)

The adverse effects reported in chronic animal studies occur at higher concentrations than effects seen in acute human exposures. For example, human irritation was reported at concentrations of 2.5-5 ppm for 15 minutes (Bhambhani and Singh, 1985), yet no effects on laboratory animals were observed at concentrations up to 80 ppm for 90 days. This suggests either that humans are more sensitive to H_2S, or that the measurements in laboratory animals are too crude to detect subtle measures of irritation. However, the uncertainty factor and HEC attempt to account for these interspecies differences.

VII. Data Strengths and Limitations for Development of the REL

Hydrogen sulfide is the leading chemical agent causing human fatalities following inhalation exposures. Although lower concentration acute exposures have been quantitatively studied with human volunteers, the dose-response relationship for human toxicity due to hydrogen sulfide exposure is not known. Thus, a major area of uncertainty is the lack of adequate long-term human exposure data. Subchronic (but not chronic) studies have been conducted with several animal species and strains, and these studies offer an adequate basis for quantitative risk assessment.

The strengths of the inhalation REL include the availability of controlled exposure inhalation studies in multiple species at multiple exposure concentrations, adequate histopathogical analysis, and the observation of a NOAEL.

Hydrogen sulfide has a strong unpleasant odor. The threshold for detection of this odor is low, but shows wide variation among individuals. A level of 7 $\mu g/m^3$, based on a 30 minute averaging time, was estimated by a Task Force of the International Programme on Chemical Safety (IPCS) (1981) to not produce odor nuisance in most situations. On the other hand, the current California Ambient Air Quality standard for hydrogen sulfide, based on a 1 hour averaging time, is 42 $\mu g/m^3$ (30 ppb).

Amoore (1985) analyzed a large number of reports from the scientific literature and found that reported thresholds for detection were log-normally distributed, with a geometric mean of 10 $\mu g/m^3$ (8 ppb). Detection thresholds for individuals were reported to be log-normally distributed in the general population, with a geometric standard deviation of 4.0, *i.e.* 68% of the general population would be expected to have a detection threshold for hydrogen sulfide between 2.5 and 40 $\mu g/m^3$ (2 and 32 ppb). Sources of variation included age, sex, medical conditions, and smoking. Training and alertness of the subject in performing the test also affected the results.

Amoore (1985) drew attention to the difference between a detection threshold under laboratory conditions, and the levels at which an odor could be recognized, or at which it was perceived as annoying. Analysis of various laboratory and sociological studies suggested that a level at which an odor could be recognized was typically a factor of three greater than the threshold for detection, while the level at which it was perceived as annoying was typically a factor of five greater than the threshold. Annoyance was characterized both in terms of esthetic or behavioral responses, and by physiological responses such as nausea and headache. He therefore predicted that, although at 10 $\mu g/m^3$ (the proposed REL) 50% of the general population would be able to detect the odor of hydrogen sulfide under controlled conditions, only 5% would find it annoying at this level. At 50 $\mu g/m^3$, 50% would find the odor annoying.

On this basis, the proposed REL of 10 $\mu g/m^3$ (8 ppb) is likely to be detectable by many people under ideal laboratory conditions, but it is unlikely to be recognized or found annoying by more than a few. It is therefore expected to provide reasonable protection from odor annoyance in practice. However, this consideration cannot be entirely dismissed due to the wide inter-individual variation in sensitivity to odors. Amoore (1985) also points out that many industrial operations generating hydrogen sulfide also generate organic thiol compounds with similar, but even more potent odors (e.g., methyl mercaptan, butyl mercaptan). Such compounds may in fact have detection thresholds as much as a hundred-fold lower than hydrogen sulfide, so even minute quantities have a powerful impact on odor perception. Because of the concurrent emission of these contaminants, the incidence of odor complaints near hydrogen sulfide emitting sites correlated poorly with the levels of hydrogen sulfide measured in the affected areas.

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CHRONIC TOXICITY SUMMARY

MANGANESE AND COMPOUNDS

Molecular Formula	*Synonyms*	*Molecular Weight*	*CAS Reg. No.*
Mn	elemental manganese; colloidal manganese; cutaval	54.94 g/mol	7439-96-5
MnO	manganese oxide; manganese monoxide; manganosite	70.94 g/mol	1344-43-0
MnO_2	manganese dioxide; black manganese oxide	86.94 g/mol	1313-13-9
Mn_3O_4	manganese tetroxide; trimanganese tetraoxide; manganomanganic oxide	228.82 g/mol	1317-35-7
$MnCl_2$	manganese chloride; manganese dichloride; manganous chloride	125.84 g/mol	7773-01-5

I. Chronic Toxicity Summary

Inhalation reference exposure level	**0.2 µg/m^3**
Critical effect(s)	Impairment of neurobehavioral function in humans
Hazard index target(s)	Nervous system

II. Physical and Chemical Properties (HSDB, 1999)

Description	Lustrous, gray-pink metal (Mn); green (MnO), black (MnO_2) or pink ($MnCl_2$) crystals; brownish-black powder (Mn_3O_4)
Molecular formula	See above
Molecular weight	See above
Density (in g/cm^3)	7.21-7.4 (Mn – depending on allotropic form); 5.43-5.46 (MnO); 4.88 (Mn_3O_4); 2.977 @ 25°C ($MnCl_2$)
Boiling point	1962°C (Mn); not available (MnO); unknown (Mn_3O_4); 1190°C ($MnCl_2$)
Melting point	1244 ± 3°C (Mn); 1650°C (MnO); 2847°C (Mn_3O_4 - NIOSH Pocket GuideTM, 1995); 650°C ($MnCl_2$)
Vapor pressure	1 torr @ 1292°C (Mn); non-volatile at room temperature (Mn_3O_4); not available (MnO; $MnCl_2$)
Solubility	Sol. in dil. acids and aq. solns. of Na- or K-

	bicarbonate (Mn); sol. in NH_4Cl, insol. in H_2O (MnO); insol. in H_2O, HNO_3, or cold H_2SO_4 (MnO_2 - Reprotext®, 1995); insol. in H_2O, sol. in HCl (Mn_3O_4); 72.3 g/100 ml H_2O @ 25°C ($MnCl_2$)
Conversion factor	Not applicable (dusts or powders)

III. Major Uses or Sources

Metallic manganese is used in the manufacturing of steel, carbon steel, stainless steel, cast iron, and superalloys to increase hardness, stiffness, and strength (HSDB, 1995). Manganese chloride is used in dyeing, disinfecting, batteries, and as a paint drier and dietary supplement. Manganese oxide (MnO) is used in textile printing, ceramics, paints, colored glass, fertilizers, and as food additives. Manganese dioxide is used in batteries and may also be generated from the welding of manganese alloys. Manganese tetroxide may be generated in situations where other oxides of manganese are heated in air (NIOSH Pocket Guide, 1995). The annual statewide industrial emissions from facilities reporting under the Air Toxics Hot Spots Act in California based on the most recent inventory were estimated to be 126,107 pounds of manganese (CARB, 1999).

IV. Effects of Human Exposure

Male workers (n=92, plus 101 matched controls) in an alkaline battery plant in Belgium exposed to manganese dioxide were the subject of a cross-sectional epidemiological investigation (Roels *et al.*, 1992). Evaluation of the subjects included tests for neurobehavioral function, lung function, hematological parameters, and urinalysis. Exposed workers showed significant differences in performance on tests of visual reaction time, eye-hand coordination, and hand tremor. Occupational-lifetime integrated respiratory dust (IRD) levels ranged from 0.04-4.43 mg Mn/m^3-yr with a geometric mean of 0.793 mg Mn/m^3-yr. Average exposure time was 5.3 years, with a range of 0.2-17.7 years. The authors grouped the workers into three exposure groups based on the IRD levels: <0.6, 0.6-1.2, and >1.2 mg Mn/m^3-yrs. Although there was an indication of a linear dose-related trend for visual reaction time and hand steadiness, the authors concluded that "analysis of the data on a group basis...does not permit us to identify a threshold effect level for airborne Mn." A daily average exposure level of 0.15 mg Mn/m^3 was derived by dividing the geometric mean of the IRD (0.793 mg Mn/m^3-yr) by the average exposure time (5.3 yr).

In an earlier study, 141 male workers plus 104 matched control workers were examined for effects of exposure to MnO_2, manganese tetroxide (Mn_3O_4), and other manganese salts (Roels *et al.*, 1987). Tests measuring visual reaction time, eye-hand coordination, hand tremor, and short-term memory were found to be significantly different in the manganese-exposed group. Statistically significant clinical symptoms (as evaluated in a questionnaire) included fatigue, tinnitus, finger trembling and irritability. Self-reported prevalence of coughs, colds and acute bronchitis were increased in the manganese exposed group relative to controls. Mean time of

employment was 7.1 years, with a range of 1-19 years. Total airborne manganese dust levels had an arithmetic mean of 1.33 mg/m^3 and a geometric mean of 0.94 mg/m^3.

Several other studies have identified neurobehavioral endpoints of manganese toxicity in human populations. A matched-pair cross-sectional study investigated 74 pairs of manganese alloy workers (Mergler *et al.*, 1994). Matched pairs were found to be discordant in reporting a number of adverse clinical symptoms including the following areas: fatigue, emotional state, memory, attention, concentration difficulty, nightmares, unusual sweating, sexual dysfunction, lower back pain, joint pain, and tinnitus. Motor function tests also revealed deficits in the manganese exposed group. Olfactory perception was enhanced in the manganese exposed group. Exposure levels were estimated at a geometric mean of 0.035 mg Mn/m^3 for respirable dust and 0.225 mg Mn/m^3 for total dust. Mean duration of exposure was 16.7 years.

Workers in two Swedish foundries were evaluated for potential neurobehavioral effects from exposure to manganese (Iregren, 1990). Exposure levels ranged from 0.02-1.4 mg Mn/m^3 with a mean of 0.25 mg Mn/m^3. Simple reaction time, standard deviation of reaction time, finger-tapping speed, digit-span short term memory, speed of mental addition, and verbal understanding were significantly different from controls among manganese exposed workers.

Further reporting of the workers described by Iregren (1990) evaluated more neurobehavioral and electrophysiological endpoints of toxicity from manganese exposure (Wennberg *et al.*, 1991; Wennberg *et al.*, 1992). Although many of the parameters measured showed differences (increased self-reported health symptoms, increased abnormal EEGs, abnormal extrapyramidal function), these results were not statistically significant.

The workers reported on by Roels *et al.* (1987) were examined for potential reproductive toxicity (Lauwerys *et al.*, 1985). These investigators found that for workers divided into certain age groups (16-25 and 26-35), there was a decrease in the number of children born to these workers.

Evaluation of reproductive toxicity in the workers reported by Roels *et al.* (1992) showed no difference in the probability of live birth in a comparison of manganese exposed workers with controls (Gennart *et al.*, 1992). Comparison of reproductive hormones (FSH, LH, prolactin) also showed no differences between the groups.

Junior high school students exposed to manganese were examined for potential effects on the respiratory system (Nogawa *et al.*, 1973). Measurement of atmospheric manganese levels showed a 5-day average level of 0.0067 mg Mn/m^3 300 m from the school.

V. Effects of Animal Exposure

Toxic effects have been described in animals exposed to manganese compounds by inhalation (Shiotsuka, 1984; Suzuki *et al.*, 1978; Moore *et al.*, 1975). Shiotsuka *et al.* (1984) demonstrated increased incidence of pneumonia among rats exposed for 2 weeks to manganese dioxide concentrations ranging from 68-219 mg/m^3. Monkeys exposed to manganese dioxide concentrations ranging from 0.7-3.0 mg/m^3 for 10 months showed increased incidence of

pulmonary emphysema (Suzuki *et al.*, 1978). Hamsters and rats exposed for 56 days to 0.117 mg Mn_3O_4/m^3 showed bronchial lesions (Moore *et al.*, 1975).

High concentrations of manganese (>10 mg/m^3) have decreased host resistance in exposed animals (Adkins *et al.*, 1980; Bergstrom, 1977; Maigetter *et al.*, 1976).

Nine month inhalation toxicity studies in rats and monkeys exposed to levels as high as 1.15 mg Mn_3O_4/m^3 produced no significant pulmonary effects (Ulrich *et al.*, 1979a; Ulrich *et al.*, 1979b; Ulrich *et al.*, 1979c). Monkeys and rats were continuously exposed over nine months to 11.6, 112.5, or 1152 µg Mn/m^3 as Mn_3O_4 aerosol (aerodynamic diameter of approximately 0.11 µm) (Ulrich *et al*., 1979a). Body weight gain was accelerated in rats exposed to the highest dose. Hemoglobin concentrations were slightly increased for both sexes and both species exposed to 1152 µg Mn/m^3. No significant effects on organ weights or histopathologic findings were reported (Ulrich *et al*., 1979b). No significant effects on pulmonary function, limb tremor, or electromyographic activity were noted (Ulrich *et al*., 1979c).

Specific uptake of manganese through the olfactory mucosa to olfactory bulbs of the brain followed by widespread brain distribution has been reported (Tjalve *et al*., 1996). This effect complicates the use of animal inhalation data in estimating human health effects.

VI. Derivation of Chronic Reference Exposure Level

Study	Roels *et al.*, 1992
Study population	Occupationally-exposed humans
Exposure method	Discontinuous occupational inhalation exposure to manganese dioxide (0.2, 1.0, and 6.0 mg/m^3)
Critical effects	Impairment of neurobehavioral function
LOAEL	0.15 mg respirable manganese $dust/m^3$ (geometric mean from exposures of 0.040 to 4.4 mg Mn/m^3-years)
NOAEL	Not observed
Study continuity	8 hours per day, 5 days per week
Average occupational exposure	0.054 mg/m^3 for LOAEL group (based on an 8-hour TWA occupational exposure to 10 m^3 manganese contaminated air per day out of 20 m^3 total air inhaled per day over 5 days/week)
Human equivalent concentration	0.054 mg/m^3 for LOAEL group
Study duration	5.3 years (average; range = 0.2-17.7))
LOAEL uncertainty factor	10
Subchronic uncertainty factor	3
Interspecies uncertainty factor	1
Intraspecies uncertainty factor	10
Cumlative uncertainty factor	300
Inhalation reference exposure level	0.2 $\mu g/m^3$

OEHHA used the same study on which USEPA based its RfC of 0.05 $\mu g/m^3$. USEPA included a Modifying Factor (MF) of 3 for database deficiencies (lack of developmental data and potential differences in toxicity for different forms of manganese). The criteria for use of modifying factors are not well specified by U.S. EPA. Such modifying factors were not used by OEHHA.

In the derivation of its reference concentration for manganese and compounds, the U.S. EPA selected the Roels *et al.* (1992) study for establishing the exposure level associated with adverse health effects. Although this study did not establish a no-observed-adverse-effect-level (NOAEL), clear evidence of toxicity was established at the level of exposure, which was found in the facility studied, and was therefore taken to be a LOAEL. This study offers several advantages over the other available studies of manganese toxicity. (1) The study population was human. (2) The workers were only exposed to a single manganese compound. (3) The study population was well controlled for with matching for age, height, weight, work schedule, coffee and alcohol consumption, and smoking. (4) The exposure duration was relatively long and work practice continuity suggests exposure levels changed little over time. (5) The effects observed were consistent with those observed among other workers occupationally exposed to manganese.

VII. Data Strengths and Limitations for Development of the REL

The strengths of the inhalation REL for manganese include the use of human exposure data from workers exposed over a period of years. Major areas of uncertainty are the lack of observation of a NOAEL, the uncertainty in estimating exposure and the potential variability in exposure concentration, the lack of chronic inhalation exposure studies, and the lack of reproductive and developmental toxicity studies.

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CHRONIC TOXICITY SUMMARY

METHANOL

(methyl alcohol, wood spirit, carbinol, wood alcohol, wood naphtha)

CAS Registry Number: 67-56-1

I. Chronic Toxicity Exposure Level

Inhalation reference exposure level	**4,000** m**g/m^3** (3,000 ppb)
Critical effect(s)	Increased incidence of abnormal cervical ribs, cleft palate, and exencephaly in mice
Hazard index target(s)	Teratogenicity

II. Chemical Property Summary (HSDB, 1999; CRC, 1994)

Description	Colorless liquid
Molecular formula	CH_3OH
Molecular weight	32.04 g/mol
Boiling point	64.6°C
Melting point	–97.6°C
Vapor pressure	92 torr at 20°C
Solubility	Methanol is miscible with water, ethanol, ether and many other organic solvents.
Conversion factor	1 ppm = 1.31 mg/m^3

III. Major Uses and Sources

Originally distilled from wood, methanol is now manufactured synthetically from carbon oxides and hydrogen. Methanol is used primarily for the manufacture of other chemicals and as a solvent. It is also added to a variety of commercial and consumer products such as windshield washing fluid and de-icing solution, duplicating fluids, solid canned fuels, paint remover, model airplane fuels, embalming fluids, lacquers, and inks. Methanol is also used as an alternative motor fuel (HSDB, 1999). The annual statewide industrial emissions from facilities reporting under the Air Toxics Hot Spots Act in California based on the most recent inventory were estimated to be 3,009,776 pounds of methanol (CARB, 1999b).

IV. Effects of Human Exposure

The majority of the available information on methanol toxicity in humans relates to acute rather than chronic exposure. The toxic effects after repeated or prolonged exposure to methanol are

believed to be qualitatively similar but less severe than those induced by acute exposure (Kavet and Nauss, 1990). These effects include CNS and visual disturbances such as headaches, dizziness, nausea and blurred vision. The role of formate, a metabolite of methanol, in chronic toxicity is unclear.

In one study, symptoms of blurred vision, headaches, dizziness, nausea and skin problems were reported in teachers aides exposed to duplicating fluid containing 99% methanol (Frederick *et al.*, 1984). Individual aides worked as little as 1 hr/day for 1 day a week to 8 hrs/day for 5 days/wk. The workers' total exposure duration was not mentioned. A dose-response relationship was observed between the self-reported amount of time spent at the duplicator and the incidence of symptoms. The concentrations of methanol in the breathing zones near the machines in 12 schools ranged from 485 to 4096 mg/m^3 (365 to 3080 ppm) for a 15 minute sample.

Forty-five percent of duplicating machine operators experienced blurred vision, headache, nausea, dizziness and eye irritation (NIOSH, 1981). Air concentrations of methanol for 25 minutes near the machines averaged 1330 mg/m^3.
Employees working in the proximity of direct process duplicating machines complained of frequent headaches and dizziness (Kingsley and Hirsch, 1954). Air concentrations of methanol ranged from 15 ppm (20 mg/m^3) to 375 ppm (490 mg/m^3).

Thirty young women, who had polished wood pencils with a varnish containing methanol, all experienced headaches, gastric disorders, vertigo, nausea and blurred vision (Tyson, 1912; as cited in NIOSH, 1976).

None of the above studies specified the workers' total duration of exposure.

Ubaydullayev (1968) exposed 3 to 6 subjects to methanol vapor for short durations (40 minutes for some subjects and others for an unspecified amount of time). Electrical reflex activity in the cortex of the brain was significantly altered upon exposures to 1.17 mg/m^3 (0.89 ppm) or 1.46 mg/m^3 (1.11 ppm). No effect was observed at 1.01 mg/m^3 (0.77 ppm).

V. Effects of Animal Exposure

With the exception of non-human primates, the signs of methanol toxicity in commonly used laboratory animals are quite different from those signs observed in humans (Gilger and Potts, 1955). The major effect of methanol in non-primates (rodents, dogs, cats, etc) is CNS depression similar to that produced by other alcohols. Metabolic acidosis and ocular toxicity are not observed. The differences in toxicity are attributed to the ability of non-primates to metabolize formate more efficiently than humans and other primates (Tephly, 1991).

Two chronic studies have been conducted with monkeys. In one study, ultrastructural abnormalities of hepatocytes indicating alteration of RNA metabolism were observed in rhesus monkeys given oral doses of 3 to 6 mg/kg methanol for 3 to 20 weeks (Garcia and VanZandt, 1969). In a study aimed at examining ocular effects, cynomolgous monkeys were exposed by

inhalation to methanol concentrations ranging from 680 mg/m^3 (520 ppm) to 6650 mg/m^3 (5010 ppm) for 6 hours per day, 5 days per week for 4 weeks (Andrews *et al.*, 1987). No deaths occurred and no treatment-related effects were found upon histopathologic examination. However, Andrews *et al.* did not examine possible neurologic or reproductive effects which have been observed in other species at lower concentrations (see Sections IV and V).
Exposure to a mixture of methanol and other solvents has been associated with central nervous system birth defects in humans (Holmberg, 1979). However, because of mixed or inadequate exposure data, methanol is not considered a known human teratogen.

In two separate studies in male rats, inhalation exposure to methanol ranging from 260 to 13,000 mg/m^3 for 6 to 8 hours per day either for 1 day or for 1, 2, 4 or 6 weeks resulted in a significant reduction in testosterone levels (Cameron *et al.*, 1984; Cameron *et al.*, 1985).

Ubaydullayev (1968) exposed rats (15 per group) to 0, 0.57, or 5.31 mg/m^3 methanol continuously for 90 days. Chronaxy ratios of flexor and extensor muscles were measured in addition to hematologic parameters and acetyl cholinesterase activity. No changes were apparent in the 0.57 mg/m^3 group. Effects observed in the 5.31 mg/m^3 group included decreased blood albumin content beginning 7 weeks after exposure, slightly decreased acetylcholinesterase activity, decreased coproporphyrin levels in the urine after 7 weeks, and changes in muscle chronaxy. (Chronaxy is the minimum time an electric current must flow at a voltage twice the rheobase to cause a muscle to contract. The rheobase is the minimal electric current necessary to produce stimulation (Dorland, 1981).

Pregnant rats were exposed by inhalation to methanol at concentrations ranging from 5000 to 20,000 ppm for 7 hours per day on days 1-19 gestation, and days 7-15 for the highest dose group (Nelson *et al.*, 1985). A dose-related decrease in fetal weight, an increase in extra or rudimentary cervical ribs, and urinary or cardiovascular defects were observed. Exencephaly and encephalocoele were observed in the 20,000 ppm dose group. The no-observed-adverse-effect level (NOAEL) was 5000 ppm.

Pregnant mice were exposed to methanol vapors at concentrations ranging from 1000 to 15,000 ppm for 7 hours per day on days 6-15 of gestation (Rogers *et al.*, 1993). Increased embryonic and fetal death, including an increase in full-litter resorptions, was observed at 7500 ppm and higher. Significant increases in the incidence of exencephaly and cleft palate were observed at 5000 ppm and higher. A dose-related increase in the number of fetuses per litter with cervical ribs (usually small ossification sites lateral to the seventh cervical vertebra) was observed at 2000 ppm and above. The NOAEL was 1000 ppm.

VI. Derivation of Chronic Reference Exposure Level (REL)

Study	Rogers *et al.* (1993)
Study population	Pregnant mice
Exposure method	Discontinuous inhalation, 7 hours/day on days 6-15 of gestation
Critical effects	Abnormal cervical ribs, exencephaly, cleft palate
LOAEL	5000 ppm
NOAEL	1000 ppm
Benchmark Concentration (BMC_{05})	305 ppm
Exposure continuity	7 hr/day
Exposure duration	10 days
Average experimental exposure	89 ppm at BMC_{05} (305 ppm x 7/24)
Human equivalent concentration	89 ppm atBMC_{05} (gas with systemic effects, based on RGDR = 1.0 using default assumption that lambda (a) = lambda (h))
Subchronic uncertainty factor	1 (see below)
LOAEL uncertainty factor	1
Interspecies uncertainty factor	3
Intraspecies uncertainty factor	10
Cumulative uncertainty factor	30
Inhalation reference exposure level	3 ppm (3,000 ppb, 4 mg/m^3, 4,000 µg/m^3)

A NOAEL of 1000 ppm for developmental malformations was observed in mice exposed for 7 hours/day on days 6 through 15 of gestation (Rogers *et al.*, 1993). Although not a chronic study, the endpoint, teratogenicity, is a function of exposure only during gestation, especially in the case of a non-accumulating compound such as methanol. Therefore, an uncertainty factor to account for differences between subchronic and chronic exposures was not required. The investigators calculated maximum likelihood estimates (MLEs) using a log-logistic model for both 1% and 5% added risks above background. The most sensitive developmental toxicity endpoint was an increase in the incidence of cervical ribs. The MLE_{05} and BMC_{05} for cervical ribs were 824 ppm (1079 mg/m^3) and 305 ppm (400 mg/m^3), respectively.

VII. Data Strengths and Limitations for Development of the REL

The major strengths of the REL for methanol are the observation of a NOAEL and the demonstration of a dose-response relationship. The major uncertainties are the lack of human data for chronic inhalation exposure, the lack of comprehensive, long-term multiple dose studies, and the difficulty in addressing reproductive short-term effects within the chronic REL framework.

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CHRONIC TOXICITY SUMMARY

NAPHTHALENE

(naphthene, NCI-C5290, albocarbon, dezodorator, moth balls, moth flakes, tar camphor, white tar, naphthalin, naphthaline)

CAS Registry Number: 91-20-3

I. Chronic Toxicity Summary

Inhalation reference exposure level	**9 mg/m^3** (2 ppb)
Critical effect(s)	Respiratory effects (nasal inflammation, olfactory epithelial metaplasia, respiratory epithelial hyperplasia) in mice
Hazard index target(s)	Respiratory system, blood systems

II. Physical and Chemical Properties (HSDB, 1995; 1999 except as noted)

Description	White crystalline powder; odor of mothballs
Molecular formula	$C_{10}H_8$
Molecular weight	128.6 g/mol
Density	4.42 g/cm^3 @ 20ºC
Boiling point	218ºC
Melting point	80.5 ºC
Vapor pressure	0.078 torr @ 25ºC (Sonnenfeld *et al*., 1983); 0.10 torr @ 27ºC (CRC, 1994)
Conversion factor	5.26 µg/m^3 per ppb at 25ºC

III. Major Uses or Sources

Naphthalene is a natural constituent of coal tar (approximately 11%) (HSBD, 1995). It is present in gasoline and diesel fuels. Naphthalene is used as a moth repellent, though this use is decreasing in favor of p-dichlorobenzene (HSDB, 1995). It has also been used in the manufacture of phthalic anhydride, phthalic and anthranilic acids, naphthols, naphthylamines, 1-naphthyl-n-methylcarbamate insecticide, beta-naphthol, naphthalene sulfonates, synthetic resins, celluloid, lampblack, smokeless powder, anthraquinone, indigo, perylene, and hydronaphthalenes (NTP, 1992; HSDB, 1995). The statewide emissions from facilities reporting under the Air Toxics Hot Spots Act in California, based on the most recent inventory, were estimated to be 164,459 pounds of naphthalene (CARB, 1999).

IV. Effects of Human Exposure

Nine persons (eight adults and one child) were exposed to naphthalene vapors from several hundred mothballs in their homes. Nausea, vomiting, abdominal pain, and anemia were reported (Linick, 1983). Testing at one home following the incident indicated an airborne naphthalene concentration of 20 ppb (105 $\mu g/m^3$). Symptoms abated after removal of the mothballs.

Workers occupationally exposed to naphthalene fumes or dust for up to five years were studied for adverse ocular effects (Ghetti and Mariani, 1956). Multiple pin-point opacities developed in 8 of 21 workers. Vision did not appear to be impaired.

Cataracts and retinal hemorrhage were observed in a 44 year old man occupationally exposed to powdered naphthalene, and a coworker developed chorioretinitis (van der Hoeve, 1906).

Wolf (1978) reported that a majority of 15 persons involved in naphthalene manufacture developed either rhinopharyngolaryngitis and/or laryngeal carcinoma.

Ingestion of naphthalene or p-dichlorobenzene mothballs is a frequent cause of accidental poisoning of children (Siegel and Wason, 1986). Infants exposed to naphthalene vapors from clothes or blankets have become ill or have died (U.S. EPA, 1990). The effects in infants have been associated with maternal naphthalene exposure during gestation (U.S. EPA, 1990).

Deaths have been reported following ingestion of naphthalene mothballs. A 17-year old male ingested mothballs, developed gastrointestinal bleeding, hematuria, and coma, and died after five days (Gupta *et al.*, 1979). A 30-year old female ingested 30 mothballs and died after five days (Kurz, 1987).

Acute hemolytic anemia was reported among 21 infants exposed to naphthalene vapors from nearby mothball-treated materials (Valaes *et al.*, 1963). Increased serum bilirubin, methemoglobin, Heinz bodies, and fragmented red blood cells were observed. Kernicterus was noted in eight of the children, and two of the children died. Ten of these children had a genetic deficiency in glucose-6-phosphate dehydrogenase.

A 12-year old male ingested 4 g of naphthalene and 20 hours later developed hematuria, anemia, restlessness, and liver enlargement (Manchanda and Sood, 1960). The patient recovered after 8 days.

A 69-year old female developed aplastic anemia two months after several weeks exposure to naphthalene and p-dichlorobenzene (Harden and Baetjer, 1978).

V. Effects of Animal Exposure

Male and female B6C3F1 mice were exposed to naphthalene (>99% pure) vapor for 6 hours per day, 5 days per week over 104 weeks (NTP, 1992). Concentrations used were 0 (150 mice), 10 (150 mice), or 30 ppm (300 mice) naphthalene. (Table 1). Lesions were observed in the nose and lungs of exposed mice, including increased incidences of chronic nasal inflammation, olfactory epithelial metaplasia, and respiratory epithelial hyperplasia.

Table 1. Incidence of respiratory tract lesions in mice (male and female combined) chronically exposed to naphthalene vapors (NTP, 1992).

	0 ppm	*10 ppm*	*30 ppm*
Nasal inflammation	3/139	34/134	108/270
Olfactory epithelial metaplasia	0/139	131/134	269/270
Respiratory epithelial hyperplasia	0/139	131/134	269/270

CD-1 mice were administered 5.3, 53, or 133 mg/kg/day naphthalene by gavage over 90 days (Shopp *et al.*, 1984). The only effect noted was inhibition of aryl hydrocarbon hydroxylase activity. No increase in mortality or changes in body weight were noted. Reduced spleen weights were noted in females exposed to the highest dose. No changes were noted in serum enzyme levels or electrolytes. The researchers did not conduct a histopathological examination.

B6C3F1 mice were administered 200 mg naphthalene/kg/day by gavage for 5 days per week over 13 weeks. No adverse effects were observed (U.S. EPA, 1990).

Developmental effects of naphthalene ingestion in Sprague-Dawley CD rats was studied by Navarro and associates (1991). The lowest dose tested (50 mg/kg/day by gavage) was associated with signs of CNS depression for the first 3 days. Fetal growth, survival, and morphological development were not significantly affected at 450 mg/kg/day compared with control animals, although a trend toward decreased fetal weight and increased malformations was observed.

Harris and associates (1979) intraperitoneally administered 395 mg/kg/day naphthalene to Sprague-Dawley rats over days 1 though 15 of gestation. Fetuses had a 50% increase in incidence in delayed cranial ossification and heart development.

New Zealand white rabbits were given 0, 40, 200, or 400 mg/kg/day by gavage over days 6 through 18 of gestation (U.S. EPA, 1986a). A dose-dependent increase in grooming, vocalization, aggression, diarrhea, dyspnea, and ocular and nasal discharge were noted at all doses. No statistically significant increase in malformations or developmental abnormalities was observed.

Sprague-Dawley rats were administered 0, 100, 300, or 1000 mg/kg/day of naphthalene via dermal application (U.S. EPA, 1986b). No effects were reported at 100 or 300 mg/kg/day. At the high dose a slight decrease in testes weight was noted.

VI. Derivation of Chronic Reference Exposure Level (REL)

Study	NTP (1992)
Study population	B6C3F1 mice (75 or 150/group/sex)
Exposure method	Discontinuous whole-body inhalation exposures to 0, 10, or 30 ppm naphthalene vapor
Critical effects	Nasal inflammation, olfactory epithelial metaplasia, and respiratory epithelial hyperplasia
LOAEL	10 ppm (96% incidence for males and 100% incidence for females)
NOAEL	Not observed
Exposure continuity	6 hours/day for 5 days/week
Average experimental exposure	1.8 ppm (10 ppm x 6/24 x 5/7) for LOAEL group
Exposure duration	104 weeks
Subchronic uncertainty factor	1
LOAEL uncertainty factor	10
Interspecies uncertainty factor	10 (see below)
Intraspecies uncertainty factor	10
Cumulative uncertainty factor	1000
Inhalation reference exposure level	0.002 ppm (2 ppb, 0.009 mg/m^3, 9 μg/m^3)

The NTP study was chosen for the REL derivation since it is the only available lifetime animal inhalation bioassay and because no adequate epidemiological studies of long-term human exposure are available. The study was judged to be of adequate study design. The complete lack of nasal effects among control animals and the nearly total effect among animals exposed at 2 different concentrations strongly indicates a causal relationship between naphthalene exposure and nasal effects. The effects seen are consistent with those reported among exposed workers, who developed rhinopharyngolaryngitis or laryngeal carcinoma (Wolf, 1978). However, the hematological effects observed in humans have not been reported in laboratory animals, which raises the possibility that humans may be significantly more sensitive to naphthalene.

The most important limitation of the study is that the lowest concentration tested caused adverse effects in most (≥96%) of the animals tested. Thus the study amply demonstrates the risk of lifetime exposures to 10 ppm, but is uninformative regarding the concentration-response relationship at lower concentrations. Only a general assumption can be drawn on the magnitude of uncertainty factor needed to predict a concentration at which adverse effects would most likely not be observed. Lacking specific guidance or relevant research for this situation, the default 10-fold factor was applied. U.S. EPA also used the NTP study to develop its RfC of 3 μg/m^3 with slightly different assumptions and a cumulative uncertainty factor of 3000 (U.S. EPA, 2000). OEHHA followed the U.S. EPA precedent in using an intraspecies UF of 10 for naphthalene, rather than using the HEC/RGDR approach. According to U.S. EPA (2000), because of its low water solubility and low reactivity, naphthalene-related effects on the nasal epithelium are expected to result following absorption of naphthalene and its metabolism to reactive oxygenated metabolites, not from direct contact. This is supported by data on

naphthalene metabolism indicating that toxic effects on the respiratory tract are due to a naphthalene metabolite that may be formed either in the liver or in the respiratory tract. Necrosis of bronchial epithelial (Clara) cells in mice and necrosis of olfactory epithelium in mice, rats, and hamsters occur following intraperitoneal injection of naphthalene. The nasal effects from inhalation exposure to naphthalene were considered to be extra-respiratory effects of a category 3 gas (U.S. EPA, 1994). The assumption is made that nasal responses in mice to inhaled naphthalene are relevant to humans; however, it is uncertain that the RfC for naphthalene based on nasal effects will be protective for hemolytic anemia and cataracts, the more well-known effects from naphthalene exposure in humans.

VII. Data Strengths and Limitations for Development of the REL

The strengths of the REL for naphthalene include the large number of animals in the key study on which the REL is based and the 2 year length of the study. The limitations include the very high incidence of lesions at the lowest level tested in the key study, the absence of a NOAEL in the key study, the absence of other animal studies by the inhalation route, and the paucity of human data.

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CHRONIC TOXICITY SUMMARY

PHENOL

(Carbolic acid, phenylic acid, phenyl hydroxide)

CAS Registry Number: 108-95-2

I. Chronic Toxicity Summary

Inhalation reference exposure level	**200 µg/m^3** (50 ppb)
Critical effect(s)	Twitching, muscle tremors, neurological impairment; elevated serum liver enzymes in rats
Hazard index target(s)	Alimentary system; circulatory system; kidney; nervous system

II. Physical and Chemical Properties (From HSDB, 1995, 1999; ATSDR, 1989)

Description	Colorless to light pink solid
Molecular formula	C_6H_5OH
Molecular weight	94.11 g/mol
Density	1.0576 g/cm^3 @ 20° C
Boiling point	181.75° C
Melting point	40.9° C
Vapor pressure	0.3513 torr @ 25° C
Odor threshold	40 ppb (150 µg/m^3) (Amoore and Hautala, 1983)
Solubility	86,000 ppm in water, very soluble in alcohol, carbon tetrachloride, acetic acid and liquid sulfur dioxide; soluble in chloroform, ethyl ether, carbon disulfide; slightly soluble in benzene
Henry's Law Constant	3.97 x 10^{-7} ATM-m^3 /mol (25 °C)
Conversion factor	1 ppm = 3.85 mg/m^3

III. Major Uses or Sources (HSDB, 1995)

Phenol is obtained from coal tar and is widely used as a disinfectant for industrial and medical applications. It also serves as a chemical intermediate for manufacture of nylon 6 and other man-made fibers and for manufacture of epoxy and other phenolic resins and as a solvent for petroleum refining. Approximately half of the U.S. consumption is directly related to the housing and construction industries, in applications such as germicidal paints and slimicides. Phenol is present in the atmosphere as an emission from motor vehicles and as a photooxidation product of

benzene. The annual statewide industrial emissions from facilities reporting under the Air Toxics Hot Spots Act in California, based on the most recent inventory, were estimated to be 234,348 pounds of phenol (CARB, 1999).

IV. Effects of Human Exposures

The information that is available on the health effects of phenol exposure to humans is almost exclusively limited to case reports of acute effects of oral exposure (Bruce *et al.*, 1987), dermal exposure (Griffiths, 1973), or occupational exposures, including some exposure by inhalation (Dosemeci *et al.*, 1991; Ohtsuji and Ikeda, 1972; Connecticut Bureau of Industrial Hygiene, undated). Data in animals are consistent with human data and show phenol to be well absorbed by oral, dermal, and inhalation routes of exposure. Severe chronic poisoning manifests in systemic disorders such as digestive disturbances including vomiting, difficulty swallowing, ptyalism (excess secretion of saliva), diarrhea, and anorexia (Bruce *et al.*, 1987; Baker *et al.*, 1978). Phenol poisoning is associated with headache, fainting, vertigo, and mental disturbances (Bruce *et al.*, 1987; Gosselin *et al.* 1984) which are likely symptoms of neurological effects well documented in animal studies. Ochronosis, or discoloration of the skin, and other dermatological disorders may result from dermal phenol exposure (Deichmann and Keplinger, 1962; Bruce *et al.*, 1987). Several investigators (Truppman and Ellenby, 1979; Warner and Harper, 1985) have reported that the use of phenol in the surgical procedure of skin peeling can produce cardiac arrhythmias although specifics of dose received were not determined and would be expected to be high.

Human exposure studies in which populations were exposed to phenol over longer periods of time (subchronic and chronic) are limited and have serious deficiencies including multiple chemical exposures, in many cases small size of exposed populations, and lack of information on dose received.

Occupational studies make up the majority of subchronic/chronic studies available on human health effects associated with phenol exposure. Merliss (1972) described muscle pain and weakness of unknown etiology, enlarged liver, and elevated serum enzymes (LDH, GOT, and GPT) characteristic of liver damage in an individual with intermittent inhalation and dermal exposures to phenol, cresol and xylenol. Bruze (1986) noted that a number of phenol-formaldehyde based resins are dermal irritants and contact sensitizers. Johnson *et al.* (1985) examined 78 iron and steel foundry workers with multiple chemical and aerosol exposures that included phenol and found more respiratory symptoms in the phenol exposed group. However, multiple exposure to diphenyl methane diisocyanate, formaldehyde, and silica containing aerosols prevented determination of the effects of phenol. Baj *et al.* (1994) examined twenty-two office workers exposed for six months via inhalation to a commercial product containing formaldehyde, phenol and chlorohydrocarbons. At the end of the six month period the indoor air of the workers contained 1,300 $\mu g/m^3$ of formaldehyde and 800 $\mu g/m^3$ of phenol. The eight workers with the highest concentrations of phenol in their urine had decreased erythrocyte and T-helper lymphocyte numbers and increased numbers of eosinophils and monocytes compared to controls. The multiple chemical exposure of this study prevents concluding that these effects are attributable to phenol exposure. In a study of hospital workers Apol and Cone (1983) documented dermal effects in workers exposed to a number of chemicals including phenols

contained in disinfectants. This study however could not document any differences in urinary levels of phenol metabolites between control populations and exposed populations and could not assign any of the dermal effects seen to phenol or other substances in the work environment. Dosemeci *et al*. (1991) conducted a follow-up study to evaluate mortality in 14,861 workers in five manufacturing facilities producing or using phenol and formaldehyde. Arteriosclerotic heart disease, emphysema, disease of the digestive system, and cirrhosis of the liver were inversely related to the extent of phenol exposure. Due to multiple chemical exposures the effects of phenol alone could not be identified with any certainty.

Baker *et al.* (1978) completed a study of 39 individuals exposed to drinking water contaminated with phenol for a period of 4-8 weeks. Doses of phenol were estimated to range between 10 mg/day and 240 mg/day. Effects seen included increased incidence of diarrhea, mouth sores and irritation of the oral cavity.

Two occupational studies are of note since they reported NOAELs. Workers exposed continuously for an unspecified period of time to an average air concentration of 4 ppm phenol experienced no respiratory irritation (Connecticut Bureau of Industrial Hygiene, undated). No adverse effects were reported among workers in a Bakelite factory who were exposed to levels of phenol up to 12.5 mg/m^3 (3.3 ppm) (Ohtsuji and Ikeda, 1972). In this study urinary phenol levels were measured and were observed to return to pre-exposure levels within 16 hours after exposure indicating a relatively rapid clearance of phenol from the body that was confirmed in a study by Piotrowski (1971). Ohtsuji and Ikeda (1972) did not clearly indicate the number of workers sampled or the duration of exposure.

V. Effects of Animal Exposures

In animal studies, a number of subchronic and chronic studies employing oral and inhalation routes of exposure are available as well as shorter term studies using the dermal route of exposure. Responses observed in animal studies include: pulmonary damage (inhalation exposure), myocardial injury (inhalation and dermal exposure), liver damage (inhalation exposure), renal damage (inhalation exposure), neurological effects (inhalation exposure), developmental effects (oral exposure) and dermal effects (dermal exposure). Comparison of the three routes of exposure found that oral exposure was less effective at producing systemic toxic effects possibly due to the rapid metabolism of phenol to sulfate and glucuronide conjugates by the gastrointestinal tract. Comparison of health effects among studies using dermal, oral and inhalation routes of exposure finds that inhalation is a sensitive route of exposure for laboratory animals.

Several subchronic inhalation studies of health effects from phenol exposure are available but no inhalation studies longer than 90 days could be identified. Deichmann *et al.* (1944) exposed guinea pigs, rats, and rabbits to concentrations of phenol between 26 and 52 ppm for 28-88 days depending on species. Guinea pigs exposed for 7 hours per day, five days per week, for four weeks, displayed signs of respiratory difficulty and paralysis primarily of the hind quarters, indicating neurological effects. Five of twelve animals exposed at this concentration died at 28 days. At necropsy, extensive myocardial necrosis, lobular pneumonia, fatty degeneration of the

liver, and centrilobular hepatocellular necrosis were observed in all animals exposed at this level. Guinea pigs that were necropsied at 41 days also exhibited pulmonary inflammation, pneumonia, bronchitis, endothelial hyperplasia, and capillary thrombosis. Rabbits exposed at these same concentrations did not exhibit any signs of discomfort, but showed similar findings at necropsy at 88 days. Rats were less sensitive in this study with an apparent NOAEL of 26 ppm phenol for these effects. In this study, guinea pigs were the most sensitive species. Limitations of the Deichmann study include the range of exposure concentrations and the lack of a control group.

Sandage (1961) exposed Sprague-Dawley rats, mice and rhesus monkeys for 90 days continuously to 5 ppm phenol. Sandage found no effects on pulmonary, cardiovascular, hematological, hepatic, or renal systems, thus defining free-standing NOAELs for these systemic effects in these species. Limitations of this study include absence of guinea pigs (previously identified as the most sensitive species in the Deichmann study) and lack of a demonstrated dose response to the effects of phenol.

Dalin and Kristofferson (1974) examined the effects of phenol on the nervous system in rats exposed continuously for 15 days to a concentration of 26 ppm phenol and found muscle tremors, twitching and disturbances in walking rhythm and posture after 3-5 days exposure. After 15 days exposure, severe neurological impairment as measured by decreased performance on tilting plane test was found. The Dalin and Kristofferson (1974) study also documented elevated serum concentrations of LDH, GOT, GPT, and GDH indicative of liver damage in animals exposed to 26 ppm phenol continuously for 15 days.

The NCI (1980) study of the carcinogenicity of phenol is the most complete chronic study using the oral route of exposure. Mice and rats were exposed for 103 weeks to concentrations of phenol in their drinking water of 100, 2500, 5000, and 10,000 ppm. NOAELs in the mouse of 523 mg/kg/day (5000 ppm in drinking water) and NOAELs in the rat of 630 mg/kg/day (5000 ppm in drinking water) were observed for effects on the respiratory system, cardiovascular system, gastrointestinal system, hepatic system, renal system, and the brain based on histological examination of tissues. Male rats exposed to the 5000 ppm had a higher incidence of kidney inflammation (94%) than controls (74%). No tests of kidney function were performed in this study.

Boutwell and Bosch (1959) reported on the results of a chronic study in mice involving skin painting of 1.2 mg phenol or 2.5 mg phenol for a 52 week period. A NOAEL of 1.2 mg/animal for a 52 week exposure for dermal effects was found.

No multi-generational studies evaluating reproductive or developmental effects under chronic exposure conditions could be identified. Jones-Price *et al.* (1983a) reported that pregnant rats dosed orally with 0, 30, 60, and 120 mg/kg/day on gestation days 6-15 exhibited reduced fetal weight in a dose-related manner. However, no teratogenic effects or fetal deaths were observed. In a following study Jones-Price *et al.* (1983b) reported that pregnant mice dosed orally with 0, 70, 140, and 280 mg/kg/day on gestation days 6-15 exhibited decreased maternal weight gain, tremors, and increased maternal mortality at the 280 mg/kg/day dose. In the fetus reduced growth, decreased viability, and increased incidence of cleft palate were seen at the 280 mg/kg/day dose.

VI. Derivation of Chronic Reference Exposure Level (REL)

Study	Sandage, 1961; Dalin and Kristofferson, 1974
Study population	Mice, Sprague Dawley rats and rhesus monkeys
Exposure method	Continuous inhalation
Critical effects	Systemic effects including liver and nervous system effects
LOAEL	26 ppm (Dalin and Kristofferson, 1974)
NOAEL	5 ppm (Sandage, 1961)
Exposure continuity	Continuous
Average exposure concentration	5 ppm for NOAEL group
Human equivalent concentration	5 ppm for NOAEL group (gas with systemic effects, based on RGDR = 1.0 using default assumption that lambda (a) = lambda (h))
Exposure duration	90 days
Subchronic uncertainty factor	3
LOAEL uncertainty factor	1
Interspecies uncertainty factor	3
Intraspecies uncertainty factor	10
Cumulative uncertainty factor	100
Inhalation reference exposure level	0.05 ppm (50 ppb; 0.2 mg/m^3 (200 μg/m^3)

No suitable human studies were available for use since exposures were short term or occupational in nature with insufficient ancillary information (e.g., duration of exposure) or did not determine dose. Of the three routes of exposure available, inhalation appears to be the most sensitive based on the number and intensity of systemic effects noted (Deichmann *et al.*, 1944) relative to oral exposure (NCI, 1980). In support of this, ATSDR (1989) notes that the gastrointestinal tract has a large capacity to metabolize phenol to sulfate and glucuronide conjugates which appear likely to be less toxic than the parent compound, thus NOAELs derived from oral studies may not be applicable for other routes of exposure. The Deichmann *et al.* (1944) study identified guinea pigs as the most sensitive species. However, this study had a number of serious deficiencies including absence of controls, significant variability in the concentrations of phenol used in their exposure, and exposure that was not continuous. Since alternative studies using guinea pigs could not be identified, the rat was chosen as an alternative species since the rat has the most similar metabolic profile for metabolism of phenol to that of humans (ATSDR, 1989; Capel *et al.*, 1972). The Sandage (1961) study was chosen over other available studies since it was the longest in duration (90 days), had a continuous exposure, and evaluated three species (rats, mice, monkey). NOAELs determined in the Sandage study for systemic effects in all three species examined were 5 ppm, consistent with the idea that 5 ppm is a NOAEL for a number of species. Although this is a free-standing NOAEL, a subsequent study in rats indicated that nervous system and hepatic effects occur at a concentration of 26 ppm after several days (Dalin and Kristofferson, 1974).

The 5.0 ppm standard for phenol in the workplace (ACGIH, 1988; OSHA, 1985; NIOSH, 1976) is considered protective of the health of workers exposed occupationally but does not consider sensitive populations and is not for continuous exposure conditions. The workplace standard is

consistent with reports indicating that no respiratory irritation occurred among workers exposed regularly to 4 ppm phenol (Connecticut Bureau of Industrial Hygiene, undated) and no adverse effects were mentioned among workers exposed to 3.3 ppm (Ohtsuji and Ikeda, 1972). Neither report was considered appropriate to be the basis of a REL. However, for the sake of comparison adjusting the reported NOAEL of 4 ppm to continuous exposure and dividing by an intraspecies uncertainty factor of 10 results in an estimated chronic REL of 140 ppb, in reasonable agreement with the proposed REL of 50 ppb.

VII. Data Strengths and Limitations for Development of the REL

The major strength of the key study is the observation of a NOAEL from a continuous exposure study involving exposure of several different species. The primary uncertainties are the lack of adequate human health effects data, the lack of multiple concentration inhalation exposure studies demonstrating a dose-response relationship, the lack of animal studies longer than 90 days, and the lack of studies with guinea pigs, which have previously been identified as a sensitive species for phenol.

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CHRONIC TOXICITY SUMMARY

PROPYLENE

(1-propene; 1-propylene; propene; methylethene; methylethylene)

CAS Registry Number: 115-07-1

I. Chronic Toxicity Summary

Inhalation reference exposure level	**3,000** m**g/m^3** (2,000 ppb)
Critical effect(s)	Squamous metaplasia (males and females), epithelial hyperplasia (females only), and inflammation (males only) of the nasal cavity in Fischer 344/N rats
Hazard index target(s)	Respiratory system

II. Chemical and Physical Properties (HSDB, 1995; CRC, 1994)

Description	Colorless gas; practically odorless.
Molecular formula	C_3H_6
Molecular weight	42.08
Boiling point	–47.6 °C
Melting point	–185.2°C
Vapor pressure	8690 torr at 25°C
Solubility	Soluble in alcohol and ether.
Conversion factor	1.72 µg/m^3 per ppb at 25°C

III. Major Uses and Sources (HSDB 1995)

Propylene is produced primarily as a by-product of petroleum refining and of ethylene production by steam cracking of hydrocarbon feedstocks. Propylene is a major chemical intermediate. The most important derivatives of chemical and polymer grade propylene are polypropylene, acrylonitrile, propylene oxide, isopropanol and cumene. Use of polypropylene in plastics (injection moulding) and fibers (carpets) accounts for over one-third of U.S. consumption. It is also used in the production of synthetic rubber and as a propellant or component in aerosols. In 1994, propylene was ranked seventh among the top 50 chemicals produced domestically (C&EN, 1995). In the environment, propylene occurs as a natural product from vegetation. It is also a product of combustion of organic matter (biomass burning, motor vehicle exhausts and tobacco smoke) and is released during production and use. The most probable route of exposure to humans is by inhalation. Propylene has been detected in the atmosphere over both metropolitan (2.6 to 23.3 ppb) and rural (0.007 to 4.8 ppb) areas (Cox *et al.*, 1976; Leonard *et al.*, 1976). The annual statewide emissions from facilities reporting under

the Air Toxics Hot Spots Act in California, based on the most recent inventory, were estimated to be 696,350 pounds of propylene (CARB, 1999).

IV. Effects of Human Exposures

No data were available on the absorption, distribution or excretion of propylene in humans. However, hemoglobin (Hb) adducts of the metabolite intermediate propylene oxide have been used to monitor the internal dose of propylene (Tornqvist and Ehrenberg, 1990). The background level of the 2-hydroxylpropyl adduct to the N-terminal valine of hemoglobin was found to be about 2 pmol/g Hb. This was estimated to be equivalent to smoking 10 cigarettes per day; cigarette smoking is a source of propylene. Occupational exposure to propylene at 1 ppm (1.72 mg/m^3) was assumed to be associated with an increment of 5 pmol/g Hb (Kautiainen and Tornqvist, 1991).

No data were available on the chronic effects of propylene in humans.

V. Effects of Animal Exposures

In rats and mice, most propylene inhaled into the lungs is exhaled again and does not reach the blood to become systemically available (Golka *et al.*, 1989; Svensson and Osterman-Golkar, 1984). Once absorbed, a major route of metabolism for propylene is through the cytochrome P-450 system to propylene oxide, a known carcinogen in experimental animals. Cytochrome P-450 enzymes in both the liver and nasal epithelium (Maples and Dahl, 1991) can convert propylene to its toxic metabolite. However, in rats, propylene metabolism becomes increasingly saturated at concentrations above 50 ppm (86 mg/m^3) in the atmosphere (Golka *et al.*, 1989), which limits the amount of propylene oxide produced. Therefore, the amount of absorbed propylene may not reach high enough levels in classical long-term inhalation studies (Quest *et al.*, 1984) to show positive carcinogenic or serious chronic effects.

The only chronic toxicity investigation found for propylene was a comprehensive 2-year study in F344/N rats and B6C3F$_1$ mice (Quest *et al.*, 1984; NTP, 1985). Groups of 50 rats and 50 mice of each sex were exposed to concentrations of 0, 5000, and 10,000 ppm for 6 hr/day, 5 days/week, for 103 weeks. (Mean daily concentrations were 0, 4985, and 9891 ppm, respectively, for the rat study; and 0, 4999, and 9957 ppm, respectively, for the mouse study.) In exposed rats, treatment-related chronic effects were observed in the nasal cavity. In female rats, epithelial hyperplasia occurred in the high dose group and squamous metaplasia occurred in both dosage groups. In male rats, squamous metaplasia was seen only in the low dose group, but both dosage groups had inflammatory changes characterized by an influx of lymphocytes, macrophages and granulocytes into the submucosa and granulocytes into the lumen (see below). Nasal lesions were not observed in mice. The inflammatory lesions were more severe in the high dose group. Very mild focal inflammation was observed in the kidneys of treated mice but the relationship to propylene exposure was unclear. No other treatment-related effects, including clinical signs, mortality, mean organ and body weights, and histopathology, were observed.

Incidences of epithelial changes in nasal cavities of rats (Table 2 from Quest *et al*., 1984)

Observation	Control	5000 ppm	10,000 ppm
Epithelial hyperplasia			
Male	2/50 (4%)	2/50 (4%)	5/50 (10%)
Female	0/49 (0%)	4/50 (8%)	9/50 (18%)*
Squamous metaplasia			
Male	2/50 (4%)	19/50 (38%)*	7/50 (14%)
Female	0/49 (0%)	15/50 (30%)*	6/50 (12%)*
Inflammation			
Male	11/50 (22%)	21/50 (42%)*	19/50 (38%)
Female	8/49 (16%)	10/50 (20%)	13/50 (26%)

* Significantly ($p < 0.05$) higher than control values

In a long-term carcinogenicity study, Sprague-Dawley rats and Swiss mice (100-120 animals/group/sex) were exposed by inhalation to 0, 200, 1000 and 5000 ppm propylene 7 hr/day, 5 days/week, for 104 weeks (rats) or 78 weeks (mice) (Ciliberti *et al*., 1988). No body weight differences were observed between treated and control animals of either species. Mortality was reported to be slightly increased in male rats in the 1000 and 5000 ppm groups and in male mice in the 5000 ppm group, but numerical values of mortality were not presented in the report. Therefore, it is assumed that mortality differences were insignificant. Other possible general body system or nonneoplastic effects were not reported and assumed to have not been investigated.

VI. Derivation of Chronic Reference Exposure Level (REL)

Study	Quest *et al.*, 1984
Study population	50 rats/group/sex, 300 total.
Exposure method	Discontinuous whole body inhalation exposure (0 or 4,985 or 9,891 ppm).
Critical effects	Respiratory system; squamous metaplasia (males and females), epithelial hyperplasia (females only), and inflammation (males only) of the nasal cavity
LOAEL	4,985 ppm (8,570 mg/m^3)
NOAEL	Not observed
Exposure continuity	6 hr/day, 5 days/week
Exposure duration	2 years
Average experimental exposure	890 ppm for LOAEL group (4985 x 6/24 x 5/7)
Human equivalent concentration	190 ppm (gas with extrathoracic respiratory effects, RGDR = 0.21, based on BW = 305 g, MV = 0.21 L/min, SA(ET) = 15 cm^2)
LOAEL uncertainty factor	3 (low severity)
Subchronic uncertainty factor	1
Interspecies uncertainty factor	3
Intraspecies factor	10
Cumulative uncertainty factor	100
Inhalation reference exposure level	2 ppm (2,000 ppb, 3 mg/m^3, 3,000 μg/m^3)

VII. Data Strengths and Limitations for Development of the REL

Strengths of the propylene REL include the availability of a long-term, controlled exposure study in large groups of experimental animals that included extensive histopathological analyses.

Lifetime exposure of rats and mice to propylene resulted in adverse effects in the nasal cavity of rats at both exposure levels. Therefore, a NOAEL was not observed. However, the effects observed were mild.

Other weaknesses of the database for propylene include the lack of lifetime toxicity studies in any non-rodent species. Also, no long-term human toxicity or epidemiology studies were located in the literature. Human pharmacokinetic studies to compare with experimental animal pharmacokinetic studies were absent. Another uncertainty is the lack of reproductive and developmental toxicity studies. A comprehensive multi-generation study in an experimental animal species would enhance the development of a REL for propylene.

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CHRONIC TOXICITY SUMMARY

STYRENE

(ethenylbenzene, phenylethylene, vinylbenzene)

CAS Registry Number: 100-42-5

I. Chronic Toxicity Summary

Inhalation reference exposure level	**900** mg/m^3 (200 ppb)
Critical effects(s)	Neuropsychological deficits in humans as measured by memory and sensory/motor function tests
Hazard index target(s)	Nervous system

II. Chemical Property Summary

Description	Colorless to slightly yellow liquid with sweet, floral odor (HSDB, 1999)
Molecular formula	C_8H_8
Molecular weight	104.16
Boiling point	145.2 ^oC
Melting point	–31^oC (HSDB, 1999)
Vapor pressure	10 torr at 31^oC, polymerizes at 82^oC and above (Weast, 1979)
Solubility	310 µg/ml (Dean, 1985)
Conversion factor	4.26 µg/m^3 per ppb at 25^oC

III. Major Uses and Sources

The major source of styrene is industrial synthesis in which ethylbenzene is the starting material (ATSDR, 1992). The major uses of styrene are in polystyrene manufacturing, the butadiene-styrene rubber industry, and in the reinforced plastics industry (RPI) (WHO, 1983). Major non-styrene contaminants in the butadiene-styrene rubber industry are butadiene, benzene, carbon disulfide, and trichloroethylene, whereas the main co-contaminants associated with the RPI are glass fibers and acetone (WHO, 1983). Environmental exposures to styrene may result from mainstream cigarette smoke (Newhook and Caldwell, 1993) and newly installed carpets containing a styrene-butadiene rubber latex adhesive (Hodgson *et al.*, 1993). The Third National Health and Nutrition Examination Survey (NHANES) (Ashley *et al.*, 1994) reported a mean blood styrene level among ≥ 600 individuals as 0.074 ppb. In 1996, the latest year tabulated, the statewide mean outdoor monitored concentration of styrene was less than 0.1 ppb (CARB, 1999a). The annual statewide industrial emissions of styrene from facilities reporting under the

Air Toxics Hot Spots Act in California based on the most recent inventory were estimated to be 2,365,873 pounds (1999b).

IV. Effects of Human Exposure

Chronic exposures to styrene (to be discussed below) result in central nervous system (CNS) and peripheral nervous system effects, although the latter are not as pronounced (ATSDR, 1992; Rebert and Hall, 1994; Murata *et al.*, 1991). Irritation or discomfort of the upper respiratory tract resulting from styrene exposure has not been reported in long-term occupational studies (Foureman, 1994). However, sensory irritation and neurological impairment does occur in acute human studies at concentrations above 100 ppm (Stewart *et al*., 1968). The evidence for styrene induced hepatic changes is either negative or equivocal (ATSDR, 1992). Evidence for nephrotoxicity due to long-term occupational exposure is also negative or equivocal (ATSDR, 1992; Verplanke and Herber, 1998; Kolstad *et al.*,1995). Some human studies suggest that chronic exposure to styrene results in reproductive effects, but the limited data are difficult to interpret because of the small sample numbers (Brown, 1991; Lindbohm, 1993). Immunologic alterations (e.g., altered phenotypic profiles among lymphocyte subsets, decreased natural killer cell activity, and decreased chemotaxis) have also been observed, but the limited data prevent quantitative interpretation (Bergamaschi *et al.*, 1995; Governa *et al.*, 1994).

The CNS depressant effects of acute exposures to high styrene levels are probably mediated by the direct effect of the lipophilic, unmetabolized styrene on nerve cell membranes. Long-term effects of styrene exposure may result from the action of one or more metabolites of styrene (Savolainen, 1977; Mutti *et al.*, 1988). In humans, styrene metabolism is initiated by cytochrome P450 (P450)-mediated oxidation of styrene to a reactive metabolite, styrene oxide. The reaction takes place in human liver and, to a minor extent, in lung (Nakajima *et al.*, 1994). The P450 enzymes responsible for the epoxidation of styrene to stryene oxide are also found in human brain, but the brain isozymes have not been tested specifically with styrene as a substrate (Bhamre *et al.*, 1993). Styrene may also be oxidized to styrene oxide by enzymes which share specific iron and porphyrin components with P450 and those that utilize active oxygen species (Belvedere *et al.*, 1983; Tursi *et al.*, 1983; Miller *et al.*, 1992).

The major end product of styrene metabolism in humans is urinary mandelic acid (MA) and phenylglyoxylic acid (PGA) (Bardodej and Bardodejova, 1970; Leibman, 1975; Guillemin and Bauer, 1979). Other pathways that may be present in other animals are either absent or are quantitatively negligible in humans, except when high styrene levels are encountered (Guillemin and Berode, 1979; Chakrabarti *et al.*, 1993; Hallier *et al.*, 1995). Confounders of the quantitative relationship between styrene exposure and urinary MA+PGA are the consumption of ethanol (Berode *et al.*, 1986) and exposure to ethylbenzene (Bardodej and Bardodejova, 1970). An important consequence of ethanol related decreased levels of urinary mandelic acid is the potential underestimation of exposure to styrene (Guillemin and Bauer, 1979; Berode *et al.*, 1986). However, the urinary metabolite levels return to control values 4-5 hours after the ethanol consumption (Berode *et al.*, 1986).

Indicators of human styrene exposure include exhaled styrene, blood styrene, urinary MA, and urinary MA+PGA (Guillemin and Berode, 1988). Exposure to styrene by inhalation results in 89 percent absorption (Guillemin and Berode, 1988). In the occupational studies that are the basis

for quantifying the relationship between chronic styrene exposure and health effects, end-of-shift or next-morning MA+PGA have been used. The next-morning measurements are more reflective of past exposures due to the high fat solubility of styrene (fat:blood partition coefficient = 94 (Csanady *et al.*, 1994)), the presence of a second, long biological half-life for MA = 25 hours., and a long biological half-life for PGA = 11 hours (Guillemin and Bauer, 1979). Following inhalation, the half-life for styrene is 41 minutes in blood (Wigaeus *et al.* 1983) and 32-46 hours in fat tissue (Perbellini *et al.*, 1988).

One postulated mechanism for the chronic non-cancer toxicity of styrene is the binding of the highly reactive styrene oxide to components of nervous tissue. Another postulated mechanism is an alteration in the levels of circulating catecholamines (e.g., dopamine) due to the binding of PGA to these biogenic amines (Mutti, 1993; Mutti *et al.*, 1984a; Checkoway, 1994) and the subsequent changes in physiological functions that are under biogenic amine control. Although long-term exposures to styrene are associated with decrements in physiological functions, the exact mechanism(s) for these effects have not been clearly established (see reviews by ATSDR, 1992; Mutti, 1993; Rebert and Hall, 1994).

Kolstad *et al.* (1995) estimated excess deaths due to four major non-malignant disease groups for 53,847 male workers in the Danish RPI. Low and high styrene exposures were based on companies with less than 50% (low) and those with 50% or more (high) employees involved with reinforced plastics. An internal comparison was made with workers unexposed to styrene to account for more similar activities and lifestyles. Statistically significant ($p < 0.05$) excess deaths due to pancreatitis and degenerative disorders of the myocardium and non-significant excess deaths due to degenerative diseases of the nervous system were observed. Non-significant excess deaths due to glomerulonephritis were also observed.

Checkoway *et al.* (1994) described a cross-sectional study of 59 male boat plant workers exposed to <1 to 144 (mean = 37.2) ppm styrene. Monoamine oxidase B (MAO-B) activity in platelets was measured as an indicator of catecholamine metabolism. When the styrene exposed workers were divided into quartile exposures, a dose dependent decrease in MAO-B activity was observed after adjustments were made for age, smoking, alcohol and medication use.

Female workers employed in the reinforced plastics industry (RPI) were studied for levels of substances associated with neuroendocrine function (Mutti *et al.*, 1984a**).** Serum prolactin, thyroid stimulating hormone, human growth hormone, follicle stimulating hormone, and luteinizing hormone were measured in 30 women who were between the 5th and 15th day of the menstrual cycle. Exposure was based on the next-morning MA+PGA, and levels of the neuroendocrine substances were measured in venous blood samples taken the next morning before the start of work. On the basis of a relationship (not detailed in the report) between urinary metabolites and styrene air concentration, the authors estimated that the average styrene TWA/8 hr was about 130 ppm. Controls consisted of women factory workers living in the same area as the styrene-exposed women, but not knowingly exposed to styrene. After controlling for age and exposure time, the increased prolactin and thyroid stimulating hormone levels were correlated with the concentration of next-morning urinary MA+PGA, although only the increased prolactin levels were statistically significant. Numerous occupational studies have noted CNS disturbances in styrene-exposed workers. Decreased manual dexterity, increased reaction times, and/or abnormal vestibuloocular reflex (ability to track moving objects) were observed by Gotell *et al*. (1972), Gamberale *et al*. (1975), Lindstrom *et al*. (1976), Mackay and

Kelman (1986), Flodin *et al.* (1989), Moller *et al.* (1990), and Cherry and Gautrin (1990) for air styrene levels of about 12 ppm to more than 100 ppm. However, in each of these studies, there were difficulties in quantifying the effect. The difficulties included small sample size, unknown exposure duration, lack of concurrent control group, lack of dose-response data, and either unknown ethanol consumption or lack of adjustment for ethanol consumption. In the Cherry and Gautrin (1990) investigation, however, the authors determined that accounting for ethanol consumption did not reduce the correlation between increased reaction time and exposure.

Decrements in other CNS functions were observed among workers in the well-controlled studies of Fallas *et al.* (1992), Chia *et al.* (1994), and Mutti *et al.* (1984b). Fallas *et al.* (1992) studied 60 male workers (average age = 29.5 years, average air styrene = 24.3 ppm). The styrene-exposed population was compared to non-exposed worker controls and matched for age, intellectual level, and ethnic origin. The results from a standardized test battery showed decrements in the aiming response and 22/60 styrene exposed workers exhibited increased reaction times compared to 7/60 controls. Acquired color vision loss (dyschromatopsia) was also observed in the styrene-exposed workers compared to controls. Chia *et al.* (1994) also observed decrements in CNS function as defined by altered visual retention, audio-digit recognition, and digit recognition. However, a dose-response relationship did not exist. These workers also exhibited a statistically nonsignificant dose-dependent dyschromatopsia.

In the most comprehensive occupational study to date on CNS effects of styrene exposure, Mutti *et al.* (1984b) assessed memory and sensory/motor function in a group of 50 male styrene-exposed workers (average exposure = 8.6 years) and a control group of 50 manual workers. In addition to matching for age, sex, and educational level, a vocabulary test was included to match for general intelligence. Eligibility criteria included absence of metabolic, neurologic, or psychiatric disorders, limited ethanol intake, and limited cigarette usage. All subjects were instructed to avoid intake of alcohol and drugs for two days prior to testing. Styrene exposure was assessed from urinary MA+PGA levels the morning after the last workday in the week, followed immediately by participation in a battery of 8 neuropsychological tests designed to measure CNS function. The tests included reaction time, short and long term logic memory, short and long term verbal memory, digit-symbol association (using a reference code), block design (reproducing a displayed design using colored blocks), and embedded figures (timed identification of figures in Rey's table). The mean $\pm$ 2 SDs of the values found in the control group was set as the normal range limit for each neuropsychological test. The results were expressed as continuous and quantal data. Expressed as continuous data, styrene-exposed workers exhibited significantly poorer performances than controls in all tests, except in the digit-symbol test. Also, urinary metabolite concentration and duration of exposure were found to be significantly correlated with the scores of several tests. As a subgroup, workers with metabolite levels of up to 150 mmoles MA+PGA/mole creatinine (mean = 75 mmoles/mole creatinine $\pm$ 33 [SD], which is equivalent to a mean styrene concentration of 15 ppm) appeared to have no significant effects. The authors state that this level of urinary metabolites corresponds to a mean daily 8-hour exposure to air styrene of 25 ppm (106 mg/m^3). Based on greater urinary excretion of styrene metabolites, significantly poorer performances in four or more neuropsychological tests were recorded in the other three subgroups (150-299, 300-450, and > 450 mmoles MA + PGA/mole creatinine).

Mutti *et al.* (1984b) expressed the quantal data as the fraction of tested subjects who responded abnormally to ≥ 1, ≥ 2, and ≥ 3 tests (see Table 1). Positive dose-response relationships existed between intensity of styrene exposure (mmoles MA + PGA/mole creatinine) and abnormal scores, whether it was expressed as abnormal responses in at least one, at least two, or at least three neuropsychological tests. The chi-square test and validity calculations were performed by constructing 2 x 2 tables selecting different levels of urinary excretion of MA and PGA as a cut-off point. The highest values for chi-square and predictive validity were found when the cut-off of 150 mmol/mol creatinine was chosen, suggesting that the quantal isolation of the low dose subgroup from the next subgroup is appropriate. When the quantal data for the low dose subgroup were analyzed by OEHHA using the Fisher's Exact Test, a significant level of abnormal responses were observed for ≥1 ($p = 0.005$) and ≥3 ($p = 0.04$) tests. The abnormal responses for ≥2 tests were statistically marginal ($p = 0.06$). For each of the remaining exposure groups, the p-values were <0.05. Unlike the assumptions made concerning the continuous data, quantal data results suggest that the low dose subgroup represents a LOAEL, and that a NOAEL is not available from the data. Mutti *et al*. (1984b) also expressed the data in a quantal three-way representation including prevalence (number of respondents for at least one, two or three abnormal tests), duration (years at work), and intensity (metabolite level). This representation revealed a positive correlation of neuropsychological deficits with duration as well as intensity.

Table 1. Subjects Classified Positive on Neuropsychological Tests as a Function of Styrene Exposure [a].

MA+PGA, mmoles per mole creatinine[b]	Total Subjects	Number of Abnormal Tests		
		≥ 1	≥ 2	**≥ 3**[c]
Controls	50	4/50	2/50	**0/50**
< 150 (mean = **75** ± 33)[d]	14	6/14	3/14	**2/14**
150-299 (mean = **216** ± 45)	9	6/9	5/9	**3/9**
300 - 450 (mean = **367** ± 49)	14	10/14	7/14	**5/14**
> 450 (mean = **571** ± 108)	13	11/13	8/13	**6/13**

[a] Data from Table IV in Mutti *et al.* (1984b).
[b] "Next-morning" styrene urinary metabolites.
[c] The quantal grouping of the number of subjects that performed abnormally in ≥3 tests based on their styrene urinary metabolite concentrations, both shown in bold, were used in a benchmark concentration (BMC) analysis for the derivation of the REL (see Section VI below).
[d] Based on Guillemin *et al.* (1982), a linear relationship exists for converting the urinary metabolite concentrations to ppm air styrene levels (4.97 mmoles MA+PGA/mole creatinine is equivalent to 1 ppm styrene). Thus, the mean styrene concentrations per group are 0, 15, 44, 74, and 115 ppm.In addition to dyschromatopsia observed by Chia *et al*. (1994), Gobba and Cavalleri (1993) and Campagna *et al*. (1995) also reported this visual dysfunction among styrene workers in the reinforced plastics industry. Workers (n=36) exposed to an average of 16 ppm styrene exhibited significantly greater dyschromatopsia than controls, matched for age, ethanol consumption and tobacco smoking (Gobba and Cavalleri, 1993). Among the study population, only 1/36 styrene-exposed workers (compared to 16/36 controls) performed the test with 100 percent

accuracy. When a different group of styrene-exposed workers was tested, those exposed to > 50 ppm styrene exhibited greater dyschromatopsia than those exposed to ≤ 50 ppm, and within this group, a subset exhibited a similar decrement after returning from a one month vacation. In the Campagna *et al.* (1995) study, the test for dyschromatopsia was given to 81 reinforced plastics industry workers (79 male and 2 female) exposed to 4.6, 10.1, and 88.8 ppm styrene (first quartile, median, and third quartile, respectively). No control group was used in this study. Statistical analysis revealed a correlation of color vision loss with exposure to styrene (defined as next-morning urinary mandelic acid), age, and ethanol consumption.

Exposure to styrene may affect the peripheral nervous system (PNS). In a case report (Behari *et al.*, 1986), a man working for 5 years with a photostat process that used styrene was diagnosed with peripheral neuropathy. However, in occupational studies, the relationship between exposure to styrene and PNS effects has been inconsistent (Triebig *et al.*, 1985; Cherry and Gautrin, 1990). A major difficulty in understanding the potential for this relationship is the lack of knowledge about the appropriate surrogate for dose that leads to PNS disturbance (Murata *et al.*, 1991). In one study, however, chronic exposure indices were developed which included work method, years at work, time spent laminating (source of high exposure), styrene air concentration, and end-of-shift urinary mandelic acid (Matikainen *et al.* (1993). Numbness in the extremities increased with the exposure index, although statistically the effect was marginally insignificant ($p < 0.1$). The styrene TWA/8 hr was 32 ppm for the 100 study subjects.

Female reproductive toxicity has been inconsistently reported among humans (Brown, 1991; Lindbohm, 1993). These studies are difficult to interpret because of the high background rates of endpoints such as spontaneous abortion and menstrual disorders in combination with confounding exposures. In those studies that showed no reproductive effects due to styrene exposure, the power of the studies was low due to the small numbers of women. Hence the evidence for any adverse effects of exposure to styrene on female reproductive function is inconclusive.

Male workers employed in the reinforced plastics industry were examined for effects on sperm chromatin structure and semen quality (Kolstad *et al*., 1999a) and time to pregnancy (Kolstad et al., 1999b). No indications of an exposure-response relationship were seen when individual changes in semen quality were related to the postshift urinary mandelic acid concentrations among 23 exposed workers. A weak increase in sperm DNA-susceptibility to *in situ* denaturation as a function of mandelic acid concentration was indicated, but was within the interassay variability. No detrimental effect of styrene exposure was observed with regard to male fecundity among 188 exposed workers when compared to 353 unexposed workers.

Immune system alterations were reported in a study conducted by Bergamaschi *et al.* (1995). Reinforced plastics industry workers (n=32 female/39 male, average age = 32 years, average exposure duration = 7 years) were compared with non-styrene exposed factory workers and matched for age, sex, tobacco use and ethanol consumption. Air styrene levels, among the different factories, varied between 10 - 50 ppm, and individual worker exposure was measured by urinary metabolites the morning after the last shift (15 hours post-exposure). Among all workers in the study (median exposure = 16 ppm - according to the data of Guillemin *et al.* (1982)), the proportion of 12/18 lymphocyte subsets and the prevalence of abnormal values of immunologic phenotypes for 11/18 subsets were statistically different from the controls (p <

0.001 to < 0.05). When the workers were placed into three exposure groups (0, < 25 ppm, and > 25 ppm styrene), dose-response relationships were observed for prevalences of abnormal responses for four lymphocyte subsets and, in the case of two subsets, abnormal responses were observed in the group exposed to < 25 ppm styrene. Natural killer cell activity (a lymphocyte function), measured in a different group of workers in the same study, was decreased compared to unexposed worker controls. The median exposure, given in terms of urinary metabolites, was calculated as 21 ppm based on the data of Guillemin *et al.* (1982). The data show that exposure of these workers to air styrene levels below 50 ppm, and probably at levels near 25 ppm, resulted in alterations of the immune system.

Governa *et al.* (1994) observed reduced chemotactic responses of polymorphonuclear lymphocytes (PMNs) obtained from 21 styrene-exposed workers. However, the lack of exposure data prevents a quantitative assessment. In the same study, 0.1 - 0.6 mM styrene inhibited the chemotaxis of isolated healthy PMNs.

V. Effects of Animal Exposure

In a subchronic study, carried out under the auspices of NTP (NTP, 1992), mice and rats were exposed by inhalation to styrene vapors to establish a maximum tolerated dose for chronic studies. Mice were exposed to 0, 62.5, 125, 250, or 500 ppm styrene (6 hr/d, 5 d/wk, 13 wks). Among males deaths occurred in the 250 ppm group. Body weights among all exposed mice were lower than controls, and the difference was about 9 percent. Lung, olfactory epithelial, and forestomach lesions were observed in females and males. In females, degeneration of the adrenal gland cortex was observed. An effect not discussed in the chairperson's report, but recorded in the original laboratory report, was an increased estrous cycle length among the female mice at all styrene doses. A LOAEL of 62.5 ppm is indicated by the olfactory epithelial, forestomach and respiratory tract lesions in mice of both sexes and for lesions in the adrenal cortex in the female mice. Rats were exposed to 0, 125, 250, 500, 1000, or 1500 ppm styrene (6 hr/d, 5 d/wk, 13 wks). No deaths occurred, but reduced body weights were observed at the two highest doses. Lesions of the respiratory tract were observed at all dose levels. A LOAEL of 125 ppm is therefore indicated for the rats.

Rats were exposed by ingestion for 2-years to styrene in drinking water (0, 125, and 250 ppm). (The water solubility of styrene is 310 ppm.) The only effect was a styrene-related reduction in water consumption (Beliles *et al.*, 1985).

Kishi *et al.* (1995) carried out a developmental study on rat pups born to dams exposed by inhalation to styrene (0, 50, 300 ppm; 6-hr/d; gestation days 7-21). Although the small number of litters (n=2) at the 50 ppm dose prevented detailed statistical analysis, the data suggest that exposure of the dams to 50 ppm styrene resulted in deficits and delays in some motor and coordination abilities among the pups. Pups born to dams exposed to 300 ppm exhibited statistically significant increases in spontaneous activity and in the delay of some neurobehavioral functions. Many of the effects became diminished as the pups aged. Measurements of reproductive toxicity (maternal weight gain, length of gestation, number of live births) did not change. Postnatal body weights were lower among the styrene-exposed pups, but the differences became less as the pups aged to 125-days.

A follow-up developmental study by the same research group investigated neurochemical levels in rat pups born to dams exposed by inhalation to styrene (0, 50, 300 ppm; 6 hr/day on gestation days 6-20) (Katakura *et al*., 1999). Cerebrum weights of day 0 pups were significantly lower when compared to cerebrum weights of *ad libitum* fed animals, but not pair-fed animals. At the highest dose, occasional reductions in neuroamines, i.e. 5-hydroxytryptamine, homovanillic acid, and 5-hydroxyindoleacetic acid, were seen in various parts of the brains of rat pups compared to one or both control groups on day 0 and day 21. No reproductive or histopathological changes were seen.

Rosengren and Haglid (1989) investigated whether long term inhalation exposure (three months) to styrene (90 and 320 ppm) could induce long lasting astroglial alterations in Sprague Dawley rats, traceable four months after exposure ceased. Styrene exposure at 320 ppm induced the alterations as shown by raised concentrations of the glial cell marker, glial fibrillary acidic protein (GFA), in the sensory motor cortex and in the hippocampus. GFA is the structural protein of the astroglial filaments. These filaments form after damage to the central nervous system from any cause. The authors concluded that exposure to styrene at moderate exposure levels induces regional, long lasting astroglial reactions that serve as an indicator of solvent induced brain damage.

Mice, exposed acutely (14 days) by inhalation to 125 - 500 ppm styrene, exhibited decreased spleen / body weight, splenic hypocellularity, altered lymphocyte proportions among subsets, and increased proliferative response to mitogens (Corsini *et al.*, 1994). Mice and rats, exposed by gavage to high levels of styrene (18, 27, 45 mg/kg - mouse; 118, 177, 294 mg/kg - rat) for 5 days/week for 4 weeks, exhibited decreased resistance to encephalomyocarditis virus, to *Plasmodium berghie* (a malaria parasite), and to *Nippostrongylus braseleinisi* (a parasitic worm) (Dogra *et al.*, 1992).

Groups of 70 male and 70 female Charles River CD (Sprague-Dawley-derived) rats were exposed whole body to styrene vapor at 0, 50, 200, 500, or 1000 ppm 6 h/day 5 days/week for 104 weeks (Cruzan *et al*., 1998). A battery of hematologic and clinical pathology examinations was conducted at 13, 26, 52, 78, and 104 weeks. Nine or 10 rats per sex per group were necropsied after 52 weeks of exposure and the remaining survivors were necropsied after 104 weeks. Control and high-exposure rats received a complete histopathologic examination, while target organs, gross lesions, and all masses were examined in the other 3 groups. Styrene had no effect on survival in males, but females exposed to 500 or 1000 ppm had a dose-related increasein survival. Levels of styrene in the blood at the end of a 6-h exposure during week 95 were proportional to exposure. Levels of styrene oxide in the blood of rats exposed to 200 ppm or greater styrene were proportional to styrene exposure concentration. The authors found no changes of toxicologic significance in hematology, clinical chemistry, urinalysis, or organ weights. Styrene-related non-neoplastic histopathologic changes were confined to the olfactory epithelium of the nasal mucosa. (The authors also found no evidence of cancer induction.)

Groups of 70 male and 70 female CD-1 mice were exposed in whole body inhalation chambers to styene vapor concentrations of 0, 20, 40, 80, and 160 ppm 6 hrs/day, 5 days/week, over a period of up to 2 years (Huntingdon Life Sciences, 1998). Ten mice per sex per group were necropsied after 52 and 78 weeks of exposure, and the remaining survivors necropsied after 104 weeks. Due to increased mortality in female control mice, terminal sacrifice for this group

occurred at 98 weeks. Two female mice exposed to 160 ppm styrene died during or immediately following the first week of exposure. Histopathology revealed liver necrosis that was a likely contributor to the deaths. Reduced body weight gain and increased food consumption were observed in male mice at the two highest exposure levels and in female mice at the highest exposure level. Both styrene monomer and styrene oxide in blood increased with exposure concentration. No changes of toxicologic significance in hematology, ophthalmology, clinical chemistry, urinalysis, or organ weights were noted. Styrene-related non-neoplastic histopathologic changes were seen in the lungs (bronchiolar-alveolar hyperplasia) and nasal olfactory epithelium (respiratory metaplasia, degeneration or necrosis, and changes to the underlying Bowman's glands) from all exposure groups. The nasal lesions showed progression with time. Focal loss of bone from the turbinate was also seen more frequently as the study progressed. In addition, atrophy of the olfactory nerve fibers was present in mice at the three highest exposure concentrations.

VI. Derivation of the Chronic Reference Exposure Level (BMC Approach)

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Study	Mutti *et al.* (1984b)
Study populations	Human (occupational)
Exposure method	Inhalation
Critical effects	Central nervous system
LOAEL	15 ppm
NOAEL	Not established
BMC_{05}	1.7 ppm
Exposure continuity	8 hr/d (10 m^3 per 20 m^3 day), 5 d/wk
Exposure duration	8.6 years (average years at work)
Average occupational exposure	0.61 ppm (1.7 x 10/20 x 5/7)
Human equivalent concentration	0.61 ppm
LOAEL uncertainty factor	Not needed in the BMC approach
Subchronic uncertainty factor	1 (average exposure 12.3% of lifetime)
Interspecies uncertainty factor	1
Intraspecies uncertainty factor	3
Cumulative uncertainty factor	3
Inhalation reference exposure level	0.2 ppm (200 ppb; 0.9 mg/m^3; 900 $\mu g/m^3$)

The most relevant chronic noncancer effect due to styrene exposure is neurotoxicity. The Mutti *et al*. (1984b) occupational study presented convincing dose-response information and was well designed and executed in terms of experimental protocol and statistical evaluation, which included tests for false positive and false negative responses. While not all confounders could be ruled out (e.g., compensatory mechanisms, biorhythms, workers who leave because of styrene related illness), careful attention was paid to include eligibility criteria for the control group that correct for confounders unique for this population (e.g., limited ethanol intake, a control work-force not exposed to neurotoxic substances, and a test to allow a match for general intelligence). The use of urinary metabolites to measure exposure dose is based on the observation that the next-morning urinary MA+PGA is directly related to the air level of styrene. The Guillemin *et*

al. (1982) study provides the basis for the conversion of urinary MA+PGA levels to styrene exposure levels used by Mutti *et al.* (1984b).

The quantal dose-response data by Mutti *et al*. (1984b) is applicable for use in a benchmark concentration (BMC) approach. The quantal grouping of the number of subjects that performed abnormally in $\geq$3 tests based on their urinary metabolite concentrations was chosen for a BMC analysis (see Table 1). Basing the BMC on abnormal responses to >3 tests reduces the complexity of multiple test comparisons and the potential for inappropriate comparison of different neuropsychological tests between control and exposure groups for statistical purposes. Also, the potential for false positive responses is reduced due to the zero background level of abnormal responses in the control group when the criteria are >3 abnormal tests. Using a log-normal probit analysis (Tox-Risk, version 3.5; ICF-Kaiser Inc., Ruston, LA) with the data (emphasized in bold typeface) in Table 1 (above) the maximum likelihood estimate (MLE) for a 5% response was 4.0 ppm. The resulting 95% lower confidence limit at the MLE provided a BMC_{05} of 1.7 ppm. A BMC_{05} is considered to be similar to a NOAEL in estimating a concentration associated with a low level of risk. Following adjustment for exposure continuity (10 m^3 per 20 m^3 day for 5 d/wk) and application of an UF of 3 to account for human intraspecies variability, a REL of 0.2 ppm (0.9 mg/m^3) was attained. For exposure data that utilizes healthy human subjects, the resulting BMC represents a less than 10% incidence in the general population. When combined with an UF of 3, as carried out above, the resulting REL will be protective of the vast majority of individuals.

This analysis of the quantal data is supported by recognizing that, in a population of 50 subjects, individual test-specific effects that occur at low doses may not have been observed. If the criterion for abnormality is expressed in terms of CNS dysfunction, defined by all tests, the sensitivity of the testing procedure is increased and the low dose effects are more easily observed. The quantal data of Mutti *et al.* (1984b), i.e., the proportion of subjects responding abnormally to the tests, therefore provide a more sensitive approach to detecting low dose effects. Collasping a battery of test data to increase sensitivity may introduce the dilemma of multiple test comparisons, as noted above. However, OEHHA believes that a statistical method to correct for this, known as a Bonferroni correction, is unnecessary. The REL development is based on calculating a statistic of one effect of a complex of responses (or a syndrome) that results from CNS dysfunction, and not based on calculating a statistic for each test within the group of tests. The apparent global nature of the neurological syndrome resulting from long-term styrene exposure, in addition to basing the BMC on abnormal responses to >3 tests, should more than adequately address any concerns that may result from combining neurological test data.

Applying NOAEL/LOAEL methodology to the Mutti *et al*. (1984b) quantal data yields an exposure value similar to that attained with the BMC approach. The LOAEL of 15 ppm is adjusted to an equivalent continuous exposure of 5.36 ppm (15 ppm x 10/20 m^3 x 5/7 d/wk). Use of a LOAEL UF of 3 and an intraspecies UF of 10 resulted in an estimated REL of 0.2 ppm (0.8 mg/m^3).

The U.S. EPA (1996) calculated a reference concentration (RfC) of 0.3 ppm (1 mg/m^3), which is slightly higher than the OEHHA-derived chronic REL of 0.2 ppm (0.9 mg/m^3). The RfC for styrene is also based on the findings of Mutti *et al*. (1984b), but utilized the continuous data for

development of the RfC and used standard NOAEL methodology for the RfC derivation. U.S. EPA (1996) established a NOAEL for the lowest exposure group (<150 MA+PGA mmole/mole creatinine; equivalent to < 25 ppm styrene). However, OEHHA staff believe that the use of the continuous data to establish a NOAEL overlooks the advantages of using the BMC approach using the quantal data. These advantages are that the BMC_{05} reflects the shape of the dose-response curve and takes into account the number of subjects involved in the study. In addition, OEHHA staff evaluated the quantal data with the Fisher's Exact Test and determined the probabilities of abnormal responses among the exposed subjects based on the unexposed subjects whose responses were assumed to be normal. At the lowest exposure, the probability that the proportion of subjects responding abnormally to $\geq$1 and $\geq$3 tests was within the expected range was p = 0.005 and p = 0.04, respectively, indicating that neuropsychological deficits due to styrene occur in the low dose subgroup. Thus, the quantal data indicate that a NOAEL was not established in this study.

With regard to application of uncertainty factors, U.S. EPA (1996) applied a UF of 3 for intraspecies variability and a partial UF of 3 for lack of information on chronic studies because the critical study was considered intermediate, i.e., between subchronic and chronic duration (Foureman, 1994). OEHHA applied a UF of 1 because the mean exposure duration, 8.6 years, was greater than 12 percent of expected lifetime (8.6/70 = 12.3%). The U.S. EPA (1996) also included a modifying factor of 3 for database deficiencies. The criteria for use of modifying factors are not well specified by U.S. EPA. Such modifying factors were not used by OEHHA.

In addition to the OEHHA and the U.S. EPA hazard assessments, the Agency for Toxic Substances and Disease Registry (ATSDR) also calculated a chronic inhalation minimal risk level (MRL) for styrene (ATSDR, 1992). The calculation was based on the same Mutti *et al.* (1984b) worker study. ATSDR (1992) identified the lowest exposure group as a LOAEL and assigned an air styrene level of 25 ppm. To derive the MRL, ATSDR corrected the LOAEL for discontinuous exposure and applied uncertainty factors (UFs) for the use of a LOAEL and for intraspecies variability. The MRL was calculated as: 25 x (8/24 x 5/7) / 10 x 10 equal 0.06 ppm (ATSDR, 1992). The MRL was a factor of 3 different from the proposed REL.

For comparison, chronic exposure levels for styrene can be developed from chronic inhalation studies in rats (Cruzan *et al*., 1998) and mice (Huntingdon Life Sciences, 1998). The mice were more sensitive to the styrene vapors than were rats, and a LOAEL of 20 ppm was identified based on lesions in various organs in both sexes. The adjustment factor for discontinuous exposure is (6/24 x 5/7) = 0.18. The uncertainty factors are: 10 for intraspecies variability, 3 for interspecies sensitivity, and 10 for adjustment from a LOAEL to a NOAEL. The resultant exposure level is (20 ppm x 0.18) / 300 which equals 0.01 ppm or 10 ppb (40 $\mu g/m^3$). Besides the different toxic endpoints between the chronic mouse exposure study and human occupational studies, the well designed human study of Mutti *et al.* (1984b) is preferable for REL development because it does not introduce the uncertainties associated with interspecies extrapolations.

The NOAEL of 50 ppm from the chronic rat study of Cruzan *et al*. (1998) may be adjusted to an equivalent continuous exposure of 8.9 ppm. Use of an RGDR of 1, an interspecies UF of 3, and an intraspecies UF of 10 resulted in an estimated REL of 300 ppb (1300 $\mu g/m^3$) for styrene.

VII. Data Strengths and Limitations for Development of the REL

The strengths of the REL for styrene include the excellent database available on styrene effects and the availability of a suitable human study for use as the key study. Limitations include the lack of direct exposure data and selection bias. Although a NOAEL was not observed in the key study, the BMC_{05} is considered to be similar to a NOAEL in estimating a concentration associated with a low level of risk.

Use of urinary metabolite concentrations to indirectly determine styrene exposure, while an accepted approach, still introduces another level of uncertainty in the hazard assessment. In addition, potential absorption of styrene via dermal exposure in the reinforced plastics industry has not been addressed and may overestimate the air concentration determined by urinary metabolite levels. However, unlike air levels, the presence of urinary metabolites of styrene gives an unequivocal indication that an individual has been exposed to styrene. At the present time, a system does not exist to obtain direct exposure information, although a recent report suggests a methodology is being developed (Jensen *et al.*, 1995).

A potential bias in the key study was the finding that general intelligence, as measured by the vocabulary test, appeared to be negatively correlated with both age and exposure intensity. This finding suggests that age may also be a factor in poor neuropsychological test scores of highly exposed subgroups. Another source of uncertainty is that the reinforced plastics industry, from which the workers in the Mutti *et al.* (1984) study were taken, is characterized by a large turnover of highly exposed workers (Wong, 1990; Kogevinas *et al.*, 1993). This possible selection bias may result in more sensitive workers leaving employment while more tolerant workers remain.

VIII. References

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CHRONIC TOXICITY SUMMARY

TOLUENE

(Methyl benzene; methyl benzol; phenyl methane; toluol)

CAS Registry Number: 108-88-3

I. Chronic Toxicity Summary

Inhalation reference exposure level	**300 µg/m^3** (70 ppb)
Critical effect(s)	Neurotoxic effects (decreased brain [subcortical limbic area] weight, altered dopamine receptor binding).
Hazard index target(s)	Nervous system; respiratory system; teratogenicity

II. Physical and Chemical Properties (HSDB (1999) except as noted)

Description	Colorless liquid
Molecular formula	C_7H_8
Molecular weight	92.13 g/mol
Density	0.8661 g/cm^3 @ 20°C
Boiling point	110.6 ° C (CRC, 1994)
Melting point	–94.9° C (CRC, 1994)
Vapor pressure	28.1 torr @ 25°C (U.S. EPA, 1984)
Solubility	miscible in most organic solvents
Conversion factor	1 ppm = 3.76 mg/m^3 @ 25°C

III. Major Uses or Sources

Toluene occurs naturally as a component of crude oil and is produced in petroleum refining and coke oven operations; toluene is a major aromatic constituent of gasoline (HSDB, 1999). It is used in household aerosols, nail polish, paints and paint thinners, lacquers, rust inhibitor, adhesives and solvent based cleaning agents. Toluene is also utilized in printing operations, leather tanning and chemical processes. Benzene and other polycyclic aromatic hydrocarbons are common contaminants of toluene. Toluene is considered a sentinel chemical for benzene in the context of air and water sample monitoring. In 1996, the latest year tabulated, the statewide mean outdoor monitored concentration of toluene was approximately 2.2 ppb. For 1998, annual statewide industrial emissions of toluene from facilities reporting under the requirements of the Air Toxics Hot Spots Act in California were estimated to be 5,176,626 pounds (CARB, 1999). Note that this estimate is for stationary sources, and does not include emissions from mobile sources.

IV. Effects of Human Exposures

Neurological Effects

Most studies reporting adverse effects due to chronic toluene exposures involve either toluene-containing solvent abuse or occupational exposure to toluene. Solvent abusers are generally exposed to higher levels of toluene than are workers. A continuum of neurotoxic effects ranging from frank brain damage to degraded performance on psychometric tests which roughly track exposure levels has been observed.

Solvent abusers

Chronic toluene abuse has been shown to cause permanent changes in brain structure (loss of grey and white matter differentiation; cerebral, cerebellar and brainstem atrophy) which correlated with brain dysfunction as measured by magnetic resonance imaging (MRI) and brainstem auditory evoked response (BAER) evaluations (Caldemeyer *et al.*, 1996; Filley *et al.*, 1990; Ikeda and Tsukagoshi, 1990; Rosenberg *et al.*, 1988a; Rosenberg *et al.*, 1988b; Yamanouchi *et al.*, 1995; reviewed by Agency for Toxic Substances and Disease Registry (ATSDR), 1999).

Eleven chronic solvent (spray lacquer; ≈ 60% toluene, 10% dichloromethane) abusers were examined using MRI and BAER tests (Rosenberg *et al.*, 1988b). Neurological abnormalities were seen in four of 11 subjects and included brainstem, cerebellar, cognitive and pyramidal findings. Brain MRIs were abnormal in three of 11 subjects and indicated the occurrence of diffuse cerebral, cerebellar, and brainstem atrophy and loss of differentiation between the gray and white matter throughout the CNS. BAERs were abnormal in five of 11 individuals. All three individuals with abnormal MRI scans also had abnormal neurological examinations and BAERs. However, two of five individuals with abnormal BAERs had normal neurological examinations and MRI scans. The authors suggested that BAERs may detect early CNS injury from toluene inhalation, even at a time when neurological examination and MRI scans are normal.

Two subjects of a group of 22 hospitalized solvent abusers (primarily abusing toluene-based solvents) demonstrated decreases in intelligence quotient (IQ) as measured by the comparison of tests administered before the commencement of solvent abuse with tests administered during hospitalization for long-term solvent abuse (Byrne *et al.*, 1991).

Filley *et al.* (1990) studied 14 chronic toluene abusers using MRI and neuropsychological evaluations. The neuropsychological testing indicated that three patients functioned normally, three were in a borderline range, and eight were impaired. Independent analyses of white matter changes on MRI demonstrated that the degree of white matter abnormality was strongly correlated ($p < 0.01$) with neuropsychological impairment. The authors concluded that dementia in toluene abuse appears to be related to the severity of cerebral white matter involvement.

Six chronic toluene abusers were examined using MRI by Caldemeyer *et al.* (1996). All patients examined demonstrated white matter atrophy and T2 hyperintensity (T2: “Spin-spin” relaxation time; a time constant that reflects the rate at which protons stop rotating in phase with each other

because of the local magnetic fields of adjacent nuclei; OTA, 1984), and five of six demonstrated T2 hypointensity of the basal ganglia and thalami. The authors noted a correlation between the severity of white matter degeneration and degree of neurological dysfunction. However, there was no correlation between the severity of imaged white matter changes and the presence of T2 hypointensity or duration of toluene abuse. Additionally, no definite clinical evidence of damage to the basal ganglia and thalami was found despite the MR imaging finding of T2 hypointensity..

Ungar *et al*. (1994) developed a physical bilayered model of dipalmitoylphosphatidylcholine (DPPC) and toluene, and subjected DPPC control and toluene-mixed bilayers to MRI. T1 (T1: "Spin-lattice" relaxation time; a time constant that reflects the rate at which excited protons exchange energy with the surrounding environment; OTA, 1984) and T2 were measured as a function of toluene and lipid concentrations. Measurements of the DPPC-toluene model indicated that toluene-containing lipid bilayers substantially shortened T2 and had little effect on T1. By comparison, DPPC alone had little effect on either T1 or T2. The authors believe that these results suggest that partitioning of toluene into the lipid membranes of cells in cerebral tissue may be responsible for the hypointensity of basal ganglia noted on T2-weighted MR images of brains of toluene abusers.

Occupational exposure

Solvent workers exposed to 42.8 ppm toluene (estimated as a time-weighted average) for an average duration of 6.8 years reported a significantly greater incidence of sore throat, dizziness and headache than controls; the sore throat and headache incidence demonstrated a rough dose-response (Yin *et al.*, 1987).

Orbaek and Nise (1989) examined the neurological effects of toluene on 30 rotogravure printers, 33-61 years of age (mean 50), employed at two Swedish printing shops for 4-43 years (median 29) in 1985. Mean exposure levels at the two printing shops were 43 and 157 mg/m^3 of toluene, respectively; however, before 1980 the exposure levels had exceeded 300 mg/m^3 in both shops. The authors noted that rotogravure printing provides an occupational setting with practically pure toluene exposure. Comparisons were made to a reference group of 72 men aged 27-69 (mean 47). The alcohol consumption of both the workers and referents was also determined (< 200 g/week or > 200 g/week). Neurological function in the workers and referents was evaluated using interviews and psychometric testing; the results from each of the two printing shops were pooled. The printers reported statistically significantly higher occurrences of fatigue (60%), recent short-term memory problems (60%), concentration difficulties (40%), mood lability (27%), and other neurasthenic symptoms. The printers also scored significantly worse than referents in a number of psychometric tests, including synonym, Benton revised visual retention and digit symbol tests, even after adjustment for age. For all comparisons, tests of interaction between the effects of toluene exposure and alcohol consumption were not statistically significant.

A battery of neurobehavioral tests was performed in 30 female workers exposed to toluene vapors in an electronic assembly plant (Foo *et al.*, 1990). The average number of years worked was 5.7 ± 3.2 for the exposed group and 2.5 ± 2.7 years for the controls. Study subjects did not smoke tobacco or drink alcohol, were not taking any medications, and had no prior history of

central or peripheral nervous system illness or psychiatric disorders. The exposed group of workers inhaled a time-weighted average (TWA) of 88 ppm (330 mg/m^3) toluene while the control workers inhaled 13 ppm (49 mg/m^3). A significant decrease in neurobehavioral performance was observed in the exposed workers in 6 out of 8 tests. Irritant effects were not examined, and concurrent exposures to other chemicals were not addressed. In this study, 88 ppm was considered a LOAEL for central nervous system effects. However, the workers designated by the authors to be controls did not comprise a true control group, since they were exposed to 13 ppm toluene. This may have resulted in an underestimation of the effects of exposure to 88 ppm toluene. Similar effects were noted in a follow-up study by Boey *et al.* (1997).

Abbate *et al.* (1993) evaluated alterations induced in the auditory nervous system by exposure to toluene in a group of rotogravure workers. A sample of 40 workers of normal hearing ability was selected from a group of 300 workers who were apparently in good health but were professionally exposed to toluene (12 – 14 years exposure, 97 ppm average exposure, exposure assessment not described). They were subjected to an adaptation test utilizing a BAER technique with 11 and 90 stimulus repetitions a second. The results were compared with an age and sex-matched control group not professionally exposed to solvents. A statistically significant alteration in the BAER results was noted in the toluene-exposed workers with both 11 and 90 stimuli repetitions. The authors suggested that these results can be explained as a toluene-induced effect on physiologic stimulus conduction mechanisms, even in the absence of any clinical sign of neuropathy. Furthermore, this effect could be observed in the responses of the entire auditory system, from peripheral receptors to brainstem nuclei.

A group of 49 printing-press workers occupationally exposed to toluene for approximately 21.6 years was studied by Vrca *et al.* (1997). Toluene exposure levels were determined from blood toluene and urinary hippuric acid levels, and were estimated to range from 40-60 ppm. No control group was used. Brain evoked auditory potential (BEAP; similar to BAER) and visual evoked potential (VEP) measurements were performed on a Monday morning after a nonworking weekend. There was a significant increase in the latencies of all the BEAP waves examined, except for P2 waves, as well as in the interpeak latency (IPL) P3-P4, while IPL P4-P5 decreased significantly with the length of exposure. No correlation was noted between the amplitude of BEAP waves and the length of exposure. The amplitude but not the latency of all the VEPs examined decreased significantly with the length of exposure.

The effects of acute and chronic toluene exposure on color vision were studied in a group of eight rotogravure printing workers (Muttray *et al.*, 1999). The workers had been employed as printers for an average of 9.8 years. The color vision acuity of the workers before and after an acute toluene exposure (28 – 41 minutes in duration, concentration 1115 – 1358 mg/m^3) was evaluated using the Farnsworth panel D-15 test, the Lanthony desaturated panel D-15 test, and the Standard Pseudoisochromatic Plates part 2. A control group of 8 unexposed workers was also tested. Acute toluene exposure had no effect on color vision. Print worker performance prior to acute toluene exposure (chronic effects) was similar to controls on the Farnsworth panel D-15 and Standard Pseudoisochromatic Plates part 2 tests. Print worker performance on the Lanthony desaturated panel D-15 test was worse than that of controls (median scores of 1.18 and 1.05 for exposed and controls (higher number indicates degraded performance), respectively,

but not significantly ($p = 0.06$). The authors noted that the small number of subjects limited the statistical power of the study.

Zavalic *et al.* (1998) examined the effects of chronic occupational toluene exposure on color vision using a group of 45 exposed workers (mean toluene exposure concentration = 120 ppm) and 53 controls. Color vision was evaluated using the Lanthony desaturated panel D-15 test; test scores were age and alcohol consumption-adjusted. Color vision was significantly impaired in toluene-exposed workers ($p < 0.0001$) compared to controls. It was also observed that there was no significant difference between test scores on Monday morning (prework) and Wednesday morning. The authors stated that the effect of toluene on color vision can be chronic and that the possible recovery period is longer than 64 hours.

Hepatic Effects

Greenburg *et al.* (1942) reported liver enlargement in 32 of 106 (30.2%) painters employed in an aircraft factory compared to 7% in a control group. However, there was some exposure to other solvents (ethanol, ethyl acetate, butyl acetate) and paint ingredients such as zinc chromate.

Liver toxicity has been reported in toluene solvent abusers (Fornazzari *et al.*, 1983). Eight of 24 solvent abusers demonstrated abnormal results in three liver function tests; however, the tests used were not specified. The test parameters returned to normal after two weeks of toluene abstinence, suggesting that any liver damage caused by toluene abuse in those patients was not long lasting.

A cross-sectional study by Boewer *et al.* (1988) showed no significant correlation between toluene exposure and the levels of serum enzymes (serum aspartate aminotransferase (ASAT), alanine aminotransferase (ALAT), γ-glutamyltransferase (GGT)) considered to be indicators of hepatic damage. In another cross-sectional study of 289 printing workers exposed to less than 200 ppm for 8 hours/day, 8 workers had significantly elevated serum enzymes (ALT/AST ratio, mean = 1.61) potentially indicative of liver damage. In each case, liver biopsy indicated a mild pericentral fatty change (Guzelian *et al.*, 1988). However, the mean toluene exposure concentration was not reported (only an upper bound), and no control group was included in the study.

V. Effects of Animal Exposures

Neurotoxic Effects

Sprague-Dawley rats (15/sex/group) were exposed to 0, 100, or 1481 ppm toluene for 6 hours/day, 5 days/week for 26 weeks (API, 1981). Neurohistopathological examinations were conducted in 3-5 rats/sex/group at weeks 9, 18, and 27. No significant treatment-related effects were reported. The study usefulness was limited because there were no other neurohistopathological examinations or organ weight measurements conducted on the animals.

Forkman *et al.* (1991) studied the potential neurotoxicity of toluene inhalation exposure (3700 mg/m^3 (1000 ppm), 21 hours/day, 5 days/week for 4 weeks) in male Sprague-Dawley rats. The

rats were either trained in behavior meant to be performance tested and then exposed to toluene, or exposed and then trained. The rats were then subjected to several behavioral tests, including an operant test with baseline performance and extinction, motor coordination, and exploratory activity. All tests were performed from 11 to 35 days after the end of the exposure. Exposure of trained rats to toluene resulted in a significantly different overall test performance when compared to controls. Rats trained after toluene exposure also had test performances different from controls, but the difference was not statistically significant.

Rats exposed to toluene concentrations of 1000 ppm or 100 ppm, 6 h/day, 5 days/week, for 3 or 6 months, respectively, demonstrated statistically significant decreased motor function as measured by degraded performance (approximately 60% and 65% of control at 1000 and 100 ppm toluene, respectively) on a rotarod performance test and decreases in spontaneous motor activity (approximately 62% of control at 100 ppm toluene) (Korsak *et al*., 1992).

von Euler *et al.* (1993) studied the effects of subchronic toluene inhalation exposure (80 ppm, 4 weeks, 5 days/week, 6 hours/day) on spatial learning and memory, dopamine-mediated locomotor activity and dopamine D_2 agonist binding in rats. Spatial learning (postexposure days 3-6) and memory (postexposure day 14) were tested using a water maze. Spontaneous and apomorphine-induced locomotor activity was evaluated on postexposure day 17. Effects on binding parameters of the dopamine D2 agonist S(-)[N-propyl- ^{3}H(N)]-propylnorapomorphine ([H]NPA) were determined using membrane preparations of the neostriatum of the rat brain. Toluene exposure caused a statistically significant impairment in spatial learning and memory. Toluene also significantly increased apomorphine-induced locomotion and motility but not rearing. Spontaneous locomotion, motility and rearing were not affected by toluene. Toluene exposure significantly increased the B_{max} and K_D values for [^{3}H]NPA binding. These results indicate that subchronic toluene exposure of rats to toluene causes persistent deficits in spatial learning and memory, a persistent increase in dopamine-mediated locomotor activity and an increase in the number of dopamine D_2 receptors in the neostriatum.

Male rat exposure to toluene (0, 40, 80, 160 or 320 ppm, 4 weeks, 6 hours/day, 5 days/week) , followed by a postexposure period of 29-40 days, resulted in decreased brain wet weights of the caudate-putamen (trend test for dose-response significant at $p < 0.05$) and subcortical limbic areas (trend test for dose-response significant at $p < 0.01$; significantly less than controls ($p < 0.001$) at concentrations of 80 ppm and higher) (Hillefors-Berglund *et al.*, 1995). Toluene exposure also significantly altered dopamine receptor activity (trend test for dose-response) as indicated by decreased IC_{50} (inhibition constant) (significantly less than controls ($p < 0.05$) at 80 ppm), K_H (inhibition constant for high-affinity receptor sites), K_L (inhibition constant for low-affinity receptor sites), and R_H% (high-affinity receptor site specific binding) values for dopamine competitive inhibition of [^{3}H]raclopride-binding in the caudate-putamen. Toluene exposure did not significantly affect the wet weights of the whole brain, serum prolactin levels, the K_D (disassociation constant) or the B_{max} (maximal specific binding) values of [^{3}H]raclopride-binding in the caudate-putamen and the subcortical limbic area, or the effect of dopamine on IC_{50} values at [^{3}H]raclopride-binding sites in the subcortical limbic area. Exposure to xylene or styrene (80 and 40 ppm, respectively; 4 weeks, 6 h/day, 5 days/week) followed by a postexposure period of 26-32 days had no effect on the parameters described above. The authors concluded that long-term exposure to low concentrations of toluene (≥ 80 ppm), but not xylene

(80 ppm) or styrene (40 ppm), leads to persistent increases in the affinity of dopamine D_2 agonist binding in the rat caudate-putamen. The authors also suggested that the enhancement of apomorphine-induced locomotor activity seen after toluene exposure by von Euler *et al.* (1993) may be related to the increased D_2 agonist activity described above (IC_{50}, K_H, K_L values).

Respiratory Effects

A study of the chronic effects of toluene in rats (5-20 animals per group) exposed for 106 weeks to 0, 30, 100, or 300 ppm (0, 113, 375, or 1125 mg/m^3) toluene showed no treatment-related effects on histopathology of major organs, including the nasal turbinates (CIIT, 1980). In this study, the nasal histopathology examination sampling may have been inadequate to demonstrate the nasal lesions reported by the NTP (1990).

Rats (20 per group) exposed for 2 years to 0, 600, or 1200 ppm (0, 2261, or 4523 mg/m^3) toluene 6.5 hours/day, 5 days/week for 103 weeks were examined for hematological and histopathological effects in addition to gross observations of toxicity (NTP, 1990). Significant erosion of the olfactory epithelium was observed in male rats while degeneration of the respiratory and nasal epithelium was observed in both sexes at 600 ppm.

Mice were exposed chronically to 0, 120, 600, or 1200 ppm (0, 452, 2261, or 4523 mg/m^3) toluene 6.5 hours/day, 5 days/week, for 2 years (NTP, 1990). The only treatment-related effect was a significant increase in the number of animals with hyperplasia of the bronchial epithelium in the 1200 ppm exposure group.

Reproductive and Developmental Toxicity

Reproductive toxicity to maternal rats was observed during exposure to 1500 ppm toluene, 24 hours/day on days 9 to 14 of gestation (Hudak and Ungvary, 1978). Two dams out of 19 died during exposure. Fetuses from the 1500 ppm group showed increased incidence of sternebral alterations, extra ribs and missing tails. The same exposure on days 1 through 8 of gestation resulted in 5 deaths out of 14 dams. Fetuses in this regimen showed increased incidence of hydrocephaly and growth retardation compared to controls. A third regimen that exposed maternal rats to 1000 ppm on days 1 through 21 of gestation resulted in no maternal deaths or toxicity, and an increase in the incidence of skeletal variations in the fetuses. When exposed to 1500 ppm continuously, maternal mice died within 24 hours of exposure whereas exposure to 500 ppm had no apparent effect. Examination of the fetal mice showed significant growth retardation in the 500 ppm group.

A 2-generation study of the effects of 0, 100, 500, or 2000 ppm (0, 377, 1885, or 7538 mg/m^3) toluene in rats (males, 10-40 per group; females, 20-80 per group) was done by the American Petroleum Institute (API)(1985). Rats were exposed for 6 hours/day, 7 days/week for 80 days and a 15 day mating period. The mated females were then exposed to the same concentrations during days 1-20 of gestation and days 5-20 of lactation. After weaning, the F_1 pups were exposed 80 times to the appropriate exposure level and then randomly mated to members of the same exposure group. The F_1 generation showed significantly decreased body weight which

persisted throughout lactation. No effects were observed on histopathology. No data were presented for the F_2 generation.

Da Silva *et al.* (1990) exposed rats and hamsters to 0 or 800 mg/m^3 toluene for 6 hours/day on gestation days 14-20 (rats), or days 6-11 (hamsters). Exposed rats demonstrated a significant exposure-related decrease in birth weight compared with controls. In addition to low birth weight, the number of live pups was significantly lower in the 800 ppm group. No deficits in any parameter were noted in the hamsters. In this study, no neurobehavioral effects were noted in the offspring.

Hass *et al.* (1999) exposed rats to 0 or 1200 ppm toluene for 6 h per day from day 7 of pregnancy until day 18 postnatally. Developmental and neurobehavioral effects in the offspring were investigated using a test battery including assessment of functions similar to those in the proposed Organization for Economic Cooperation and Development (OECD) Testing Guidelines for Developmental Neurotoxicity Study (physical development, reflex development, motor function, motor activity, sensory function, and learning and memory). The exposure did not cause maternal toxicity or decreased offspring viability. However, lower birth weight, delayed development of reflexes, and increased motor activity in the open field was noted in the exposed offspring. The exposed female offspring had poorer scores on a Morris water maze test (they took longer to locate a hidden platform after platform relocation) at the age of 3.5 months indicating impaired cognitive function. The difference was not related to impaired swimming capabilities since swim speeds were similar to control values. The authors stated that exposure to 1200 ppm toluene during brain development caused long-lasting developmental neurotoxicity in rats.

Toluene has been listed under Proposition 65 as being known to the State of California to cause reproductive toxicity (OEHHA, 1999). Its NSRL is 7,000 micrograms per day.

VI. Derivation of Chronic Reference Exposure Level (REL)

Study	Hillefors-Berglund *et al*. (1995); supported by Orbaek and Nise (1989), Foo *et al.* (1990)
Study population	Male Sprague-Dawley rats
Exposure method	Inhalation
Critical effects	Decreased brain (subcortical limbic area) weight, Altered dopamine receptor (caudate-putamen) binding
LOAEL	80 ppm
NOAEL	40 ppm
Exposure continuity	6 hours/day, 5 days/week
Exposure duration	4 weeks, followed by 29-40 days recovery
Average experimental exposure	7 ppm (40 × 6/24 hours × 5/7 days)
Human equivalent concentration	7 ppm (gas with systemic effects, based on RGDR = 1.0 using default assumption that $\lambda_a = \lambda_h$
Subchronic uncertainty factor	10
Interspecies uncertainty factor	1 (see below)
Intraspecies uncertainty factor	10
Cumulative uncertainty factor	100
Inhalation reference exposure level	0.07 ppm (70 ppb; 0.3 mg/m^3; 300 µg/m^3)

Supportive human study	Foo *et al.*, 1990
Study population	30 female workers in an electronic assembly plant
Exposure method	Occupational inhalation
Critical effects	Neurobehavioral deficits in 6 out of 8 tests
LOAEL	88 ppm
NOAEL	Not observed
Exposure continuity	10 m^3/day occupational inhalation rate, 5 days/week
Average occupational exposure	31.4 ppm (88 ppm x 10/20 x 5/7)
Exposure duration	5.7 $\pm$ 3.2 years (exposed group); 2.5 $\pm$ 2.7 years (controls)
LOAEL uncertainty factor	10
Subchronic uncertainty factor	3
Interspecies uncertainty factor	1
Intraspecies uncertainty factor	10
Cumulative uncertainty factor	300
Inhalation reference exposure level	0.1 ppm (100 ppb; 0.4 mg/m^3; 400 µg/m^3)

The critical animal study (Hillefors-Berglund *et al*., 1995) used to derive an REL for toluene describes adverse neurological effects in rats after a well characterized inhalation exposure to toluene. The study results contain both a LOAEL and a NOAEL. Decreased brain (subcortical limbic area) weight and altered dopamine receptor binding compared to controls were noted at the NOAEL, but the changes were not statistically significant; this suggests that if a threshold for

adverse neurological effects exists in this study, it would be at or below the observed NOAEL. The study LOAEL for altered dopamine receptor binding agrees qualitatively with results from similar studies (von Euler *et al.*, 1994). Additionally, toluene-induced neurotoxicity has been described in many studies by a variety of endpoints in both animals and humans (ATSDR, 1999). The adverse neurotoxic effects associated with toluene exposure in the rat study by Hillefors-Berglund *et al*. (1995), decreased brain (subcortical limbic area) weight and altered dopamine receptor binding, occur in areas of the rat brain that are structurally and functionally similar to brain areas (basal ganglia, thalami) of some human toluene abusers that demonstrate MRI alterations (T2 hypointensity). The altered MRI parameters may be the result of the partitioning of toluene into the lipid membranes of brain cells (Ungar *et al*., 1994). Table 1 lists several Reference Exposure Levels (RELs) calculated from the most sensitive animal and human neurotoxicity studies available. These RELs are also protective for other adverse endpoints, such as respiratory tract damage and teratogenicity.

Table 1: Reference Exposure Levels (RELs) from Selected Neurotoxicity Studies

Study	Duration	Effect	LOAEL (ppm)	LOAEL (ppm) (TWA)	NOAEL (ppm)	NOAEL (ppm) (TWA)	total UF[a]	REL (ppb)	REL (µg/m^3)
VonEuler *et al*. (1988)	4 weeks	rat: altered brain dopamine receptor binding	80	14.3			1000	14	54
Orbaek and Nise[b] (1989)	29 years	human: impairment on neuropsychometric tests	11.2 - 41	4 - 14.6			100	40 - 146	150 - 551
Foo (1990)	5.7 years	human: neurobehavioral tests	88	31.4			300	105	394
Korsak (1992)	6 months	rat: impaired motor function	100	17.9			100	179	671
Hillefors-Berglund (1995)	4 weeks	rat: decreased brain (subcortical limbic area) weight; altered brain dopamine receptor binding	80	14.3	40	7.1	100	71	271

LOAEL: Lowest Observable Effect Level; NOAEL: No Observable Effect Level
REL: Reference Exposure Levels; TWA: time-weighted average

a: Uncertainty Factors used to derive RELs

VonEuler *et al*. (1988)	LOAEL to NOAEL UF = 10, subchronic to chronic UF = 10, animal to human UF = 1, intraspecies variability = 10; total UF = 1000.
Orbaek and Nise (1989)	LOAEL to NOAEL UF = 10, intraspecies variability = 10; total UF = 100
Foo *et al*. (1990)	LOAEL to NOAEL UF = 10, subchronic to chronic UF = 3, intraspecies variability = 10; total UF = 300
Korsak *et al*. (1992)	LOAEL to NOAEL UF = 10, animal to human UF = 1, intraspecies variability = 10; total UF = 100.
Hillefors-Berglund *et al*. (1995)	subchronic to chronic UF = 10, animal to human UF = 1, intraspecies variability = 10; total UF = 100.

b: Pooled psychometric data from two printing plants with different toluene concentrations (11.2 and 41 ppm) were used to determine significant neurotoxic effects by Orbaek and Nise (1989). The range of RELs derived from that study lists the upper and lower bounds for risk associated with the pooled population exposures. ATSDR (1999) used the Orbaek and Nise (1989) study data, assuming an exposure concentration of 11.2 ppm, to derive a chronic inhalation minimal risk level (MRL).

If both human and animal adverse effect data on a chemical are available, OEHHA prefers to use the human data to develop a REL when possible. However, the study by Hillefors-Berglund *et al*. (1995) provides data (decreased brain [subcortical limbic area] weight and altered brain dopamine receptor binding) which are specific and sensitive measures of neurotoxicity that would not be obtainable in human studies. In contrast, the psychometric tests used to generate the neurotoxicity data in the human occupational exposure studies described above tend to be less sensitive and suffer from greater measurement uncertainty. Additionally, the Hillefors-Berglund *et al*. (1995) study has better exposure characterization than the human occupational exposure studies. Nonetheless, the human studies are useful in supporting the derivation of the REL for toluene. Ordinarily, an interspecies uncertainty factor of 3 would be applied, in addition to the human equivalent concentration calculation, to reflect the uncertainty associated with extrapolating from animals to humans. However, in this case the uncertainty in the interspecies extrapolation is reduced by the availability of human epidemiological data with generally consistent effect levels, after appropriate duration corrections. Based on comparison of the data in both animals and humans, it appears that a REL of 271 $\mu g/m^3$ (rounded to 300 $\mu g/m^3$ in the final derivation) would protect exposed humans from experiencing chronic neurotoxic effects.

VII. Data Strengths and Limitations for Development of the REL

The major strength of the REL for toluene is the use of an animal study with accurate exposure characterization and both LOAEL and NOAEL observations for an effect (neurotoxicity), supported by observations from other animal and human studies. A weakness is the uncertainty in predicting human health risk from animal adverse effect data. However, this is mitigated by the availability of human data showing effect levels that are, after appropriate corrections, broadly consistent with the animal data.

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CHRONIC TOXICITY SUMMARY

TRICHLOROETHYLENE

(trichloroethylene; 1,1-2-trichloroethylene, 1,1-dichloro-2-chloroethylene, acetylene trichloride, and ethylene trichloride))

CAS Registry Number: 79-01-6

I. Chronic Toxicity Summary

Inhalation reference exposure level	**600 µg/m^3** (100 ppb)
Critical effect(s)	Neurotoxicological effects (drowsiness, fatigue, headache) and eye irritation in workers.
Hazard index target(s)	Nervous system; eyes

II. Physical and Chemical Properties (Fan, 1988; CRC, 1994)

Description	Colorless liquid/vapor; sweetish, chloroform-like odor
Molecular formula	C_2HCl_3
Molecular weight	131.4
Density	1.47 g/cm^3 @ 20°C
Boiling point	87.2 °C
Melting point	–84.7°C
Vapor pressure	77 torr @ 25°C
Vapor density	4.5 (air = 1)
Solubility	Soluble in alcohol, ethers, petroleum distillates and other halogenated solvents
Conversion factor	1 ppm = 5.37 mg/m^3 @ 25° C

III. Major Uses or Sources

Trichloroethylene was once used as an extractant in food processing and has been used as an anesthetic and analgesic for medical purposes (Waters *et al.* 1977). Currently, it is widely used as a solvent in the industrial degreasing of metals, with secondary solvent uses in adhesive paint and polyvinyl chloride production (U.S. EPA, 1985). Trichloroethylene is used as a solvent in the textile industry, as a solvent for adhesives and lubricants, and as a low-temperature heat transfer fluid (IARC, 1979). Trichloroethylene is also implemented in the manufacturing of pesticides and other chemicals (Feldman, 1979). In 1996, the latest year tabulated, the statewide mean outdoor monitored concentration of trichloroethylene was approximately 0.035 ppb (CARB, 1999a). The annual statewide emissions from facilities reporting under the Air Toxics Hot Spots Act in California based on the most recent inventory were estimated to be 176,908 pounds (CARB, 1999b)

IV. Effects of Human Exposure

An occupational study of trichloroethylene (TCE) vapor emissions in a pump room was conducted by Vandervort and Polnkoff (1973). Workers were an average age of 40 and had been employed for an average of 8 years. For 11-day shift workers, individual 8 hour time weighted average (TWA) TCE exposure concentrations were extrapolated from two area samples; these averages ranged from 170-420 mg/m^3 (32-78 ppm). Nineteen workers (including the 11 workers whose work areas were sampled) completed a questionnaire and reported the following symptoms: 73% eye irritation, 70% drowsiness, 58% heart palpitations, 58% cough, 53% weakness and 52% dizziness. About half of the 19 exposed workers reported that consumption of small amounts of alcohol outside of work resulted in changes of skin color and severe intoxication. One worker of the 19 reported no adverse effects from the occupational exposure. Nine control workers experienced none of the above symptoms. Urine samples from the 19 exposed and 9 unexposed workers were collected before and after the work shift and examined for the TCE metabolites trichloroacetic acid (TCA) and trichloroethanol (TRI). TRI levels ranged from 4-260 mg/l and TCA levels ranged from 4-197 mg/l. Results of the urine assays showed a range of TCE metabolite concentrations and, therefore, confirmed that the workers were exposed to a variety of concentrations in their environments.

Nomiyama *et al.* (1977) examined 36 trichloroethylene workers, of whom 9 males and 12 females were occupationally exposed to a constant concentration of trichloroethylene (TCE) and 18 males were exposed to variable concentrations (duration of exposure unspecified). The control group consisted of 6 males and 10 females who were of similar educational, sociologic and economic status to the trichloroethylene workers. Researchers used urinary excretion of TCE metabolites as an indicator of the level of TCE exposure in the working environment; total excreted trichloro-compounds of 100 mg in 4 hours corresponded to 100 ppm TCE present in the working environment (Bardodej, 1958; Medek, 1958). Of the 36 exposed workers, 5 were exposed to 0-25 ppm; 14 were exposed to 25-50 ppm; 6 were exposed to 50-100 ppm; 8 were exposed to 100-150 ppm; and 3 were exposed to 150-200 ppm TCE. In the low exposure group, workers experienced mucous membrane irritation in the eyes, nose and throat, in addition to drowsiness, fatigue and headache. These symptoms were persistent through the higher concentration exposures with an increase in eye irritation, headache, fatigue, and nasal obstruction above 100 ppm TCE. Increases in rhinorrhea and drowsiness were seen above 50 ppm TCE exposure.

Kimmerle and Eben (1973) exposed 4 human subjects (3 males and 1 female) to a subacute regimen of 48 $\pm$ 3 ppm trichloroethylene (TCE) for 4 hours a day over a period of 5 days. Levels of TCE and the metabolites trichlororethanol (TRI) and trichloroacetic acid (TCA) were determined. Trichloroethanol-blood levels were elevated immediately after exposure, and detection of trichloroethanol occurred up to 7 days after the last exposure. TCE-blood concentration increased slightly over the 5 days. Levels of urinary excreted trichloroethanol, as well as the TCA-concentration, increased throughout the study, with the female showing a significantly higher excretion of TCA. Levels of TCA were detected up to 12 days after the final exposure.

Okawa and Bodner (1973) studied the occupational exposure of 24 electrical plant workers to trichloroethylene (TCE). The plant worker group consisted of 22 males and 2 females ranging in age from 21-52 years old. Environmental samples of TCE were collected over three days and yielded varying concentrations of TCE related to the task performed in certain areas (duration of exposure unspecified). Spray booth operators were exposed to an average of 25.3 ppm TCE (13-40 ppm range) in addition to averages of 15.2 ppm n-propyl acetate (NPA) and 6 ppm toluene (TOL). Workers involved in washing board units were exposed to an average value of 39 ppm (6-82 ppm range) TCE. Although the workers wore respiratory protection during the washing procedure, the overall average of airborne TCE in this area was 48.3 ppm. In the testing area of the plant, researchers report that the amounts of toluene and n-propyl acetate were insignificant. Here, TCE levels were an average of 24.4 ppm (range = 8-44 ppm). The solder machine operators were exposed to an average of 44.0 ppm TCE (range = 23-87 ppm) with no NPA or TOL present. During the cleaning of the soldering machines, TCE levels rose to an average of 70.5 ppm (range = 30-106 ppm). Concentrations were only at these elevated levels for 20-30 minutes a day. Researchers note that although other agents were used in the work area, TCE was the only chemical found in significant amounts throughout the work area and that the levels of NPA and TOL were insignificant. An analysis of urinary TCE metabolites indicated that the workers were exposed to a time weighted average concentration of <50 ppm TCE. Three of the 24 workers reported that they were unaffected by their working conditions, but the most prominent complaints consisted of 70.8% workers experiencing nausea, 54.2% headache, 33.3% dizziness, 25.0% fatigue, 25% nose and throat irritation, and 20.8% eye irritation. Workers reported that these symptoms were alleviated hours after leaving the work environment. Researchers collected 8 hour urine samples from 20 of the workers and from 9 controls and analyzed them for TCE metabolites. Results of urinary analysis showed that the controls had exposure to an unspecified amount of TCE. TCA levels in exposed workers were elevated from that of the controls and correlated to the different exposures in specific work areas.

Phoon *et al.* (1984) reported on 5 cases of Stevens-Johnson syndrome (erythema multiforme major) with liver involvement which followed exposure to TCE. In two cases, reactions to the exposure began with a fever followed by an itchy rash on the face spreading over the body. Lesions were observed on the face, arms and in the mouth. Liver function tests were abnormal. One of the two developed jaundice with hepatomegaly. Case #3 developed a similar reaction after 5 weeks of exposure to 216-912 mg/m^3 TCE (40-170 ppm) as did case #5 after two weeks of exposure to 370 mg/m^3 TCE (69 ppm). Case #4 involved a 39 year old man exposed to <50 mg/m^3 TCE (< 9.3 ppm) for three weeks who developed the characteristic rash, lesions and jaundice with slight hepatomegaly. Upon returning to work over the next three weeks, he developed generalized erythrodermia and facial oedema, hepatosplenomegaly and liver failure with septicemia from which he died 14 days later.

Stewart *et al.* (1974) studied the effects of subacute trichloroethylene (TCE) exposure in combination with alcohol consumption. Seven men exposed to 200 ppm TCE ingested 1 quart of beer or 90 ml of 100-proof vodka and developed red blotches on their faces 30-40 minutes later. These lesions enlarged with time until they reached a peak intensity, whereupon they faded. One subject experienced facial flush with the consumption of alcohol for three weeks after the last TCE exposure, while another showed flushing six weeks after the last exposure.

V. Effects of Animal Exposure

Kjellstrand *et al.* (1983) studied the effects of both intermittent and continuous exposures of various concentrations of trichloroethylene on male and female mice over a period of 30 days. The concentrations used range from 37-3600 ppm, and 7 of the 14 groups were continuously or intermittently exposed to lower concentrations of 37, 75, 150, 225 and 300 ppm TCE. Continuous exposure studies were conducted over a period of 30 days for exposure groups of 37, 75, 150 and 300 ppm TCE. All groups consisted of 10 males and 10 females (except the 37 ppm group, consisting of 20 males and 20 females) and were compared to identical groups of air-exposed controls. Liver weights increased in a non-linear fashion as the concentration level of TCE increased. All groups exhibited statistically significant increases in liver weights as compared to the controls. In both the 37 and the 75 ppm groups, the increase in females was less than in males. No increase in spleen weight was detected at either the 37 or 75 ppm exposure level. At the 37 ppm level, a slight increase in plasma butyrylcholinesterase (BuChE) activity (not statistically significant) was also detected. A significant increase in kidney weight was seen in the male 75 ppm group and was more pronounced with increasing concentration. Male mice in the 75 ppm group also showed statistically significant increases in BuChE activity. In the 150 ppm group, male and female liver weight increases were statistically significant and of equal magnitude. A statistically significant increase was seen in the BuChE activity of the 150 ppm male mice. It was not until female mice were exposed to 300 ppm, that they showed slight increase in BuChE activity, while the males increased 3.5 times the controls. Liver weight increases for the 300 ppm group were close to the maximum with females showing greater increase than the males. Ten male and 10 female mice were continuously exposed to 150 ppm TCE for 30 days, but then allowed a 120 day rehabilitation period. Following rehabilitation, liver weights returned to levels comparable to the controls. The elevated BuChE activity returned to a normal level. No significant effects were seen after the period of rehabilitation. A continuous study was performed on 10 male and 10 female mice for 120 days at an exposure level of 150 ppm TCE. No further increase in liver weight occurred beyond the level reached in the 30 day study. Body weight gain was slightly decreased, and the same level of BuChE activity was seen as in the 30 day exposure. The intermittent study consisted of 30 days exposure to 225 ppm TCE for 16 hours a day, 7 days a week. A significant increase was seen in the BuChE activity of male mice, while females did not exhibit an increase in BuChE activity. Both males and females showed statistically significant increases in liver weight. Kidney weight increased in the same manner as in the continuous exposures. The authors noted that "extrapolation of the concentration-effect curve suggests that both liver weight and BuChE activities are influenced at still lower concentration."

Briving *et al.* (1986) examined neurotoxicity as a result of chronic trichloroethylene (TCE) inhalation exposure. Two groups of gerbils (6 in each group) were exposed to 50 or 150 ppm TCE for a period of 12 months. Two equivalent groups were used as controls. Two areas of the brain were specifically observed, the hippocampus and the posterior part of the cerebellar vermis. These discrete brain areas were previously shown to be sensitive towards chlorinated aliphatic solvents (Haglid *et al.*, 1981). Following exposure, gerbils were decapitated and measurements were made of total free tissue amino acids as well as high-affinity uptake and release of ^{3}H-aminobutyric acid (GABA) and ^{14}C-glutamate. A significant increase in

glutathione was seen in the hippocampus of the 150 ppm gerbils, but amino acid levels were not significantly affected. In the posterior part of the cerebellar vermis, glutamate and GABA accumulation levels increased in a dose-dependent manner, with significant increases seen at both 50 and 150 ppm TCE. Evaluation of the hippocampus revealed no significant changes. The authors suggest that the stimulation of transport functions for GABA and glutamate may be triggered by the presence of the TCE metabolite, trichloroethanol. Therefore, the levels of GABA and glutamate are indicative of the amount of trichloroethanol from TCE in the brain.

Kligerman *et al.* (1994) exposed 20 male CD rats to 0, 5, 50, or 500 ppm trichloroethylene (TCE) for 6 hours a day, over a period of 4 days. Groups at each concentration consisted of 5 rats. One of the cytogenetic effects measured was peripheral blood lymphocytes (PBLs), abnormal with regard to sister chromatid exchanges. Also analyzed were the cell cycle, bone marrow micronuclei in polychromatic erythrocytes (MN-PCEs/1000) and micronuclei in cytochalasin B-blocked binucleated cells (MN-BN/1000). The 5 ppm and 500 ppm exposure groups showed a decrease (not statistically significant) in cell cycle. In addition, the 50 ppm group exhibited a statistically significant decrease in cell cycle. For all concentrations, there was an overall increase in the PCE percentage. The number of PCEs with micronuclei also rose with the increasing concentrations of 50 ppm and 500 ppm TCE (not statistically significant due to high control values). The researchers conclude that the resulting increase of MN in exposed rats is indicative of aneuploidy induction as opposed to chromosomal breakage, and that the lack of chromosome aberrations corresponds to spindle effects such as aneuploid induction. Concurrent results of increased levels of leukocyte aneuploidy were also found by Konietzko *et al.* (1978) in degreasing workers occupationally exposed to TCE.

Haglid *et al.* (1981) continuously exposed gerbils to 60 ppm or 320 ppm trichloroethylene (TCE) for 3 months. Following the exposure period, gerbils were maintained for 4 months in TCE-free conditions in order to observe any restoration of neuronal function. Both of the exposed groups as well as the control group consisted of six pairs of males and females. Brain samples were collected from the gerbils after the 4 month non-exposure period and used for determination of DNA and proteins. In order to determine areas of the brain that were sensitive to TCE, researchers examined biochemical and morphological changes in the hippocampus, the posterior part of the cerebellar vermis, and the brain stem. In addition to the biochemical tests, the cerebellum, brain stem, and cerebrum of two gerbils from each group, including the control, were used for neuropathological examination. Brain tissue from 2 gerbils in the control group and the 320 ppm group were examined under the electron microscope. No difference was seen in the body and brain weights of the exposed gerbils compared with controls. A slight but significant increase in soluble proteins was detected in the frontal cerebral cortex of the 60 ppm group, and a more significant elevation was seen in the visual cerebral cortex of both the 60 ppm and 320 ppm groups. In the 60 ppm group, a slight but significant decrease was seen in the soluble proteins of the sensory-motor cortex. Both groups exhibited significant decreases in levels of soluble proteins in the hippocampus, the brain stem, and in the posterior part of the cerebellar vermis. Soluble protein levels in the cerebellar hemisphere and anterior part of the vermis of gerbils in both exposed groups did not differ from the controls. The 320 ppm group showed significantly increased DNA levels in the posterior part of the sensory motor cortex and cerebellar vermis. The glial cytoplasmic protein (S 100 fraction) level of the 60 ppm group was decreased in the frontal and visual cerebral cortex, but increased in the posterior part of the

cerebellar hemisphere and the sensory-motor cortex. However, only a slight decrease of S 100 protein was observed in the visual cerebral cortex of 320 ppm exposed gerbils. The most notable S 100 increase occurred in the hippocampus, brain stem and the posterior part of the cerebellar vermis, indicating that either the glial cells were directly affected or that damage to surrounding neuronal cells caused an indirect response. There was an increase in DNA in the posterior part of the cerebellar vermis in the exposed gerbils, suggesting that TCE induced astroglial cell mitosis. Light microscopy revealed shrinkage of cell bodies and axon swelling occurred in various parts of the brain. The electron microscopy performed on control and 320 ppm brain tissues revealed increased levels of filament bundles in the cytoplasm of some Purkinje and Golgi cell perikarya, lysosomes, myelin bodies and lipid containing lysosomal structures in the exposed gerbils. Unique arrangements of filament bundles were seen in Purkinje and Golgi cell dendrites of the exposed group. A significant decrease in the number of microtubules was observed as well as a decrease in the number of synaptic vesicles in the granular layer. Also, the granular layer had decreased maximal nerve cell surface area. Nerve cells were affected by the exposure as several types were reduced in size with fewer organelles and more lysosomes and myelin bodies. Many axons and dendrites had reduced numbers of microtubles, and there were filament bundles observed that were not present in the controls. Lysosomal structures were increased in the synaptic terminals.

Kimmerle and Eben (1973) performed a subchronic study on 20 male rats for a period of 14 weeks. Rats were exposed to a mean concentration of 55.0 $\pm$4 ppm trichloroethylene (TCE) for 8 hours a day, 5 days a week. The control group consisted of 20 rats who in similar inhalation chambers under similar conditions to that of the exposed rats. Ten exposed rats were analyzed for TCE metabolite excretion on a daily basis. Blood levels of trichloroacetic acid (TCA), trichloroethanol (TRI) and chloral hydrate (CH) were measured during the 2nd, 3rd, 4th, 6th, 9th and 14th weeks. Weekly measurements of body weights were recorded. Macroscopic examinations were performed on the thyroid gland, heart, lungs, liver, kidneys, testes and adrenal glands. Hematological evaluations, liver function tests, and renal function tests were also conducted following exposure. Urinary levels of TRI varied individually among the rats, but a continuous increase in TRI was observed through the 10th week. TCA levels remained fairly constant throughout the duration of the experiment. TCE was not detectable in the blood or the tissues of exposed rats. Although liver and renal function tests did not reveal abnormalities, there was an increase in the liver weights of the exposed rats. The weights of the other organs examined were similar to the controls.

Norpoth *et al.* (1974) observed an increase in liver cytochrome P_{450} activity in 9 rats exposed to 50 ppm trichloroethylene for 28 days, compared with 9 control rats.

VI. Derivation of Chronic Reference Exposure Level

Study	Vandervort and Polnkoff (1973)
Study population	19 workers and 9 controls
Exposure method	Discontinuous occupational inhalation exposure
Critical effects	Drowsiness, fatigue, headache, and eye irritation
LOAEL	32 ppm (170 mg/m^3) in the heavy assembly area
NOAEL	Not observed
Exposure continuity	8 hours a day (10 m^3/day occupational inhalaton rate), 5 days a week
Exposure duration	8 years
Average occupational exposure	11.4 ppm for LOAEL group (32 x 10/20 x 5/7)
Human equivalent concentration	11.4 ppm for LOAEL group
LOAEL uncertainty factor	10
Subchronic uncertainty factor	1
Interspecies uncertainty factor	1
Intraspecies uncertainty factor	10
Cumulative uncertainty factor	100
Inhalation reference exposure level	0.1 ppm (100 ppb; 0.6 mg/m^3; 600 µg/m^3)

The Vandervort and Polnkoff (1973) study accounted for 8 years of human occupational exposure to TCE vapors. Sensitive, non-specific neurotoxicological endpoints were exhibited by a majority of those workers exposed. Although the time-weighted averages (TWAs) included a wide range of concentrations, the TWA of 32 ppm (170 mg/m^3) was shown to contribute to the high incidence (52 - 73%) of adverse effects experienced by the workers. Many of the symptoms reported by the workers may have been due to short-term fluctuations in the concentrations in the workplace. The symptoms were not reported separately for the various TWAs, therefore, the lowest TWA (32 ppm) was chosen as a LOAEL. Uncertainty includes the small number of workers studied, the limited extent of the effects mentioned, and the lack of a NOAEL. Strengths include the use of human data, the demonstration of a dose-response relationship, and exposure estimates correlated with urinary excretion measurements.

This study was the best chronic account of the non-carcinogenic effects of TCE on humans, but several other studies show similar results. Nomiyama *et al.* (1977) found similar endpoints of drowsiness, fatigue and eye irritation in 36 workers occupationally exposed to trichloroethylene. Okawa *et al.* (1973) also saw non-specific neurological endpoints in 24 electrical plant workers who were similarly exposed to TCE.

For comparison with the proposed REL of 100 ppb based on human studies, the LOAEL of 50 ppm trichloroethylene obtained by Briving *et al*. (1986) in gerbils exposed continuously for 12 months was used to estimate a REL based on animal data. Use of a LOAEL UF of 3, a subchronic UF of 1, an interspecies UF of 10, and an intraspecies UF of 10 resulted in an estimated REL of 200 ppb for trichloroethylene.

VII. Data Strengths and Limitations for Development of the REL

The strengths of the inhalation REL for trichloroethylene include the use of human exposure data from workers exposed over a period of years. Major areas of uncertainty are the lack of reproductive and developmental toxicity studies and the lack of observation of a NOAEL.

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CHRONIC TOXICITY SUMMARY

XYLENES

(Xylol or commercial xylenes (mixture of 60-70% m- and remaining percentage is mix of o- and p- xylenes), technical grade xylenes or mixed xylenes (20% o-xylene, 40% m-xylene, 20% p-xylene, 20% ethyl benzene, and traces of toluene and C9 aromatics), o-xylene (1,2-dimethylbenzene or 2-xylene), m-xylene (1,3-dimethylbenzene or 3-xylene), p-xylene (1,4-dimethylbenzene or 4-xylene), also noted as methyltoluene, benzene-dimethyl, dimethylbenzene)

CAS Registry Numbers.: 1330-20-7 (technical mixture of o-, p-, and m-xylene); 95-47-6 (o-xylene); 108-38-3 (m-xylene); 106-42-3 (p-xylene)

I. Chronic Toxicity Summary

Inhalation reference exposure level	**700 µg/m^3** (200 ppb) (for technical or mixed xylenes or sum of individual isomers of xylene)
Critical effect(s)	CNS effects in humans; irritation of the eyes, nose, and throat
Hazard index target(s)	Nervous system; respiratory system

II. Physical and Chemical Properties (ATSDR, 1995; HSDB, 1995; CRC, 1994)

Description	Colorless liquid
Molecular formula	C_8H_{10}
Molecular weight	106.16 g/mol
Density	0.864 g/cm^3 @ 20°C(technical mixture); 0.881 (o-); 0.860 (m-); 0.861 (p-)
Boiling point	137-140°C @ 760 torr (technical mixture); 144.5 °C (o-); 139.1°C (m-); 138.3 °C (p-)
Melting point	–25.2 °C (o-); –47.8°C (m-); +13.2 °C (p-)
Vapor pressure	6.6 torr (o-); 8.39 torr (m-); 8.87 torr (p-) all @ 25°C.
Solubility	Practically insoluble in water; miscible with absolute alcohol, ether and many other organic solvents
Conversion factor	1 ppb = 4.34 µg/m^3

III. Major Uses or Sources

Mixtures of o-, p-, and m-xylenes are extensively used in the chemical industry as solvents for products including paints, inks, dyes, adhesives, pharmaceuticals, and detergents (HSDB, 1995). In the petroleum industry xylenes are used as antiknock agents in gasoline, and as an intermediate in synthetic reactions. Of the three isomers, p-xylene is produced in the highest

quantities in the U.S. for use in the synthesis of phthalic, isophthalic, and terephthalic acid used in manufacture of plastics and polymer fibers including mylar and dacron. In 1996, the latest year tabulated, the statewide mean outdoor monitored concentration of meta/para-xylene was approximately 1 ppb (CARB, 1999a). The annual statewide emissions from facilities reporting under the Air Toxics Hot Spots Act in California based on the most recent inventory were estimated to be 3,568,318 pounds of xylenes (CARB, 1999b). Also reported were speciated emissions of p-xylene - 51,203 pounds, of o-xylene - 34,573 pounds, and of m-xylene - 30,440 pounds. (Xylenes are also present in motor vehicle exhaust.)

IV. Effects of Human Exposure

Information on the toxicity of xylenes to humans is almost exclusively limited to case reports of acute exposures and studies of occupational exposures in which persons often inhaled a mixture of hydrocarbon solvents 8 hours per day, 5-6 days per week. These studies often have incomplete information on the airborne concentrations of xylene and other hydrocarbons. One study examining chronic effects in humans from inhalation of predominantly mixed xylenes was identified (Uchida *et al.*, 1993) and one 4-week controlled exposure study examining the effects of p-xylene exclusively was identified (Hake *et al.*, 1981). No studies examining the chronic effects of oral or dermal xylene exposure in humans were identified.

Pharmacokinetic studies have documented the absorption of xylene in humans through inhalation, oral, and dermal routes of exposure. Approximately 60% of inspired xylene is retained systemically (Sedivec and Flek, 1979). The majority of ingested xylene (~90%) is absorbed into the systemic circulation (ATSDR, 1995). Xylene is also absorbed dermally; the rate of absorption of xylene vapor is estimated as 0.1-0.2% of that by inhalation (Riihimaki and Pfaffli, 1978). Loizou *et al*. (1999) exposed human volunteers to 50 ppm *m*-xylene for 4 hours and determined that the dermal route of exposure contributed 1.8% of the total body burden. Measurement of the rate of absorption through direct contact with the skin produced variable results ranging from 2 $\mu g/cm^2/min$ (Engstrom *et al.*, 1977) to 75-160 $\mu g/cm^2/min$ (Dutkiewicz and Tyras, 1968).

Xylene exposure has been associated with effects in a number of organ systems including the lungs, skin and eyes; neurological system; heart and gastrointestinal system; kidney; and possibly the reproductive system.

Pulmonary effects have been documented in occupational exposures to undetermined concentrations of mixed xylenes (and other solvents) and include labored breathing and impaired pulmonary function (Hipolito 1980; Roberts *et al.*, 1988). High levels of xylene exposure for short periods are associated with irritation of the skin, eyes, nose and throat (ATSDR, 1995). Chronic exposure to xylenes has been associated with eye and nasal irritation (Uchida *et al.*, 1993).

The central nervous system is affected by both short term and long term exposure to high concentrations of xylene. Levels of 100-200 ppm are associated with nausea and headache; 200-500 ppm with dizziness, irritability, weakness, vomiting, and slowed reaction time; 800-10,000

ppm with lack of muscle coordination, giddiness, confusion, ringing in the ears, and changes in sense of balance; and >10,000 ppm with loss of consciousness (HESIS, 1986). Other documented neurological effects include impaired short term memory, impaired reaction time, performance decrements in numerical ability, and impaired equilibrium (dizziness) and balance (Carpenter *et al.*, 1975; Dudek *et al.*, 1990; Gamberale *et al.*, 1978; Riihimaki and Savolainen, 1980; Savolainen and Linnavuo, 1979; Savolainen and Riihimaki 1981; Savolainen *et al.*, 1979; 1984; 1985).

Chronic exposure to xylenes (with other hydrocarbons) has been associated with cardiovascular and gastrointestinal effects. Heart palpitations, chest pain, and abnormal electrocardiogram were noted (Hipolito, 1980; Kilburn *et al.*, 1985) as were effects on the gastrointestinal system producing nausea, vomiting and gastric discomfort in exposed workers (Goldie, 1960; Hipolito, 1980; Uchida *et al.*, 1993; Klaucke *et al.*, 1982; Nersesian *et al.*, 1985).

Results of studies of renal effects of xylene are mixed and come from case reports and occupational studies where multiple chemical exposures are common. The effects from subchronic exposure documented by Hake *et al.* (1981) and from chronic exposure documented by Uchida *et al.* (1993) did not include renal effects. However, Morley *et al.* (1970) found increased BUN and decreased creatinine clearance; Martinez *et al.* (1989) found distal renal tubular acidemia; Franchini *et al.* (1983) found increased levels of urinary β-glucuronidase; and Askergren (1981, 1982) found increased urinary excretion of albumin, erythrocytes, and leukocytes.

Reproductive effects were documented by Taskinen *et al.* (1994) who found increased incidence of spontaneous abortions in 37 pathology and histology workers exposed to xylene and formaldehyde in the work place. The multiple chemical exposures and the small number of subjects in this study limit the conclusions that can be drawn as to reproductive effects of xylene in humans.

No hematological effects have been identified in studies where exposure was to xylene only. Previous studies identifying hematological effects included known or suspected exposure to benzene (ATSDR, 1995; ECETOC, 1986). One series of case reports identified lowered white cell counts in two women with chronic occupational exposure to xylene (Hipolito, 1980; Moszczynsky and Lisiewicz, 1983; 1984), although they may also have had multiple chemical exposures.

Groups of male volunteers (1 to 4 subjects/group) were exposed to p-xylene in a controlled-environment chamber for 7.5, 3, or 1 hr/day, 5 days/week for 4-weeks (Hake *et al*., 1981). The p-xylene concentration was changed on a weekly basis starting at 100 ppm the first week, followed by 20 ppm, 150 ppm, and 100 ppm (average, with a range of 50 to 150 ppm) over subsequent weeks. In addition, groups of female volunteers (2 or 3/group) were exposed to 100 ppm p-xylene for 7.5, 3, or 1 hr/day for 5 days. The volunteers acted as their own controls, with exposure to 0 ppm p-xylene occurring for two days (males) or one day (females) the week before and the week after the xylene exposures. No serious subjective or objective health responses, including neurological tests, cognitive tests and cardiopulmonary function tests were observed. Odor was noted, but the intensity decreased usually within the first hour of exposure. The

authors concluded that p-xylene may have a weak irritating effect on the soft tissues starting at 100 ppm, but overall, the small sample size and high variability among the volunteers made all results difficult to interpret.

The Uchida *et al.* (1993) study included a relatively large number of workers studied, exposure for an average of 7 years to xylenes predominately and a comprehensive set of medical examinations to document potential effects. A survey of 994 Chinese workers involved in the production of rubber boots, plastic coated wire and printing processes employing xylene solvents was carried out. The survey consisted of fitting individual workers with diffusive samplers for an 8 hour shift. At the end of the 8 hour shift the samplers were recovered for analysis of solvent exposure, and urine samples were collected for analysis of xylene metabolites. The following day workers answered a questionnaire concerning subjective symptoms, and blood and urine were collected for analysis. Out of this group of xylene-exposed workers, 175 individuals (107 men and 68 women) were selected for further study and analysis based on completion of their health examinations and on results from diffusive samplers showing that xylene constituted 70% or more of that individual's exposure to solvents in the workplace. The control population consisted of 241 (116 men and 125 women) unexposed workers from the same factories or other factories in the same region, of similar age distribution, of similar time in this occupation (average of 7 years), and having a similar distribution of alcohol consumption and cigarette usage. The xylene-exposed and unexposed groups were given health examinations which evaluated hematology (red, white, and platelet cell counts, and hemoglobin concentration), serum biochemistry (albumin concentration, total bilirubin concentration, aspartate aminotransferase, alanine aminotransferase, gamma-glutamyl transferase, alkaline phosphatase, leucine aminopeptidase, lactate dehydrogenase, amylase, blood urea nitrogen, creatinine), and subjective symptoms (survey of symptoms occurring during work and in the previous three months).

Results of analysis of the diffusive samplers showed that workers were exposed to a geometric mean of 14.2 ± 2.6 ppm xylene (arithmetic mean of 21.3 ± 21.6 ppm). This was broken down into geometric means of 1.2 ppm o-xylene, 7.3 ppm m-xylene, 3.8 ppm p-xylene, 3.4 ppm ethyl benzene, and 1.2 ppm toluene. N-Hexane was rarely present and no benzene was detected. Analysis of data from the health examinations found no statistically significant difference ($p<0.10$) between hematology and serum biochemistry values for xylene-exposed and unexposed populations. The frequency of an elevated ratio of aspartate aminotransferase to alanine transferase and of elevated ratio of alkaline phosphatase to leucine aminopeptidase was significantly ($p<0.01$) higher in exposed men than in the control population of men. Results of the survey of subjective symptoms found differences in symptoms occurring during work and during a similar analysis over the preceding three month period, apparently related to effects on the central nervous system and to local effects on the eyes, nose and throat. The frequency of five symptoms experienced during work was significantly ($p<0.01$) elevated in either xylene-exposed men or women including: dimmed vision, unusual taste, dizziness, heavy feeling in the head, and headache. The frequency of four symptoms experienced during work were significantly ($p<0.01$) elevated in both men and women including irritation in the eyes, nasal irritation, sore throat, and floating sensation. Ten subjective symptoms occurring in the previous three months were significantly ($p<0.01$) elevated in exposed men and women including nausea, nightmare, anxiety, forgetfulness, inability to concentrate, fainting after suddenly standing up,

poor appetite, reduced grasping power, reduced muscle power in the extremities, and rough skin. Dose dependency appeared to exist for 3 subjective symptoms noted during work: irritation in the eyes, sore throat, floating sensation, and for one symptom occurring in the last three months, poor appetite.

V. Effects of Animal Exposure

A limited number of chronic toxicity studies are available for xylene including two inhalation studies with o-xylene (Tatrai *et al.*, 1981; Jenkins *et al.*, 1970) and one oral chronic study with mixed xylenes (NTP, 1986). No chronic dermal studies could be identified. A spectrum of adverse effects has been documented in shorter term studies which potentially could occur with chronic exposure. These studies are presented here along with a brief description of the three chronic studies identified. Xylene affects a number of organ systems including the pulmonary system, the cardiovascular system, the gastrointestinal system, the hepatic system, the renal system, the dermis, and the eye, and it has numerous neurological effects and developmental effects.

Animal data are consistent with human data in documenting respiratory effects from xylene exposure. Acute and subacute exposures in mice, rats, and guinea pigs have been associated with decreased metabolic capacity of the lungs; decreased respiratory rate; labored breathing; irritation of the respiratory tract; pulmonary edema; and pulmonary inflammation (Carpenter *et al.*, 1975; De Ceaurriz *et al.*, 1981; Elovaara *et al.*, 1987; 1989; Furnas and Hine, 1958; Korsak *et al.*, 1988; 1990; Patel *et al.*, 1978; Silverman and Schatz, 1991; Toftgard and Nilsen, 1982).

Limited evidence is available in animal studies for cardiovascular effects resulting from xylene exposure. Morvai *et al.* (1976; 1987) conducted two studies. The first study observed rats following acute and intermediate duration inhalation exposure to very high (unspecified) levels of xylene and recorded ventricular repolarization disturbances, atrial fibrillation, arrhythmias, occasional cardiac arrest and changes in electrocardiogram (Morvai *et al.*, 1976). In a subsequent study morphological changes in coronary microvessels were seen in rats exposed to 230 ppm xylene (isomer composition unspecified) (Morvai *et al.*, 1987). However the chronic toxicity studies conducted by the National Toxicology Program (NTP, 1986) and by Jenkins *et al.* (1970), as well as other shorter term studies (Carpenter *et al.*, 1975; Wolfe, 1988), have not identified histopathological lesions of the heart.

Studies identifying adverse gastrointestinal effects, hematological effects, or musculoskeletal effects in animals were not identified. Studies reporting no hematological effects include Carpenter *et al.* (1975) (rats exposed to 810 ppm of mixed xylenes for 10 weeks, 5 days/week, 6 hours/day and dogs exposed for 13 weeks to 810 ppm mixed xylenes, 5 days/week, 6 hours/day) and Jenkins *et al.* (1970) (rats, guinea pigs and dogs exposed for 6 weeks to 780 ppm o-xylene, 5 days/week, 8 hours per day). Carpenter *et al.* (1975) and the NTP (1986) reported no effects on the musculoskeletal system.

Hepatic effects have been documented after acute exposure to high concentrations of xylene (2,000 ppm) or subacute exposure to lower concentrations (345-800 ppm) of mixed xylene or individual isomers. These effects include increased cytochrome P-450 and b5 content, increased

hepatic weight, increased liver to body weight ratios, decreased hepatic glycogen, proliferation of endoplasmic reticulum, changes in distribution of hepatocellular nuclei, and liver degeneration (Bowers *et al.*, 1982; Condie *et al.*, 1988; Elovaara, 1982; Elovaara *et al.*, 1980; Muralidhara and Krishnakumari 1980; Patel *et al.*, 1979; Pyykko 1980; Tatrai and Ungvary, 1980; Tatrai *et al.*, 1981; Toftgard and Nilsen, 1981; 1982; Toftgard *et al.*, 1981; Ungvary *et al.*, 1980).

Renal effects have been identified in studies with rats, guinea pigs, dogs, and monkeys exposed to 50-2,000 ppm of xylenes. These effects include increased cytochrome P-450 content and increased kidney to body weight ratios (Condie *et al.*, 1988; Elovaara 1982; Toftgard and Nilsen, 1982). Condie *et al.* (1988) also noted tubular dilation, atrophy, and increased hyaline droplets in the kidney of Sprague-Dawley rats administered 150 mg/kg/day orally of mixed xylenes. This response is consistent with early nephropathy.

Xylene has been found to affect the dermis and eyes of animals. Hine and Zuidema (1970) found skin erythema and edema, epidermal thickening, and eschar formation in response to xylene exposure. Direct instillation of xylenes into the eyes of rabbits produces eye irritation (Hine and Zuidema, 1970; Smyth *et al.*, 1962)

Numerous neurological effects have been documented in response to acute and subchronic xylene exposures ranging from 100 to 2,000 ppm. This is consistent with effects on neurofunction documented in humans. These effects include narcosis, prostration, incoordination, tremors, muscular spasms, labored respiration, behavioral changes, hyperactivity, elevated auditory thresholds, hearing loss, and changes in brain biochemistry (Andersson *et al.*, 1981; Carpenter *et al.*, 1975; De Ceaurriz *et al.*, 1983; Furnas and Hine, 1958; Ghosh *et al.*, 1987; Gralewicz *et al.*, 1995; Kyrklund *et al.*, 1987; Molnar *et al.*, 1986; NTP, 1986; Pryor *et al.*, 1987; Rank 1985; Rosengren *et al.*, 1986; Savolainen and Seppalainen, 1979; Savolainen *et al.*, 1978; 1979a; Wimolwattanapun *et al.*, 1987).

Developmental effects have been documented in pregnant animals exposed to xylenes. ATSDR (1995) concluded that the body of information available for developmental effects is consistent with the hypothesis that xylene is fetotoxic and many of the fetotoxic responses are secondary to maternal toxicity. However, the ATSDR also observed that there was a large variation in the concentrations of xylene producing developmental effects and of those producing no developmental effects. The ATSDR thought that these differences were influenced by a number of factors (strain and species of animal, purity of xylene, method of exposure, exposure pattern and duration, etc.). The two most common test species have been the rat and the mouse.

With respect to rats, Mirkova *et al*. (1983) exposed groups of pregnant rats (unspecified strain of white rats) to clean air or 2.3, 12, or 120 ppm of xylene (unspecified composition) for 6 h/day on days 1-21 of gestation. They reported increased postimplantation losses and fetotoxicity (reduced fetal weights) as well as a statistically increased incidence of visceral abnormalities (including ossification defects in bones of the skull) at xylene air concentrations of 12 ppm and above. The ATSDR has suggested that the Mirkova *et al*. (1983) study results may have been influenced by poor animal husbandry as indicated by the low conception rates and the high incidence of fetal hemorrhages seen in the controls. Hass and Jakobsen (1993) attempted to replicate the findings of Mirkova *et al*. (1983). Hass and Jakobsen (1993) exposed groups of 36

pregnant Wistar rats to clean air or 200 ppm of xylene for 6 h/day on days 4-20 of gestation. Unlike Mirakova *et al*. (1983), there was no sign of maternal toxicity and no decrease in fetal weights and no increase in soft-tissue or skeletal malformations. A large increase in the incidence of delayed ossification of the *os maxillare* of the skull, however, was observed (53% of experimental fetuses as opposed to 2% of the controls). Potential neurological/muscular changes measured as performance on a rotorod were also noted upon testing of 2-day-old rat pups.

Ungvary *et al*. (1985) exposed CFY rats by inhalation to air concentrations of xylene (60 ppm, 440 ppm, 800 ppm) for 24 h/day on days 7-15 of gestation. Maternal toxicity was described as moderate and dose-dependent. They observed weight retarded fetuses at all air concentrations. However, there was no increase in malformations, and an increase in minor anomalies and resorbed fetuses occurred only at the highest concentration. In a separate study investigating the interactions between solvents and other agents, Ungvary (1985) exposed CFY rats to either 140 ppm or 440 ppm of xylene on days 10-13 of gestation and also reported increases for either condition in weight retarded and skeletal retarded fetuses without any increase in malformations. Hudak and Ungvary (1978) had earlier examined the effect of 230 ppm xylene (24 h/day, days 9-14 of pregnancy) in the CFY rat and reported effects on skeletal development (e.g., fused sternebrae). In contrast to the other Ungvary findings, no effect on fetal weight was observed. Bio/dynamics (1983) conducted an inhalation exposure study in the rat (CrL-CD (SD) BR strain). Rats were exposed 6 h/day during premating, mating, gestation and lactation. Exposure concentrations were 0, 60, 250, and 500 ppm. Most measures for adverse effects on fetal development were not significantly increased. Mean fetal weights at the highest exposure level were lower than controls, but this difference was significant only for the female fetuses. These depressed weights were, however, still significant on day 21 of lactation. Other adverse effects (such as increased soft tissue and skeletal abnormalities, increased fetal resorptions) were not increased significantly at any of the test concentrations.

Ungvary *et al*. (1980a) tested by inhalation the individual ortho, meta, and para isomers of xylene in the CFY rat. Pregnant rats were exposed 24 h/day on days 7 –14 of pregnancy to 35, 350, or 700 ppm of each isomer. An increased incidence of weight retarded fetuses was observed for each isomer at the 700 ppm level, and for the ortho isomer at the 350 ppm level. Post implantation losses were increased only at the 700 ppm level in the para-xylene exposed group. Skeletal anomalies were increased only at the 700 ppm level for the meta and para isomers of xylene. Rosen *et al*. (1986) evaluated the effects of prenatal exposure to para-xylene in the rat. They exposed pregnant Sprague-Dawley rats by inhalation to either 800 ppm or 1600 ppm of p-xylene from days 7-16 of gestation. Despite the high concentrations, no effects were seen on litter size or weight at birth or on the subsequent growth rates of the pups.

Hass *et al.* (1995) examined postnatal development and neurobehavioral effects in rats following prenatal exposure to 0 or 500 ppm technical xylene 6 hr/day on gestation days 7-20 of pregnancy. Xylene exposure caused no signs of maternal toxicity and no difference in the number of live or dead fetuses. The mean birth weight in exposed litters was about 5% lower compared to control litters but the difference was not statistically significant. Body weights were similar between groups during the preweaning and postweaning period but lower absolute brain weights were observed in exposed animals. Exposed offspring showed a delay in the ontogeny

of the air righting reflex and exhibited impaired performance in behavioral tests for neuromotor abilities (Rotorod) and for learning and memory (Morris water maze). In a follow-up study under the same exposure conditions, exposed offspring exhibited impaired performances in the Morris water maze at 16, 28, and 55 weeks of age, although the difference was not statistically significant at 55 weeks (Hass *et al*., 1997). These data indicate that xylene exposure during development may cause long-lasting deficits on learning and memory in offspring.

With respect to mice, Ungvary *et al*. (1985) exposed CFLP mice by inhalation to air concentrations of xylene (120 ppm, 230 ppm) for 24 h/day on days 7-15 of gestation. In the mouse, they observed increased incidences of weight-retarded fetuses and increased skeletal retarded fetuses at 230 ppm. Shigeta *et al*. (1983) exposed pregnant ICR mice to approximately 0, 120, 230, 460, and 920 ppm of xylene in an exposure chamber for 6 h/day on days 6-12 of gestation. Shigeta *et al.* (1983) reported significant decreases in fetal weight in the 460 ppm and 920 ppm dose groups only. There was no difference in the number of live or dead fetuses. Decreased weight gains and delayed development of body hair and teeth were observed at the 920 ppm exposure level. Dose-response relations were reported for delayed ossification of the sternebrae. Marks *et al*. (1982) noted that 2060 mg/kg/day of mixed xylene administered orally is associated with cleft palate and decreased fetal weight in the mouse.

Ungvary *et al*. (1985) also tested the individual ortho, meta, and para isomers of xylene at 120 ppm in the CFLP mouse. Each isomer of xylene also increased the incidence of weight-retarded fetuses and skeletal retarded fetuses at 120 ppm. There was no increase in malformations.

Of the three chronic studies available (Tatrai *et al.*, 1981; Jenkins *et al.*, 1970; NTP 1986) none comprehensively examined systemic effects. The study by Tatrai *et al.* (1981) exposed rats for one year, 7 days/week, 8 hours per day to 1096 ppm o-xylene. This exposure was a LOAEL for body weight gain in males and a NOAEL for hepatic effects in male rats. Jenkins *et al.* (1970) exposed rats, guinea pigs, squirrel monkeys, and beagle dogs for 90-127 days continuously to 78 ppm of o-xylene. The study examined body weight gain; hematological parameters including white cell counts, red blood cell counts, and hematocrit; serum biochemistry including bromosulfophthalein retention, blood urea nitrogen, alanine aminotransferase, aspartate aminotransferase, alkaline phosphatase, and creatinine and liver function including alkaline phosphatase, tyrosine aminotransferase, and total lipids. No effects were observed in any of the parameters examined in this study. This study found a NOAEL for all effects examined of 78 ppm o-xylene. The NTP (1986) study administered 0, 250, or 500 mg/kg/day doses of mixed xylene in corn oil by gavage 5 days/week for 103 weeks to groups of F344/N rats of both sexes, 50 animals per group. B6C3F1 mice were treated in a similar manner but given 0, 500 or 1000 mg/kg/day of mixed xylenes in corn oil by gavage. A complete histopathological examination of all tissues was made as well as determination of body weight gain. Based on histopathology of all organ systems, a NOAEL of 500 mg/kg/day was observed for rats and a NOAEL of 1000 mg/kg/day was observed for mice.

VI. Derivation of Chronic Reference Exposure Level (REL)

Study	Uchida *et al.* (1993)
Study population	175 xylene-exposed factory workers and control population of 241 factory workers
Exposure method	Inhalation
Critical Effects	Dose related increase in the prevalence of eye irritation, sore throat, floating sensation, and poor appetite.
LOAEL	14.2 ppm (geometric mean of exposure concentrations)
NOAEL	Not applicable
Exposure continuity	8 hr/d (10 m^3/day occupational inhalation rate), 5 d/wk
Exposure duration	Occupational exposure for an average of 7 years
Average occupational exposure	5.1 ppm for LOAEL group (14.2 x 10/20 x 5/7)
Human equivalent concentration	5.1 ppm for LOAEL group
LOAEL uncertainty factor	3
Subchronic uncertainty factor	1
Interspecies uncertainty factor	1
Intraspecies uncertainty factor	10
Cumulative uncertainty factor	30
Inhalation reference exposure level	0.2 ppm (200 ppb; 0.7 mg/m^3; 700 µg/m^3) for mixed xylenes or for total of individual isomers

A number of issues are important in considering the uncertainty associated with this REL. For ATSDR (1995), the animal and human toxicity data suggest that mixed xylenes and the different xylene isomers produce similar effects, although different isomers are not equal in potency for producing a given effect. Therefore exposure of workers to a mix of xylenes in the Uchida *et al.* (1993) study would be expected to generate a similar spectrum of responses as exposure to single isomers, however the intensity of particular effects could be different. The use of a neurological endpoint for derivation of a REL is supported by the large number of inhalation and oral studies, which associate neurological effects with xylene exposure. ATSDR (1995) indicates that neurological effects are a sensitive endpoint. The observation that floating sensation is apparently related to dose further supports the concept that this subjective symptom related to neurological effects was due to xylene exposure.

A UF of 3, rather than 10, was applied for the LOAEL to NOAEL extrapolation due to the generally mild adverse effects observed and the principally low incidence (<50%) of the effects. A factor of 1 was used for subchronic uncertainty. Although the average occupational exposure was only 7 years, there were 176 xylene-exposed workers of average age 29.7 ± 9.0 years (arithmetic mean ± SD) for whom, according to the report, there had been essentially no change in workplace in their working life. Thus, many workers would likely have been exposed for more than 8.4 years, the cut-off point for chronic human exposure. Another issue is the use of diffusive samplers in the Uchida *et al.* (1993) study. These samplers provide a time weighted

average concentration of hydrocarbon and cannot indicate the maximum concentrations a worker is exposed to. It is unknown whether peak concentrations alter the response to xylenes in humans.

For comparison with the proposed REL of 200 ppb based on human studies, (1) the free-standing NOAEL of 78 ppm o-xylene obtained by Jenkins *et al*. (1970) in rats and guinea pigs continuously exposed for 90 days was used to estimate a REL based on animal data. Use of an RGDR of 1, a subchronic UF of 3, an interspecies UF of 3, and an intraspecies UF of 10 results in a REL of 800 ppb for o-xylene for systemic effects. (2) Tatrai *et al*. (1981) found a free standing LOAEL of 1096 ppm o-xylene for body weight gain in male rats exposed every day for 8 hours. Time adjustment to continuous exposure and use of an RGDR of 1, a LOAEL UF of 3 for a mild effect, an interspecies UF of 3, and an intraspecies UF of 10 result in a REL of 4000 ppb. (3) Ungvary *et al*. (1985) exposed mice by inhalation continuously to 120 ppm or 230 ppm xylene for 24 h/day on days 7-15 of gestation. The LOAEL was 230 ppm and the NOAEL was 120 ppm. No time adjustment is needed. Use of an RGDR of 1, a subchronic UF of 1, an interspecies UF of 3, and an intraspecies UF of 10 results in a REL of 4000 ppb for xylene for developmental effects.

VII. Data Strengths and Limitations for Development of the REL

The strengths of the inhalation REL for xylene include the use of human exposure data from 175 workers exposed over a period of years. Major areas of uncertainty are the uncertainty in estimating exposure, the potential variability in exposure concentration, and the lack of observation of a NOAEL in the key study.

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Response to Comments on the October 1997 Draft of the

Air Toxics Hot Spots Risk Assessment Guidelines Part III: Determination of Noncancer Chronic Reference Exposure Levels

Responses to Comments on the Methodology and the First Forty Chemicals

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Allied Signal - Naphthalene

Allied Signal Chemicals of Morristown, New Jersey, submitted comments on December 15, 1997 regarding the draft chronic reference exposure level for **naphthalene** presented in the OEHHA Technical Support Document for the Determination of Noncancer Chronic Reference Exposure Levels. The proposed chronic REL was based on a 2-year NTP study (1992) with mice. Olfactory epithelial metaplasia and respiratory epithelial hyperplasia were noted in most (>95%) exposed animals at the lowest concentration (10 ppm) tested but not in any of the control animals. Adjustment for discontinuous exposure and a cumulative 1000-fold uncertainty factor for interspecies differences, intraspecies variability, and lack of a NOAEL resulted in a proposed REL of 9 $\mu g/m^3$ (2 ppb).

Comment 1. In general, additional details of the study should be provided, such as species used, methods of administration, and all results (not just selected findings).

Response. The presentation of research findings has been reviewed and additional details have been added where warranted.

Comment 2. In addition, the results of an unpublished 13-week inhalation study in rats (Coombs, D.W., Kiernan, P.C., Hardy, C.J., Crook, D., Lewis, D.J., and Gopinath, C. Naphthalene: 13-Week Inhalation Study in Rats, Huntingdon Research Centre Ltd., England, Report No. LDA 2/930704, April 28, 1993) is not mentioned.

Response. The document cited has been requested from the study authors and, if a copy of this unpublished study is obtained, a review will be added to the naphthalene section of the OEHHA document.

Comment 3. The summary of the chronic inhalation bioassay in $B6C3F_1$ mice (NTP, 1992) should also include the results/conclusion for carcinogenicity, a primary objective of this study. The lesions mentioned in this summary should be identified as "nonneoplastic" lesions.

Response. The potential carcinogenicity of naphthalene was evaluated separately by OEHHA. OEHHA does not currently consider naphthalene to be a carcinogen, thus any lesions mentioned would be noncarcinogenic. The Technical Support Document focuses on noncancer endpoints as noted in the Introduction.

Comment 4. The data presented in Table 1 do not appear accurate, as the incidence for 0 ppm is for female mice and the incidences for 10 and 30 ppm are for male mice. It seems that the table should include consistent data for both sexes in order to be complete.

Response. OEHHA thanks the commentator for pointing out errors in the table. Corrections have been made.

Comment 5. The draft OEHHA document does not include results from an unpublished subchronic inhalation toxicity study in male and female Sprague-Dawley rats which was conducted by Huntingdon Research Centre Ltd., and then reviewed and found acceptable by the U.S. EPA (Coombs et al., 1993). Rats were exposed to naphthalene vapor for 13-weeks (snout only, 6 hours/day, 5 days/week) at nominal concentrations of 0, 2, 10 or 60 ppm. Compared to controls, treatment-related effects were observed in all groups. In the low-dose group (2 ppm or 0.01 mg/L), male and female rats had minimal degenerative changes and proliferative lesions in the nasal passages. In the mid-dose group (10 ppm or 0.052 mg/L), moderate degenerative and proliferative nasal passage lesions were produced, along with hypertrophy of the respiratory epithelium and decreased body weight gain and food consumption (males only). In the high-dose group (60 ppm or 0.315 mg/L), marked degenerative and proliferative nasal passage lesions were produced, as well as degenerate fibers in the spinal cord and sciatic nerve in one male, and decreased body weight gain and food consumption. Based on this information, the systemic NOEL for both sexes was estimated to be < 2 ppm (0.01 mg/L).

Response. As noted previously, OEHHA thanks the commentator for providing information about this unpublished study. OEHHA is attempting to obtain a copy for review. Based on the data summary provided by the commentator, the results obtained in this 13-week are consistent with those observed in the 104 week NTP bioassay.

Comment 6. The summary of the Shopp et al. study (1984) focuses only on a single finding for which the toxicological significance is not clear (dose-related inhibition of liver aryl hydrocarbon hydroxylase activity in both sexes). The summary should include results of all other parameters evaluated. At a daily dose level of up to 1/4 the LD50 (133 mg/kg) for 90 days, there was no treatment-related mortality, no significant effects on body weight, and no significant changes in organ weights, with the exception of reduced spleen weights in females at 133 mg/kg. Although an organ (spleen) associated with immune function showed decreased weight, there was no evidence of immunotoxicity in any treatment group of either sex. No treatment-related effects were seen in serum enzyme and electrolyte levels. A screen of the effects of the 90-day naphthalene treatment on various aspects of the liver drug metabolizing system indicated no alterations, with the exception of the specific dose-related inhibition of aryl hydrocarbon hydroxylase activity.

Response. Text has been added describing these additional findings of the study of Shopp and associates.

Comment 7. The summary of the Navarro et al. study (1991) does not mention the species used, the method of oral administration, or the number of days the animals were dosed. However, this information can be derived from the reference title. Pregnant female Sprague-Dawley CD rats were administered naphthalene (0, 50, 150, or 450 mg/kg/day) by gavage during gestational days 6-15. The adverse maternal effects observed at the low dose (50 mg/kg/day) consisted of transient clinical signs indicative of CNS depression. By the third day of dosing the dams

acquired a tolerance to the low dose and did not show maternal toxicity thereafter. However, the maternal toxicity (including CNS depression, reduced body weight, and altered food & water consumption) in dams receiving 150 and 450 mg/kg/day was greater and longer lasting.

Response. OEHHA thanks the commentator for this clarifying comment. Additional text has been added to the document.

Comment 8. The summary of the Harris et al, study (1979) does not indicate the method of administration of naphthalene or the species of animal used. Sprague-Dawley rats were administered naphthalene by ip injection.

Response. Clarifying details of this study have been added to the document.

Comment 9. The reference (U.S. EPA, 1986a or b) is confusing in the last two summaries (NZ white rabbit developmental study and rat dermal application study). The rabbit developmental study and the rat dermal application study are listed separately in the reference section as U.S. EPA, 1986 and U.S. EPA, 1986b, respectively.

Response. OEHHA thanks the commentator for noting this error; corrections have been made to the document.

Comment 10. It is our opinion that the use of an interspecies uncertainty factor of 10 and intraspecies uncertainty factor of 10 in the calculation of the REL for naphthalene (9 $\mu g/m^3$) is overly conservative and unrealistic based on the endpoint of upper respiratory system irritation. Rats and mice are far more susceptible to upper respiratory irritation than humans "based on (1) physiological differences in mode of breathing (obligate nasal for rat; oronasal for humans); (2) differences in overall geometry of the nasal passages, including the turbinate profiles; (3) the enormous difference in relative nasal cavity surface areas between rats and humans; (4) differences in the proportion of nasal cavity surface area covered by different epithelia; (5) differences in mucociliary clearance routes, especially in the anterior portion of the nasal cavity; and (6) differences in the inspiratory airflow routes. In rodents, almost 100% of a volatile chemical is absorbed or trapped in the tissues of the nasal passages.

Response. The data on naphthalene effects in the respiratory system suggest that the observed effects are <u>not</u> due to direct irritancy, but rather due to absorption and activation to a reactive metabolite. Thus interspecies comparisons of responses to direct acting irritants is not helpful. Naphthalene has low reactivity and low water solubility. Necrosis of olfactory epithelium (Plopper CG, Suverkropp C, Morin D, Nishio S, Buckpitt A, 1992, Relationship of cytochrome P-450 activity to Clara cell cytotoxicity. I. Histopathologic comparison of the respiratory tract of mice, rats and hamsters after parenteral administration of naphthalene, J Pharmacol Exp Ther 261(1):353-63) and of bronchial Clara cells (O'Brien et al., 1989, Tolerance to multiple doses of the pulmonary toxicant, naphthalene, Toxicol. Appl. Pharmacol. 99(3):487-500) have been noted

in rodents following intraperitoneal injection of naphthalene. Respiratory epithelial cells, as well as liver, appear to be major sites of activation of naphthalene to toxic intermediates.

Comment 11. For example, 10 ppm of naphthalene vapor, which is the NIOSH 10-hour TWA value recommended to protect humans from exposure to naphthalene vapor in the work place, produced an incidence of 96% and 100% nasal effects in male and female mice, respectively in the NTP study (1992). Furthermore, ACGIH recommends an 8-hour TLV TWA value of 10 ppm in view of the fact that irritation is experienced in humans at 15 ppm and that continued exposure at that concentration may result in fairly serious eye effects. (See American Conference Governmental Industrial Hygienists (ACGIH). Documentation of the Threshold Limit Values (TLVs) for Chemicals in the Work Environment, 5th Ed., 1986).

Response. Comparison of the REL with an occupational standard is not particularly informative. OEHHA agrees with the recommendations of USEPA and ACGIH itself that the occupational exposure limits such as NIOSH RELs and ACGIH TLVs are not an appropriate basis for the derivation of RELs to protect the general public, including sensitive subgroups, from exposures over a lifetime.

Comment 12. Taking into account the well documented interspecies differences with respect to nasal or upper respiratory irritation, an interspecies uncertainty factor of 3 is recommended as a more realistic uncertainty factor to use in the calculation of the REL. In fact one could easily justify an uncertainty factor of one.

Response. As noted previously, the data on naphthalene effects in the respiratory system suggest that the observed effects are <u>not</u> due to direct irritancy, but rather due to absorption and activation to a reactive metabolite. It is true that a default interspecies uncertainty factor of 3 was used by OEHHA for most chemicals for which a human equivalent concentration (HEC) was estimated. However, in the case of naphthalene, a factor of 3 was considered to be inadequate, because the major effects noted in human populations exposed to naphthalene, namely hemolytic anemia and cataracts, were not noted in animal studies. Thus available animal studies may underpredict human risks because of the relative insensitivity of rodents to these effects. Therefore a 10-fold interspecies uncertainty factor was used.

Comment 13. In addition, it is thought that the intraspecies uncertainty factor of 10 is much too conservative with materials that are irritants. The distribution from normal to sensitive populations is generally approximately 3-fold. (See Rotman, H.H., Fliegelman, M.J., Moore, T., Smith, R.G., Anglen, D.M., Kowarski, C.J. and Weg, J.G. Effects of low concentration of chlorine on pulmonary function in humans. J. Appl. Physiol. 54: 1120-1124, 1993).

Response: As noted in the previous response, since the mechanism of naphthalene respiratory toxicity differs from that for direct irritants, the variability in human responses to such irritants is not relevant to assessing the variability in human response to naphthalene exposure. In addition, the range of interindividual response may be broad for some irritants (e.g., formaldehyde).

Comment 14: In summary, a more practical REL of 0.022 ppm (~100 $\mu g/m^3$) is calculated when an uncertainty factor of 3 is used for both interspecies and intraspecies variability. Considering that only marginal effects were seen in a subchronic inhalation study at 2 ppm in rats, a sensitive species for nasal irritants, 0.022 ppm would clearly represent a safe level for chronic exposure to the general population.

Response: As described above, OEHHA can not support the changes suggested by the commentator in this case. Recently USEPA developed an inhalation reference concentration (RfC) for naphthalene. The USEPA RfC is in fact 3-fold *lower* than that proposed by OEHHA. USEPA used the same study, endpoint, and uncertainty factors as those proposed in 1997 by OEHHA. USEPA, however, added an additional 3-fold database uncertainty factor because of the lack of a two-generation reproductive toxicity study and chronic data for additional species.

Allied Signal – Allied Engineered Materials

Comments on the methodology used in the chronic REL TSD were made by Dr. George Rusch of Allied Signal – Allied Engineered Materials in a letter dated December 19, 1997.

Comment 1. I have reviewed the Technical Support Document dated October 1997, pages 1 - 46. In my judgement, it is well thought out and clearly presents the process to be used for the calculation of RELs. The consideration given to existing guidelines such as the RfDs, ADI and various occupational exposure guidance levels is well described as is the utilization of both toxicology and epidemiology studies. Again, in the area of risk assessment, the utilization of NOAELs and LOAELs are well described. I strongly support the use of variable uncertainty factors which take into consideration the type and severity of the effects observed and their relevance to man. Table 2 on page 21 is most informative. Utilization of all the considerations described in Section 3.2 - 3.6 on pages 22 -31 can lead to robust risk assessments. Table 7 presents a valuable, flexible approach in the calculation of uncertainty factors.

It is, however, important in reviewing the data on specific chemicals that uncertainty factors be carefully selected to most precisely estimate the true uncertainty. There can be a tendency, when looking at multiple uncertainties, to treat each in a conservative fashion such that the combined uncertainty factors is not reflective of the true uncertainty.

Response. The issue of treatment of multiple areas of uncertainty is an area of ongoing evaluation by both OEHHA and US EPA. Multiplying several uncertainty factors could yield unnecessarily conservative exposure guidelines. Thus US EPA has set the maximum uncertainty factor at 3,000 to partially offset such concerns. On the other hand, there are numerous areas of uncertainty not specifically addressed with conventional uncertainty factor approaches. Analysis of data on human variability, including genetic variability, by Dale Hattis and others indicate that human interindividual variability may be much greater than 10-fold for some chemicals. A factor of 10 for intraspecies variability will not be adequate for those chemicals. If an animal model is insensitive to a certain chemical, such as the rabbit model was to thalidomide, the standard interspecies uncertainty factor of 10 would also not protect humans. These uncertainties have been considered to potentially offset any over-conservatism arising from aggregation of multiple uncertainty factors. OEHHA's goal is to develop better data-based approaches in the future, but such methods are time-consuming and data-intensive.

Comment 2. It is also important, in the selection of key studies as the basis for the REL, that the most significant studies be given the greatest consideration. Again, there can be a tendency to select studies with low NOAELs over others that may be of higher quality and greater relevancy to the endpoint being evaluated. Focusing on key studies of good scientific quality will result in meaningful, valuable risk assessments. In contrast, risk assessments that use poor data and large or unsupported uncertainty factors, lack credibility and ultimately are of limited value for protecting the public.

Response. OEHHA agrees that human relevance and data quality are key issues. Human relevance was emphasized and human exposure data were used wherever possible. In numerous cases the key study did not involve the lowest exposure concentration for which adverse effects

have been claimed. In one case a chemical under review was dropped from the document because the scientific database was judged to be inadequate. Numerous other chemicals of concern to California air quality agencies were not included in the current document because of the poor quality of the scientific database. The need for large uncertainty factors can best be addressed by the development of better data. Most toxicity studies have been designed to acquire data for purposes other than health risk assessment and thus are not optimal for that purpose. But by the same token, failing to address potential health impacts because there are limitations in the scientific database would be imprudent public health policy.

Comment 3. My concern is based in part on a brief review of the "Proposed OEHHA Chronic Inhalation REL Summary" which lists RELs for many chemicals. Many of the actual values presented in this table appear to be very conservative. For example, the value of 100 $\mu g/m^3$ for ammonia, a normal biological metabolite, is well below the odor and irritation threshold; that for fluoride is an order of magnitude below the typical dose received by ingestion; and the level for dichlorodifluoromethane is one thousand times below the occupational exposure limit and over 50 thousand times below even marginal effect levels. I would suggest that those conducting the risk assessments review the guidance in the Technical Support Document and reconsider their calculations and approach where the values do not appear supported by the data. This will greatly increase the value of these guidance levels.

Response. The use of uncertainty factors was judicious and compares favorably with those used by US EPA in the derivation of Reference Concentrations (Table 9 of the Chronic Reference Exposure Level TSD). Occupational exposure limits (OELs) were by design not incorporated into this document. This is in accord with the developers of the OELs, who have cautioned against use of such values for protection of the general public. OEL values lack a consistent basis, are designed for healthy workers, and frequently approximate more closely a LOAEL rather than a NOAEL. In addition, frank toxic effects occur in some workers at some TLVs.

The ammonia RfC was adopted as the proposed chronic REL because the US EPA evaluation was considered adequate by OEHHA.

In the case of fluoride, differences in toxicity by route of exposure are known for several chemicals. For example, nickel, chromium VI, beryllium, and cadmium are much more toxic by the inhalation route than by the oral route.

In the case of dichlorodifluoromethane, OEHHA used the maximum uncertainty factor of 3,000 because staff based the REL on a LOAEL from an animal 90-day study (defined by USEPA as a “subchronic study” in rats and mice). To use different UFs we would have to change the default procedure in some way such as considering a guinea pig 90 day experiment to be chronic (and thus using a lower UF) or to have evidence that humans were not more sensitive to dichlorodifluoromethane than animals or that there was limited intraspecies variability to dichlorodifluoromethane. If we had the latter type of information, we would probably also have human data on which to base the chronic REL.

California Mining Association

Comments on the chronic REL for **hydrogen cyanide** were made by Denise M. Jones, Executive Director of the California Mining Association. The proposed chronic REL for HCN is the USEPA RfC of 3 $\mu g/m^3$.

Comment 1. Based on our review of this document, the California Mining Association strongly recommends against adoption of the proposed Chronic Reference Exposure Level (REL) for hydrogen cyanide (HCN). Several of CMA's member companies use dilute sodium cyanide solutions as an essential component of their ore extraction processes. Potential emission sources include heap leach pads, leaching tanks, solution retention ponds, carbon circuit tanks, and electrowining processes. The results of numerous studies investigating potential HCN emissions from the preceding sources are regulated by the Mine Safety and Health Administration (MSHA) at 10 ppm or less in the work place. The MSHA HCN exposure limit is based on peer-reviewed data and was ratified during a formal rulemaking process.

Response. The proposed chronic REL for HCN is the USEPA RfC of 3 $\mu g/m^3$, which has been in use since 1994. All USEPA Reference Concentrations (RfCs), available when the Technical Support Document (TSD) on chronic Reference Exposure Levels was drafted in October 1997, are being used as chronic RELs. RfCs are already used by the USEPA and by California's Department of Toxic Substances Control and were earlier incorporated by reference in Appendix F of the Emissions Inventory Criteria and Guidelines for the Air Toxics "Hot Spots" Program for use in screening risk assessments in the Hot Spots Program. These Guidelines were effective July 1, 1997. The Risk Assessment Advisory Committee (RAAC) recommended that CalEPA harmonize where possible with USEPA on risk assessment. Governor Wilson's Executive Order W-137-96 concerned the enhancement of consistency and uniformity in risk assessment between Cal EPA and USEPA. Use of RfCs as chronic RELs was one action that OEHHA took to address the RAAC recommendation and to implement the Executive Order. RfCs released after October 1997, including ones that are revisions of those in the October 1997 draft, will be evaluated for use in the Hot Spots program by reviewing the scientific basis of each RfC when it becomes available and by determining whether the scientific literature cited in the RfC is appropriate. Appropriate RfCs will be submitted yearly to the SRP for their review and possible endorsement.

The MSHA HCN level is for healthy workers exposed during a normal work-week. The chronic REL is for continuous ambient exposure for the entire population including sensitive individuals, such as infants, children, the elderly, and the respiratory impaired. The MSHA HCN value is not relevant for such exposure, other than to indicate that the chronic REL should be lower than it. OEHHA staff note that the ACGIH has a STEL (ceiling limit) of 5 ppm (5.5 mg/m^3) for HCN, somewhat lower than the MSHA value of 10 ppm.

Comment 2. OEHHA has proposed adoption of the U.S. EPA reference concentration (RFC) for HCN, as published in the IRIS database. Supporting documentation provided for the proposed HCN Chronic REL indicates that U.S. EPA's RFC is based on <u>only</u> one study, performed in Egypt and published in 1975. The Egyptian study evaluated 36 male electroplating

workers in three factories using a mixture of chemical compounds containing cyanide. Based on our review of available literature, and our members considerable experience in the evaluation of the potential health effects of chronic compounds used in the mining industry, the U.S. EPA adopted the HCN RFC without sufficient scientific study. The following summarizes our specific concerns regarding the Egyptian study and U.S. EPA's RFC. No NOAEL was identified. It is uncertain as to whether HCN exposure levels were accurately characterized. No information was supplied to evaluate potentially conflicting factors, such as food supply, lifestyle or working conditions. As a result of the preceding issues, it is not possible to objectively determine whether OEHHA's proposed Chronic HCN REL is more protective of human health than the existing HCN REL. In conclusion, based on our review of available data, no compelling scientific evidence has been supplied by U.S. EPA or OEHHA to justify adoption of the proposed HCN chronic REL. As a result, we strongly recommend against adoption of the proposed HCN chronic REL.

Response. As stated above the chronic REL is for ambient exposure of the general population. The comment points out some of the problems with using human data. Although the REL is based on a single study of 36 exposed humans, hydrogen cyanide is a known metabolic poison and needs a chronic REL. However the commentator does not suggest an alternative study for the development of the REL. The existing animal studies would likely result in a much lower chronic REL due to their subacute/subchronic duration and to the HCN levels tested. OEHHA would be pleased to review any superior study and would also encourage USEPA to do the same.

Chemical Manufacturers Association (CMA) - Ethylene Glycol Panel

Comments on the chronic REL for **ethylene glycol** were received from the Ethylene Glycol Panel of the Chemical Manufacturers Association (CMA). Panel members include BASF Corporation, the Dow Chemical Company, Eastman Chemical Company, Huntsman Corporation, Occidental Chemical Corporation, Shell Chemical Company, and Union Carbide Corporation. OEHHA staff developed a chronic REL of 400 $\mu g/m^3$ based on a 37 day inhalation exposure of prison volunteers to ethylene glycol.

Comment 1. The technical support document provides an ethylene glycol chronic inhalation reference exposure level (REL) of 400 $\mu g/m^3$. However, the Panel notes that the calculations used to derive the REL are flawed. In addition, the uncertainty factors used in the ethylene glycol assessment are overly conservative and should be lowered. As outlined below, using the appropriate values in the calculations and more appropriate uncertainty factors, the chronic inhalation REL for ethylene glycol should be set at 730 $\mu g/m^3$, or higher.

In the technical support document, there appears to be an error in the formula used to derive "ppm" exposure values from "mg/m^3"values. For example, the document indicates that the proposed REL of 0.2 ppm ethylene glycol would be equivalent to 400 $\mu g/m^3$. However, based on the appropriate formula, indicated below, 0.2 ppm ethylene glycol would actually be equivalent to 0.508 mg/m^3, or 508 $\mu g/m^3$.

$$mg/m^3 \text{ at } 25`C \text{ and } 760 \text{ mm Hg} = \frac{\text{ppm x molecular weight (EG = 62.07)}}{24.45}$$

$$1 \text{ ppm} = 2.53865 \text{ mg/m}^3$$

$$0.2 \text{ ppm} = 0.508 \text{ mg/m}^3 \text{ or } 508$$

Response. The formula used to convert ppm to mg/m^3 in the document is correct. The human equivalent concentration of 16.7 ppm was divided by a UF of 100 to get an REL of 0.167 ppm or 423 $\mu g/m^3$. Unfortunately both values were subsequently rounded separately to 0.2 ppm and 400 $\mu g/m^3$. When the 0.167 ppm is rounded first to 0.2 ppm, the equivalent value is 508 $\mu g/m^3$, which is rounded to 500 $\mu g/m^3$. Based on this comment OEHHA staff will revise the chronic REL to 500 $\mu g/m^3$.

Comment 2. The exposure levels used as the basis for the REL are inappropriately reported in the technical support document. According to the support document, the basis of the REL is the Wills *et al.* (1974) study, with the REL calculated from the average exposure level from the No Observed Adverse Effect Level (NOAEL). The Wills study, "Inhalation of Aerosolized Ethylene Glycol by Man," is a human study that was conducted by NASA to evaluate the irritant and systemic effects from continuous exposure to ethylene glycol aerosols. During the main study, "twenty volunteers were exposed during 20 to 22 hours per day to aerosolized ethylene glycol in mean daily concentrations between 3 and 67 mg/m^3." However, in its review of the Wills study, the technical support document indicated that the upper end of the exposure concentrations was only 49 mg/m^3 (reported as 20 ppm in the technical document), rather than 67 mg/m^3 (or 26.4 ppm).

Using the correct NOAEL value of 67 mg/m^3 in the derivation equation and the appropriate conversion factor between "mg/m^3 and "ppm", the calculated human equivalent concentration should be 22 ppm. (See footnote 2.)

Response. OEHHA staff used the highest mean value of 49 mg/m^3 (20 ppm) as the No-Observed-Adverse-Effect-Level (NOAEL), not the highest high value of 67 mg/m^3 (rounded from 66.8). (See Table 1 from Wills et al. below.) OEHHA staff believe that 49 mg/m^3 is the appropriate NOAEL.

Table 1. Concentrations of Ethylene Glycol in the Air within the Exposure Chamber

	Concentration of ethylene glycol in air, mg/m^3		
Days	Low	High[a]	Mean
1-7	3.6	75.0	37
8-14	18.8	44.8	29
15-21	0.8	41.6	17
22-28	3.5	49.2	23
29-35	20.6	**66.8**	**49**
36-37	14.4	39.0	31

[a] This column does not include the very high concentrations maintained for comparatively brief periods.

Comment 3. The subchronic uncertainty factor should be reduced from 10 to 3. EPA's IRIS database states that an uncertainty factor "of 3 is used for extrapolation from subchronic to chronic duration due to limited progression between short-term and subchronic exposure and due to rapid metabolism." Ethylene glycol has been shown to have rapid metabolism. Moreover, ethylene glycol respiratory irritation response is due to short-term exposure and does not appear to worsen at subchronic exposure duration, which is supported by the observation in the Wills study with humans exposed up to 30 days.

Response. OEHHA staff consider 30 days to be too short to be considered for a factor less than 10. This length of 30 days is considered by OEHHA staff to be subacute for humans. It is only 0.12% of the 70-year human life span versus 4.2% of a rodent's 2-year life span. Although respiratory irritation might not worsen, the factor of 10 protects against other known systemic effects that may occur over long-term exposure to ethylene glycol in the other 99+% of the human life span.

Comment 4. In addition, it should be noted that the American Conference of Governmental Industrial Hygienists (ACGIH) also considered the Wills study when recommending an occupational exposure level for ethylene glycol. Because of the reported irritation of the upper respiratory tract at 140 mg/m^3 (the LOAEL), ACGIH selected 100 mg/m^3 as the ethylene glycol TLV ceiling value. To the Panel's knowledge, no systemic toxic or irritant effects have been reported in humans from inhalation of ethylene glycol at concentrations less than 100 mg/m^3.

With the corrected human equivalent concentration and the appropriate uncertainty factors applied, the chronic inhalation REL for ethylene glycol should be at least 730 $\mu g/m^3$ (0.287 or 0.3 ppm).

Response. Comment noted. The TLV is for healthy workers and may not protect sensitive individuals in the general population. Furthermore, as stated above, OEHHA staff do not consider 30 days to be a subchronic exposure for humans, although respiratory irritation might not worsen with longer exposure.

Comment 5. The summary section indicates that the critical effects include eye irritation in humans. However, the Wills study did not report any eye irritation, but only respiratory irritation.

Response. The Wills et al. (1974) study did not report any eye irritation. OEHHA staff will delete the reference.

Comment 6. SECTION III - MAJOR USES AND SOURCES: The Panel is concerned with some of the listings in this section which appear to be incorrect, and potentially, dangerously misleading. For example, the technical support document indicates that ethylene glycol is used as a vehicle in some pharmaceutical preparations or as a flavoring, which are inappropriate and certainly not uses recognized by the Panel members. Attached is an information sheet developed by the Panel that provides an overview of recognized uses of ethylene glycol.

Response. This information is from the ethylene glycol file in HSDB and is based on the 1965 edition of Ethel Browning's Toxicology and Metabolism of Industrial Solvents. The file was reviewed by a scientific review panel in 1990. The Ethylene Glycol Panel should make its information available to the National Library of Medicine, maintainers of the HSDB.

Comment 7. SECTION IV - EFFECTS OF HUMAN EXPOSURE. The technical support document includes a review of the Laitinen *et al* study (1995). Attached is the Panel's critique of the Laitinen study. As you will note, given the concerns raised on the clinical chemistry and the analytical methodology; and the findings of ethylene glycol in the control samples, the findings of this study would have to be considered questionable at best. The Panel is also providing a copy of its recent information sheet, "Ethylene Glycol: Research Shows that Normal Skin Contact Is Not Expected to Cause a Health Hazard", which provides an overview of available dermal data on ethylene glycol. In fact, the American Conference of Governmental Industrial Hygienists concluded that a "skin notation", a designation referring to potential exposure by the dermal route, was not necessary for ethylene glycol, given its negligible absorption through the skin

Response. Laitinen et al. made a tentative conclusion from their study of 10 car mechanics: "Therefore, it seems that ethylene glycol is absorbed by skin contact." The study was published in a respected occupational journal and may be questioning the accepted belief about skin

absorption. The point is not critical to the development of the chronic REL. The critique should be sent to the authors of the article or to the journal *Occupational Medicine (Oxford)*.

Comment 8. SECTION V - EFFECTS OF ANIMAL EXPOSURE. The first paragraph of this section includes a review of the DePass study, which indicates that the exposure levels for both rats and mice were 0, 0.04, 0.2, or 1 g/kg/day, and that there were no effects observed in mice. Therefore, the NOAEL for mice would be 1 g/kg/day (1000 mg/kg/day), and not 40 mg/kg/day, as reported in the last sentence.

Response. OEHHA staff regret the error and will change the sentence.

Comment 9. The fourth paragraph of this section includes a review of the Tyl mouse nose-only inhalation study. The technical support document indicates that the NOAEL for maternal effects in this study was 500 mg/m^3, based on increased kidney weights. However, the significance of the kidney weight change has been questioned. The study author herself has concluded "The absence of any treatment-related maternal renal lesions is not unexpected since the mouse appears resistant to ethylene glycol-induced nephrotoxicity after short term exposure, especially since the systemic exposure in the present study is lower than that previously employed (see for example, Price et al., 1985). There is no apparent explanation for the increased maternal kidney weights observed at 1000 and 2500 mg/m^3 by nose-only exposure, in the absence of any microscopic lesions." The Panel believes that the control values were abnormally low in this study. There were no statistically significant differences for relative kidney weights at 1000 mg/m^3 and there were no absolute or relative weight differences in three other inhalation studies at concentrations as high as 2500 mg/m^3. Given that there were no microscopic lesions in the kidneys, that the findings have not been reproduced, and that the author has indicated no apparent explanation for the findings, the kidney weight change should not be used as the basis for the maternal NOAEL in the Tyl study.

Response. Comment noted. OEHHA staff will review the Tyl study in mice, especially the data on controls. However, the true NOAEL for the Tyl study in mice will not influence the chronic REL, which is based on a NOAEL of 50 mg/m^3 (20 ppm) found in a study of respiratory tract irritation in people. The Tyl study also will not influence the respiratory irritation endpoint.

Chemical Manufacturers Association – Isopropanol Panel

Comments on the chronic REL for **isopropanol** (IPA) were made by the Isopropanol Panel of the CMA in a letter from Courtney M. Price dated January 29, 1998. The IPA Panel includes all major U.S. manufacturers of IPA, including: Exxon Chemical Company, BP Chemical Company, ARCO Chemical Company, Shell Chemical Company and Union Carbide Corporation. OEHHA developed a chronic REL of 2,000 $\mu g/m^3$ based on a subchronic (13 week) inhalation study in mice and rats by Burleigh-Flayer et al. (1994).

The Panel believes that IPA should not be listed as an air toxic. Accordingly, the Panel has filed a petition with the California Air Resources Board requesting that IPA be removed from the California list of air toxics. The petition explains that an extensive database exists for IPA and demonstrates that this chemical poses low toxicological concerns. Indeed, IPA is not regulated by the federal government under any environmental statute based on toxicity concerns. A copy of the petition is attached to the comments.

The Panel further asserts:

- OEHHA's chronic toxicity summary for IPA omits discussion of the results of the rat and mouse chronic inhalation studies, which, along with numerous other studies, were conducted by the Panel pursuant to a test rule under Section 4 of the Toxic Substances Control Act (TSCA).

- Any chronic REL for IPA should be derived from the rat chronic inhalation study. This study was of chronic duration by the most relevant route of exposure and demonstrates the lowest "critical toxic effect" of the IPA studies - exacerbation of chronic progressive nephropathy - with a NOAEL of 500 ppm. Applying OEHHA's REL methodology to this NOAEL results in an REL of 7.3 mg/m^3 (3.0 ppm).

- The toxicity summary as currently written bases the critical effect for the chronic REL on changes that are not adverse or relevant to human health. Specifically, OEHHA relies on hyaline droplets in the kidneys of male rats, although the toxicity summary states that these effects are not relevant to humans, and on increased relative liver weights in female mice, although the summary states that this effect may be considered more of a metabolic response than a toxic effect.

- The toxicity summary also credits several questionable findings that the Panel believes should not be included in the toxicity summary. Specifically, the IPA chronic toxicity summary lists three indicators of chronic adverse effects: the development of tolerance of narcosis, blood chemistry changes and reduced fetal body weights. The Panel, however, questions the relevance of the first two effects and the validity of the third effect. The Panel therefore believes that OEHHA should remove references to these questionable findings from its chronic toxicity summary for IPA.

The following Appendices were included:

APPENDIX A. OECD SIDS Dossier for Isopropanol
APPENDIX B. OECD SIDS Initial Assessment Report (SIAR) for Isopropanol
APPENDIX C. Petition of the CMA Isopropanol Panel to Remove Isopropanol from the California List of Air Toxics, Submitted to the California Air Resources Board January 29, 1998
APPENDIX D. Kapp *et al.*, (1996). Isopropanol: Summary of TSCA Test Rule Studies and Relevance to Hazard Identification. *Regulatory Toxicology and Pharmacology* 23: 183-192.
APPENDIX E. Burleigh-Flayer, H., Garman, R., Neptun, D., Bevan, C., Gardiner, T., Kapp, R.,. Tyler, T., and Wright G. (1997). Isopropanol vapor inhalation oncogenicity study in Fischer 344 rats and CD - 1 mice. *Fundam. Appl.* Toxicol., 36:95-111.

Comment 1. Under a Toxic Substances Control Act (TSCA) test rule, Panel members have sponsored extensive studies of IPA, including chronic inhalation studies in rats and mice. All of these studies have been published in the peer-reviewed literature. In addition, the Panel recently worked collaboratively with the United States Environmental Protection Agency (EPA) to prepare a SIDS dossier and SIDS Initial Assessment Report (SIAR) for IPA. Copies of the SIDS dossier and SIAR are attached to these comments as Appendices A and B, respectively.

IPA should not be regulated as an air toxic. The California Air Toxic "Hot Spots" Program lists over 400 chemicals, including IPA, as air toxics for which emissions must be reported and a risk assessment may be required. The Panel believes, however, that IPA is not properly listed as an air toxic. An extensive database exists on the toxicity of IPA and demonstrates that this chemical poses low toxicological concerns. Indeed, IPA is not regulated by the federal government under any environmental statute based on toxicity concerns. IPA is not listed as a federal hazardous air pollutant (HAP), nor is it listed as a "hazardous waste" under the Resource Conservation and Recovery Act (RCRA), a "hazardous substance" under the Comprehensive Environmental Response, Compensation and Liability Act (CERCLA), or a "toxic chemical" under Section 313 of the Emergency Planning and Community Right-to-Know Act (EPCRA). 2 IPA's workplace exposure limits (OSHA permissible exposure limit of 400 ppm) confirm that it is relatively non-toxic.

Moreover, IPA has relatively low photochemical reactivity and has been approved as a substitute for ozone-depleting substances (ODSs). Thus, the removal of IPA from the California air toxics list would facilitate pollution prevention efforts in California, while its retention on that list merely results in unnecessary and wasteful expenditures. Because no toxicological basis exists for listing IPA as an air toxic, and because its listing may have an adverse effect on pollution prevention efforts in California, the Panel has submitted a petition to the California Air Resources Board (CARB) requesting that IPA be removed from the air toxics list. A copy of that petition is included as Appendix C. At a minimum, however, OEHHA should revise its chronic toxicity summary and REL for IPA as described below.

As described in the Panel's petition to remove IPA from the California list of air toxics, EPA has determined that IPA does not meet the criteria for listing under Section 313 of EPCRA; only "isopropyl alcohol (manufacturing - strong acid process") is included on this list. However, the strong acid process is no longer used in the United States.

Response. Isopropanol is currently listed as a Hot Spots chemical and therefore a chronic REL is being proposed. If isopropanol is delisted in California as a Toxic Air Contaminant, then it would likely be removed from the Hot Spots list and the chronic REL would be withdrawn.

Comment 2. The chronic toxicity summary for IPA should discuss the chronic inhalation studies in mice and rats. Because of the extensive studies of IPA conducted by the Panel under a TSCA Section 4 test rule, IPA's human health hazard potential has been extremely well-characterized. The studies that were included in this testing program are identified in Table 1. A review article, which includes citations to several publications of individual studies, is attached as Appendix D.

The EPA's Office of Pollution, Prevention and Toxics (OPPT) has already carefully reviewed all of these studies, and the Agency's assessment of these studies is reflected in a risk management (RM 1) review of IPA, as well as in the SIDS Initial Assessment Report (SIAR) prepared by the Panel and approved by EPA as part of the OECD SIDS program. Moreover, the Panel has striven to ensure that all data produced from its studies are published in the peer-reviewed literature.

TABLE 1. Health Effects Testing for IPA TSCA Section 4 Test Rule Studies

	Test Submission Of Final Report
Mutagenicity Study: Mammalian Cells in Culture	06/90
Developmental Toxicity Studies in Rats and Rabbits	12/90
Subchronic Inhalation Toxicity Studies in Rats and Mice	03/91
Mutagenicity Study: In Vivo Cytogenetics: Micronucleus	03/91
Acute and Subchronic Neurotoxicity Studies in Rats	03/91
Oral and Inhalation Pharmacokinetics Studies in Rats and Mice	03/91
Developmental Neurotoxicity Study in Rats	08/91
Reproductive Toxicity Study in Rats	05/92
Chronic Studies in Rats and Mice	06/94

OEHHA's chronic toxicity summary discusses several of these studies but fails to include the chronic inhalation studies conducted on rats and mice. Indeed, the summary states that a "weakness of the data base for" IPA is that "[n]o long-term studies, spanning a majority of the life span of the test animal, have been performed with isopropyl alcohol." OEHHA's incorrect statement, coupled with its failure to include a discussion of the most recent and relevant studies in the IPA chronic toxicity summary, creates the misleading impression that IPA is a high-production volume chemical that has not been adequately tested. That impression is wrong and should be corrected. In the end, the risk assessments conducted under the Hot Spots program will only be as good as the underlying assumptions and chemical-specific data on which OEHHA relies. It is critical that the most accurate and reliable health hazard information be used. The Panel therefore believes strongly that, if IPA is included in the Hot Spots program, then the technical support document should utilize the chronic studies sponsored by the Panel under TSCA Section 4.

Response. OEHHA prefers to base its RELs on peer-reviewed articles that have been published in the medical and toxicological literature. The chronic study appeared in the peer-reviewed literature after staff had completed its literature review. A summary of the chronic study has been added to the section on Effects of Animal Exposure. The chronic study has been used as the basis of a revised chronic REL.

Comment 3. Any chronic REL for IPA should be derived from the rat chronic inhalation study conducted under TSCA Section 4. Because OEHHA apparently was unaware of the chronic studies of IPA, the proposed IPA REL was calculated using a subchronic inhalation study. Clearly, however, the recent chronic inhalation studies in mice and rats (Burleigh-Flayer et al., 1997), which were conducted in accordance with EPA test guidelines, are more suitable for calculating a chronic REL than the subchronic studies. The results of the chronic studies are summarized below. [OEHHA staff have omitted the commentator's summary of the (negative) results about carcinogenicity since they are not relevant to the chronic REL.]

With regard to systemic chronic toxicity, Burleigh-Flayer *et* al. report that equivocal minimal to mild kidney effects, including renal tubular dilation, were observed in mice. The incidence of renal tubular proteinosis was generally significantly increased for all male and female treatment groups relative to controls; however, the majority of affected animals showed minimal degrees of tubular proteinosis *(i.e.*, only a few tubules affected), there was no concentration-related gradient in either the frequency or severity of this change, and there was no corresponding evidence of alterations to the glomeruli. Mild to moderate degrees of tubular dilation were observed in a small number of females in the 2500 and 5000 ppm groups, but were significantly increased only for the 5000 ppm group. Moreover, this finding was not duplicated in male mice (a significant increase was seen only for the 500 ppm group, but not at the higher dose levels), nor was it accompanied by evidence of tubular cell degeneration or urinary outflow obstruction.

Kidney effects also were observed in the rat following chronic exposure. These effects included some organ weight changes and an exacerbation of chronic progressive nephropathy that occurred in both male and female rats at 2500 and 5000 ppm. Chronic progressive nephropathy is a spontaneous kidney disease of unknown etiology that occurs commonly in aged rats. Although the human health relevance of this condition is unknown, the study researchers considered the effect to be treatment-related and adverse to the rat. (It was considered to be the likely cause of early mortality in some male rats). Exposure to 500 ppm IPA did not produce any effects on the kidney, and is considered the NOAEL for this study.

The Panel believes that rat chronic inhalation study (Burleigh-Flayer *et al, 1997)* should be considered the "critical study" for derivation of an REL for IPA. The study was of chronic duration (two years), by the most relevant route of exposure (inhalation) and demonstrates the lowest "critical toxic effect" of the IPA studies - the exacerbation of chronic progressive nephropathy that occurred in both male and female rats at 2500 and 5000 ppm. This effect should be considered the "critical toxic effect" because it was the likely cause of early mortality in some rats and the study researchers considered the effect to be treatment-related and adverse.

As described above, equivocal effects to the kidney (tubular proteinosis and dilation) were also reported in mice following chronic exposure to IPA vapor. However, it was not clear whether these effects were treatment-related, nor was it clear whether they were adverse effects. Accordingly, the Panel does not consider these effects to be as appropriate for consideration as the "critical toxic effect."

As noted above, OEHHA has generally adopted EPA's RfC methodology for calculating chronic RELs. *See* EPA, *Methods for Derivation of Inhalation Reference Concentrations and Application of Inhalation Dosimetry*, EPA/600/8-90/066F (October, 1994).

As described above, at 2500 and 5000 ppm, the rats experienced chronic progressive nephropathy. Thus, 2500 ppm should be considered the LOAEL for kidney effects, and 500 ppm can be considered the NOAEL. The NOAEL then must be converted into mg/m , and dosimetrically adjusted to provide a human equivalent concentration (HEQ NOAEL, accounting for the non-continuous duration of the dosing in the inhalation study. Id. at 4-2 1.

Critical Study:	IPA Chronic Rat Inhalation Study (Burleigh-Flayer *et al* 1997)
Critical Toxic Effect:	Chronic progressive nephropathy
Study NOAEL (ppm):	500 ppm
Study NOAEL (mg/m3):	500 ppm x 60.11 */24.45 = 1229 mg/m^3
NOAEL[ADJ]:	1229 x 6/24 x 5/7 = 220 mg/m^3
NOAELWCI:	NOAEL[ADJ] x [b:a lambda(a)/b:a lambda(h)]**
	220 mg/m^3 x 1 = 220 mg/m^3

The molecular weight of IPA is 60.11. For water-soluble compounds such as IPA, a factor of one is used.

Consistent with EPA's RfC methodology, OEHHA's methodology involves the application of uncertainty factors to the NOAELWC1 to arrive at an REL that is designed to be protective of the general population receiving repeated daily exposures over the course of a lifetime. Uncertainty factors can range from one (no uncertainty) to ten (the highest value), with a median of three, for five separate categories of uncertainty: (1) protection of sensitive human subpopulations; (2) extrapolating from animal data to humans; (3) extrapolating from subchronic to chronic exposure levels; (4) extrapolating from a LOAEL to a NOAEL; and (5) accounting for an incomplete data set. [According to EPA, however, a composite (maximum) uncertainty factor involving four areas of uncertainty would be 3,000, reflecting the fact that these factors are interdependent.]

An uncertainty factor of 10 typically is used to protect sensitive human subpopulations. For extrapolation from animal data to humans, EPA recommends, and OEHHA has used, an uncertainty factor of three when relying on default dosimetric adjustments in deriving a NOAEL [HEC] from the NOAEL. The Panel has applied these default adjustments to the chronic IPA study. No uncertainty factor is necessary to extrapolate from a LOAEL to a NOAEL, as a clear NOAEL was identified in the IPA study and was used in these calculations.

Similarly, the inhalation study was of chronic duration, so no uncertainty factor is necessary to extrapolate from subchronic to chronic. [Nor is an uncertainty factor needed to account for an incomplete database. As identified in these comments, and generally described in OEHHA's chronic toxicity summary for IPA, a large database exists for IPA, covering a broad range of endpoints and routes of exposure.] Accordingly, an uncertainty factor of 30 would be applied to the NOAEL [HEC, to derive an REL for IPA.

$$\text{REL} \quad 220\ \text{mg/m}^3 / 30 = 7.3\ \text{mg/m}^3\ (3.0\ \text{ppm})$$

Response. OEHHA agrees with the commentator that the 1997 chronic inhalation study in rats and mice by Burleigh-Flayer et al., which became available too late to be included in the October 1997 draft, is superior to the 1994 subchronic study originally used. OEHHA has based a revised chronic REL for isopropanol on the 1997 study and arrived at the same REL calculated by the commentator. The critical effects occurred in the kidney. Therefore we are revising our document accordingly.

Comment 4. OEHHA should remove references to questionable findings from its chronic toxicity summary for IPA. For the reasons discussed above, the Panel believes that, if an REL is to be derived for IPA, OEHHA should use the chronic inhalation study. However, the Panel also believes that modifications to the IPA chronic toxicity summary are necessary to ensure that the document accurately describes the potential human health effects of IPA. As noted above, the chronic toxicity summary should include a discussion of the chronic inhalation studies. In addition, the Panel believes that the toxicity summary as currently written bases the critical effect for the chronic REL on changes reported in the subchronic inhalation study that OEHHA itself has acknowledged are not adverse or relevant to human health. The toxicity summary also credits several questionable findings from the subchronic inhalation and other studies that the Panel believes should not be included in the toxicity summary. OEHHA derived its chronic REL for IPA from a subchronic inhalation study in rats and mice (Burleigh-Flayer *et al.* 1994). OEHHA states that the critical effects are increased relative liver weight (10 percent over controls) in female mice and hyaline droplets in the kidneys of male rats. Elsewhere in the toxicity summary, however, OEHHA states that the "hyaline droplets found in kidneys of male rats has been shown to be a male rat-specific phenomenon and is not considered to be relevant to human risk assessment." The toxicity summary also states: “Many studies also noted increased liver and kidney weights in exposed animals but with no observable relevant pathology. This change may be considered more of a metabolic response, rather than a toxic effect, of the alcohol.”

The Panel agrees that the kidney effect (hyaline droplets) is not relevant to humans, and that the increased liver weights (and kidney weights in other studies) are more properly considered a metabolic response to exposures to high doses of the alcohol. Because of their equivocal nature, the Panel believes that the increased liver weights and hyaline droplets should not be listed as critical effects for derivation of the chronic REL for IPA.

The toxicity summary also discusses results of some studies that the Panel believes are of questionable validity. Specifically, the IPA chronic toxicity summary lists three sensitive

indicators of IPA chronic adverse effects: the development of tolerance to narcosis, blood chemistry changes and reduced fetal body weights. The Panel, however, questions the relevance of the first two effects and the validity of the third effect, and therefore believes that these effects should not be discussed in the summary.

The first effect, tolerance to narcosis, is questionable as an indicator of chronic adverse effect. Narcosis is clearly adverse, but the diminishing of this effect following repeated exposure (likely through enzyme adaptation) would appear to be a positive adaptation for the animal and not an adverse effect. The second effect, hematological changes (anemia) reportedly was observed in the cited studies, Burleigh-Flayer *et al* (1994) and USEPA/OTS (1986) (actually a BIBRA study). However, anemia was not observed in the IPA chronic study, even though the chronic study involved concentrations as high as that at which the effect was observed by Burleigh-Flayer *et al.* (1994) and the higher equivalent concentration that produced the effect in USEPA/OTS (1986). The fact that the chronic study did not corroborate this finding calls into question the reliability of the anemia effect as an indicator of chronic toxicity.

Response. As indicated above, a summary of the chronic inhalation study has been added to the section on Effects of Animal Exposure. The summary notes the lack of anemia in the chronic study, although it occurred in the subchronic study. OEHHA has reviewed the chronic REL summary for other possible revisions.

Comment 5. The third effect, reduced fetal body weight, is an appropriate indicator of chronic adverse effects. Nonetheless, the conclusion in the toxicity summary that this effect occurred at doses lower than those that caused maternal toxicity is questionable when the data are examined as a whole. Specifically, the OEHHA chronic toxicity summary concludes that the LOAEL for fetal body weight effects in the Tyl *et* al. (1994) study was 400 mg/kg. The data presented in the manuscript do not support this conclusion, however, showing statistically significant changes in fetal body weight only at 800 and 1200 mg/kg, doses which also produced maternal lethality. There was no effect on fetal body weight at 400 mg/kg; thus, this dose level should be considered the NOAEL.

Moreover, although Nelson *et* al. (1988) reported reduced fetal weights at all vapor concentrations (3500, 7500, 10000 ppm) and effects on maternal body weight at only the top two vapor concentrations, this study's assessment of maternal toxicity is suspect given that the recent subchronic and chronic inhalation toxicity studies clearly identified narcotic effects in animals (non-pregnant) at 2500 ppm (Burleigh-Flayer *et* al., 1994, 1997). Nelson may have simply missed these narcotic effects in his study. Although Nelson *et* al. (1988) reported a very slight reduction in fetal weight in the 3500 ppm fetuses (approximately 3%), the study researchers discounted this finding in their discussion as not selective developmental toxicity.

However, except for a small but statistically significant decrease in fetal weight in the case of isopropanol, no effect was detected with either solvent at 3500 ppm. These data indicate that neither of these alcohols is a selective developmental hazard to the developing conceptus.

Similarly, in the two BIBRA studies (reported by OEHHA as US EPA/OTS studies), maternal toxicity also apparently was present at the dose levels at which reduced fetal body weights were reported.

The OEHHA toxicity summary further cites Beyer (1992) (or Bevan *et al.,* 1995) as showing a LOAEL for developmental effects at a dosage that did not produce parental toxicity (1000 mg/kg). In fact, however, several parental effects were observed in this study at 1000 mg/kg, including increases in body weights in females, increases in liver weights without structural changes in females, increases in liver weights with hepatocellular hypertrophy in a few males, and increases in kidney weight and structural changes in males. Nonetheless, OEHHA's interpretation may be correct in that these effects may not be adverse to the animal such that fetal effects were seen in this two-generation reproductive toxicity study in the absence of clear parental toxicity. It should be noted, however, that the developmental toxicity study in rats (Tyl *et al.,* 1994) found clear maternal toxicity (lethality, decreased body weight) at only a slightly higher dosage than was used in Beyer (1200 mg/kg).

(The Panel has access only to abstracts of these studies, rather than to the studies themselves. Thus, it is difficult to interpret the study results and assess fully the adequacy of the studies. Nonetheless, the abstracts report that in both studies, animals had reduced food and water intake at the middle and high dose levels (1.0 and 2.0, and 1.25 and 2.5 percent IPA in drinking water in the reproductive and teratogenicity studies, respectively). The reproductive and embryotoxicity study reports decreased body weights in the middle and high dose female animals, while the teratogenicity study reports decreased body weights only in the high dose females. These results are somewhat surprising in that the middle dose animals in the teratogenicity study were exposed to higher concentrations of IPA than the middle dose animals in the reproductive and embryotoxicity study.)

The Panel believes that a weight-of-the-evidence evaluation of these studies leads to a conclusion that IPA may produce only equivocal minimal selective toxicity to the developing fetus at high doses, but that delays in development generally occur only at levels that produce parental toxicity. The Panel requests that OEHHA reevaluate its discussion on developmental toxicity in light of these comments. The Panel further requests that OEHHA revise its final toxicity summary for IPA to remove references crediting questionable findings to ensure that the public is not misled about the potential human health effects of IPA exposure.

Response. As indicated above, a summary of the chronic inhalation study has been added to the section on Effects of Animal Exposure. OEHHA has reviewed the chronic REL summary for possible revisions. The chronic REL has been revised and the critical effect for the revised REL is kidney lesions as described in the 1997 chronic inhalation study.

In regard to developmental studies, especially fetal body weight effects, OEHHA is very concerned about the possible biological importance of even small weight decrements in (small) animal fetuses, even when the decrements may not be statistically significant. In humans, the logarithm of infant mortality (death before the infant's first birthday) increases linearly as birth weight decreases from 3500 to 1000 grams (Hogue *et al*., 1987; Rees and Hattis, 1994). This log-linear relationship exists on both sides of the birthweight of 2500 g conventionally used as a

cutoff defining low birth weight. There is no evidence for a threshold or break in the curve in this region. Thus any reduction in human birth-weight is a cause for concern since it increases the risk of infant mortality. (Hogue CJ, Buehler JW, Strauss LT, Smith JC. Overview of the National Infant Mortality Surveillance (NIMS) project--design, methods, results. Public Health Rep 1987 Mar-Apr;102(2):126-138; Rees DC, Hattis D. Chapter 8. Developing Quantitative Strategies for Animal to Human Extrapolation. In: Principles and Methods of Toxicology. Third Edition. AW Hayes, editor. New York: Raven, 1994). In the absence of certainty, OEHHA staff take the health protective approach that the reduced weight effect in animal fetuses may be biologically significant and may have import for humans. Tyl et al. (1994) used Dunnett's test to show statistically significant diffrerences between the controls and the 800 and 1200 mg/kg dosed animals (Table 1 of Tyl et al., 1994). Analysis of the data by a trend test showed a highly statistically significant trend ($p<0.001$). In each comparison of rat fetal weight (combined, males only, females only) the fetuses in the 1200 mg/kg dosed group weighed less than those in the 800 mg/kg group, which in turn weighed less than the 400 mg/kg fetuses, which weighed less than the controls. Based on the Hogue et al. results, OEHHA considers the 400 mg/kg group to set a biological LOAEL even if not statistically a LOAEL by a test such as Dunnett's.

Similar to the commentator, OEHHA staff have difficulty when faced with reviewing only the abstracts of studies. It is difficult to base a REL on a one paragraph abstract.

Comment 6. (CONCLUSION). For the reasons set forth in these comments, the Panel believes that IPA should be removed from the list of "air toxics" subject to the "Hot Spots" program. At a minimum, however, OEHHA should derive a chronic REL for IPA using the chronic rat inhalation study, rather than the subchronic study. OEHHA also should remove language from its chronic toxicity summary for IPA that suggests that this chemical has not been adequately studied, and should remove references crediting questionable findings from this document.

Response: OEHHA appreciates the thoroughness of the comments. OEHHA presently has no authority to remove IPA from the Hot Spots list. However, OEHHA has revised its chronic REL for IPA using the rat chronic inhalation study, as suggested by the comment. OEHHA has also reviewed the chronic REL summary and made possible revisions. For example, since a chronic inhalation study is now available, OEHHA included a summary of the study and deleted the statement that no long-term chronic study was available.

Chemical Manufacturers Association - Ketones Panel

The Chemical Manufacturers Association (CMA) Ketones Panel submitted comments on January 28, 1998 in response to the draft chronic reference exposure level (REL) for **methyl ethyl ketone (MEK)**. In the draft TSD OEHHA proposed use of the USEPA Reference Concentration (RfC) of 1,000 $\mu g/m^3$ (0.3 ppm) as the chronic REL. The RfC is based on decreased mean fetal body weight in mice.

Comment 1. OEHHA should re-calculate the REL for MEK using current EPA methodology for deriving inhalation reference concentrations (RfCs). Using current EPA methodology, the REL should be 3.3 mg/m^3 (slightly greater than 1 ppm). The uncertainty factor for MEK for interspecies extrapolation should be 3 and not 10. In the Technical Support Document, OEHHA states that the RELs were developed using the United States Environmental Protection Agency's (EPA) methodology for deriving RfCs. EPA's RfC calculation for MEK is reported in the Integrated Risk Information System (IRIS), an EPA on-line database containing health risk and EPA regulatory information. OEHHA thus based its REL calculations for MEK on the methodology reflected in IRIS. EPA derived the RfC for MEK using a developmental toxicity study (Schwetz et al.; Mast et. al. 1989). RfCs are calculated by applying various "uncertainty factors" (UFs) to account for the uncertainty of applying results from animal testing to humans and for any lack of unequivocal data. The RfC calculation listed in IRIS for MEK was performed in 1992, and uses a default UF of 10 for interspecies extrapolation. In 1994, EPA published new guidance for deriving RfCs. *See* EPA Office of Research and Development, "Methods for Derivation of Inhalation Reference Concentrations and Application of Inhalation Dosimetry," EPA No. 600/8-90/066F (Oct. 1994). The new guidance states that, if standard default dosimetric adjustments have been made, an UF of 3 should be used for interspecies extrapolation rather than an UF of 10. Since the 1994 RfC Guidance was issued, the Agency has used this approach for setting a number of RfCs in the IRIS database. The IRIS database clearly indicates that default dosimetric adjustments were made in the case of MEK. However, because the RfC was established before the 1994 RfC Guidance was adopted, an UF of 10 for interspecies extrapolation, rather than an UF of 3, was used to derive the RfC that is currently listed in IRIS. Using the UF of 3 for interspecies extrapolation reduces the total uncertainty factor from 3,000 to 900, and produces a corrected RfC value of 3.3 mg/in^3 (slightly greater than 1 ppm).

Response. All USEPA Reference Concentrations (RfCs), available when the Technical Support Document (TSD) on chronic Reference Exposure Levels was drafted in October 1997, are being proposed as chronic RELs. RfCs are already used by the USEPA and by California's Department of Toxic Substances Control and were earlier incorporated by reference in Appendix F of the Emissions Inventory Criteria and Guidelines for the Air Toxics "Hot Spots" Program for use in screening risk assessments in the Hot Spots Program. These Guidelines were effective July 1, 1997. The Risk Assessment Advisory Committee (RAAC) recommended that CalEPA harmonize where possible with USEPA on risk assessment. Governor's Executive Order W-137-96 concerned the enhancement of consistency and uniformity in risk assessment between Cal EPA and USEPA. Use of RfCs as chronic RELs was one action that OEHHA took to address the RAAC recommendation and to implement the Executive Order. RfCs released after October 1997, including ones that are revisions of those in the October 1997 draft, will be evaluated for

use in the Hot Spots program. OEHHA staff will review the scientific basis of each RfC when it becomes available and determine whether the scientific literature cited in the RfC is appropriate. Appropriate RfCs will be submitted to the SRP for their review and possible endorsement.

In the case of the MEK RfC, OEHHA agrees that the use of a 10-fold intraspecies factor in this case differs from the method more recently supported by USEPA.

Comment 2. This corrected RfC should be considered a conservative value because it is designed to allow continuous exposure for a lifetime of 70 years without adverse effect. Moreover, in the case of MEK, a modifying factor of 3 for incomplete database probably is excessive. A 2-generation reproductive effects study in rats using 2-butanol, which is rapidly converted metabolically to MEK, has been conducted and in fact was used by EPA to derive an oral reference dose (RfD) for MEK. Further, experience with other compounds shows that an UF of 10 for lack of a chronic study usually is higher than necessary (Dourson, M.L. and Stara, L.F., 1983, "Regulatory history and experimental support of uncertainty (safety) factors", *Regulatory Toxicology and Pharmacology,* 3: 224-238).

Response. OEHHA agrees that there is reason to eliminate a modifying factor of 3 for incomplete database. OEHHA has not used modifying factors in the development of its chronic RELs.

Comment 3. EPA has not revised the MEK IRIS data since 1992, and therefore has not re-calculated the RfC for MEK using the 1994 Guidance methodology. The IRIS database RfC for MEK, as a result, is outdated and inaccurate. Because OEHHA used an UF of 10 for MEK in its calculations for interspecies extrapolation, it appears that OEHHA relied on the outdated RfC methodology as reflected in IRIS. The 1994 Guidance clearly dictates that the correct UF for interspecies extrapolation for MEK should be 3. OEHHA should therefore re-calculate the REL for MEK using an UF of 3 for interspecies extrapolation.

Response. OEHHA reevaluated the RfC and proposed REL, which were derived from the data of Schwetz and associates (1991). Using slight differences in approach preferred by OEHHA, a chronic REL value of 10 ppm (30,000 $\mu g/m^3$) was derived. However, this reanalysis presents a situation where the short-term exposure study of Schwetz is no longer the most appropriate study to evaluate potential chronic health effects, since the 90 day inhalation study of Cavender and associates (1983) reported adverse effects at levels close to that reported by Schwetz. From the Cavender study a revised chronic REL of 4 ppm (10,000 $\mu g/m^3$) was derived (table below).

OEHHA revised chronic REL

Study	Cavender *et al*., 1983
Study population	Rats
Exposure method	Inhalation for 90 days
Critical effects	Increased liver weight and relative kidney weight in males and females
LOAEL	5,041 ppm
NOAEL	2,518 ppm
Exposure continuity	6 hours/day; 5 days/week
Average experimental exposure	449.6 ppm for NOAEL group
Human equivalent concentration	449.6 ppm for NOAEL group (gas with systemic effects, based on RGDR = 1.0 using default assumption that lambda (a) = lambda (h))
Exposure duration	90 days
LOAEL UF	1
Subchronic UF	3
Interspecies UF	3
Intraspecies UF	10
Cumulative UF	100
Chronic inhalation reference exposure level (REL)	4 ppm (4,000 ppb; 10 mg/m^3; 10,000 µg/m^3)

Chemical Manufacturers Association (CMA) - Metal Catalysts Panel

Comments on the Determination of Chronic Toxicity Reference Exposure Levels were received from the Metal Catalysts Panel of the Chemical Manufacturers Association (CMA) in a letter dated January 29, 1998. The CMA Metal Catalysts Panel represents firms manufacturing, using, or reprocessing metal-bearing catalysts, including nickel and nickel compounds. Member firms were Akzo Nobel Chemicals Inc., CRI International, Inc., Criterion Catalyst Co., LP, Crosfield Catalysts, Engelhard Corporation, Gulf Chemical & Metallurgical, Haldor Topsoe, Inc., OM Group, Inc., United Catalysts Inc., and W.R. Grace & Co.

The Panel supported comments separately submitted on the Guidelines and proposed REL for **nickel** by the Nickel Development Institute, the Nickel Producers Environmental Research Association, and Inco United States, Inc (see below). OEHHA proposed a chronic REL of 0.05 $\mu g/m^3$ for nickel based on respiratory system and immune system toxicity.

Chemical Manufacturers Association - Olefins Panel

Comments on the chronic REL for **propylene** were received from Courtney M. Price, on behalf of the Olefins Panel of the Chemical Manufacturers Association (CMA), in a letter dated January 29, 1998.

In addition to the comments below, the commentator provided a list of the references cited. This list is available upon request. The commentator also provided two slides of data in an appendix. These slides were presented by Dr. James Swenberg of CMA in March of 1996 regarding ethylene and ethylene oxide research. The appendix is also available upon request.

OEHHA developed a chronic inhalation REL of 3,000 $\mu g/m^3$ for propylene based on an inhalation study in rats.

Comment 1. The OEHHA summary for propylene should emphasize the minimal severity and species-specificity of the nasal effects and the NOEL of 10,000 ppm in a 14-week study. The observed nasal effects for propylene are minimal, reversible, and have been observed in only one species. OSHA does not regulate propylene inhalation, and ACGIH has classified propylene as a simple asphyxiant [ACGIH 1997, p.34] -- that is, an essentially inert gas that can cause asphyxiation at high levels due to dilution of oxygen in the atmosphere, but which otherwise does not have significant physiologic effects [ACGIH, 1997]. The nasal effects in the rat provide a basis for deriving a chronic REL simply because they are the only observed adverse effects. OEHHA should emphasize to users of the REL document, however, the minimal severity of the effects and the fact that they have been reported only for one species in one study.

Quest *et al*. (1984; NTP, 1985) reported the appearance of nasal cavity changes in rats following chronic exposure to both the low exposure level of 5,000 ppm and the high exposure level of 10,000 ppm, while there were no such effects observed in mice exposed to the same levels. Based on a review of these data, it is clear that chronic exposure to 5,000 ppm propylene can cause some minimal effects in the nasal cavity of rats (but not mice). These effects include increased incidence of inflammation, not otherwise specified (NOS), with no obvious dose-response relationship, and of squamous metaplasia, again with no obvious dose-response relationship; and increased incidence of epithelial hyperplasia in females only. The lack of a clear dose-response effect complicates the interpretation of the significance of these observations, in particular for inflammation, NOS, where the high exposure level incidences were similar to control values. In addition, epithelial hyperplasia, which was observed in one sex only, generally is reversible upon cessation of treatment.

Overall, the minimal severity of these effects, the general reversibility of epithelial hyperplasia, and the lack of dose-response relationship for the other effects indicate that 5,000 ppm represents a borderline LOAEL/NOAEL. In addition, the 14-week subchronic exposure data (NTP, 1985) demonstrated a clear NOEL of 10,000 ppm, with no effects in nasal cavity of rats, at either 5,000 or 10,000 ppm exposure levels, thus emphasizing the minimal response following chronic exposure to 5,000 ppm. The Panel believes that the OEHHA summary for the propylene REL should emphasize these points.

Response. OEHHA staff agree with the commentator that the effects observed at 5000 ppm in rats, as reported by Quest *et al.*, are of low severity. Staff also agree that the lack of a clear dose-response for some of the observed toxic effects (e.g., squamous metaplasia and inflammation) complicates the interpretation of those particular findings. However, it is important to note that with the squamous metaplasia, a statistically significant response was obtained at the low dose in both sexes and at the high dose in females, therefore a propylene-related effect cannot be ruled out. In addition, while the inflammatory lesions were statistically significant only for low-dose male rats, the incidence among high-dose males was of similar magnitude, suggesting that a plateau effect had occurred over the range of concentrations tested.

OEHHA acknowledges that the toxicity effects observed by Quest *et al.* are of low severity, both in the text of OEHHA's document and in the calculation of the REL, where an uncertainty factor of 3 is used instead of the usual 10 to account for the magnitude of the effect observed at a LOAEL compared with a NOAEL. In regards to epithelial hyperplasia, it may be generally reversible upon cessation of exposure. This is consistent with the determination that this LOAEL is of lower severity. However, in the Quest/NTP study, animals were not observed following exposure, so it is unclear what would have happened in this case. It is important to note that chronic RELs are based on continuous life-time exposures, where reversiblity of tissue damage upon cessation of exposure would not come into play.

The study by Quest/NTP, conducted in rats and mice, was the only chronic inhalation toxicity investigation found for propylene. There are no data on toxic responses from chronic inhalation to propylene from any other species, including humans. While the results of this study do suggest that a species difference exists between rats and mice in response to the inhalation of propylene, the authors also suggest that the difference could be due to a better compensatory reflex apnea defense mechanism, as documented in B6C3F1 mice exposed to formaldehyde. If so, the mice may not have taken in as much of the compound into the respiratory tract, thereby resulting in less tissue damage and a lesser degree of respiratory tract toxicity than that observed in rats.

In regards to OSHA and ACGIH, these organizations develop guidance for healthy workers exposed to chemicals during a normal workweek. The chronic REL is intended to protect against continuous lifetime ambient exposure for the entire population, including those individuals who may be more sensitive to the toxic effects of airborne chemicals. Those individuals include infants, children, the elderly, and those with compromised respiratory systems.

Comment 2. OEHHA should calculate the Human Equivalent Concentration based on the total respiratory tract surface area. OEHHA calculated a Human Equivalent Concentration (HEC) by using a calculated regional gas dosage ratio (RGDR) of 0.21, based on extrathoracic respiratory surface area and a minute volume derived from body weight. The Panel believes that using an RGDR is a plausible method to estimate an equivalent human exposure, but that the RGDR should be based on total respiratory tract surface area, not just on extrathoracic respiratory tract surface area.

The nasal cavity effects seen in rodents are expected to be, in large part, due to the fact that rats are obligate nose-breathers. Humans, however, are not obligate nose-breathers, and generally are mouth- and nose-breathers. The equivalent target tissue for irritation effects in humans would be the entire respiratory tract. Therefore, the Panel believes that if one is to estimate an HEC with an RGDR, the RGDR should be calculated using the surface area for the entire respiratory tract to better approximate the conditions of dose to humans. Thus, based on the methodology described in the Technical Support Document, the calculation of an RGDR for a gas with respiratory effects would be as follows:

$$\text{RGDR} = \frac{(\text{Minute volume})_{rat}/(\text{Minute volume})_{human}}{(\text{Surface area})_{rat}/(\text{Surface area})_{human}}$$

The values used by EPA for minute volumes of rats and humans are 2.3996×10^{-4} m^3/min and 0.0138 m^3/min, respectively (EPA, 1994). Using the values cited in the OEHHA Technical Support Document, the total respiratory tract surface areas are 3,440 cm^2 and 543,400 cm^2, for rat and human, respectively. Thus, the calculation for the RGDR would be:

$$\frac{(2.3996 \times 10^{-4})/(0.0138)}{(3{,}440/543{,}400)} = \frac{.017388406}{.006330512} = 2.746761399$$

Use of this factor (2.75) would imply that the exposure level in humans required to result in an equivalent dose per surface area is higher than the exposure level in rats. This is logical as the surface area for the human respiratory tract is much larger than the rat one, about 158 times larger, compared with the 59-fold difference in minute volume. Therefore it would take a much higher exposure concentration in humans than in rats to result in an equivalent dose per surface area for humans. However, to be conservative, the Panel recommends that OEHHA use a factor of 1 to convert the rat LOAEL to a human equivalent concentration.

Response. OEHHA staff determined that the critical effects in the Quest et al. (1984) report were in the extrathoracic portion of the respiratory system, especially the nasal cavity. Thus staff made the HEC adjustment for a gas with extrathoracic respiratory effects and thus calculated a RGDR of 0.21. Staff then used an interspecies UF of 3 since some of the uncertainty/variability in the interspecies extrapolation was subsumed in the HEC correction. That "the equivalent target tissue for irritation effects in humans would be the entire respiratory tract" is possible but since there are no data in humans it is only a guess. In the absence of human data OEHHA staff take a public health protective approach and assume that the target tissue is the same one seen in animals, the nasal epithelium, and use the suggested approach for such an effect.

Comment 3. OEHHA should consider eliminating some uncertainty factors and should acknowledge the conservatism of the propylene REL. Using the foregoing recommendations to derive a chronic inhalation REL for propylene would result in the following values:

Study	Quest *et al.*, 1984; NTP, 1985
Study population	50 rats/group/sex, 300 total

Exposure method	Discontinuous whole body inhalation exposure (0 or 4,985 or 9,891 ppm)
Critical effects	Respiratory tract irritation: species-specific, non-dose-related squamous metaplasia (males and females); epithelial hyperplasia (females only); non-dose-related inflammation (males only) of nasal cavity.
LOAEL/NOAEL	4,985 ppm
NOEL 14-week	10,000 ppm
Exposure continuity	6 hr/d, 5 d/wk
Exposure duration	2 years
Average experimental exposure	890 ppm
Human equivalent concentration	890 ppm (based on entire respiratory tract surface area comparison)
LOAEL uncertainty factor	3 (minimal effects, low severity, reversible or non-dose related)
Subchronic uncertainty factor	1
Interspecies uncertainty factor	3
Intraspecies uncertainty factor	10
Cumulative uncertainty factor	100
Inhalation reference exposure level	8.9 ppm

This value is very conservative. OEHHA applied an uncertainty factor of 3 for use of a low severity LOAEL. The Panel believes that the minimal effects noted at the borderline LOAEL/NOAEL exposure level of 5,000 ppm, the reversibility of the epithelial hyperplasia, and the lack of dose-response for effects such as squamous metaplasia, would support a lower uncertainty factor than the factor of 3 for use of a LOAEL uncertainty factor. Indeed, OEHHA has noted the need to develop a more sophisticated method to address low severity effects. In the case of propylene, the Panel believes that application of the LOAEL uncertainty factor is very conservative.

Furthermore, OEHHA applied an uncertainty factor of 3 for interspecies variation, in addition to the adjustment of the exposure level to a human equivalent concentration. As discussed above, however, the Panel believes an appropriate rat to human conversion would give a HEC that is higher than the rat LOAEL by a factor of 2.75. Thus, use of a factor of 1 for the HEC is already conservative.

The cumulative effect of these uncertainty factors provides an extremely conservative REL. The Panel believes that OEHHA should consider eliminating the uncertainty factors for the LOAEL and for interspecies variation. If OEHHA nevertheless persists in using uncertainty factors of 3 for these parameters, then the REL discussion should point out the very conservative nature of the propylene REL.

Response. OEHHA has used a modified LOAEL to NOAEL uncertainty factor of 3. A factor of 1 would indicate no effect while the data clearly show some effect. OEHHA acknowledges the

need for additional research in order to implement a more sophisticated approach than what we are doing at present. This is stated in our document.

The lower interspecies UF of 3 is used in those cases where an HEC adjustment has been applied since part of the interspecies adjustment involves different configurations of the respiratory system.

The chronic REL must address uncertainties in the available data. Unfortunately, there are very limited data available on the toxicity of propylene. No long-term human toxicologic or epidemiologic studies were located in the literature. No reproductive/developmental data for humans or animals are available. If a better study becomes available, we will use it as the basis for a better health value.

Chemical Manufacturers Association - Phenol Regulatory Task Force

Comments on the chronic REL for **phenol** were made by the CMA's Phenol Regulatory Task Group (Task Group) in a letter dated January 29, 1998. The Task Group is comprised of the major domestic manufacturers of phenol that represent approximately 95 percent of United States production of the chemical. Task Group members include: Allied Signal Inc., Aristech Chemical Corporation, Dakota Gasification Company, Dow Chemical Company, Georgia Gulf Corporation, GE Plastics, GIRSA, Inc., JLM Industries, Inc., Kalama Chemical, Inc., Merichem Company; Shell Chemical Company; and Texaco Refining & Marketing. Associate members are: Borden Inc. and Procter & Gamble. OEHHA proposed a chronic REL of 600 $\mu g/m^3$ for phenol based on reports of systemic effects in mice, rats and monkeys inhaling phenol.

The Task Group urges OEHHA to withdraw its draft chronic toxicity summary and proposed reference exposure level (REL) for phenol. The studies on which OEHHA has relied are inadequate to derive a REL, and the draft chronic toxicity summary does not reflect accurately phenol's potential health effects. All data bearing on phenol's chronic health effects, including data recently generated by members of the Task Group, should be reviewed before OEHHA publishes its chronic toxicity summary or issues a final REL. In restricting its comments to the toxicity summary and related REL, however, the Task Group does not endorse the risk assessment practices, policies, and methods set forth in those Guidelines in whole or in part. Moreover, the Task Group reserves the right to challenge OEHHA's use of the Guidelines to assess or regulate any chemical, including phenol. OEHHA staff provide detailed responses to the appended comments below.

Comment 1. *The studies on which OEHHA relies are not adequate to derive a REL*. OEHHA has based its proposed REL of 0.2 parts per million (ppm) (600 micrograms per cubic meter ($\mu g/m^3$)) for phenol on the findings of two subchronic inhalation studies performed in animal species: Sandage (1961) and Dalin and Kristofferson (1974). These studies are an inadequate basis for deriving a REL. The Sandage (1961) study in rhesus monkeys, rats, and mice detected no adverse effects following continuous exposure to phenol at 5 ppm for 90 days.

The Dalin and Kristofferson (1974) study in rats exposed continuously to phenol concentrations of 26 ppm reported some signs of neurological impairment, but the signs did not last during the whole exposure period and therefore were not considered to be severe. Moreover, the results are inconsistent with those of Deichmann et al. (1944). In the Deichmann et al. study involving inhalation exposure to 26 to 52 ppm phenol, no overt neurological signs were found in mice or rats after 88 and 74 days of exposure. For these reasons, the Task Group believes, that studies on which OEHHA relies are not adequate to derive a REL for phenol, and the proposed REL should be withdrawn.

Even if the studies on which OEHHA relies were an adequate basis for deriving a REL, the approach OEHHA uses to estimate risk does not accurately reflect phenol's potential chronic inhalation hazard. Using the no observed adverse effect level (NOAEL) of 5 ppm

referenced in its draft summary, a comparable human equivalent concentration of approximately 28 ppm would be derived, which, in turn, gives rise to a REL of approximately 1.0 ppm (using the identical uncertainty factors employed by OEHHA).

Moreover, based upon the available toxicity database for phenol, the occupational Safety and Health Administration (OSHA) has established an exposure limit of 5 ppm 8-hour time-weighted average. Identical standards have been established by the National Institute for Occupational Safety and Health (NI0SH) and the American Conference for Governmental Industrial Hygienists (ACGIH). Adjustment of the occupational exposure standard of 5 ppm for continuous rather than workday exposure would derive a REL of 1.0 ppm - a value which is, the Task Group believes, far more defensible than the standard of 0.2 ppm proposed by OEHHA.

REL calculations using human equivalent concentrations are appended as Attachment 1. The Panel notes that OEHHA has computed an HEC of 5 ppm, which is equivalent to the NOAEL. Because OEHHA has not set forth its HEC calculations, the Panel is unable to comment upon these calculations or their effect on that HEC.

Accordingly, RELs derived using existing occupational standards, or animal studies together with appropriate human equivalent concentrations, would yield roughly the same value - one that is considerably higher than that proposed by OEHHA. The Task Group believes that OEHHA's proposed REL is not supported by the data on phenol's toxicity, and will mislead the public about the health significance of exposure to low levels of phenol in the ambient air.

Response. OEHHA staff do not understand the basis for the commentator converting the animal NOAEL of 5 ppm into an HEC of 28 ppm. OEHHA determined that phenol is a gas with systemic effects. We used an RGDR of 1 using default assumptions. Thus the Human Equivalent Concentration (HEC) is the same as the average animal exposure concentration, in this case 5 ppm. The methodology is explained in the introductory chapter of the Technical Support Document and in the 1994 document U.S. Environmental Protection Agency Methods for Derivation of Inhalation Reference Concentrations and Application of Inhalation Dosimetry (EPA/600/8-90/066F. Office of Research and Development, Washington, DC). With this methodogy it is rare for the HEC to be greater than the average animal exposure.

In regard to TLVs and PELs, neither USEPA nor OEHHA use work-place standards to calculate RfCs and RELs. In regard to the TLV of 5 ppm, time adjustment to continuous exposure from 40 to 168 hours would result in a value of 1.2 ppm. However, TLVs are usually not NOAELs. They are usually LOAELs and in some cases FELs (Frank Effect Levels) for healthy workers. In the case of phenol the TLV is based on a study of 8 human volunteers who were exposed to from 1.6 to 5.2 ppm phenol by face mask for 8 hours on one day (Piotrowski, 1971). The study did not report any irritation of the eyes, nose and throat. Thus based on this experiment 5.2 ppm (20,002 $\mu g/m^3$) is a NOAEL for an acute exposure. OEHHA used this study as the basis of its acute REL for phenol. For a chronic REL, allowances for extrapolating from a 1 day acute exposure to chronic exposure (for which

precedents are lacking) and for exposing sensitive individuals such as infants, children, asthmatics and the elderly would need to be made.

Other human studies have reported workplace levels of 3.3 ppm phenol (Ohtsuji and Ikeda, 1972), 4 ppm phenol (Connecticut Bureau of Industrial Hygiene, undated), and 1.22-4.95 ppm phenol (Ogata *et al*., 1986). The studies do not mention adverse health effects or years of exposure. For comparison with the REL proposed, assume, for example, that 4 ppm is a human chronic NOAEL. A worker inhales 10 cubic meters of air per workday, so the level of phenol averaged over 7 days would be 1.43 ppm (4 x 10/20 x 5/7). Applying an uncertainty factor of 10 to account for variability in sensitivity in the human population results in a REL of 143 ppb or 550 $\mu g/m^3$, in good agreement with the 600 $\mu g/m^3$ REL derived from animal studies. The comment time-adjusted the TLV of 5 ppm to 1 ppm (3,850 $\mu g/m^3$) but did not make any allowance for variability in sensitivity in the human population. Thus OEHHA is still proposing 600 $\mu g/m^3$ as the chronic REL for phenol.

Comment 2. *Before publishing its chronic toxicity summary and REL, OEHHA must consider all information on phenol's health effects including data being generated by task group members*. The California Toxic Air Contaminant Program provides that OEHHA "shall evaluate the health effects of and prepare recommendations regarding ... toxic air contaminants." In conducting its evaluation, OEHHA must "consider all available scientific data," including, but not limited to, data provided by state and federal agencies, private industry, and public health and environmental organizations. The evaluation must include an assessment of the availability and quality of data on health effects, including potency, mode of action, and other biological factors. OEHHA's Guidelines are intended to help implement this statutory requirement. OEHHA's draft toxicity summary does not, however, review and evaluate all available information on phenol's health effects. Studies or information not reviewed by OEHHA include:

Argus Research Laboratories (1997): In this study conducted in rats exposed by gavage to high levels of phenol, which has been provided to OEHHA by the Non-Prescription Drug Manufacturers Association (NDMA) the only evidence of developmental toxicity observed was a decrease in fetal body weight and an increased incidence of one minor skeletal variation at the high dose level (360 mg/kg/day) only, a dose level associated with serious maternal toxicity. No developmental toxicity was observed at 60 or 120 mg/kg/day, despite the occurrence of significant maternal toxicity at the 120 mg/kg/day level.

ATSDR Toxicological Profile for Phenol (Sept. 1997): OEHHA's chronic toxicity summary for phenol relies heavily on an eight-year old ATSDR toxicological profile. OEHHA apparently has not reviewed, and does not reference, the updated profile for phenol, which was released by ATSDR for public comment in September 1997. The1997 draft profile summarizes a large number of studies addressing phenol's potential health effects that are not included in the 1989 profile.

CMA Pharmacokinetic Study: In this study, provided to EPA by Task Group members in *1994*, no phenol was detected in the blood of rats exposed to phenol in the ambient air at concentrations of 25 ppm, or in drinking water at concentrations of 5,000 ppm. This study demonstrated that, under both exposure conditions, phenol is readily conjugated and detoxified.

The Task Group also urges OEHHA not to publish its chronic toxicity summary, or issue a final REL for phenol, until after it has reviewed additional data generated recently by Task Group members, as well as data that are being generated and which will be available soon. The most recent schedule for completing these data is appended as Attachment 3. These studies are being conducted pursuant to an enforceable consent agreement entered into between the United States Environmental Protection Agency (EPA) and some members of the Task Group to satisfy certain testing proposed by EPA. The testing is intended to characterize phenol's potential for subchronic neurotoxicity, developmental toxicity, reproductive toxicity, and respiratory toxicity and will provide additional data upon which to base a REL. Data completed to date, pursuant to the ECA, include the following:

Subacute Rat Inhalation Study: No toxic effects were detected in the respiratory system of rats exposed to phenol concentrations up to 25 ppm for two weeks. A copy of this study is appended as Attachment 3.

Subchronic Drinking water Study in the Rat: No neurotoxic effects were reported in rats exposed to phenol in drinking water at doses up to 5,000 ppm.

Task Group members also are conducting a two generation reproductive toxicity study in the rat. In its chronic toxicity summary, OEHHA commented that "[n]o multi-generational studies evaluating reproductive or developmental effects under chronic exposure conditions could be identified. " The ongoing CMA study will provide definitive data on this endpoint. CMA is also conducting a neurotoxicity study. These studies will be available no later than, and October 17, 1998, respectively. The Task Group urges OEHHA to defer publishing the chronic toxicity summary, or issuing a final REL for phenol, until it has received and reviewed the data. Publication of the summary and REL at this time would be contrary to the intent embodied in the Health and Safety Code that OEH11A use all available information to characterize toxic air contaminants.

Response. OEHHA appreciates the additional information on phenols. But their relevance to developing a chronic inhalation REL is limited.

Argus Research Laboratories (1997). This is not an inhalation study but a recent gavage study (not yet published in the peer-reviewed literature), that addresses developmental endpoints. It would appear to have limited relevance to developing a chronic inhalation REL.

ATSDR Toxicological Profile for Phenol (Sept. 1997). The chronic REL Technical Support Document was released in October 1997 and thus staff did not have the opportunity to

thoroughly review the ATSDR profile. However, OEHHA did its own literature searches for phenol and referred to ATSDR as a convenient summary document.

CMA Pharmacokinetic Study. In this unpublished study, no phenol was detected in the blood of rats ex posed to phenol in air at 25 ppm, or in drinking water at 5,000 ppm. This study indicates that phenol is readily conjugated and detoxified, but it is not applicable to developing a chronic inhalation exposure level.

Subacute Rat Inhalation Study. No toxic effects were detected in the respiratory system of rats exposed to phenol concentrations up to 25 ppm for two weeks. This result is consistent with the results of the subchronic studies OEHHA staff used to develop the chronic REL.

Subchronic Drinking Water Study in the Rat. It is not clear how a subchronic drinking water study in the rat is relevant to the chronic REL especially since the length of the study is not specified.

Task Group's two generation reproductive toxicity study in the rat due June 17, 1999. OEHHA staff is not willing to delay release of the chronic REL until this report is released. However, we will be happy to review it when it is released to determine if the chronic REL should be revised.

Task Group's a neurotoxicity study due October 17, 1998. As of February 1999 OEHHA staff have not received a copy of this study. However, we will be happy to review it when it is released to determine if the chronic REL should be revised.

Comment 3. *OEHHA should revise its draft toxicity summary to describe more accurately phenol's potential chronic health effects*. The Task Group urges OEHHA to revise its draft chronic toxicity summary for phenol to characterize accurately phenol's potential chronic health effects. In particular, OEHHA has not accurately described phenol's potential developmental and reproductive effects. In its summary, OEHHA reviewed two studies conducted by Jones-Price et al. (1983) ("Teratologic evaluation of phenol in CD-1 rats" and "Teratologic evaluation of phenol in CD-1 mice. Research Triangle Institute) and notes that, in the first study, pregnant rats dosed with 0, 30, 60, and 120 mg/kg/day phenol on gestation days 6-15 exhibited reduced fetal body weight in a dose-related manner. The second study notes that in the fetus, reduced growth, decreased viability, and an increased incidence of cleft palate was also observed at the highest dose.

The Jones-Price et al. (1983a) study does not support the conclusion that phenol causes developmental /reproductive toxicity, for the following reasons.

- EPA has expressed "low confidence" in the Jones-Price et al. studies in its Integrated Risk Information System (IRIS) database because of the gavage nature of dosing.

- A seven percent decrease in fetal body weight (the only endpoint apparently affected in the study, which evaluated dozens of endpoints) is close to the limits of statistics to discern a significant decrease in body weight in a study of this type (five-six percent is the limit). Thus, the effect is at the border of being detectable.

- A seven percent decrease in fetal body weight may not be biologically significant. Decreases in fetal body weight of this small magnitude are usually readily reversible and of no functional consequence.

The average fetal body weight of the control group in this study was high compared to historical controls. This observation calls into question whether the apparent decrease in fetal body weight at the high dose was real and repeatable. The authors did not consider the historical control data in their interpretation of the fetal body weight data.

The authors did however consider historical control data in the interpretation of malformation data. The incidence of malformations was also unusually high in the controls in this study. Based on the comparison with historical controls, the authors concluded phenol had no significant effect on malformation data. A similar comparison with historical controls would show that phenol had no significant effect on fetal weight.

The preliminary range-finding study for the NCTR (1983) rat study showed no effect on fetal body weight at much higher doses. Although the statistical power of the preliminary study was less than that of the full study, one would expect to see some indication of a reduction in fetal weight at the higher doses.

A statistically significant difference was observed at the high dose only. Thus, a clear dose-response relationship was not demonstrated.

The litter size was 12 percent greater in the high dose group than in the control group. An inverse relationship between fetal weight and litter size is well recognized. While a 12 percent increase in litter size is not normally sufficient to explain a significant decrease in fetal weight, given that the decrease was at the limit of statistical significance, one cannot rule out the possibility that increased litter size may have played some role in the difference in fetal weight. Additionally, the combination of the heavier weight control group, plus the increased litter size, may have been sufficient to result in a statistically significant (although minimal) decrease in fetal weight.

Response. In regard to fetal weight differences, the weight decrement of 7% was statistically significant. A weight difference of 7% may be biologically meaningful in a very small, developing animal. The weight decrement of 7% might not be biologically significant if the loss is generally distributed. If it were specific to some organ or system, it could be. In the absence of certainty, OEHHA takes the health protective approach that the effect may be biologically significant. However, the difference in fetal body weights between the experiment cited and historical controls could mean that there was really no difference. Also, as pointed out in the comment, the increase in litter size may also have affected the fetal body

weight. OEHHA prefers to use articles from the peer-reviewed scientific literature to develop RELs since such discrepancies are often noticed by peer reviewers. USEPA's low confidence in the study is a conclusion of their peer review.

A further concern with fetal weight reduction is that in humans the logarithm of infant mortality (death) increases linearly as birth weight decreases from 3500 to 1000 grams (Hogue *et al*., 1987; Rees and Hattis, 1994). This log-linear relationship exists on both sides of the weight (2500 g) conventionally used as a cutoff defining low birth weight. There is no evidence for a threshold. Thus any reduction in fetal weight is a cause for concern. (Hogue CJ, Buehler JW, Strauss LT, Smith JC. Overview of the National Infant Mortality Surveillance (NIMS) project--design, methods, results. Public Health Rep 1987 Mar-Apr;102(2):126-138; Rees DC, Hattis D. Chapter 8. Developing Quantitative Strategies for Animal to Human Extrapolation. In: Principles and Methods of Toxicology. Third Edition. AW Hayes, editor. New York: Raven, 1994)

Comment 4. The chronic toxicity summary should also be revised to correct or clarify the following: *Include Phenol Exposure Levels for End-Points*: In the section of the toxicity summary entitled Effects of Animal Exposures, OEHHA describes a number of subchronic and chronic studies conducted with phenol and concludes that their findings indicate pulmonary damage, liver damage, renal damage, neurological effects, as well as various other chronic effects. The introductory paragraph does not, however, reference the exposure at which these findings were induced. Absent these data, the public cannot accurately assess whether phenol presents any health risk. OEHHA should revise its discussion of the animal studies to indicate levels of exposure at issue and thereby provide a more meaningful and less misleading summary of phenol's potential health effects.

Response. Many toxicological effects occur at fairly high concentrations. OEHHA staff look for the most sensitive toxic endpoint in humans or in an animal that is considered to react like humans to the chemical. Thus, the concentrations used or estimated in the key study are regularly included as well as our estimate of what the LOAEL and NOAEL are. In addition phenol concentrations are given for many of the other studies cited. In OEHHA's chronic toxicity summary for phenol the concentrations used in many of the studies are specifically mentioned.

Comment 5. *OEHHA's Conclusion About the Greater Toxicity of Inhalation Exposure is Not Borne Out by CMA's Study:* OEHHA states that "[c]omparison of the three routes of exposure found that oral exposure was less effective at producing systemic toxic effects possibly due to the rapid metabolism of phenol to sulfate and glucuronide conjugates by the gastrointestinal tract," and that "inhalation is a sensitive route of exposure for laboratory animals." Data from CMA's pharmacokinetic study, however, indicate that under either oral or inhalation exposure, phenol is readily conjugated and detoxified. No phenol was detected in the blood of rats at inhalation concentrations of 25 ppm or after drinking water exposure to concentrations of 5,000 ppm. The Task Group therefore urges OEHHA to revise its

conclusion regarding phenol's purported greater sensitivity through the inhalation route to reflect the findings of CMA's study.

Response. OEHHA based its conclusion on the publications of Deichmann et al. (1944) and NTP (1980) concerning toxic effects of phenol. The comment does not state whether in the CMA study toxic effects were seen in rats ex posed to phenol in air at 25 ppm or in drinking water at 5,000 ppm. The CMA study itself does not state adverse effects by these routes. In fact 25 ppm was probably chosen for inhalation since Deichmann et al. reported no effects at 26 ppm. The CMA study did find transient muscle twitching in rats administered phenol by a third route, gavage, at 150 mg/kg phenol. In any case two free standing NOAELs for different routes of exposure are not an adequate basis to conclude that the routes do not differ in effects. Although other organ systems are more sensitive, phenol would be irritating to the respiratory system at high levels, an effect not likely to be ameliorated by rapid sulfation or glucuronidation. The phenomenon of irritancy would not be tested by measuring phenol concentrations in the blood. There is no indication given that objective measures of irritancy were taken in the CMA rat study. It is difficult to know when a laboratory animal is experiencing irritation until it is rather pronounced.

Comment 6. *OEHHA Should Provide a Statistical Analysis of Study Endpoints:* OEHHA does not apply any statistical analyses of study endpoints, or otherwise describe the strength of the association between exposure to phenol and the relevant effect. Absent such information, the public cannot properly assess the predictive power of the study and its relevance to human exposure.

Response. It has not been customary to provide a statistical analysis of all study endpoints and to mention all concentrations used. Usually an endpoint is mentioned only if it has been adversely affected consistently in exposures to the chemical under study. The toxicity summaries are brief summaries of the literature. It would not be useful to entertain statistical analyses of every study mentioned.

Comment 7. *Correct the Reference to the Jones-Price et al. (1983b) Study:* OEHHA states that increased mortality was detected in rats in this chronic developmental effect study. The study in which increased maternal mortality was detected was conducted with mice, not rats, and the draft should be corrected accordingly.

Response. OEHHA has changed the animal from rats to mice in the summary of the Jones-Price et al. (1983b) study.

Chemical Manufacturers Association – Phthalate Ester Panel

Comments on the chronic REL for **diethylhexylphthalate** (DEHP) were made by the Phthalate Ester Panel of the Chemical Manufacturers Association in a letter dated January 29, 1998. OEHHA proposed a chronic REL for DEHP of 10 $\mu g/m^3$ based on a rat subchronic inhalation study by Klimisch et al. (1992) which found increased liver weight plus the appearance of lung alveolar thickening and foam-cell proliferation.

Comment 1. D*iethylhexylphthalate***.** OEHHA should adjust the DEHP REL to reflect the known greater sensitivity of the rat and should revise its discussion of the effects of DEHP.

Response. OEHHA staff are not aware of available data useful in assessing the relative susceptibility of humans and rats to inhaled DEHP. The commentator does not specify any. It has been the practice of OEHHA as well as USEPA and other authoritative bodies to consider the most sensitive species when estimating potential human health risks based on animal data.

Comment 2. DEHP has very low vapor pressure, so that the study (Klimisch *et al*., 1992) used to derive the REL was performed using a specially-generated aerosol. OEHHA should make that distinction clear to readers.

Response. OEHHA agrees that the vapor pressure of DEHP is low. However air contaminants, such as DEHP, that exist mostly or entirely as particulates rather than vapors may still be present at levels hazardous to human health. The low vapor pressure of DEHP was indicated or reflected at several locations in the document. (1) Mass concentration units ($\mu g/m^3$) were not converted to volume concentration units (ppb) in the Chronic Toxicity Summary (p. A-186). (2) The vapor pressure is reported in the Physical and Chemical Properties Summary (p. A-186). (3) The particulate nature of DEHP administered by Klimisch and associates (1992), as well as by Schmezer and colleagues (1988), was described in the experimental summary (p. A-187). The review of the study of Merkle and associates (1988) did not specifically mention the particulate nature of the administered DEHP. Text is being added at several locations in the document to emphasize the particulate nature of DEHP at concentrations experimentally studied.

Comment 3. OEHHA also should emphasize that the effects observed in Klimisch, et al., were reversible upon cessation of treatment.

Response. The apparent resolution of adverse findings after an eight-week post-exposure period was noted in the document (A-187). Additional text is being added to "Section VI. Derivation of Chronic Reference Exposure Level" to clarify this point. However, it should be noted the OEHHA chronic reference exposure levels specifically address the potential health effects from continuous lifetime exposures. Issues specific to

intermittent or less-than-lifetime exposures such as resolution of adverse effects over time are not addressed in this document.

Comment 4. OEHHA has incorrectly identified liver weight increase as a critical effect in this study, and should list only the lung effects as a critical effect. In addition, OEHHA has incorrectly characterized other studies as demonstrating a potential for DEHP inhalation to cause pulmonary effects. OEHHA should revise its discussion to correct these mischaracterizations.

Response. Relative liver weights were significantly increased in males and females at the LOAEL dose in the Klimisch et al. (1992) study, and absolute liver weights were significantly increased at that dose. No histopathological evidence of liver toxicity was noted, and the authors considered the effects secondary to toxicity at other sites. The text in the document is being changed to emphasize that the primary adverse effects observed at the LOAEL dose were increased lung weights, accompanied by foam-cell proliferation and alveolar septi thickening.

Comment 5. OEHHA should not apply an interspecies uncertainty factor to derive the REL. Extensive data demonstrate that primates are far less susceptible to DEHP effects than the rat. Indeed, OEHHA should adjust the REL by a factor of 0.2 to account for the known difference between primates and rats.

Response. The existing database of relative toxicity of substances toward different species does not support assuming species more closely linked evolutionarily will respond to chemical exposures more similarly than distantly related species. Among the two most commonly studied rodent species, rats and mice, large differences in susceptibility have frequently been reported. Also relative species susceptibility observed for oral exposures may differ from that observed for inhalation exposures, in part because of large species difference in lung anatomy.

Chemical Manufacturers Association (CMA) - Propylene Glycol Methyl Ether Panel

Comments on the chronic REL proposed for **propylene glycol methyl ether** (PGME) were received from the Propylene Glycol Methyl Ether Panel of the Chemical Manufacturers Association (CMA). The proposed chronic REL is the USEPA RfC of 2,000 $\mu g/m^3$ based on neurotoxicity.

Comment 1. The TSD proposes an REL for PGME of 2,000 $\mu g/m^3$ (0.6 ppm) by explicitly accepting U.S. EPA's Reference Concentration (RfC) for the compound. EPA derived this RfC by applying an uncertainty factor of 300 (factors of 10 each for the absence of a chronic study and intraspecies variability, and a factor of 3 for interspecies differences) to the 1,000 ppm No Effect Levels (NOEL's) in the Landry (1983) rat and rabbit 13-week inhalation studies. The observed effects of note at 3,000 ppm were

transient sedation in both species that resolved after the first one-two weeks of exposure and an adaptive hepatocellular swelling in rats with no evidence of degenerative changes.

Response. USEPA Reference Concentrations (RfCs), available when the chronic REL TSD was drafted in October 1997, are being used as chronic Reference Exposure Levels (RELs). RfCs are already in use by California's Department of Toxic Substances Control and by the USEPA and were earlier incorporated by reference in Appendix F of the Emissions Inventory Criteria and Guidelines for the Air Toxics "Hot Spots" Program for use in screening risk assessments in the Hot Spots Program. These Guidelines were effective July 1, 1997. The RfCs were recommended for use by OEHHA by the Risk Assessment Advisory Committee (RAAC) and used in response to Governor Wilson's Executive Order W-137-96 which concerned the enhancement of consistency and uniformity in risk assessment. RfCs released after October 1997 will be evaluated for use by reviewing each new RfC as it becomes available. Acceptable RfCs will be submitted yearly to the SRP for review and possible endorsement.

Comment 2. The Panel has recently completed lifetime studies of PGME in rats and mice exposed to 0, 300, 1,000 or 3,000 ppm PGME vapor for two years. As in the Landry study, sedation was observed at 3,000 ppm for both species, but, again, the effects had resolved by the second week of the study. PGME did not cause a dose-related increase in tumors in males or females of either species. Lifetime NOELs of 300 ppm were determined for both rats and mice.

We recommend employing this chronic study in the REL determination to eliminate the need for a subchronic vs. chronic uncertainty factor of 10 in the RfC/REL calculation. Because this study has just recently been completed, the final laboratory report has not yet been issued. However we will send the full report to you as soon as possible.

Response. Staff appreciates receiving the documentation of a chronic study of exposure to PGME and looks forward to the full report. A lifetime NOEL of 300 ppm from an exposure continuity of presumably 6 hours per day, five days per week results in an average experimental exposure of 54 ppm and a human equivalent concentration (HEC) of 54 ppm. Applying a total uncertainty factor of 30 (3 for interspecies UF and 10 for intraspecies UF) to the HEC results in a REL of 1.8 ppm, 3-fold higher than the RfC of 0.6 ppm. Staff will reconsider the proposed REL when the study is finalized. OEHHA staff encourage the commentator to submit the study to a peer-reviewed journal in order to increase the acceptability of the results.

Comment 3. The Panel has also recently completed a two-generation reproduction study of PGME in rats. We enclose that study report. As you will see, the NOEL for fertility and reproductive effects was 1,000 ppm; no effects were seen in the parents at 300 ppm. Decreased female fertility and reproductive effects were found at the highest concentration tested, 3,000 ppm PGME. These effects were associated with general toxicity and apparent resultant nutritional stress by the mothers and offspring at this high

concentration of PGME and not thought to be due to direct toxicity to reproductive organs.

Response. Staff appreciates the information on these endpoints. Both the proposed REL/RfC and a REL based on the chronic study described above should be protective against adverse reproductive effects.

Chemical Manufacturers Association – Hydrocarbon Solvents Panel (Xylenes)

Comments on the chronic REL for **xylenes** were made by the Hydrocarbon Solvents Panel of the Chemical Manufacturers Association. The Panel's members include: CITGO Petroleum Company, Exxon Chemical Company, Koch Chemical Company, Mobil Chemical Company, Phillips 66 Chemical, Shell Chemical Company, and Sun Company. OEHHA developed a chronic REL of 700 $\mu g/m^3$ from a study of 175 xylene-exposed factory workers by Uchida et al. (1993). Critical effects were nervous system effects as well as irritation of the eyes, nose and throat.

Comment 1. The Chemical Manufacturers Association (CMA) Hydrocarbon Solvents Panel has reviewed the Reference Level (REL) proposed for xylenes. We find the human study upon which that value is based seriously flawed and urge California not to establish an REL based on that study. We also include comments on the Technical Support Document's (TSD's) discussion of animal developmental studies of xylenes.

Response. Responses to the substantive issues are provided below.

Comment 2. THE PROPOSED REL. California proposes an REL of 0.05 ppm (200 $\mu g/m^3$) for xylenes. That value is derived from the Uchida et al. (1993) study of factory workers, which OEHHA interprets as finding a Lowest Observed Adverse Effect Level (LOAEL) of 14.2 ppm and no NOAEL. The critical effects specified are increases in the prevalence of eye irritation, sore throat, floating sensation and poor appetite. OEHHA converts the reported average 14.2 ppm workplace exposures to a continuous lifetime exposure of 5.1 ppm and then applies an uncertainty factor of 100 (10 for the absence of a NOAEL and 10 for intraspecies difference) to obtain the 0.05 ppm REL.

SIGNIFICANT FLAWS IN THE UCHIDA STUDY. The Uchida study suffers from a number of serious deficiencies and limitations in the design and reporting that render it unreliable for risk assessment.

Absence of Well-Documented Health Effects Data. First, Uchida, *et al.* used a subjective symptom questionnaire to assess health effects and thus did not obtain well-documented or reliable data on health effects. Symptom questionnaires may be substantially influenced by response bias and are therefore not reliable indicators of adverse health effects. The authors reported no control for such a potential bias (e.g., blinded interview), nor any validation of the subjective survey (e.g., a neurobehavioral or irritancy assessment).

Response. As noted by the comment, the subjective reports are not objectively verified by other measures. We agree that such verification would provide additional confidence in the subjective reports. The Uchida et al. (1993) article does not indicate whether the survey was blinded. We also agree that blinded interviews reduce the likelihood of inadvertent bias; the comment therefore raises a substantial potential limitation of this study. Here, the simplicity of the task and of the questions mitigates the potential for

such bias. Furthermore, and more importantly, the overall prevalence of subjective symptoms during work was greatly increased in the workers exposed to xylene as compared to the controls (19.2% versus 4.0%). With respect to many individual symptoms (e.g., eye irritation, nasal irritation, sore throat, floating sensation, and headache) the differences between exposed and unexposed workers were as great or greater than the overall prevalence. The magnitude of the observed differences makes such bias an unlikely explanation. The magnitude of the response differences also reduces concerns regarding the lack of an objective validation of the subjective complaints.

Comment 3. Other apparent problems with the symptom survey are an inherent bias to irritation and CNS symptoms (especially Part 1) and a duplication of like symptoms (e.g., dizziness, floating sensation, drunken feeling). This problem may have resulted in inflated prevalence results in the exposed workers.

Response. The comment asserts that there was an inherent bias toward irritation and CNS symptoms in the questionnaire. The sensory and subjective symptoms of Part 1 of the questionnaire (unusual smell, unusual taste, and face flushing aside) solely relate to irritative and CNS depressant effects. The Part I survey results are therefore experimentally limited to finding only effects related to irritancy and CNS depression. This limitation is not a bias that would affect the validity of the results as to the health effects covered by the survey. This limitation was appropriate given the known ability of many solvents to cause irritation and CNS depression. However, the absence (except for facial flushing) from the list of other symptoms not associated with the known health effects of xylene exposure is of some relevance. The presence of other unrelated symptoms on the list could have served as an internal control for false positive results. Here, the prevalence of the likely unrelated facial flushing symptom was not increased. In addition, the prevalence of the drunkenness symptom (the most severe symptom of CNS depression) was not increased.

As the comment points out, the duplication of like symptoms in the questionnaire has the potential to inflate the <u>overall</u> prevalence rates. However, this duplication is substantially mitigated by the means of calculating prevalence rates which takes the number of questions into account. The prevalence rate for a group is calculated by dividing the number of affirmative answers by the group by the number of people in the group and dividing that result by the number of questions asked.

Here, the key question is whether or not the inflation in overall prevalence rates could have biased the results so as to produce a false positive rate for the study as a whole. This clearly is not the case. If one considers only those workers who report no symptoms, duplicative symptoms would not be an issue. For symptoms during work, the great majority of controls (189 out of 241 unexposed workers or 78%) report no symptoms; by contrast only a very small percentage of the exposed workers (37 out of 175 or 21%) report no symptoms. Although the overall prevalence rates at issue here include unusual smell and unusual taste as symptoms, OEHHA would not consider these

sensations as symptoms of toxic injury. However, the individual results for these two 'symptoms' suggests that they do not importantly contribute to the overall prevalence rates. Nevertheless, while the overall prevalence rates are positive, our draft document did not base its analysis on the overall prevalence rates alone. Uchida et al. (1993) also presented the prevalence results for each individual symptom that was significantly elevated. Our conclusions were also based on those data. Duplication of like symptoms does not affect those prevalence rates.

Comment 4. Odor may also have contributed to the subjective response results of the exposed workers. Dalton et al. (1997) recently demonstrated that both perceived odor and cognitive expectations about a chemical can significantly affect the reporting of health symptoms.

Response. In Dalton et al. (1997) subjects with a *positive* bias (having been told that the test substance was beneficial) reported less irritation from short-term exposures to acetone (800 ppm) or phenylethyl alcohol. However, subjects with a *negative* bias (having been told the substances were harmful over the long term) evinced no consistent differences from subjects with a neutral bias (having been given no health hazard information). The Uchida et al. (1993) study participant biases are likely either neutral or negative. The comment is not clear as to the source of the cognitive expectations regarding xylene or as to which symptoms they would be relevant. To the extent those expectations are based upon the actual experience of workers, they are of much less concern with respect to confounding.

The Dalton et al. (1997) analysis also posited that, if irritancy is primarily a function of both odor intensity and cognitive expectations, then odor intensity should be a predictor of irritancy. With respect to acetone, the test compound, this correlation held up. However, Dalton et al (!997) could not well control for the possibility that the irritancy of acetone (at a test concentration of 800 ppm) affected the reports of its odor intensity.

Interestingly, Doty et al. (1977) (cited in Dalton et al. (1997)) tested p-xylene for its ability to intranasally stimulate the trigeminal nerve in anosmic subjects. Trigeminal nerve stimulation relates to the irritancy or pungency of a compound as opposed to its odor. These anosmic subjects rated p-xylene on average as a 3.69 in overall intensity (the intensity scale ranged from very weak which had a value of one to very strong with a value of nine) as measured along several attributes (strength, pleasantness, warmth, safety). Thus, when p-xylene was actually tested, odor was not necessary to detect the trigeminal response to p-xylene.

The relationship between odor and pungency is apparently a complex one. Cometto-Muniz et al. (1990) (cited in Dalton et al. (1997)) reported that the odor threshold and pungency threshold for eight aliphatic alcohols (methanol to octanol) varied from 23-fold to 10,000-fold.

Furthermore, in Uchida et al. (1993), the subjective complaints of the workers over the prior three months were also elevated. While odor may arguably have contributed to the subjective responses of the exposed workers, odor is much less likely to account for the symptoms experienced while workers are not on the job. In addition, as to on-the-job complaints, the sensation of unusual smell was not reported to be significantly increased.

Comment 5. Limited Exposure Data. Second, the Uchida study's assessment of worker exposures is similarly problematic. The study relied on a single point estimate, one 8-hour air sample (time-weighted average, TWA), to characterize "chronic" solvent exposure. The TWA concentrations of xylene did not indicate maximum concentrations, which the authors admitted might have influenced the subjective symptom prevalence.

Also, no evaluations for other non-solvent exposures were included, although workers may have been exposed to such materials in rubber boot production (e.g., adhesives), plastic-coated wire production (e.g., metals), or printing work (e.g., pigments).

Response. For each exposed worker in the study, Uchida et al. (1993) assessed exposure over the period of an entire shift. The LOAEL of 14.2 ppm is based upon the geometric mean of 175 such exposure measures taken on the day before the questionnaire was administered. The measurements and survey instruments are therefore very close in time. We therefore have high confidence in the representativeness of those measurements.

With respect to the prevalence of symptoms on the job, the comment presents a substantial uncertainty as to the interpretation of the findings. Acute exposures to xylene can cause irritation of the eyes and respiratory tract as well as CNS depression. Therefore, with respect to the prevalence of symptoms reported on the job, there is uncertainty whether some of the symptoms with a potentially quick onset (eye irritation) relate to the peak exposures as much as to the 8-hour average exposures. Other symptoms (e.g., sore throat, headache) may not be as responsive to peak concentrations. With respect to symptoms outside of work, short-term variations in xylene exposure are much less a concern and the 8-hour time weighted average is more clearly a reasonable measure of exposure.

The comment suggests that perhaps other exposures could have accounted for the observed results. The study specifically addressed the potential for confounding by a variety of other solvents, which could produce a similar spectrum of effects. The spectrum of effects found here parallel closely with those previously reported for xylene; it seems improbable therefore that this same spectrum of relatively typical effects would be due to a confounding agent. Furthermore, with respect to subjective symptoms at work, very few of the exposed workers were without symptoms. Thus, each of the three workplaces studied would have to have been independently subject to such confounding. This appears particularly unlikely.

Comment 6. Failure to Assess Exposure Variations and Worker Hygiene Practices. Third, the relationship of duration of exposure to health effects was not specifically assessed, as the authors did not attempt to differentiate acute from chronic effects through evaluation of changes in symptom magnitude over time. Variations in an individual's length of time on the job or changes in work assignments over time could have resulted in differences in exposure that would have affected interpretation of chronic health effects.

Response. As noted in the comment, the Uchida et al. (1993) study does not well differentiate whether the observed adverse effects are the result of a long-term chronic exposure or are simply short term effects repeatedly occurring as the result of daily repeated exposures. The characterization of the effects as chronic or something much less than chronic is therefore uncertain.

It is not likely that symptoms away from the job, as compared to symptoms while at work, relate to peak exposures on any given day. Symptoms away from the job more likely relate to the prior cumulative exposure/duration and not peak concentrations.

The comment is correct that the available information does not distinguish whether any given symptom is more closely related to the prior shift's average exposure, the prior week's average exposure, or the prior year's average exposure. If we assume that <u>only</u> the prior day's exposure contributed to the observed symptoms, then the dose response analysis below would suggest the <u>daily</u> REL which would be protective of health. If we assume, at the opposite extreme, that the prior annual exposure accounts for the observed symptoms, the dose response analysis below provides the annual (chronic) REL value that would be protective of public health. The difference in the magnitude of these two RELs is small. Their principal practical difference relates to the time frame for which they would be applied. A daily REL of 0.07 ppm would result in a hazard index greater than one if exposures exceeded that level for any given day in a year. A chronic REL of 0.05 ppm would allow prolonged excursions above the 0.07 ppm level providing that they were balanced by exposure periods equally below 0.05 ppm such that the ground level concentration divided by the REL would be less than one.

	Daily Exposure	Annual Exposure
LOAEL:	14.2 ppm	14.2 ppm
Average exposure concentration*	7.1 ppm	5.1 ppm
LOAEL uncertainty factor	10	10
Subchronic uncertainty factor	1	1
Intraspecies uncertainty factor	10	10
Cumulative uncertainty factor	100	100
Inhalation reference exposure level	0.07 ppm	0.05 ppm
Applicable period	Daily	Annually

*For daily exposure, the standard continuity adjustment factor of 10/20 was applied. For annual exposure, the standard factors of 10/20 x 5/7 were applied.

Thus, with respect to dose response assessment, knowing whether or not the observed effects are subacute or chronic (or something in between) is not essential, in this context, to derive a reasonable and health protective chronic REL.

Comment 7. Finally, the authors did not comment on the workers' hygiene practices at the factories, specifically whether the workers wore gloves or instead had dermal contact with multiple solvents and other chemicals. Substantial dermal exposure would have complicated an accurate estimation of the worker exposure.

Response. One of the symptoms reported to be increased in both men and women was rough skin. This symptom would be consistent with solvent dermatitis and would therefore indicate the likelihood of skin contact. Thus, as noted in the comment, dermal absorption is a potential confounding effect. However, in a companion paper, Uchida et al. (1993) have extensively analyzed the relationship between urinary metabolite measurements and the actual xylene air levels for these same workers. Their analyses showed a very strong correlation between the measured air levels and the urinary metabolite levels. The parameters of the curve relating urinary metabolite levels to these air concentrations indicated that at the reported median air concentration of 14.2 ppm, the great bulk of the urinary metabolite levels would be predicted by the air concentration data. Thus, use of the air concentration data as the estimate of exposure is appropriate. Furthermore, dermal exposure relates much more to systemic effects than to such site-of-contact effects as eye and respiratory tract irritation.

Comment 8. There are a number of other less serious problems in the study. These problems, however, cannot be critically assessed because of a lack of sufficient detail given in the report. Taken together, the above problems in the health and exposure assessments of the Uchida study do not permit definitive conclusions of causality of adverse health effects associated with xylene exposure. This study should not be used for risk assessment of xylenes.

Response. The study's subjective symptom survey does have limitations. The principal limitation, for our purposes, is that the key study data comes only from the results of a subjective symptom questionnaire. However, the magnitude of the differences observed for prevalence rates for many of the on-the-job symptoms reported to be increased in exposed workers as compared to unexposed workers (e.g., eye irritation: 25.1% v. 6.6%; nasal irritation: 40.6% v. 9.1%; sore throat: 31.4% v. 4.6%; floating sensation: 49.7% v. 8.3%; and headache: 22.9% v. 6.6%) strongly supports their use. For several of the symptoms while not at work over the past three months, there were also substantial differences in symptom prevalence in both men and women and in combination (forgetfulness: 33 v. 18%; nightmare: 40% v. 19%; anxiety: 12% v. 3%; inability to concentrate: 12% v. 3%). (These numerical values are estimated from graphical data in

Uchida et al. (1993), figure 2.) Given the potential for xylene to affect the CNS system in other studies, these symptoms are also of concern.

The temporal relationship between effects and exposure is not known. For some of the effects (eye irritation, nasal irritation), very short-term exposures may be more relevant than the longer term exposure history. For effects while not on the job, this concern is not as great. However, for the purposes of protecting the public health, this uncertainty as to the relevant exposure time frame is not a practical barrier to use of the study.

The exposure information itself is strong. Each of the 175 exposed subjects exposures were monitored on the day before their survey was conducted. The companion paper indicates that dermal exposure was not a major contributor to dose. The lack of complete industrial hygiene therefore is a minor limitation.

For these reasons, the study is suitable for use in deriving a chronic REL.

Comment 9. MISCHARACTERIZATION OF DEVELOPMENTAL EFFECTS. The TSD's discussion of the xylene animal developmental toxicity database does not include a critical and complete review of the xylene developmental toxicity studies. It thus suggests the erroneous conclusion that xylenes cause adverse developmental effects. There are substantial experimental design and data interpretation flaws in the cited studies that must be considered, and the well-conducted Biodynamics (1983) study, which did not find xylene to be a developmental toxicant, is not even cited. If you would like a copy of the Biodynamics study, as referenced, please contact Barbara Francis at (703)741-5609.

Response. The documentation for xylene devoted one paragraph in the TSD to its potential developmental toxicity. OEHHA did not purport to develop and provide an overall weight-of-the-evidence determination as to the potential developmental toxicity of xylene in one paragraph. The document itself offers no conclusion of its own regarding the overall weight of the evidence as to the potential developmental toxicity of xylene. The document does however put the reader on notice as to the existence of a substantial body of evidence that bears on the developmental toxicity of xylene.

The document quotes the 1995 ATSDR review, Toxicological Profile of Xylenes, thusly: "ATSDR concluded that the body of information available for the developmental effects are consistent with the hypothesis that xylene is fetotoxic and many fetotoxic responses are secondary to maternal toxicity." The paragraph then went on to briefly summarize some of the findings of the major studies bearing on the question of developmental toxicity. A principal purpose of the document is to provide background information relevant to the selection of the key study for dose response purposes. As with other health effects, the developmental toxicity data for xylene are presented with dose response information to make clear the margin of exposure between the proposed REL and other reported adverse effects.

Any inference that OEHHA inappropriately failed to cite the Biodynamics (1983) study would be incorrect. The ATSDR addressed the Biodynamics (1983) study in its review and did not find that it indicated the absence of an adverse effect on development. The ATSDR rather included the Biodynamics (1983) study in a group of studies which "had limitations that made them difficult to assess." (ATSDR p. 56). As a general rule, OEHHA prefers information published in the peer reviewed literature.

While OEHHA does not purport to offer a complete and comprehensive review of the developmental toxicity literature in this one paragraph, OEHHA has critically examined the available scientific literature. The summary is not in error as to the findings it presents. OEHHA did not offer an overall interpretation of those findings. Those findings, on their face, however, do provide evidence tending to support a conclusion that exposure to xylene may have adverse effects on development, perhaps at levels associated with maternal toxicity.

Yet, as the comment points out, there are some animal studies that did not find substantial adverse effects on fetal development. OEHHA agrees with the comment that this important and complicated subject matter merits more detail. We will therefore expand the treatment at issue. However, it is still not OEHHA's objective to develop an overall conclusion as to the weight of the evidence bearing on the developmental toxicity of xylene.

Comment 10. Inadequate Study Design. The first serious problem with the cited studies is inadequacies in study design. Unusual exposure durations were used (e.g., oral dosing of 3 times per day; continuous 24-hour exposures), suggesting excessive handling of the animals and possible stress-induced changes that could affect body weight gain/loss and food consumption (Marks et al., 1982). Continuous treatment in 24-hour exposures generally results in a higher incidence of growth and retardation characterized by decreased mean fetal body weights (Hudak and Ungvary, 1978).

Response. In general, all experimental manipulations of laboratory animals have an assumed potential to affect the study results. Therefore, it is necessary to have controls that match the experimental group, save for the alteration in one condition, so as to allow a contrast to be made on the one altered condition. In the Marks et al. (1982) study, the potential effect of the experimental manipulation (thrice daily doses by gavage) of the animals can not be denied. However, since Marks et al. (1982) included a vehicle control group in their study, this potential source of confounding was eliminated.

Hudak and Ungvary (1978) did not report that continuous treatment in 24 hour exposures generally results in a higher incidence of growth and retardation characterized by decreased mean fetal body weights. There, the untreated controls had fetal weight outcomes essentially the same as the air controls subjected to the exposure chamber manipulations. Furthermore, Hudak and Ungvary (1978) found 230 ppm xylene to have

no effect on mean fetal weight. In that study, xylene increased the incidence of skeletal anomalies and provided some evidence for retarding skeletal development.

Thus, the study design concerns raised by the comment are not a serious problem in the two example studies offered by the comment.

Comment 11. Unreliable Exposure Information. Second, in many of the studies (i.e., Hudak and Ungvary, 1978; Haas and Jacobsen, 1993), test atmospheres were not monitored continuously, and thus the exposure data provided are not reliable. Exposure test atmospheres should be monitored continuously and the method of generation should be well-documented.

Response. These comments relate potential limitations affecting some of the studies. However, the limitations bear mostly on the question of dose response and not hazard identification. OEHHA did not use the studies in its quantitative dose response assessment.

Comment 12. Species-Specific Problems. Third, the test species utilized in some of the studies may have also influenced the test results. Rabbits are known to show inherent erratic body weight gain and loss during gestation; therefore, the effects observed in the studies with this animal (e.g., Ungvary and Tatrai, 1985) must be interpreted cautiously. Some of the studies were also conducted with mice, a species known to show more variable types and incidences of spontaneous malformations compared to rats or rabbits. It is doubtful that the laboratories conducting these studies possessed the considerable experience that is necessary to work with the evaluations on the small fetus of a mouse.

Response. With respect to species differences in the variation associated with an experimental measure, increased variability within one species reduces the statistical power of experiments with that species and therefore increases the likelihood of a null result for such experiments. Thus, in the face of such increased variability, it is particularly the null result that needs to be appraised with caution.

It is difficult to respond to the comment's declaration: "It is doubtful that the laboratories conducting these studies possessed the considerable experience that is necessary to work with the evaluations on the small fetus of a mouse." However, it seems improbable that <u>all</u> the laboratories that utilized the mouse as the test species fell below a reasonable competency standard.

Regardless, poor technique is more likely to produce false null results than false positive ones. With respect to whether or not a given laboratory possessed the requisite skill to conduct studies in the mouse, poor execution of an experimental procedure may affect the accuracy or precision of an observation. Where poor precision occurs, the variance of the measures is increased and the power to discern an affect is reduced. Thus, poor precision would reduce experimental power and tend toward the null result. Where

poor accuracy is alternatively alleged, all of the measures tend to be skewed in the same way. Thus, as the exposed and control groups would be inaccurate in the same way, the differences between them would still be informative.

The blinding of experimental procedures provides further insurance as to the validity of the experimental results. Typically, the experimenters in these studies were blinded and did not know the treatments received by the test animals at the time of observation. Thus, to the extent (if any) the experimental procedures were poor, it is unlikely that the differences would reflect poor technique compounded by a subjective bias.

Comment 13. Improper Statistical Analyses. Fourth, the appropriateness of the statistical analyses employed in the cited studies is a major concern. The statistical analyses for the majority of the studies considered only the number of fetuses affected. Reproductive toxicologists now consider the litter the appropriate independent unit for statistical evaluation (USEPA, 1991).

Response. The comment is correct. The litter is the preferred unit of analysis for statistical comparisons between groups. The unit of analysis is a factor to be considered in evaluating each study's findings. It is possible that, if most or all of the adversely affected fetuses were in one or a few litters, the statistical comparison of control and experimental groups on the basis of individual fetuses would be misleading. Where the differences between experimental and control groups are sufficiently large, or where the findings have been replicated between studies, or within parts of studies, or where there is evidence of dose response, the opportunity to be misled (as to either a false positive or a false null) is much reduced.

Comment 14. Incorrect Interpretation of Certain Variants and Malformations. Finally, OEHHA's interpretation of the data from these studies is flawed. In several of the studies (e.g., Haas and Jacobsen, 1993; Hudak and Ungvary, 1978; Ungvary et al., 1980), skeletal variants (e.g., rudimentary ribs, fused sternebrae) were observed. Skeletal variations are not adverse developmental effects and, in rats, they have been found to be reversible (Chernoff, et al., 1991; Harris and De Sesso, 1994; Kimmel and Wilson, 1973). Skeletal variants such as rudimentary ribs and fused sternebrae should not be considered biologically significant in the absence of other conventional signs of embryotoxicity (e.g., increased malformations, increased embryolethality or decreased fetal weight). These variants are usually not regarded as harmful developmental toxic effects, but instead may be indicative of non-test-material-related stress due to the exposure regimen. They would only be considered toxic effects if a significant dose-related increase above controls (historical and concurrent) were observed.

Response. OEHHA did not interpret, but only presented, the available scientific information.

Comment 15. In addition, certain malformations such as cleft palate often occur spontaneously in mice as a result of environmental changes during critical stages of development. Thus, the biological significance of increased incidence of cleft palate in teratology studies must be evaluated carefully.

Response. Comment noted. The biological significance of any malformations must be carefully addressed in their interpretation. Where unexposed controls and exposed experimental groups are compared, the spontaneously occurring adverse effects should be of similar magnitude and incidence in each group. However, we agree with the comment that the mechanism of any increase in malformations in the experimental group should be evaluated carefully, especially with respect to extrapolating any findings to humans. Whether or not a malformation or other adverse effect represents a direct action upon reproduction or is secondary to a general maternal toxicity bears more upon the characterization of the toxic insult than its practical meaning. Yet, if the mechanism of the developmental insult in the test species is well understood and not thought likely to be relevant to humans, it would be inappropriate to regard the exposure on that basis alone as a potential human developmental toxicant.

The ATSDR quotation in the document does indicate that most of the adverse fetal effects occur at doses near to or causing maternal toxicity.

Comment 16. And, finally, historical control data, which were not considered, should always be considered when interpreting the significance of skeletal variants and malformations. This will ensure the findings truly exceed the range of control values for a larger population.

Response. Historical control data bear on the replicability of the concurrent control data. In general, as to control or experimental group data, where results have been replicated they warrant a greater degree of confidence.

The absence of historical control data is not a serious limitation in general. Where test animals are randomly assigned to control and experimental groups at the start of an experiment, it is the concurrent controls that are more likely to closely match the experimental groups for all appreciated and unappreciated variables at the start of the experiment. It is the comparison between the concurrent controls and experimental groups which is most probative.

Furthermore, individual studies often incorporate more than one control group. For instance, in Hudak and Ungvary (1978), the study design incorporated three different control groups (untreated controls, 24-hour air exposures in test chambers on days 9-14 of pregnancy, and 8-hour air exposures in test chambers on days 1-21 of pregnancy). These three control groups gave closely similar results that increased the confidence in each.

In other studies, the dose response information itself supports the results achieved in the null group. For instance, in Marks et al. (1982), the dose response relationship for average fetal weight itself afforded strong support to the accuracy of the control value (0 ml/kg-d - 0.982 g; 0.06 ml/kg-d – 0.982 g; 1.2 ml/kg-d – 0.975 g; 2.4 ml/kg-d – 0.861 g; 3.0 ml/kg-d – 0.785 g; 3.6 ml/kg-d – 0.708 g).

Comment 17. The above points demonstrate there are substantial weaknesses in the cited studies that diminish reliable interpretations of the data and conclusions on developmental toxicity. There is available a more definitive and well-conducted developmental toxicity study for xylene (Biodynamics, 1983) (copy attached). This study does not indicate that xylene is a developmental toxicant. We urge California to include this study in their assessment and to take account of the issues discussed above in their discussion of the other developmental studies.

Response. The most consistent adverse effect seen in the different developmental toxicity studies is decreased fetal weights. Different studies, including the Biodynamics (1983) study, have reported different NOAELS/LOAELs for this adverse effect on fetal development:

Study	Strain/Species	Exposure Duration	NOAEL	LOAEL
Mirakova et al. (1983)	"white" rat	6 h/day	2.3 ppm	12 ppm
Hass (1993)	Wistar rat	6 h/day	200 ppm	
Bio/dynamics (1983)	CrL-CD (SD) BR rat	6 h/day	250 pm	500 ppm
Shigeta et al. (1983)	ICR mice	6 h/day	230 ppm	460 ppm
Hudak et al. (1978)	CFY rat	24 h/day	230 ppm	
Ungvary et. (1985)	CFY rat	24 h/day		60 ppm
Ungvary et. (1985a)	CFY rat	24 h/day		140 ppm
Ungvary et al. (1985)	CFLP mice	24 h/day	120 ppm	230 ppm

With respect to NOAEL and LOAEL values, the Mirakova et al. (1983) is clearly an outlier. The ATSDR has suggested that this study may have been influenced by poor animal husbandry. Partially, for these reasons, OEHHA chose not to base its chronic REL upon the Mirakova et al. (1983) study.

Overall, the available data suggest that the 24 hour exposure regimens result in lower NOAELs than the 6 h/day exposure regimens. Regardless, these other observed NOAELs are sufficiently high so as to indicate the proposed chronic REL should provide an adequate margin of exposure. Thus OEHHA is proposing 700 $\mu g/m^3$ as the chronic REL for xylenes.

Geysers Geothermal

Comments on the chronic REL for **hydrogen sulfide** were made by the Geysers Geothermal Association (GGA), a non-profit, mutual benefit corporation with a membership of almost 300 companies and individuals participating in the production and utilization of geothermal energy at The Geysers geothermal field. In the draft document OEHHA proposed use of the U.S. EPA RfC of 0.7 ppb (0.9 $\mu g/m^3$) as the chronic REL.

Comment 1. The Office of Environmental Health Hazard Assessment (OEHHA) has made a great effort to involve input from risk managers and stakeholders throughout the Reference Exposure Level (REL) promulgation process. We support your approach and believe that it is important to seek input from various sources.

Response. OEHHA staff appreciate the comment. The legislation enabling the Hot Spots program included the requirement to obtain and consider input from risk managers and stakeholders.

Comment 2. However, it appears that OEHHA has taken an extremely conservative approach in determining the proposed H_2S REL of 0.7 ppb.

Response. The chronic REL proposed in the draft is a USEPA RfC, which has been available since 1995. The approach to developing RfCs is very similar to the development of chronic RELs. All USEPA Reference Concentrations (RfCs), available when the Technical Support Document (TSD) on chronic Reference Exposure Levels was drafted in October 1997, are being proposed as chronic RELs. RfCs are already used by the USEPA and by California's Department of Toxic Substances Control and were earlier incorporated by reference in Appendix F of the Emissions Inventory Criteria and Guidelines for the Air Toxics "Hot Spots" Program for use in screening risk assessments in the Hot Spots Program. These Guidelines were effective July 1, 1997. The Risk Assessment Advisory Committee (RAAC) recommended that CalEPA harmonize where possible with USEPA on risk assessment. Executive Order W-137-96 concerned the enhancement of consistency and uniformity in risk assessment between Cal EPA and USEPA. Use of RfCs as chronic RELs was one action that OEHHA took to address the RAAC recommendation and to implement the Executive Order. RfCs released after October 1997, including ones that are revisions of those in the October 1997 draft, will be evaluated for use in the Hot Spots program by reviewing the scientific basis of each RfC when it becomes available and by determining whether the scientific literature cited in the RfC is current. Appropriate RfCs will be submitted to the SRP for review and possible endorsement.

However, based on other comments and on OEHHA's assessment of the developmental toxicity data available, OEHHA staff have reviewed the value in the draft document and have calculated a revised chronic REL for hydrogen sulfide of 9 $\mu g/m^3$ (7 ppb).

Comment 3. We are aware that final reports should be available from the Chemical Industry Institute of Toxicology (CIIT) on the reproductive effects and developmental neurotoxicity of H_2S by mid-1998. We request that a final decision on the REL not be made until these studies are completed. A decision of this magnitude should be made in a collaborative setting with input from academia, industry, and regulators. This approach is consistent with OEHHA's development of the proposed REL.

Response. OEHHA staff will be happy to review the studies when they become available and hope that USEPA will do the same. As of January 1999, OEHHA had been provided with only an abstract of a CIIT study on the neurobehavioral and neurochemical effects of hydrogen sulfide. The study involved 5 consecutive days of exposure, which would not be a study length suitable for developing a chronic REL.

Comment 4. We strongly believe that OEHHA's decision to lower the REL to 0.7 ppb should be revisited. Based on the available data, the REL should be set no lower than 7 ppb. We recommend this revisitation based on the following: (1) The California ambient air quality standard for H_2S is 0.03 ppm. (2) The proposed REL of 0.7 ppb is one thousandth of the upper concentration for H_2S naturally occurring in human breath. (3) Low levels of H_2S are rapidly metabolized and detoxified by the human body and, therefore, are unlikely to be a chronic hazard at concentrations at or below the odor threshold. (4) The excessive conservative safety factors used in deriving the USEPA Reference Concentration (RfC), on which the new H_2S REL is based, should be decreased by at least one order of magnitude. (5) New studies on the toxicity of H_2S have been published or initiated since the 1994 USEPA RfC was finalized. We urge OEHHA to delay your decision on the REL until all of the information is updated.

Response. As stated above, OEHHA is now proposing 9 $\mu g/m^3$ (7 ppb) as a chronic REL for hydrogen sulfide. The commentator should also request USEPA to revisit the RfC. To decrease the UFs by an order of magnitude, changing the existing UFs would need to be addressed. If the available reproductive and developmental toxicity data are adequate for U.S. EPA, the modifying factor of 3 can be eliminated. Also if a chronic study were produced, the subchronic uncertainty factor could be eliminated. Thus the REL might increase 3, 10 or 30 fold depending on what studies are available.

Comment 5. The California H_2S ambient air quality standard of 0.03 ppm is based upon the low threshold for odor detection by humans. The Lake County Air Basin, located downwind of The Geysers, has been in attainment with all State and Federal ambient air quality standards, including H_2S, since 1990. This means that the H_2S ambient air quality level is significantly lower than the enforced level of 0.025 ppm. This attainment was accomplished by a cooperative effort of the public, regulatory agencies, and the geothermal industry. A tremendous amount of time, energy, and money was spent achieving this significant accomplishment. The proposed REL will not provide a greater level of public safety.

Response. The California H_2S ambient air quality standard of 0.03 ppm is for a 1 hour averaging time and is being retained as the acute REL in the Hot Spots program. Thus local residents should not experience acute adverse health effects from H_2S. The chronic REL is for much longer exposures and should be less than the acute REL. Although odor is one consideration with H_2S, it is not the only effect and odor is not necessarily a sentinel for other toxicity, since some chemicals have no odor and while for other with an odor adverse effects may occur above or below the odor threshold depending upon the chemical.

Comment 6. The economic impacts of the proposed REL will be enormous throughout California. It certainly could have a significant impact for the Geysers due to the deregulation of the electrical generating industry. Using the proposed REL for Air Toxics scoring purposes could subject some Geysers facilities to perform risk assessments with no corresponding benefit to public health and safety. We are attempting to control costs and remain competitive in a deregulated market. The proposed additional regulatory requirements imperil this goal.

Response. The determination of RELs is a risk assessment process and uses the best science available at the time. The economic impacts are part of risk management considerations. The risk manager can take into account the uncertainty in the REL and the delisting of H_2S as a federal Hazardous Air Pollutant as well as the known toxicity of H_2S, a hazardous gas reported to be the most common cause of sudden death in the workplace.

Lake County Air Pollution Control District

Comments on the **hydrogen sulfide** chronic REL were received from Robert Reynolds, Air Pollution Control Officer for Lake County. In the draft document OEHHA proposed use of the US EPA RfC of 0.7 ppb (0.9 $\mu g/m^3$) as the chronic REL.

Comment 1. Hydrogen sulfide is one of the air pollutants for which an Ambient Air Quality Standard (AAQS) exists, and for which the Recommended Exposure Level (REL) proposed would raise the issue as to whether the AAQS is as comparatively protective of health. The proposed action seems to be inconsistent with Cal-EPA's goal of coordinating programs, avoiding redundancies and lessening paper work. We suggest that either the AAQS should be reviewed and updated or OEHHA and the ARB should at least reconcile and comment on the Hydrogen Sulfide REL and the requirement to be protective of health as part of our state AAQS.

Response. The Ambient Air Quality Standard is for a 1 hour averaging time and is being retained in the Hot Spots program as the acute REL. The chronic REL is a USEPA RfC for long-term exposure. In order to coordinate with USEPA as recommended by the RAAC Committee and to comply with Governor's Executive Order W-137-96, all USEPA Reference Concentrations (RfCs), available when the Technical Support Document (TSD) on chronic Reference Exposure Levels was drafted in October 1997, are being proposed as chronic RELs. RfCs are already used by the USEPA and by California's Department of Toxic Substances Control and were earlier incorporated by reference in Appendix F of the Emissions Inventory Criteria and Guidelines for the Air Toxics "Hot Spots" Program for use in screening risk assessments in the Hot Spots Program.

Comment 2. The unintended consequences of some of the poorly substantiated RELs, especially those based on little or no directly applicable data, may be an erosion of public faith in the protectiveness of historic and ongoing air quality management programs. Potentially, this could result not from "poor science," but the absence of available science and effort in reaching the recommendations proposed by OEHHA.

Response. OEHHA is limited to the available data to develop a REL. Uncertainty factors which account both for known variability between humans and animals and within the human species as well as uncertainty due to extrapolating from LOAELS to NOAEL and from subchronic to chronic are used because there are seldom chronic exposures to sensitive humans available for use. The RfC for H_2S includes uncertainty due to lack of data on reproductive harm and a factor for subchronic to chronic extrapolation. Hopefully interested parties will be motivated to obtain better data.

Comment 3. The uncertainty factors, resulting from the lack of directly applicable information, may be appropriate to accept in some instances, but in those cases where the decision has much consequence in concern for public health protection, or extensive resource and cost demand, an additional effort should be made to increase certainty and the confidence in the recommendations of OEHHA. It would be better to fund necessary

work based in good science and carry it out prior to OEHHA advocacy or required recommendations being published.

Response. Risk assessment always involves uncertainty. The risk manager is expected to include that aspect in his decisions. OEHHA does not have a research budget and thus can only point out the need for more research.

Comment 4. Present state air toxic programs, while providing benefit to the public and industry, fail drastically in that they do not consider the cumulative effect of many sources within an airshed which is typical of the real world. Additionally, they are sometimes based upon a minimal effort that has tremendous downstream costs to others. I believe the subject effort potentially falls into this category and should receive more careful consideration, especially for the RELs proposed that are poorly supported but which are likely to have substantial importance and consequence.

Response. The consideration of cumulative effects within an airshed is of interest and of concern. Such considerations are theoretically possible anywhere and have been recently approached by the USEPA on a national basis (Woodruff TJ, Axelrad DA, Caldwell J, Morello-Frosch R, Rosenbaum A. Public health implications of 1990 air toxics concentrations across the United States. Environmental Health Perspectives 1998 May;106(5):245-51.) OEHHA staff does not have the resources to do such work and doubts that many air districts do either. OEHHA also recognizes that the actual costs of compliance are often less, and even considerably less, than the predictions made by affected stakeholders when a new regulation is proposed. The South Coast Air Basin has continued to thrive in spite of some of the most stringent air quality rules.

Comment 5. OEHHA needs to better identify sources and parties likely to be interested in and affected by the proposed recommendations, and then hold meaningful meetings and considerations which affords easy input. While it is obvious OEHHA has tried to include "stakeholders", "incorporate peer review," etc., I believe the effort should be redoubled and your final action delayed as necessary. Consider having workshops on specific component RELs of concern in those geographical areas of the state where the components are an air management and public exposure concern. In the long run it may save all of us time and effort, and better serve the public.

Response. The TAC program has been in place in California since 1983. The Hot Spots program has been in place since 1987. We have 1500 individuals on our mailing list for Hot Spots and contacted them regarding our Hot Spots documents. The information has been posted on the Internet and we have held public meetings in both the northern and southern parts of the state to present and discuss the issues with stakeholders. In addition we have received public comments. Based on the comments we have revised our document and we are responding to those comments. We believe that we have made every effort to involve stakeholders that have an interest in this process.

Comment 6. The recommendations seem to establish a need to protect against an exposure level that historically has been allowed as part of public policy (AAQS) and at a level that is extremely costly or virtually impossible to measure. This aspect of your recommendations should be identified for each of the RELs. The chosen approach and lack of factual information is likely to result in a great deal of confusion when applied differently than intended, and unnecessary resources going towards paper studies that provide no real benefit to air quality or public health. The worst consequences may be unfounded: fear on the part of the exposed public, wasteful efforts and costs to the regulated, and misused resources by those whom must implement programs based on the OEHHA recommendations.

Response. The CAAQS is for a 1 hour exposure which we had proposed adopting. The chronic REL is for continuous chronic exposures. The two values are not comparable and their uses are not comparable. In a risk assessment the chronic REL is compared to an annualized average, while the CAAQS (acute REL) is compared to the maximum one-hour concentration.

Based on other comments and on OEHHA's assessment of the developmental toxicity data available, OEHHA staff have reviewed the value in the draft document and have calculated a revised chronic REL for hydrogen sulfide of 9 $\mu g/m^3$ (7 ppb).

Metal Finishing Association of Southern California

Comments on the Draft Technical Support Document for the Determination of Noncancer Chronic Reference Exposure Levels were made by the Metal Finishing Association of Southern California (MFASC). The Association commented on 13 chemicals of relevance to their work. The comments included as an attachment a table titled Comparison of Limits for Selected Substances. Most of that information is in the Table included in the response to Comments 1-3.

Comments 1 – 3. For some of the substances, the proposed RELs are considerably different from those used before. … New data (i.e. more recent than 1992) were used to determine RELs for only 3 of the 13 chemicals [of interest to the MFASC]. Thus, the main difference between the new proposed RELs and the previous ones must be the methodology with which the existing data are handled.

For most of the new substances, the new RELs represent a substantial tightening of the regulatory burden. The 1992 REL for cadmium was 350 times less stringent than the proposed 1997 REL. Thus, sources that emit cadmium for which chronic non-cancer health effects were not previously predicted could now be subject to severe new regulatory requirements as a result of the change in the REL.

Response. The previous values were provided and presented by CAPCOA in response to the original Hot Spots Act. Subsequent legislation (Health and Safety Code Sec. 44360) required OEHHA to develop risk assessment guidelines for the Hot Spots program. OEHHA has used methods similar to those of the USEPA. Many of the CAPCOA values were, by contrast, derived from preexisting health risk guidance values (e.g., route-to-route extrapolation of oral Reference Doses, occupational exposure limits). These preexisting values were not originally intended for such purposes. CAPCOA used several indirect and ad hoc methodologies to derive its guidelines from these preexisting values. The CAPCOA effort was not as rigorous or time- and effort-intensive as the OEHHA effort. The comment is therefore correct: Most of the new proposed values represent intentional differences in methodology between OEHHA and CAPCOA. Cases where there would be substantial differences between the CAPCOA values and the proposed OEHHA were to be expected from the outset.

In particular, all the new values are based upon a much more thorough search of the existing scientific literature. The CAPCOA values were derived from preexisting health risk guidance values of quite varying vintage. Therefore, in many cases, the newly derived OEHHA proposed values incorporate additional data which, even if available prior to 1992, were not incorporated in the health risk guidance values upon which the CAPCOA values were based.

For instance, with respect to the case of the cited example of cadmium, in the CAPCOA document the then USEPA IRIS oral reference dose (RfD) was adjusted to an equivalent air concentration on the assumption that the oral and inhalation routes were of similar potency. However, in order to develop the OEHHA REL, OEHHA conducted an

extensive search of the inhalation toxicology literature. Through that effort, OEHHA identified the Lauwerys et al. (1974) key study of renal toxicity in workers exposed to cadmium. Unlike the original USEPA IRIS RfD, the proposed chronic REL value is based upon that 1974 epidemiological study. In 1996, the USEPA revised and greatly lowered its oral RfD for cadmium in light of health hazard information obtained from additional epidemiological studies. This recent change in the RfD supports the original presumption that oral and inhalation toxicities for cadmium would be similar. The new value for the cadmium RfD is therefore more in line with the proposed OEHHA chronic REL value.

The proposed OEHHA REL for cadmium of 0.01 $\mu g/m^3$ is 350-fold lower than the earlier CAPCOA value of 3.5 $\mu g/m^3$. The CAPCOA values were interim guidance values and were superseded when the appropriate governmental health risk assessments were completed. As a TAC, cadmium is regulated as a carcinogen with an OEHHA cancer unit risk value for cadmium is 4.2 E-3 $(\mu g/m3)^{-1}$. (At the proposed chronic REL the estimated lifetime cancer risk would be forty in a million.)

Chemical of Interest to MFASC	OEHHA Proposed REL $\mu g/m^3$	1992 CAPCOA Guidance Value $\mu g/m^3$	Basis of 1992 CAPCOA Value	Ratio of CAPCOA to OEHHA
Beryllium	0.001	0.0048	ACGIH TLV	4.8
Cadmium	0.01	3.5	USEPA IRIS	350
Chromium VI	0.0008	0.002	USEPA HEAST	2.5
Copper	0.02	2.4	USEPA IRIS	120
Hydrogen Chloride	7	7	USEPA IRIS	1.0
Hydrogen Cyanide	3	70	USEPA IRIS	23
Hydrogen Fluoride	30	5.9	ACGIH TLV	0.2
Methylene Chloride	300	3000	USEPA HEAST	0.1
Nickel	0.05	0.24	ACGIH TLV	4.8
Nitric Acid	40	none	(Not listed)	N/A
Perchloroethylene	40	35	USEPA IRIS	0.9
Sodium Hydroxide	2	4.8	ACGIH TLV	2.4
Zinc	0.9	35	Superfund PHEM	39

HEAST: Health Effects Evaluation Summary Table
IRIS: Integrated Risk Information System
TLV: Threshold Limit Value established by the American Conference of Governmental Industrial Hygienists
PHEM: Public Health Evaluation Manual

Finally, OEHHA staff attempt to use the best study of a chemical that it can find in the peer-reviewed literature to develop a chronic REL. When Hazard Indices exceed 1, air district staff consult with OEHHA staff on a case-by-case, chemical-by-chemical basis about the likelihood of adverse health effects. Risk management is an important part of the Air Toxics Hot Spots program.

Comment 4. Many of the proposed RELs may be below detection levels. Thus, it would be impossible to prove that these RELs are not being exceeded through the use of ambient monitoring. Moreover, no future epidemiological studies with such low concentrations would be possible. These values thus represent purely theoretical concentrations with no verifiable basis in reality.

Response. The inability of epidemiological studies to "verify" a chronic REL is a general one. With respect to epidemiological studies, the chronic REL value assumes that there is a threshold concentration below which adverse effects do not occur. The OEHHA values estimate this threshold with a margin for uncertainty. Thus, if the OEHHA REL is satisfactory, epidemiological studies conducted upon populations exposed at, or somewhat above, the chronic REL (or RfC for that matter) should find no adverse effect.

The comment is correct that ambient monitoring could not be used at locations where the ambient levels fell below the analytical detection limits. However, in most circumstances, emission rate information and air dispersion modeling are used to estimate ambient exposures. In the abstract, where exposures substantially exceed the REL and so are more likely to yield evidence of an adverse effect in an epidemiological study, they are also more likely to exceed the limit of detection.

While not optimal, often epidemiological investigations are necessarily conducted in the absence of actual air concentration data. These studies use distance from a source, duration of exposure, or air dispersion modeling as surrogate exposure information that permits persons to be classified by their relative degree of exposure.

The ability of an epidemiological study to detect any difference in a particular effect is much reduced at low levels of exposure. As the magnitude of exposure declines, the magnitude and frequency of any particular effect also declines to a point where any effect becomes hard to discern. Null results from such studies are reassuring but can not meaningfully "verify" a chronic REL, absent an extraordinarily large number of exposed persons in the study. For these practical reasons, epidemiological studies usually target high exposure (e.g., occupational) populations for study. Where epidemiological studies have detected adverse effects of air pollution on sensitive subjects (PM_{10}, ozone), these studies encompassed an extraordinarily large number of exposed persons.

Comment 5. The OSHA PELs cannot be used to assess risk to the public health. Yet, they are another measure of health risk published by an agency other than OEHHA. The new RELs do not reflect the general relationship of health risks for the different compounds. For example, the PELs would indicate that copper is no more hazardous than nickel although neither of these substances are as toxic as other metals. Yet, the new REL for copper is less than half that for nickel.

Response. When OSHA was created in 1973, OSHA adopted the existing ACGIH TLVs for copper and nickel as the OSHA PELs. Those OSHA values have not been revised. Since that time, a great deal of toxicity information on nickel compounds has been generated. The OEHHA nickel REL is based upon new animal data provided by the National Toxicology Program in 1994. This information was unavailable in 1973.

The proposed REL for copper is based upon comparatively limited information. The OEHHA value is based upon the Gleason study of just 3 workers exposed to copper. The study measured copper levels on only one day for three exposure conditions. This study provided a LOAEL of 30 $\mu g/m^3$ and a NOAEL of 8 $\mu g/m^3$ for copper fumes. The only other available inhalation toxicity study was a subchronic mouse study that established a LOAEL of 130 $\mu g/m^3$. Both these studies have substantial limitations. The human data were preferred as they required a much smaller overall uncertainty factor and exposure duration adjustment. If the animal data had been used, an even smaller proposed REL would have been developed. As a result of this limited animal and human information, uncertainty factors and exposure duration adjustments particularly contributed to the very low proposed chronic REL for copper. However, in light of the extent, quality, and coherence of the available toxicity information, OEHHA has reconsidered its derivation of the chronic REL for copper.

Comment 6. Because new health data are not being used, it is important to consider the assumptions especially in light of the uncertainty factors being used by OEHHA in proposing these new RELs. In lieu of corresponding RfCs from the USEPA, OEHHA appears to have applied the most conservative assumptions with regard to uncertainty where that were possible.

Response. For most of the substances under consideration, OEHHA pioneered the development of chronic RELs for environmental exposures. Whether or not the health data for a given substance were 'new', OEHHA's use of the data for this purpose was new for most of the covered substances.

OEHHA followed a methodology closely similar to that of the USEPA. A comparative analysis of the uncertainty factors applied by OEHHA in the development of its RELs and by the USEPA in the development of the similar RfCs does not support the contention that OEHHA excessively applied uncertainty factors in lieu of an available RfC. OEHHA's average cumulative uncertain factor of 134 is in fact approximately one-half the USEPA's average cumulative uncertainty factor of 238.

	Geometric Mean of Uncertainty Factors	
Uncertainty Factor	**OEHHA REL**	**USEPA RfC**
LOAEL	2.6	1.9
Subchronic	2.2	2.1
Interspecies	2.4	2.7
Intraspecies	9.3	8.9
Modifying factor	1	2.4

Average Cumulative UF	**134**	**238**

Comment 7. It is recommended that an independent peer review committee that includes toxicological experts independent of OEHHA review all assumptions used in proposing these RELs before they are adopted for use in regulatory programs.

Response. Pursuant to Health and Safety Code Section 39670, California has established an independent scientific peer review committee to perform just this function. Under this regime, OEHHA's work is submitted to the state's independent Scientific Review Panel on Toxic Air Contaminants.

Comment 8. It is recommended that OEHHA continue to use the 1992 RELs until the USEPA adopts new RfCs or until the proposed RELs are reviewed by an independent peer review committee as suggested above.

Response. We will be submitting this work to the Scientific Review Panel along with the Public Comments and staff responses to the Public Comments. The RELs will not be used until and only if the Scientific Review Panel endorses them.

Pacific Gas and Electric (PG&E)

Comments on the chronic REL document were made by PG&E in a letter dated January 29, 1998. PG&E made comments on the general methodology, the role of California Ambient Air Quality Standards (CAAQS), nitrogen dioxide, formaldehyde, and H_2S.

Comment 1. PG&E requests that the draft chronic Reference Exposure Levels (RELs) be revised to separately identify "known effect levels" and "uncertainty elimination levels", with the idea that risk assessments could be required to calculate hazard indices with respect to both the "known effect levels" and the "uncertainty elimination levels".

Response. OEHHA has based its proposed chronic reference exposure levels (RELs) on methods developed by USEPA in its reference concentration (RfC) program. RELs and RfCs are intended as estimates of levels unlikely to result in observable adverse effects among the general public. They are definitely not "uncertainty elimination levels," and methods to determine absolutely risk-free exposure levels are unknown. The REL document presents observed effects data for most chemicals reviewed that represent examples of "known effect levels." These data give a partial picture of potential adverse effects associated with chemical exposure and are presented to inform risk managers and other readers about these observed effects. Direct comparison of various observed effect levels is difficult because of the great variability in the bases for these data. Some are observations among occupationally exposed workers while others are from experimental animal studies. Exposures may be brief, intermittent, or over an entire lifetime. Effects noted may be mild or severe. They may affect a few susceptible subjects or nearly all exposed individuals. The change in severity and incidence in effects observed may be rapid or gradual with increasing exposures. Studies vary in quality and comprehensiveness and some significant adverse effects may go undetected.

Comment 2. In the toxic air contaminant identification process, H&S 39660(c) requires the Office of Environmental Health Hazard Assessment (OEHHA) to estimate both "the exposure level below which no adverse health effects are anticipated" and "an ample margin of safety." But H&S 39660(a) requires OEHHA merely to 'Prepare recommendations." H&S 39661(a)(1) states that "the state board in consultation with, and with the participation of the office, shall prepare a report", and H&S 39661(c) stipulates that the final regulation adopted by the state board will account "for the factors described in subdivision (c) of Section 39660". State law relies upon OEHHA's technical experts to recommend levels, but also upon the Air Resources Board's (ARB's) elected and appointed "risk managers" to review those levels. But these chronic RELs are not identification documents subject to ARB risk manager control. Rather OEHHA is responding to a separate guidelines mandate in H&S 44360(b)(2) which does not even, cross reference H&S 39660(c). Instead, H&S 44362(b) clearly states that it will be up to the judgment of the districts what level of risk or hazard will be deemed significant.

Response. The Toxic Air Contaminant (TAC) mandate (H&SC Sec. 39660 et seq.) is a separate mandate from the Air Toxics Hot Spots Information and Assessment program

(H&SC Sec. 44300 et seq.). However, there is overlap. All TACs, including the 189 Hazardous Air Pollutants (HAPs) in the Clean Air Act Amendments of 1990 which became TACs in April 1993 as a result of AB2728, are subject to the Hot Spots program (H&SC Sec. 39655(d)). In addition chemicals appearing on other lists are also subject to Hot Spots (H&SC Sec. 44321). OEHHA is the state's expert on health risk assessment (e.g., see Governor's Executive Order W-137-96) and develops health guidance values. The air districts decide how to manage the risks estimated using the values.

Comment 3. The draft chronic Reference Exposure Levels (RELs) include factors of uncertainty that push the draft chronic RELs below current ambient air quality standards (for hydrogen sulfide), and below levels which the Scientific Review Panel concluded caused no adverse effects other than a cancer risk (for formaldehyde). The formaldehyde and hydrogen sulfide RELs are just two examples where high uncertainty factors have been proposed for relatively mild effects even though substantial human exposure data is available.

Response. The hydrogen sulfide ambient air quality standard addresses short-term exposures. OEHHA based its chronic REL for hydrogen sulfide on the USEPA reference concentration (RfC). The USEPA also did not adopt an ambient air quality standard for hydrogen sulfide since it has none, but rather used long-term exposure data.

All USEPA Reference Concentrations (RfCs), available when the Technical Support Document (TSD) on chronic Reference Exposure Levels was drafted in October 1997, are being proposed as chronic RELs. RfCs are already used by the USEPA and by California's Department of Toxic Substances Control and were earlier incorporated by reference in Appendix F of the Emissions Inventory Criteria and Guidelines for the Air Toxics "Hot Spots" Program for use in screening risk assessments in the Hot Spots Program. These Guidelines were effective July 1, 1997. The Risk Assessment Advisory Committee (RAAC) recommended that CalEPA harmonize where possible with USEPA on risk assessment. Governor Wilson's Executive Order W-137-96 concerned the enhancement of consistency and uniformity in risk assessment between Cal EPA and USEPA. Use of RfCs as chronic RELs was one action that OEHHA took to address the RAAC recommendation and to implement the Executive Order. RfCs released after October 1997, including ones that are revisions of those in the October 1997 draft, will be evaluated for use in the Hot Spots program. OEHHA staff will review the scientific basis of each RfC when it becomes available and determine whether the scientific literature cited in the RfC is appropriate. Appropriate RfCs will be submitted to the SRP for their review and possible endorsement.

The RfC for hydrogen sulfide was adopted by USEPA in 1995 and incoporates a 1,000-fold cumulative uncertainty factor. The RfC is derived from a 90-day inhalation study with mice conducted by CIIT (Chemical Industry Institute of Toxicology). The study was well-conducted with many appropriate endpoints examined, but the number of animals tested was small. The critical endpoint for the RfC is nasal histological changes. The RfC was reviewed by OEHHA for general adequacy and accepted, although there is

some concern that the cumulative UF may be too large. OEHHA is reevaluating the hydrogen sulfide RfC and will be discussing this issue with the Scientific Review Panel.

The cumulative uncertainty factor for the formaldehyde REL was only 10-fold. This factor was necessary because sensitive human groups such as children or the elderly may be considerably more susceptible to effects from long-term formaldehyde exposure than were the relatively healthy group of workers described in the Wilhelmsson and Holmstrom reports. Cancer is a separate issue.

Comment 4. The public and their risk managers may have differing perspectives about how feasible or necessary it is to provide so ample a margin of safety. Perspectives may also differ between districts, or over time. OEHHA does not need to obtain risk manager consensus on these uncertainty factors. But OEHHA should design its factors and its hazard identification guidelines to enhance, not reduce, risk manager flexibility.

Response. OEHHA fully acknowledged in the draft chronic REL document the many and varied uncertainties involved in the task of estimating exposure values protective against noncancer health effects. The nomenclature used by OEHHA and USEPA for its values, REL and RfC, respectively, incorporate the term "reference" because of the recognition that no single exposure value can be derived that demarcates "safe" from "hazardous." It is rather intended to be a useful risk management tool in assessing relative and cumulative risks associated with chemical exposures. Thus an important goal of the OEHHA chronic REL effort was to maintain a consistent basis for deriving the various RELs.

Comment 5. We recommend that OEHHA establish chronic "Known Effect Levels" (KELs) at the projected No Observed Adverse Effects Level (NOAEL), and separate "Uncertainty Elimination Levels" (UELs) that consider what added safety or uncertainty factors are finally adopted. We further recommend that OEHHA's chronic hazard evaluation guidelines require that the hazard index be calculated both with respect to the KELs and with respect to the UELs. This would provide the state and public with a uniform database on relative hazards posed by different sources in different districts, as well as a good indication of how much uncertainty there is about those hazards. It would also enable district risk managers to chose whether to relate their significant hazard level to either the KELs or the UELs. While most Districts may be content to relate acceptable levels to OEHHA's UELs for most compounds, some may prefer to relate significance levels for some compounds to the KELs - at least until it becomes feasible to provide the additional margins of safety that uniform adherence to the UELs would likely require.

Response. OEHHA developed its risk assessment methods for noncancer health effects from chronic exposures to be consistent with methods used by USEPA in the development of its reference concentrations. Levels associated with observed adverse effects are reviewed in the document. RELs and RfCs are intended as estimates of levels unlikely to result in observable adverse effects among the general public, but are not

"uncertainty elimination levels." RELs are presented with observed effect data (that could be termed "known effect levels") to inform risk managers and other readers. Direct comparison of various observed effect levels is difficult because of the great variability in the bases for these data. Some are observations among occupationally exposed workers while others are from experimental animal studies. Exposures may be brief, intermittent, or over an entire lifetime. Effects noted may be mild or severe. They may affect a few susceptible subjects or nearly all exposed individuals. The change in severity and incidence in effects observed may be rapid or gradual with increasing exposures. Studies vary in quality and comprehensiveness and some significant adverse effects may go undetected.

Comment 6. Exclude substances regulated by state or federal ambient air quality standards, like NO_2, from the toxic air contaminant hazard evaluations, or if hazard evaluation is deemed necessary, evaluate them only with respect to a chronic REL set at the most applicable adopted ambient standard.

Response. Chronic RELs are for use in the Hot Spots program. Many chronic RELs are for toxic air contaminants (TACs) because TACs are subject to the Hot Spots program. Other Hot Spots chemicals are not currently classified as toxic air contaminants. The ambient air quality standards are generally designed to protect against adverse effects resulting from exposures to concentrations for varying time periods which varies with the standard. The USEPA has an annual standard for nitrogen dioxide of 0.05 ppm (100 $\mu g/m^3$) to "prevent health risk and improve visibility." California has a 1-hour standard for nitrogen dioxide of 0.25 ppm (470 $\mu g/m^3$). OEHHA separately evaluated health effects of short-term exposures (1-hour time-weighted average exposure) and long-term exposures ("annual time"-weighted average exposure) for acute and chronic RELs, respectively. The focus of evaluation and the averaging time for an ambient air quality standards and a REL can differ. OEHHA used the 1-hour California nitrogen dioxide standard as its acute REL. The proposed chronic REL of 20 $\mu g/m^3$ (10 ppb) is based purely on health effects, in this case a 1993 report by Infante-Rivard in which effects in asthmatic children were observed at 15 ppb. This is tabulated in Section VI of the chronic REL summary. It would not be a responsible action for OEHHA to let the chronic REL be set at the annual Federal standard of 0.05 ppm (50 ppb) when adverse effects in children at 15 ppb nitrogen dioxide have been reported in the peer-reviewed literature.

Comment 7. In 1995, there was no place in California where either the federal annual NO_2 standard of 100 $\mu g/m^3$ or the state daily NO_2 standard of 470 $\mu g/m^3$ was exceeded. But ambient concentrations exceeded the proposed NO_2 REL of 20 $\mu g/m^3$ (0.01 ppm) at 90 of 123 monitoring sites during that year. Currently, 80% of 1995 California NO_x emissions were attributed to mobile sources not regulated under the "hot spots" program (see pp. 100-110 of the ARB's annual "Summary of 1995 Air Quality Data", and pp. 34 of the 10/10/97 statewide inventory tables available at http://arbis.arb.ca.gov/emisinv/95inven195inv.htm). Those fuel combustion sources

regulated under the "hot spots" program typically emit much more NO than NO_2, and it would be difficult for NO_x dominated "hot spot" sources to accurately estimate what percentage of their NO emissions might convert to NO_2 before their points of maximum ground level impact.

Response. Issues such as (1) whether ambient concentrations in any particular areas may exceed the health-based risk assessment values, (2) the relative sources of ambient concentrations, or (3) the technical difficulties in estimating emissions or reducing emissions to avoid exceeding such values are not relevant to the development of these values, though these certainly are additional risk management issues. Unlike other exposure values that incorporate such risk management concerns, OEHHA RELs and USEPA RfCs and RfDs are purely health data-based guidance values that ultimately will be one of a number of issues considered by risk managers.

Comment 8. Although NO destroys ozone while converting to NO_2, conversion in the center of an NO dominated plume can be incomplete 60 km downwind (page 8, Air & Waste Management Association paper 95-RAI13A.01, "The Significance of NO_x Emissions from Coal-Fired Power Plants in the Middle Tennessee Area on Tropospheric Ozone, by Prof. Wayne T. Davis, Univ. of Tenn., et al, June 18-23, 1995). Also precise calculation of NO to NO_2 requires data and models that won't be available at most locations, and very costly to acquire and use at those few locations where they might be available.

Furthermore, since the effects of ozone exposures appear more serious than those from NO_2, overestimating conversions might harm rather than protect - if the result was less NO in an area where NO helps to destroy ozone. Meanwhile, much of California is non-attainment of state ambient air quality standards for ozone or fine particulate, and as a result will already require most "hot spots" sized NO_x sources to impose either Best Available Retrofit Technology or All Feasible Control Technology. All of these factors suggest that NO_2 concerns remain more appropriately addressed under the ambient air quality programs, rather than within the chronic hazards portion of the "hot spots" program.

Response. The concerns raised by this comment should be more appropriately addressed in other settings, such as at the risk management level. It was beyond the scope of the OEHHA chronic REL document to address issues such as difficulties in estimating emissions and exposures, relative risks of chemicals, and the fate of chemicals in the ambient air. This document is focused on the development of strictly health-based exposure guidance.

Comment 9. Ambient air quality standard reviews focus immense attention upon one substance. For example, the joint ARB/OEHHA Technical Support Document "Review of the One-Hour Ambient Air Quality Standard for Nitrogen Dioxide" published in December 1992 required 232 pages to address NO_2 data. While OEHHA has prepared an excellent 8 page summary on its proposed NO_2 REL, a summary of that size is unable to

provide the detail on the key study needed to attract critical review, especially when it competes for agency/commentator attention with 750+ other pages on 119 other substances. Where a comprehensive review has already been undertaken, a briefer review should not be allowed to displace it. If desired, OEHHA could ensure NO_2 inclusion in calculated total hazard indices by simply referencing the existing federal annual standard. If a subsequent review were to result in revision of the standard, the REL could also change.

Response. The 1-hour ambient air quality standard for nitrogen dioxide is designed to protect against adverse effects resulting from short-term exposures to peak concentrations and has been endorsed by the Scientific Review Panel for use as an acute REL in the Hot Spots program. The USEPA's annual standard for nitrogen dioxide of 0.05 ppm (100 $\mu g/m^3$) is to "prevent health risk and improve visibility." The proposed chronic REL of 20 $\mu g/m^3$ (10 ppb) is based purely on health effects, in this case a 1993 report by Infante-Rivard in which effects in asthmatic children were observed at 15 ppb. This is tabulated in Section VI of the chronic REL summary. It would not be appropriate for OEHHA to let the chronic REL be set at the annual Federal standard of 0.05 ppm (50 ppb) when adverse effects have been reported in the peer-reviewed literature in children at 15 ppb.

Comment 10. If a chronic REL is proposed for hydrogen sulfide even though an ambient standard exists for that compound, then the existence of the hourly standard should at least reduce the need for a subchronic exposure uncertainty factor.

Response. OEHHA separately evaluated health effects of short-term exposures (1-hour time-weighted average exposure) and long-term exposures (1-year time-weighted average exposure). The proposed chronic REL for hydrogen sulfide is based on the USEPA RfC which was derived from subchronic exposure data. USEPA followed its RfC methodology in determining an appropriate subchronic uncertainty factor. The subchronic uncertainty factor is intended to account for potential differences in the magnitude of adverse effects between those observed in subjects exposed over less than a full lifetime and those that might be experienced by the general public over their entire lifetime. Thus short-term exposure data, even if extensive, do not eliminate uncertainties resulting from a lack of long-term exposure data.

Comment 11. For Hydrogen Sulfide, data showing no adverse effects at 5400 ppb is claimed to justify limiting exposures to 0.7 ppb. In other words, the proposed REL is 7,714 times as stringent as the No Observed Adverse Effects Level (NOAEL). This reflects the use of a factor of eight in extrapolating from mice to humans, and the multiplication of additional "uncertainty" factors amounting to 900, but tabulated as 1,000. Given that the effect that is not observed to occur at the 5400 ppb level was "inflammatory changes in the nasal mucosa", is such a high uncertainty factor necessary?

Response. The magnitude of the difference between concentrations known to cause adverse effects and those without appreciable risk can never be determined with absolute

certainty. Thus USEPA has developed and OEHHA has adopted, with some changes to ensure consistency, default and data-based methods to derive the RfC and REL "reference" levels. In some cases where overall uncertainty is low, a small or even no uncertainty factor has been used. In other cases, several areas of significant uncertainty exist. This results in a large cumulative uncertainty factor that is unsatisfying for all concerned but will require the development of better data to improve the situation. When better data become available, the RELs will be reevaluated and hopefully the use of uncertainty factors can be lessened or in some cases eliminated. Also some humans experience adverse effects of nausea and headache at the 30 ppb one-hour standard and some people may experience adverse effects at a somewhat lower concentration when exposed chronically. The point of using uncertainty factors is to get below these adverse effect levels. Comparing 5400 ppb and 30 ppb, it is no surprise that the extrapolation to the chronic REL must be at least 180-fold and probably more since 30 ppb is a LOAEL for people.

OEHHA is attempting to determine if USEPA inadvertently applied an incorrect uncertainty factor of 10 in the RfC calculation when the text indicated that 3 was appropriate. It is also not clear that a database deficiency factor is warranted. We will be discussing this issue with the Scientific Review Panel.

Comment 12. California previously adopted an ambient air quality standard for hydrogen sulfide at 30 ppb or 42 $\mu g/m^3$, averaged over one hour to protect against annoying odors. It is generally recognized that annual average concentrations are typically ten or more time lower than peak hourly concentrations. The "Toxic Air Pollutant Source Assessment Model for California Air Pollution Control Districts and Applicants for APCD permits" adopted October 1, 1987 uses a multiplying factor of 0.1 for conversion of hourly model results to annual in flat terrain downwash, and presents a similar hourly to annual ratio (4.0/0.4 = 0. 1) when 24 hour complex terrain model results are extrapolated. On page 111-5 of the 1993 California Air Pollution Control Officer's Association Risk Assessment Guidelines, the 0. 1 factor continues to be used for the conversion of hourly screening model results to annual average, and the only examples cited on page 111-13 all had peak hourly concentrations 10 to 15 times the corresponding annual average concentrations. On that basis, the state standard of 30 ppb should be sufficient to protect against chronic exposures of 3 ppb. 3 ppb would be a factor of 1800 below the reported NOAEL, and a factor of 220 below the OEHHA calculated equivalent human NOAEL.

Response. The 10-fold convention used in exposure estimation is based on only commonly observed differences in maximum 1 hour and maximum 1 year average exposure concentrations. This factor does not address differences in health effects that might be observed between a short-term exposure and those over a lifetime. OEHHA separately evaluated health effects of short-term exposures (1-hour time-weighted average exposure) and long-term exposures (1-year time-weighted average exposure). The proposed chronic REL for hydrogen sulfide is based on the USEPA RfC, which was derived from long-term exposure data. The ambient air quality standard for hydrogen sulfide is designed to protect against adverse effects resulting from short-term exposures

to peak concentrations. Therefore the focus of evaluation for the AAQS is different from the chronic REL.

Comment 13. It would be better for everyone if all available compliance resources were devoted towards ensuring continuous compliance with the existing state hourly standard, rather than dividing resources to separately evaluate compliance with separate standards. Therefore PG&E recommends that the Office of Environmental Health Hazard Assessment (OEHHA) not adopt any chronic REL for hydrogen sulfide, but instead rely upon the hourly standard to protect the public from chronic exposures as well.

Response. This concern should be more appropriately addressed in other settings, such as at the risk management level. This document is focused on the development of strictly health-based exposure guidance, and includes chemicals selected from substances of concern identified by the California Air Resources Board. Acute exposure standards are not necessarily protective of the general public exposed over a lifetime.

Comment 14. If OEHHA believes that it must adopt a chronic REL for a substance for which an hourly state standard exists, then we urge OEHHA to reconsider the proposed factors of uncertainty. For example, eliminating the subchronic uncertainty factor (because there is a separate subchronic standard), would be sufficient to raise the REL to 660 ppb / 90 = 7 ppb. If the chronic REL were set at 7 ppb, the hourly 30 ppb standard would remain the governing factor in almost all situations.

Response. OEHHA reviewed the USEPA RfC and concluded that it was adequate for use as an OEHHA chronic REL. There is no basis for eliminating a subchronic uncertainty factor because of the availability of short-term data and exposure guidelines. This factor is eliminated where adequate data on toxicity following long-term exposure are available, which was not the case for hydrogen sulfide.

Comment 15. Our chief concern with such a 7 ppb REL is whether geothermal power plants would be expected to evaluate compliance with such an REL within the secured geothermal steam supplier leasehold. If OEHHA adopts a 7 ppb REL, then we would hope that OEHHA would make provisions within its risk assessment guidelines for adjacent industrial sources to agree among themselves that risks/hazards need only be evaluated outside their common perimeters.

Response. This concern should be more appropriately addressed in other settings, such as at the risk management level. This document is focused on the development of strictly health-based exposure guidance.

Comment 16. OEHHA should use lesser uncertainty factors for natural compounds like formaldehyde for which abundant exposure data exist.

Response. From a risk assessment perspective, the natural occurrence of a chemical is not a factor in estimating risks from exposure. The large health effects database for formaldehyde was considered and is reflected in the relatively small 10-fold cumulative uncertainty factor for formaldehyde.

Comment 17. [OEHHA] should not adopt RELs in conflict with prior Scientific Review Panel identification report conclusions.

The proposed formaldehyde REL is equated to 1 ppb. This is 4.4 times below the mean annual outdoor population weighted exposure that 20 million Californians were deemed subject to in finding #6 on page 17 of the Executive Summary of the "Final Report on the Identification of Formaldehyde as a Toxic Air Contaminant" in July 1992 . On page 18 of that report, Scientific Review Panel finding #10 concludes that "Adverse health effects other than cancer are not expected to occur at mean statewide outdoor ambient concentrations". OEHHA should not adopt a REL that suggests hazards could exist at levels that more exhaustive prior identification report review concluded did not pose a hazard. There are often issues which cannot be adequately evaluated in these more generic REL reviews. OEHHA also appears to be changing an identification report finding outside the established process (see PG&E's comment #1).

The proposed formaldehyde REL is also equated to 2 $\mu g/m^3$, which is 130 times below the identified Lowest Observed Adverse Effects Level (LOAEL), and 45 times below the No Observed Adverse Effects Level (NOAEL). Given the lack of data suggesting that widespread ambient air exposures are causing problems, a lesser combined factor of uncertainty would appear appropriate. Replacing the current factor of uncertainty of 130 relative to the LOAEL with a factor of uncertainty of ten with respect to the NOAEL would raise the REL to 9 $\mu g/m^3$ (6 ppb). This level would be above recent average exposures, and protecting against above average exposures would be more consistent with the identification report findings.

Response. The health effects assessment document that is part of the "Final Report on the Identification of Formaldehyde as a Toxic Air Contaminant" focused on cancer risks associated with formaldehyde exposure, and did not extensively evaluate noncancer health effects. The proposed OEHHA REL for formaldehyde is based on a review of noncancer health effects data. The Scientific Review Panel will review the proposed formaldehyde REL. The magnitude of the REL relative to ambient exposure levels is not an appropriate factor to incorporate into the derivation of the REL, but is an issue of concern to the risk management of formaldehyde exposures. The Panel approved an acute REL for formaldehyde of 94 $\mu g/m^3$.

Comment 18. One key finding in the identification report was that the risk of cancer was the greatest concern. But adoption of a formaldehyde REL of 2 $\mu g/m^3$ would flip flop that conclusion at least in non-residential areas. Both cancer risks and chronic hazards

can be adjusted for hours worked per year of exposure. But chronic hazards are based upon the single worst year, whereas as cancer risks are based upon average exposure over 46 year working lifetime (versus a 70 year nominal lifetime). A single significant figure REL would have to be at least 3 $\mu g/m^3$ before worker risk would be more significant than chronic hazards for workers subject to non-variable exposures.

10 in a million [significant risk]		70 years [nominal]		chronic REL at
----------	x	----------	= 2.54 $\mu g/m^3$	which cancer and
6 in a million/($\mu g/m^3$) [unit risk]		46 years [working]		hazards equate

But most exposures vary from year to year. In our opinion 9 $\mu g/m^3$ offers a reasonable balance - a factor of 10 below the NOAEL, a factor of 2 above the historic exposures found not to cause adverse effects, and a factor of 3 above the level at which steady state sources would calculate more significant chronic hazard indices than cancer risks. Chronic hazards could still govern at sources with more variable emissions.

Response. The availability of relevant data was an important consideration in the development of the chronic RELs. While USEPA frequently uses its limited database factor, OEHHA did not use such a factor.

Comment 19. RELs (or KELs & UELs) should be presented with both all significant figures and with an appropriately rounded number of significant figures. It would be inconsistent to propose a cumulative factor of uncertainty of 1000 or more, while insisting upon the use of multiple significant figures. But even when numbers are highly uncertain, rounding does not render the rounded numbers more accurate, so neither would it be appropriate to insist upon rounding. If one calculates a hazard index of 0.999 relative to a significance level of 1.0 no one should ever round such "insignificant" results up to the significance level.

An OEHHA REL (or as we suggest, an OEHHA UEL) should be specified both as "originally calculated" with all available significant figures, and as rounded where the degree of rounding should reflect the assumed level of uncertainty. For example, compounds employing a factor of uncertainty of 1,000 or more could be rounded to one significant figure, while compounds employing a factor of uncertainty between 1,000 and 10 could be rounded to two significant figures, and compounds employing a factor of uncertainty of 10 or less could be rounded to three significant figures. But the risk assessment guidelines should allow hazard assessors to use either rounded or originally calculated RELs, as long as only "rounded" or only "originally calculated" numbers are used for all of the RELs employed in the same hazard index evaluation.

For NO_2, the calculated median of a 10- 15 range was 12.5, which was rounded to 10. If OEHHA were to adopt that proposed REL (KEL?) for NO_2 with no added

uncertainty factor, then all three significant figures (that is the 12.5 number) should be retained.

Response. The use of one significant figure is consistent with the practice of USEPA in its RfC program. Since OEHHA adopted many USEPA RfCs as RELs, OEHHA adopted USEPA practice of using one significant figure for chronic RELs. Furthermore, additional figures would not be meaningful given the degree of uncertainty associated with the proposed chronic REL values. The values used in the derivation of the RELs are fully presented, and risk managers using the chronic REL guidance may consider this issue as part of their evaluation of health impacts associated with chemical exposures.

Comment 20. The public and risk managers would benefit if risk assessments differentiated between levels actually associated with adverse effects (Known Effect Levels) and levels deemed necessary to provide added protection (Uncertainty Elimination Levels), and if ambient standard pollutants were evaluated as clearly separated chronic background adders rather than as part of a single initially calculated chronic hazard index.

Response. OEHHA has based its proposed chronic reference exposure levels (RELs) on methods developed by USEPA in its reference concentration (RfC) program. RELs and RfCs are intended as estimates of levels unlikely to result in observable adverse effects among the general public. The REL document presents observed effects data for most chemicals reviewed that represent examples of "known effect levels." These data give a partial picture of potential adverse effects associated with chemical exposure and are presented to inform risk managers and other readers about these observed effects. Direct comparison of various observed effect levels is difficult because of the great variability in the bases for these data. Some are observations among occupationally exposed workers while others are from experimental animal studies. Exposures may be brief, intermittent, or over an entire lifetime. Effects noted may be mild or severe. They may affect a few susceptible subjects or nearly all exposed individuals. The change in severity and incidence in effects observed may be rapid or gradual with increasing exposures. Studies vary in quality and comprehensiveness and some significant adverse effects may go undetected.

Comment 21. OEHHA should reconsider its use of the same factors of ten for each level of uncertainty for compounds with both abundant and sparse exposure data. We believe that lower factors of uncertainty should be used, particularly for naturally occurring substances like formaldehyde for which abundant exposure data exist.

Response. Uncertainty factors of between 1 and 10 were used in the chronic REL document, depending on data quality. From a risk assessment perspective, the natural occurrence of a chemical is not a factor in estimating risks from exposure. An "abundance of exposure data" does not provide information for developing a health-based Reference Exposure Level, unless accompanied by a corresponding study of the

health effects of exposure. The large health effects database for formaldehyde was considered and is reflected in the relatively small 10-fold cumulative uncertainty factor for formaldehyde.

Styrene Information and Research Center

Comments on the chronic REL for **styrene** were made by Jeffrey C. Terry, Manager for State Government Relations of the Styrene Information and Research Center (SIRC). OEHHA recommended use of the US EPA Reference Concentration of 1,000 $\mu g/m^3$ based on neurotoxicity in humans as the chronic REL for styrene.

Comment 1. Effective Exposure Level. SIRC supports the evaluation of the effective exposure level the USEPA included in determining the RfC for styrene in its IRIS review. Mutti et al. concluded that the workers with metabolites of up to 150 mmoles/mole appeared to have no significant effects. SIRC recommends, as OEHHA is adopting the USEPA RfC, that OEHHA not include any discrepancy between its analysis of the effective exposure level and that of the USEPA.

Response. While OEHHA has recommended that the USEPA RfC be adopted as the California chronic inhalation REL, OEHHA is also charged under Health and Safety Code Section 39660(c)1 with providing information on the completeness and quality of the available data. This "discrepancy" relates to determination of a LOAEL or NOAEL for use in the dose response assessment. The "discrepancy" has been disclosed as it reflects an important issue associated with California's adoption of the RfC as a chronic REL.

In the Mutti et al (1984b) study, tests for some individuals in the lowest exposure group did provide abnormal results; and, conversely, tests on some of the individuals in the highest exposed groups did not provide abnormal results. The statement that "workers with metabolites of up to 150 mmoles/mole appeared to have no *significant* effects" has meaning in so far as it pertains to statistical comparisons that bear on the experience of groups, not individuals. The mean exposure for the lowest exposure group was 75 mmoles/mole. The value of 150 mmoles/mole represents the designated upper limit of the exposures for this group. It is the mean value of 75 mmoles/mole, and not the designated upper exposure level of the lowest exposure group, which most accurately represents the exposure history of that group. Therefore, 75 mmoles/mole is the appropriate starting point for dose response assessment.

Comment 2. Uncertainty Factors. SIRC understands the difficulty in assessing the Mutti study. However, USEPA's interpretation of the study by imposing a cumulative uncertainty factor of 30 is appropriate. SIRC disagrees with an UF of 10 for intraspecies variability that OEHHA mentions. SIRC quoted the USEPA's IRIS:

" A partial UF of 3 was used for database inadequacy, including the lack of concentration-response information on respiratory tract effects. A partial UF of 3 instead of 10 was used for intraspecies variability since the lower confidence limit of the exposure extrapolation was used and because Perbellini et al. (1988) demonstrated that this biological exposure index (i.e. urinary metabolites) accounts for differences in pharmacokinetic/physiologic parameters such as alveolar ventilation rate. A partial

UF of 3 instead of 10 was also evoked for lack of information on chronic studies as the average exposure duration of the principal study of Mutti et al. (1984) was not long enough (8.6 years) to be considered chronic. The total uncertainty is therefore 30."

SIRC also disagreed with the OEHHA speculation that potential nutritional differences also supported use of the full intraspecies uncertainty factor of 10.

SIRC requested that the inconsistency with the USEPA RfC analysis be taken out of the OEHHA document.

Response. The USEPA based its rationale for a partial intraspecies uncertainty of 3 on its use of the lower 95 percent confidence limit of its estimate of the central tendency value for the air styrene concentrations predicted from the 150 mmole/mole urinary styrene metabolite concentrations observed in the Mutti et al. (1984) study. The USEPA opined that use of this partial uncertainty factor was justified since the urinary metabolites' biological index took into account differences in pharmacokinetic/physiologic parameters and also because use of the lower 95 per cent confidence limit takes into account some of the intraindividual variation in the toxicokinetics of styrene.

OEHHA does not dispute that the urinary metabolites' biological index takes into account differences in pharmacokinetic/physiologic parameters. If a chronic REL were to be expressed in terms of urinary metabolite levels, a partial uncertainty factor of 3 would be appropriate to the extent that the toxicokinetic contribution to intraindividual variance was substantially eliminated by use of a standard based upon urine metabolite levels. However, here, the chronic REL is expressed in terms of a styrene air concentration, not the concentration of styrene metabolites in the urine. The contribution of toxicokinetics to the overall variance is no longer taken into account when the standard is expressed in units of air styrene concentrations, and not urinary styrene metabolite levels.

The USEPA did, however, use the lower 95 per cent level confidence limit of the airborne styrene concentration associated with 150 mmoles/mole (mmoles styrene metabolite per mole of urinary creatinine) in its dose response assessment. The USEPA stated that the choice of this value took into account some of the population variance due to toxicokinetic differences. This value, which was 88% of the central value, is based upon the *standard error* of a mean value. Therefore, this 95% lower confidence limit is an inadequate measure of the range of individual response characteristics, which relate more reliably to the *standard deviation* of the study population. When sample sizes are large, standard errors especially convey very little information about the standard deviation of the population. The Guillemin et al. (1982) study employed a large study population (N = 90). The USEPA methodology could not capture the variability that it sought to take into account when it selected the 95% lower confidence limit of the air styrene concentration associated with the 150 mmoles/mole styrene metabolite level. Therefore, this approach may not have warranted use of a partial uncertainty factor.

While nutritional factors are known to alter the human response to other chemical species, OEHHA's opinion that an intraspecies uncertainty factor of 10 was preferable did not turn on the issue of malnutrition as a potential contributor to the variability of the human response to styrene. The study on which the RfC was based addressed the effects of styrene on an occupational cohort. Clearly, since the USEPA study is based upon a worker population, the issue of malnutrition was a secondary consideration.

Worker studies, as discussed in the OEHHA draft document, do not capture the variability of the general population, which is to be protected by the chronic REL. Working populations are typically healthier than the general population and also do not share its age distribution. Furthermore, the eligibility criteria of the Mutti et al. (1984) study excluded workers with a variety of diseases. Thus, even if the USEPA methodology had captured the magnitude of the intraindividual toxicokinetic variability of the worker population in Mutti et al. (1984), it could not have adequately captured the toxicokinetic variability of a general population comprised of the elderly and children as well as those with medical conditions.

OEHHA disagrees that mention of the Khanna et al. (1994) study indirectly imposes an interspecies uncertainty factor. No such factor was applied. The Khanna et al. (1994) study raises the potential for a greater than usual intraspecies variability due to the effects of malnutrition. Similar human data are not available. The Khanna et al. (1994) study was discussed in the context of what was the most appropriate *intraspecies* uncertainty factor to use in the risk assessment.

UNOCAL Geothermal

Comments on the **hydrogen sulfide** chronic REL were submitted by UNOCAL Geothermal, which has an operation at the Geysers. The comments are those of their consultant, Dr. Charles Lambert. OEHHA proposed use of the US EPA RfC of 0.7 ppb (0.9 $\mu g/m^3$) as the chronic REL.

Comment 1. After thorough review of the H_2S literature and the OEHHA supporting documentation for the proposed dramatic change in REL, I believe strongly that OEHHA's decision to lower the REL to 0.7 ppb should be revisited. The REL for H_2S should be set no lower than 7.0 ppb. This recommendation is based on the following conclusions: (1) The California ambient air quality standard for H_2S is 30 ppb. (2) The proposed REL of 0.7 ppb is one thousand times lower than the upper concentration for naturally occurring H2S in human breath. (3) Low levels of H_2S are rapidly metabolized and detoxified by the human body and therefore unlikely to be a chronic hazard at concentrations at or below the odor threshold. (4) The extremely conservative safety factors used in deriving the USEPA RfC, on which the new H_2S REL is based, should be decreased by at least an order of magnitude. (5) New studies on the toxicity of H_2S have been published or initiated since the 1994 USEPA reference concentration (RfC) was finalized. OEHHA should wait until all of this updated information is in before finalizing the REL.

Response. The dramatic change referred to in the comment is the change from the 1 hr CAAQS for H_2S of 30 ppb used in the CAPCOA risk assessment guidance (last updated in October 1993) to the proposed use of the USEPA RfC of 0.7 ppb in the Technical Support Document. All USEPA Reference Concentrations (RfCs), available when the Technical Support Document (TSD) on chronic Reference Exposure Levels was drafted in October 1997, are being proposed as chronic RELs. RfCs are already used by the USEPA and by California's Department of Toxic Substances Control and were earlier incorporated by reference in Appendix F of the Emissions Inventory Criteria and Guidelines for the Air Toxics "Hot Spots" Program for use in screening risk assessments in the Hot Spots Program. These Guidelines were effective July 1, 1997. The Risk Assessment Advisory Committee (RAAC) recommended that CalEPA should harmonize where possible with USEPA on risk assessment. Governor's Executive Order W-137-96 concerned the enhancement of consistency and uniformity in risk assessment between Cal EPA and USEPA. Use of RfCs as chronic RELs was one action, which OEHHA took to address the RAAC recommendation and to implement the Executive Order. RfCs released after October 1997, including ones that are revisions of those in the October 1997 draft, will be evaluated for use in the Hot Spots program by reviewing the scientific basis of each RfC when it becomes available and by determining whether the scientific literature cited in the RfC is current. Appropriate RfCs will be submitted yearly to the SRP for review and possible endorsement.

Comment 2. The odor threshold for H_2S is around 10-20 ppb, while the characteristic "rotten egg" odor associated with H_2S can be clearly noted at ambient concentrations of

30-100 ppb (odor recognition threshold). However, it is not until levels in excess of 50 ppm where irritation of the mucous membranes of the eyes and lungs may start to occur. This is an approximate 1,000 fold margin of safety between the odor threshold and signs of toxicity. The concentrations at which health effects begin to occur are well documented and are the basis for current, national, safe exposure limit concentrations. It is not until levels in excess of 50 ppm where irritation of the mucous membranes of the eyes and lungs may start to occur

Response. The national limits are for workplace exposure, not chronic ambient exposure of the general population including sensitive individuals, the target of chronic RELs. OEHHA staff notes that the ACGIH intends to lower the H_2S TLV from 10 to 5 ppm. The assertion that "it is not until levels in excess of 50 ppm where irritation of the mucous membranes of the eyes and lungs may start to occur" is not held by all observers. Concentrations of hydrogen sulfide that substantially exceed the odor threshold result in the annoying and discomforting physiological symptoms of headache or nausea (Amoore, 1985; Reynolds and Kauper 1985). The perceived intensity of the odor of hydrogen sulfide depends on the longevity of the concentration, and the intensity increases 20% for each doubling of the concentration (Amoore, 1985). Several studies have been conducted to establish the ratio of discomforting annoyance threshold to detection threshold for unpleasant odors (Winneke, 1975; Winneke and Kastka, 1977; Hellman and Small, 1974; Adams *et al.*, 1968; and NCASI, 1971). The geometric mean for these studies is 5, indicating that when an unpleasant odor reaches an average concentration of 5 times its detection threshold, the odor will result in annoying discomfort. Applying the 5-fold multiplier to the mean detectable level, 0.008 ppm, results in a mean annoyance threshold of 0.04 ppm. At the current California Ambient Air Quality Standard (CAAQS) of 0.03 ppm, the level would be detectable by 83% of the population and would be discomforting to 40% of the population. These estimates have been substantiated by odor complaints and reports of nausea and headache (Reynolds and Kauper, 1985) at 0.03 ppm H_2S exposures from geyser emissions. The World Health Organization (WHO) reports that in order to avoid substantial complaints about odor annoyance among the exposed population, hydrogen sulfide concentrations should not be allowed to exceed 0.005 ppm (5 ppb or 7 $\mu g/m^3$), with a 30-minute averaging time (WHO, 1987; National Research Council, 1979; Lindvall, 1970). The RfC of 0.9 $\mu g/m^3$ (0.7 ppb) is for a year's averaging time and is within a factor of 8 of WHO's recommendation for 30 minutes. The RfC seems rather low but was the result of following USEPA's documented procedure for developing RfCs which has evolved during the last 10 years and is compatible with WHO's recommendation for a 30 minute acute exposure. The RfC is based on a study in mice in which animals in the LOAEL group had histopathological inflammatory changes in the nasal mucosa, an endpoint compatible with respiratory irritation.

Comment 3. The major human health concern from hydrogen sulfide is from acute exposures in excess of 50 ppm. There are OEHHA acute RELs in place to deal with such exposure scenarios. There is a significant amount of literature documenting chronic human exposure to hydrogen sulfide. There is no convincing evidence that chronic low-level exposure to H_2S at levels around the odor threshold causes adverse health effects. One study of a community in Rotonia, New Zealand (an area of significant geothermal

activity) showed that no chronic health effects could be identified after long-term exposure to 5 to 1,900 ppb H_2S.

Response. If chronic low-level exposure to H_2S at levels around the odor threshold cause no adverse health effects, we should be able to develop a chronic REL based on that data. The referenced study (Siegel, S.M., Penny, P., Siegel, B.Z. et al. (1986) Atmospheric hydrogen sulfide at the Sulfur Bay wildlife area, Lake Rotonia, New Zealand. *Water Air Soil Pollut.* 28:385-391) should be submitted to the USEPA for review. However, as stated above, WHO reports that in order to avoid substantial complaints about odor annoyance among the exposed population, hydrogen sulfide concentrations should not be allowed to exceed 0.005 ppm (5 ppb) for a 30-minute averaging time.

Comment 4. H_2S has been measured in the human breath at levels of 65-698 ppb, and is the result of normal bacterial activity in the digestive tract. H_2S is also produced in various tissues, including the brain where it is thought to function as a neuromodulator, and also acts as a smooth muscle relaxant. Given the rapid metabolism of H_2S and the low levels naturally produced by the body, the lack of observations of toxicity after chronic exposure to low levels is not surprising. Even the work of Bhambhani and Singh cited in the OEHHA supporting documentation found that healthy subjects could safely exercise at their maximum at hydrogen sulfide concentrations of 5 ppm (5000 ppb) H_2S. In later studies, Bhambhani found small but statistically significant changes in oxygen uptake and increase in blood lactate after exposure to 10 ppm (10,000 ppb) H_2S, a physiologic effect, but not an adverse health effect. No subjective symptoms were reported in this study as subjects breathed either 5 or 10 ppm H_2S through a mouthpiece.

Response. Many toxic chemicals are produced by metabolism: CO, acetaldehyde, formate, NO, and H_2S. The chronic RELs are to protect against low-level, involuntary exposures. OEHHA staff are aware of the Bhambhani studies which are generally acute/subacute studies with normal individuals and thus not useful for developing a chronic REL.

Comment 5. The proposed OEHHA REL of 0.7 ppb for H_2S is based on the USEPA reference concentration (RfC) promulgated in 1994. A number of studies on the toxicity of H_2S have since been published or are near completion. This new information should be incorporated into the final REL.

Response. Comment acknowledged. OEHHA's use of RfCs was explained above. OEHHA and hopefully also USEPA will examine the new information for possible incorporation when it becomes available. We have revised our proposed REL as explained in responses to comments from Geyser's Geothermal using the same study as U.S. EPA, but different uncertainty factors. The new proposed REL is 9 $\mu g/m^3$ (7 ppb).

Comment 6. In both the derivations of the RfC and REL a large uncertainty factor of 1000 is used. A large uncertainty factor is only appropriate when there is a paucity of data. This is not the case for H_2S. Based on the significant amount of human and animal data available, this factor should be decreased by at least an order of magnitude.

The derivation of the REL uses a subchronic-to-chronic uncertainty factor of 10. This should be reduced to 3, based on available data suggesting that the types of lesions found in rodents at high sub-chronic exposures are unlikely to progress with longer duration of exposure. Moreover, given the rapid metabolism and detoxification of H_2S, these subchronic rodent studies performed at high H_2S concentrations (much higher than the OEHHA acute level II REL for H_2S) are not relevant to chronic low level human exposures.

Response. OEHHA agree that the uncertainty factor (UF) is large and close to the maximum uncertainty factor used by USEPA of 3,000. We revised our proposed REL as noted above.

Comment 7. The other significant part of the large uncertainty factor is the modifying factor of 3 used for the "lack of reproductive and developmental toxicity data". I believe this modifying factor is unwarranted. The developmental study cited in the OEHHA documentation demonstrates no developmental effects in rats, even at concentrations (150 ppm) high enough to cause slight maternal toxicity. This is clear evidence that H_2S is a compound the body is capable of metabolizing and detoxifying quite rapidly. Additionally, by mid-1998, final reports should be available from the Chemical Industry Institute of Toxicology (CIIT) on the reproductive effects and developmental neurotoxicity of H_2S. No final decision on the REL should be made until these studies are completed.

Response. This suggestion has merit and should be made to USEPA. There are other developmental studies with H_2S by S. Roth and coworkers in which adverse effects on the developing nervous system were seen at 20-25 ppm, the lowest concentration tested. As noted above, we revised our proposed REL.

Comment 8. OEHHA cites the "lack of adequate long-term human exposure data" as a major area of uncertainty and the reason for the conservativeness of the REL. Humans are constantly exposed to low levels of hydrogen sulfide, and life could not continue without the production of hydrogen sulfide. To set the REL one thousand times lower than the upper concentration for naturally occurring H_2S in human breath seems very conservative. OEHHA did not take this type of human data into consideration in its derivation of the REL. OEHHA uses a high uncertainty factor for a chemical for which there is a large amount of good animal and some human data.

If the subchronic-to-chronic uncertainty factor of 10 is reduced to 3, and a very conservative modifying factor removed, the REL uncertainty factor could be lowered by

an order of magnitude to 100. This would put the REL in the 7.0 ppb range, a concentration at the very limits of odor recognition and found naturally in the human body.

Response. This suggestion also has merit and should also be made to USEPA. However, OEHHA is not willing to unilaterally change USEPA uncertainty factors. OEHHA is harmonizing with USEPA where possible.

Comment 9. I hope the foregoing discussion and data, along with the soon-to-be-completed CIIT studies, will be taken into consideration by OEHHA before setting the final REL. The economic impacts of the proposed REL could be enormous throughout California. The final decision on the REL should therefore be made in a collaborative setting with industry. OEHHA has made a great effort to involve input from risk managers and stakeholders throughout the process

Response. Many of the comments above need to be made to the USEPA. OEHHA will certainly review the CIIT studies when they become available. However, based on this comment and other comments about the hydrogen sulfide chronic REL and on OEHHA's own assessment of the developmental toxicity data available including a study on spontaneous abortion published in 1998, OEHHA staff have reviewed the value in the draft document and have calculated a revised chronic REL for hydrogen sulfide of 9 $\mu g/m^3$.

Western States Petroleum Association (WSPA)

Comments on the Determination of Chronic Toxicity Reference Exposure Levels were received from WSPA in a letter dated January 29, 1998. WSPA made comments on the general methodology and on the chronic RELs for **benzene** and **hydrogen sulfide**. WSPA is a trade association representing members engaged in all aspects of the exploration for, and production, refining, transportation and marketing of, petroleum and petroleum products in the western United States.

Comment 1. WSPA is pleased to note the extent to which OEHHA has attempted to harmonize their approach for calculating RELs with that of USEPA. WSPA agrees that uncertainties exist in the characterization and quantification of potential health effects in humans, especially when extrapolating from animal studies. However, the potential to significantly overestimate the likelihood of these effects by compounding uncertainty factors must also be recognized. OEHHA has recognized the utility of pharmacokinetics in tempering the use of uncertainty factors in REL calculations. Indeed, information about the biochemical mechanism of the chosen toxic endpoint in key studies can be extremely important in reducing uncertainties regarding intraspecies and interspecies variability, and the likelihood of enhanced susceptibility based upon age, gender, etc. OEHHA should give greater consideration to the role of mechanistic data in the parent document and in calculating selected RELs.

Although WSPA generally agrees with OEHHA on the basic methodology for calculating inhalation RELs, we find that the choice of key study and the application of the REL methodology give rise to a number of concerns with certain chemical-specific RELs. The attached comments address WSPA's concerns with the proposed REL calculations for benzene and hydrogen sulfide. We understand that the Olefins panel of the Chemical Manufacturers Association (CMA) will submit comments on certain other RELs, some of which are also of interest to WSPA. Specifically, WSPA shares CMA's interest in the proposed RELs for ethylene, 1,3-butadiene and propylene. Rather than duplicate CMA's comments on these chemicals in this submittal, we will instead incorporate their comments herein by reference.

Response. Comment noted. OEHHA used the best study it could find in the medical and toxicological literature prior to the release of the document in October 1997. In the case of isopropanol a superior study appeared in 1997 and has been used to revise the REL. In other cases OEHHA used scientific judgment with which others might not agree. In the case of hydrogen sulfide OEHHA used the USEPA’s chronic REL as part of its effort to harmonize with USEPA. OEHHA has contacted the USEPA to determine if USEPA made an error in calculating the RfC. OEHHA addressed the comments of the Chemical Manufacturers Association - Olefins Panel in a response above.

Comments on General Methodology

Comment 2. OEHHA should not include abstracts of presentations or posters as references in this document (e.g., Alexeeff et al., 1997; Foureman, et al ., 1995; Gillis, et al., 1997; Kadry, et al., 1995; Khodair et al., 1995; Mitchell, et al., 1993; Schmidt et al., 1997 and Swartout, 1997). A number of other citations are to secondary references such as book chapters. The value of such citations is extremely limited since the methodology and conclusions cannot be evaluated. In addition, these studies and their conclusions have not been subject to peer review. OEHHA should not include such citations as supporting documentation for these guidelines.

Similarly, journals from the former Soviet Union are of little use as citations (e.g., Chizikov, 1973). These documents are not available in English and cannot be translated in time to provide the opportunity for comment. In addition the frequent use of nonstandard terminology and the generally poor quality of English used in the abstract translations when present have not been subject to peer review outside of the former Soviet Union as evidenced the studies cited in these journals.

Response. OEHHA has attempted to use the best studies it could find. We would prefer peer-reviewed journal articles for all cases. However this is not always possible. We have used well-conducted, unpublished industry studies for the development of RELs if these had the best data available. USEPA used an unpublished study (at the time of promulgation of the RfC) to develop the RfC for MTBE. OEHHA also used as the basis for different RELs a case report (one individual) published in the peer-reviewed literature, a NIOSH Health Hazard Evaluation, and research institute and government (e.g., NTP) reports. In one case we based a REL on studies published in Russian that had been summarized by NIOSH. But we did not base any REL on just an abstract.

Comment 3. P. 11, Section 1.4, Para. 3: RELs may appropriately be based upon the most sensitive endpoint unless there is data demonstrating that the endpoint in question is not relevant. This will usually not be an issue for RELs based upon human studies. In the case of RELs based upon animal studies, there may be scientifically sound reasons why the most sensitive endpoint in an animal study is not relevant to human populations. These reasons may be based upon biochemical mechanism, metabolic pathways or pharmacokinetics. This possibility should be acknowledged by OEHHA here as it is in Section 2.1.2.

Response. The introduction is only an overview. The elaboration of this specific point has been done in the Hazard Identification section as noted by the comment.

Comment 4. P. 11, Section 1.4, Para 4. Under AB-2588, the Hazard Index is based on estimated exposure levels derived from air dispersion models, not from measurements as stated in the document.

Response. Comment noted. The exposure levels could be derived from measurements, but they rarely are.

Comment 5. P. 12, Section 1.5, Para 1. In the discussion of susceptible sub-populations, the reference to increased exposures should be deleted. Differences in susceptibility are the result of variations in physiological or biochemical processes characteristic of a specific sub-population. Variations in exposure are relevant to risk calculations but should be accounted for as such. Accounting for variations in exposure within a subject population is the subject of a companion technical support document, *Exposure Assessment and Stochastic Analysis.*

Response. OEHHA staff can not totally agree with this suggestion and will not delete reference to exposure differences. At the same external exposure there may be different susceptibilities due to physiological processes such as the increased absorption of ingested lead by children compared to adults and of inhaled pollutants due to the increased breathing rates of children relative to adults.

Comment 6. P. 13, Section 1.6. The last sentence would benefit from some additional clarification.

Response. The last sentence states: "Thus, human exposures of greater than 8 years are not adjusted either in their calculation or application." OEHHA has added text clarifying the concept.

Comment 7. P. 17, Section 2.1.2, Para 2. In the discussion of relevance of animal data to human response, OEHHA should include mechanism of action with pharmacokinetics and metabolism as information useful in selecting the relevant animal model.

Response. OEHHA will include mechanism of action as information useful in selecting the relevant animal model.

Comment 8. P. 17, Section 2.1.2, Para 3. Although useful information may be obtained from studies that may not conform to every detail of sound design or comply with a rigorous application of Good Laboratory Practices, clearly regulatory standards should not be based on poorly designed or executed studies. WSPA hopes that OEHHA would agree that data from studies of questionable scientific validity should only be considered in the calculation of RELs if supported by data from separate valid studies.

Response. OEHHA would prefer to only use studies that conform to every detail of sound design or comply with a rigorous application of Good Laboratory Practices. If such a study is not available for a chemical, we must act to protect public health by using the best data we can find. Hopefully affected parties will be motivated to get better data.

Comment 9. P. 19, Section 3.1.1, Para 2. The first sentence needs to have a reference and more discussion to support the allegation of such a high potential for undetected adverse effects. Clearly the statistical power of a study to detect an incidence of an adverse effect will vary with the size of the study populations. The concern that a relevant endpoint may not have been detected is reasonable if a chronic REL is being calculated from a short term study. WSPA believes that concern is addressed adequately by the inclusion of an uncertainty factor for a less-than-chronic study, especially in the light of numerous other uncertainty factors which are applied because of concerns that *may be true,* but are not known to be true.

Response. The commentator apparently does not agree that the NOAEL may be associated with an incidence of adverse effects of 1 to 20%. One relevant reference is the paper by Leisenring and Ryan (1992) which is given at the end of the paragraph 2. They report that "average risk levels associated with the NOAEL may be substantial." Another reference is Crump (1984) which is cited elsewhere in the introduction. It is true that the sample size in part determines statistical power. That is the basis for the statements in the 2 papers cited.

OEHHA prefers to address the subchronic to chronic differences separately from the LOAEL/NOAEL consideration.

Comment 10. Studies should be evaluated for thoroughness in considering appropriate endpoints before they are used for REL calculation. This is another area in which consideration of pharmacokinetic and mechanistic information can be useful. Increasing the degree of uncertainty adjustment because one can never answer all possible "what if" scenarios is not a sound basis for calculation of useful health based standards.

Response. OEHHA has evaluated all relevant studies for use in the REL calculations. The database for most chemicals is limited. Uncertainty factors are used when insufficient data are available to support the use of chemical-specific and species-specific extrapolation factors. The human intraspecies factor is in many ways a variability factor since humans are known to be variable in response to chemicals. While the default factor of 10 may be too large for some chemicals it is probably not adequate for others (Hattis D. 1996. Variability in susceptibility – how big, how often, for what responses to what agents? Environmental Toxicology and Pharmacology. 2:133-145). As one example, in a study of DNA adducts due to PAHs the interindividual variability was about 24-fold (Dickey C et al. Variability in PAH-DNA adduct measurements in peripheral mononuclear cells: implications for quantitative cancer risk assessment. Risk Anal 1997;17(5):649-656).

Comment 11. P. 20, Para 2. Although WSPA agrees with the general approach in addressing the uncertainty in NOAEL to LOAEL relationships, the comparison of

NOAELs and LOAELs is not as straightforward as implied in the discussion. The ratio of LOAEL to NOAEL will usually be an overestimate. This is unavoidable unless a study has many dose levels. Discussions of population statistics notwithstanding, the study NOAEL will usually underestimate the true value. This is especially true when there is large variation among dose levels. Again, when there are large differences among dose levels it is also likely that the LOAEL determined will be higher than the true value. The portion of the Alexeeff et al., 1997 study that compared LOAELs for serious effects to NOAELs for all effects should not be used to justify large uncertainty corrections, since comparably based parameters were not being considered. The comparison based on mild effects has a firmer theoretical foundation and showed ratios within the 10-fold range. Unfortunately the methodology of this study could not be evaluated for these comments since the reference is to a meeting abstract.

Response. OEHHA agrees that in practice the comparison of NOAELs and LOAELs is not straightforward. Based on a review of the literature by ATES staff the SRP recommended in December 1998 that staff use a LOAEL to NOAEL default adjustment factor of 6 instead of 10 for acute RELs. The Alexeeff et al. (1997) report is available as Appendix F of the final Acute Reference Exposure Level Technical Support Document. ATES has considered whether a LOAEL to NOAEL factor other than 10 is justified for chronic RELs also and has described its approach in Section 3.1.2 of the Introduction. As examples in which a factor other than 10 was used, in the propylene chronic REL developed by OEHHA, after doing the RGDR adjustment, a LOAEL to NOAEL factor of 3 was used due to low severity of the adverse effect. For the sulfuric acid REL OEHHA used 3 due to slight, low incidence adverse effects. Finally, for silver the LOAEL to NOAEL factor was 1 because there were the effects were cosmetic without associated adverse health effects.

OEHHA disagrees with the comment that the LOAEL and NOAEL are generally underestimates. There is no basis for this statement. A NOAEL is sometimes incorrectly viewed as an estimate of a threshold level for adverse effects. However, a NOAEL could be associated with a substantial (1-20%) but undetected incidence of adverse effects among the exposed population, or alternatively it could be lower than a true population threshold (Gaylor DW. Incidence of developmental defects at the no observed adverse effect level (NOAEL). Regul Toxicol Pharmacol 1992 Apr;15(2 Pt 1):151-160; Leisenring W, Ryan L. Statistical properties of the NOAEL. Regul Toxicol Pharmacol 1992 Apr;15(2 Pt 1):161-171).

Comment 12. Page 20, Para 3. WSPA agrees with the OEHHA scheme for use of an intermediate uncertainty factor, but suggests that EPA grade 6 be the cutoff for a low severity effect since by definition the changes have no functional effect on the organism.

Response. OEHHA staff have weighed the cutoff level thoroughly and decided that level 5 was appropriate. Degenerative or necrotic tissue changes are considered serious, even though they are not accompanied by an apparent decrement in organ function.

Comment 13. Page 23, Para 1. It is not clear why 3 + 3 equals 10. OEHHA should provide some justification for adding intermediate uncertainty factors in this manner.

Response. Uncertainty factors are multiplied, not added. The factor of 3 is really 3.16, the square root of 10, which has been rounded to 3. When 2 of these are multiplied together, each is actually the square root of 10 and the product is therefore 10.

Comment 14. P. 27, Section 3.4.1.2, Para 2. OEHHA should justify the use of a 10-fold factor rather than the HEC in the absence of chemical- and species- specific information. There is no thermodynamic reason to expect that the blood:air partition coefficients between species will vary to any great extent. Therefore, unless OEHHA has data to indicate that this is not the case, the calculation of an HEC seems to be warranted.

Response. OEHHA is unable to calculate an HEC in the absence of a blood:air partition coefficient for the specific chemical. Rather than using the HEC adjustment and the lower interspecies UF of 3, the interspecies default value of 10 is used.

Comment 15. P. 28, Section 3.4.2. The use of the Schmidt et al., 1997 reference to justify the default use of a 10-fold uncertainty factor for animal to human extrapolation is inappropriate. This reference is to an abstract and neither the methodology nor the interpretation of the results can be adequately evaluated.

Response. The report of Schmidt et al. is consistent with known data on interspecies uncertainty factors. The report was presented at the 1997 Annual Meeting of the Society of Toxicology. It is unfortunate that there are limited analyses of this type in the peer-reviewed literature.

Comment 16. P. 29, Section 3.5, Para 1. The phenomenon is not indicative of hyper-susceptibility and should not be part of this discussion. Idiosyncratic response refers to a response, which is qualitatively different than that seen in study populations. The true idiosyncratic response does not necessarily occur at doses or exposures below those at which the "normal" response is seen.

Response. OEHHA staff believe that the inclusion of idiosyncratic response is appropriate. According to Casarett and Doull's Toxicology (4th edition, p. 16), the chemical idiosyncratic response "is usually qualitatively similar to that observed in all individuals but may take the form of extreme sensitivity to low doses or extreme insensitivity to high doses of the chemical." In addition, allergic hypersensitivity is also considered by OEHHA to be an idiosyncratic response.

Comment 17. P. 30, Para 1. While the default use of a 10-fold uncertainty factor is consistent with USEPA default methodology, OEHHA should recognize that this is an area in which the consideration of basic mechanisms of toxicity can reduce uncertainty. OEHHA should consider an intermediate uncertainty factor for those chemicals for which the chronic REL is based upon direct irritation as the endpoint. Since direct irritation responses do not involve sources of population variation such as metabolism, pharmacokinetic considerations or enzyme mediated responses, the opportunity for inter-individual variation within a population is much smaller than for systemic effects. An intermediate UF of 3 would be consistent with OEHHA's approach for developing intermediate UFs for LOAEL to NOAEL conversions.

Response. There are a number of studies indicating wide variability in the population in response to irritant chemicals (e.g., formaldehyde). There are no data indicating less variability for irritants than for other toxicants that we are aware of. The commentator presents an attractive hypothesis but without supporting data.

Comments on Specific Chemical RELs: Benzene

For benzene OEHHA developed a chronic REL of 60 $\mu g/m^3$ based on hematologic effect seen in the Tsai et al. (1983) study of 303 male refinery workers.

Comment 18. WSPA takes issue with a number of points in the calculation of the REL for benzene and believes that the REL should be at least 2-fold higher than the OEHHA estimate. While we agree on the choice of toxicological endpoint we disagree with the exposure estimate chosen as the NOAEL in the Tsai et al. study and with some of the supporting documentation for the resultant REL. In addition, we believe that there is biochemical information supporting the use of an UF for intraspecies variation less than the default value of 10.

The Tsai et al. (1983) retrospective epidemiology study of refinery workers exposed to benzene was chosen by OEHHA as the key study and the endpoint of depressed red and white blood cell counts as the critical effect for this REL. An examination of the WBC and RBC counts of a subset of 303 workers, approximately 75% of the total cohort, indicate that all counts, including multiple counts on many workers over the course of their employment in a benzene-exposed job, were within normal limits. The study authors stated that the overall median benzene exposure of this group as determined by personal monitors was 0.53 ppm. OEHHA chose this value as the NOAEL for the group. If the value of the median exposure is 0.53 ppm, then that means that 50% of all exposures were greater than that value. Since Tsai et al. reported that all blood counts were within normal limits, 0.50 ppm is clearly an underestimate of the NOAEL for this group. Tsai et al also determined, as reported by OEHHA, that 85% of all exposures to benzene in their study were less than or equal to 1.0 ppm. It is this latter figure that WSPA believes should be used as the NOAEL for the hematologic effects of benzene in this cohort. Other considerations notwithstanding, making this adjustment would increase the REL by an effective value of 2.

Response The comment is correct in pointing out that Tsai et al. (1983) determined that 84% of all exposures to benzene in their study were less than or equal to 1.0 ppm. This statistic applies to the entire study population. Tsai et al. (1983) reported the central tendency for each of the three reported subgroups as median exposure values: "benzene-related" subgroup - 0.53 ppm; "other benzene" subgroup - 0.24 ppm; and "all others" - 0.07 ppm. The central tendency of a value, and not its upper exposure range, most accurately represents the population exposure history. The benzene chronic REL is based upon the median (half higher/half lower) exposure value (0.53 ppm) reported for the highest exposed subgroup ("benzene-related") without a reported adverse health effect in the Tsai et al. (1983) study.

The chronic REL value is based upon the medical surveillance program results obtained for the subset of workers who worked in the benzene areas. These workers mostly included those assigned to the "benzene related" category (benzene, aromatic distillate hydrogenation (ADH), ethylene, and cumene) with a median exposure of 0.53 ppm. However, workers assigned to the "other benzene" category (pumps, docks) with a median exposure of 0.24 ppm were also included. Therefore, it appears that the choice of 0.53 ppm to represent the median exposure history of the medical surveillance population is likely to be somewhat above the actual median for this group. However, since the "benzene-related" subgroup included in that surveillance population could reasonably be considered to have shown no ill effects since the whole group showed no ill effects, the use of the 0.53 ppm value is justified.

Comment 19. Citing the existence of a healthy worker effect, OEHHA applies the default 10-fold UF for intraspecies variation to the NOAEL adjusted for continuous exposure to calculate the final REL. While acknowledging that a healthy worker effect for mortality or all causes and cardiovascular diseases existed, with regard to those factors known to be important for the hematotoxicity of benzene there is no reason to believe that the variation within the population of the Tsai et al., study was less than in the general population, including the very young and the elderly. To produce a toxic effect, benzene must first be metabolized to active metabolites by cytochrome P-450 system, specifically Cyp2EI (Medinsky et al., 1997). In addition it has also been reported by Martyn Smith (but not yet peer-reviewed) that the enzyme DT-diaphorase, or reductase-NQO 1, is important in the detoxification of quinones such as those generated as metabolites of benzene. In neither case can WSPA imagine a medical condition caused by a variant form (or degree of activity) of either of those enzymes that would disqualify an individual from employment in a refinery and through its omission contribute to a "healthy worker" effect. In other words, there is no reason to believe that the worker population studied by Tsai et al., differed from the general population in these two critical aspects. In fact, if the very young or very old differ from the general population in the activity of Cyp-IIE I, it is most likely in the direction of having lower than average levels of this enzyme, since P-450 mediated metabolism is generally agreed to be decreased in infants and the elderly. In the absence of other plausible bases for the

full 10 fold default intraspecies UF, WSPA believes that an intermediate factor of 3 or 5 could be used and remain health protective of sensitive individuals.

Response. The application of the 10-fold intraspecies uncertainty factor was not predicated upon the existence of a healthy worker effect in the study populations. The parenthetical statement following the listing of the intraspecies uncertainty factor in the derivation section was misleading and will be deleted. The intraspecies uncertainty factor reflects the fact that the study populations typically do not and can not (given study size limitations and the non-random selection of workers pursuant to occupational qualifications and requirements) capture the variability of the general population's susceptibility to toxic injury. The healthy worker effect observed in the Tsai et al. study suggests the presence of prior selection. However, the absence of a healthy worker effect would not demonstrate that the workers were, in fact, representative of the broader population. Here, for instance, the Tsai et al. (1983) study reported the noncancer chronic endpoints only for males.

The Tsai et al. (1983) study also lacked a comparison of the exposed group to matched controls. Thus, although none of the observed hematology parameters were considered clinically abnormal, there was limited ability to detect changes in mean values attributable to exposure. By way of illustration, in the Rothman study[1] which compared a control group to exposed groups, even where the ranges of the reported hematology parameters were similar for control and exposed groups, the mean values for several health effects (e.g., reduced white blood cell counts, reduced absolute lymphocyte counts) were statistically different. Rothman et al. (1996) reported a LOAEL of 7.6 ppm which is just slightly more than 10-times the NOAEL from Tsai et al. (1983). However, even considering the lack of a comparison group, the Tsai et al. (1983) study remains suitable for use in risk assessment. It followed a large number of workers over a long period of time and included up to four hematological tests per year for each worker. However, since the Tsai et al. (1983) study did not compare an exposed to an unexposed population, and especially since it did not provide dose response information, the Tsai et al. (1983) study provides limited information as to population variability. These limitations militate against use of a smaller intraspecies uncertainty factor.

As indicated by the comment, recent research by Rothman et al.[2] (published after the comments were submitted) indicates that known genetic variants in the enzyme pathways which activate benzene (CYP2E1) and then detoxify (NQ01) its metabolites affect the potential to develop benzene poisoning. In this Rothman et al. (1997) case-control study, individuals with benzene poisoning (abnormal blood counts) were 7.8-fold more likely than other workers to have enzyme genotypes which would result in fast activation and slow detoxification. Since the majority of the workers in the case control study were not fast activators and slow detoxifiers, other factors are likely important to the development of benzene poisoning. It is improbable that these enzymes are the only substantial source of variation in the human poisoning response to benzene exposure.

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[1] Rothman et al. American Journal of Industrial Medicine 29:236-246 (1996).
[2] Rothman et al. Cancer Research 37, 2839-2842, 1977

Given uncertainties as to the range of human susceptibility, an uncertainty factor of ten for intraspecies variation is appropriate.

Comment 20. A number of studies have been cited by OEHHA as support for the accuracy of the proposed REL of 0.02 ppm. The Cody et al. (1993) study was mentioned as correlating decreased WC and RBC counts in a subset of the Pliofilm cohort with median, job-specific exposures to benzene in the range of 30 to 54 ppm. For the comparison, the lower value of 30 ppm was selected by OEHHA and a hypothetical REL of 0.01 ppm calculated as if this study had been selected as the critical study. The Cody et al, (1993) study used the Crump and Allen exposure estimates for the Pliofilm cohort as a basis for the 30-54 ppm median exposure estimates. Again, by definition, 50% of the exposure estimates for the subject workers were above 30 ppm, using the Crump and Allen estimates. In addition, the exposure estimates for the Pliofilm cohort calculated by Paustenbach et al. (1992) are significantly higher than those of Crump and Allen for most job categories and years and have been acknowledged as superior by the senior author of the Crump and Allen reference, Crump (1994). Typically, Paustenbach et al. (1992) exposure estimates were 50% to 100% higher than those of Crump & Allen on a job-specific basis. This strongly suggests that the 30 ppm exposure estimate used by OEHHA for Cody et al. (1993) should be considered a significant underestimate of the majority of exposures in that study, and the calculated REL should be revised upward accordingly.

Response. The comment is correct that OEHHA used the 30 ppm value which is the low end of the range of median values (30 – 54 ppm) used in the Cody et al. (1993) study. OEHHA notes this fact in our document. The purpose of the discussion and related dose response calculation from Cody et al. (1993) was to assess our use of the Tsai et al. (1983) free standing NOAEL as the basis for the proposed chronic REL. The dose response analysis of the Cody et al. (1993) data resulted in a derived comparative REL of 0.01 ppm. This value was close enough to the proposed chronic REL of 0.02 ppm to indicate that reliance upon the free standing NOAEL reported for Tsai et al. (1983) study, a study which lacked a control group for comparison, would not yield results which were inconsistent with those which might be derived from the Cody et al. (1993) study. For these purposes, 30 ppm was selected as the most health conservative median value. However, whether the dose response analysis of the Cody et al. (1993) data was based upon the lowest or highest end of the range, or something in between, would not affect our conclusion that the results achieved with either the Cody et al. (1993) or the Tsai et al. (1983) data would be consistent. This conclusion satisfied prudential concerns regarding the selection of a study with a free-standing NOAEL as the key study.

Utterback and Rinsky[3] (1995) have critically evaluated in detail the Paustenbach et al. (1992) reanalyses of the Pliofilm cohort exposures. They reported that the Paustenbach et al. (1992) exposure estimates are greatly skewed by the use of unrealistically large dermal absorption factors and unrealistically large estimates of the exposed body surface area. They also provided substantial evidence also that the

[3] Utterback, David F. and Rinsky, Robert A. American Journal of Industrial Medicine 27:661-676 (1995).

Paustenbach et al. (1992) reanalyses inadequately incorporated the available historical airborne exposure information. Furthermore, Utterback and Rinsky (1995) noted that the Paustenbach et al. (1992) reconstructions entailed exposures which were on the order of 100 to 200 ppm and lasted as long as a decade. Yet, there was no epidemic of hematopoietic disorders which would be expected from chronic exposures to these high doses. Furthermore, given the repeated visits by industrial hygiene inspectors from the State of Ohio to evaluate the benzene exposures, it seems unreasonable to conclude that there were persistent exposures to benzene grossly over the recommended level.

Nevertheless, ultimately the exposure history uncertainties for the Pliofilm cohort favor our use of the Tsai et al. (1983) study for purposes of dose-response assessment. To adjust the proposed chronic REL upward, as suggested by the comment, on the basis of analyses of the Pliofilm cohort would in fact negate the selection of the Tsai et al. (1983) cohort as the basis of the dose response assessment and import the uncertainties associated with analyses of the Pliofilm cohort into the proposed chronic REL.

Comment 21. OEHHA briefly mentions the study of Kipen et al. (1988), continues on to a discussion of the Cody et al. (1993) study and never returns to Kipen. The reason for this is unclear. The Cody study clearly identifies itself as a sequel to the earlier Kipen et al. study which was designed to respond to criticisms of the earlier study (See Hornung et al., 1989). The results of the Kipen et al study were never invalidated and should be more thoroughly considered by OEHHA. That study identified a median exposure for benzene exposed workers subject to WBC & RBC counts as 75 ppm during the early 1940s. The Cody et al. study was limited to workers for whom pre-employment physicals were available as a baseline. The results of the Kipen et al. study indicate that the effect on WBC and RBC counts disappeared after the 1940's when regulatory levels and additional engineering controls decreased benzene exposures below 50 ppm. The longer term of follow-up for workers in this study versus those in the Cody study support the use of a smaller UF for subchronic to chronic extrapolation and support a higher REL than the current proposal.

Response. The Kipen et al. (1988) study reported that as benzene exposure levels fell during the 1940's worker blood counts went up. However, Hornung et al. (1988) challenged the Kipen analysis on the basis that similar temporal trends showed up in preemployment blood samples. Thus, the analyses in Kipen et al. (1988) with respect to the time period in question were subject to confounding. Kipen et al. (1988) did not closely analyze the reported effect of benzene exposure for later periods when exposures were lower and the preemployment blood count trends were stable. However, the Ward et al. (1996) study did apply a more sensitive nested case control methodology to the same study population and reported a relationship between benzene exposure and reduced white blood cell counts whether or not the pre-1947 data was included. Thus, the Ward et al. (1996) study reported an association for exposures occurring after 1947, a time when the pre-employment blood values had stabilized and when the benzene exposures were much reduced and less uncertain. Given, the Ward et al. (1996) study

findings and the primary focus of the Kipen et al. (1993) study on the earlier exposure period, there was no reason to use the Kipen et al. (1993) study as the basis for the REL.

The proposed chronic REL for benzene of 0.02 ppm was derived using a subchronic to chronic uncertainty factor of one. The derivation of the comparative chronic REL from the Cody et al. (1993) study used a subchronic to chronic uncertainty factor of ten. This factor of ten was based upon the fact that the Cody et al. (1993) study focused upon the blood dyscrasias developing over the first year of exposure.

Comment 22. The Ward et al. (1996) study is cited in support of the 0.53 ppm NOAEL chosen by OEHHA. This study analyzed the same data set as that of Kipen et al. (1988) and Cody et al. (1993) with the important exception that the exposure estimates for the individuals from whom the blood count data were obtained were based upon the work of Rinsky et al. (1987). This was the earliest set of exposure estimates for the Pliofilm rubber-worker cohort and used the most simplistic set of exposure assumptions, i.e., that the exposures of the workers in the early 1940s were essentially the same as those in the 1970s. A later exposure estimate was made by Crump and Allen in 1984 for the OSHA benzene risk assessment and is the one used by Cody et al., 1993 cited by OEHHA. The most recent assessment is that of Paustenbach et al. (1992) which has been accepted by Crump as the most thorough and best assessment to date (Crump, 1994). Both the Crump and Allen and the Paustenbach exposure assessments conclude that the workers in this cohort had much higher exposures during the 1940s than estimated by Rinsky et al. (Paustenbach, et al., 1992). Accordingly, the Ward et al. (1996) study cannot be used for accurate quantitative assessment of exposures or any calculations based on those exposures.

Response. The proposed chronic REL is not based upon the Ward et al. (1996) study. Our document briefly describes the Ward et al. (1996) study and concludes that the results are not inconsistent with each other. While the comparisons at issue are directed toward the proposed chronic REL value, the principal value of these comparisons was in assessing the reasonableness of OEHHA's choice of the Tsai et al. (1983) study as the source of the NOAEL value to be used in deriving the chronic REL. This confirmation was of particular importance as the Tsai et al. (1983) study provided a free-standing NOAEL with respect to frank toxicity. Given that the key study lacked a control group for internal comparison, the power to detect an adverse effect based upon a comparison of means was reduced. These factors reduced confidence in null results reported for the Tsai et al. (1983) study. Therefore, OEHHA sought to compare the proposed chronic REL obtained using the Tsai et al. (1983) study to those obtained from other studies which reported LOAELs but not NOAELs.

As stated above, Utterback and Rinsky[4] (1995) have critically evaluated in detail the Paustenbach et al. (1992) reanalyses of the Piofilm cohort exposures. They reported that the Paustenbach et al. (1992) exposure estimates are greatly skewed by the use of unrealistically large dermal absorption factors and unrealistically large estimates of the

[4] Utterback, David F. and Rinsky, Robert A. American Journal of Industrial Medicine 27:661-676 (1995).

exposed body surface area. They also provided substantial evidence that the Paustenbach et al. (1992) reanalyses inadequately incorporated the available historical airborne exposure information. Furthermore, Utterback and Rinsky (1995) noted that the Paustenbach et al. (1992) reconstructions entailed exposures which were on the order of 100 to 200 ppm and lasted as long as a decade. Yet, there was no epidemic of hematopoietic disorders which would be expected from chronic exposures at these high levels. Furthermore, given the repeated visits by industrial hygiene inspectors from the State of Ohio to evaluate the benzene exposures, it seems unreasonable to conclude there were persistent exposures to benzene grossly over the recommended level.

Comment 23. In the general discussion of animal data and in the last paragraph of Page A-63, the work of Farris et al., (1997) is not discussed. Specifically, with respect to the report of Baarson et al., (1984) the Farris study demonstrated that 10 ppm was a NOAEL for any hematologic effects after 8 weeks of inhalation exposure in mice. The response of the study animals was assessed at 1, 2, 4, and 8 weeks at 4 exposure concentrations with recovery groups. Although not 13 weeks in length, the pattern of the data in this study strongly supports 10 ppm as a true NOAEL for benzene in this species.

Response. OEHHA thanks the commentator for pointing out the more recent Farris et al. (1997) reference. The document will be updated to include a summary of the information provided by Farris et al. (1997). The Baarson et al. (1984) study utilized C57Bl mice. The Farris et al. (1997) study utilized $B6C3F_1$ mice. Any differences in the results of these two studies may reflect the difference in the strains used. Baarson et al. reported marked depression in erythropoietic colony formation after 178 days of exposure to benzene. The Baarson et al. (1984) study also reported decreases in circulating red blood cells and lymphocytes at 60 and 178 days of exposure. The NOAEL for a species is determined by the most sensitive strain. Here, C57B1 is the most sensitive strain. Although the pattern of the data in the Farris et al. (1997) study may support 10 ppm as a true NOAEL for benzene in mice (or at least this strain), 8 weeks is not a chronic exposure.

Comment 24. In summary, WSPA believes that evaluation of the full data set, including the most recent animal data, available mechanism-based information and a full assessment of the Pliofilm worker hematologic data supports the assertion that the chronic REL for benzene should be 2- to 6-fold higher than the current proposal.

Response. OEHHA staff appreciate the thoroughness of the comments and hope that we have adequately addressed them. In accordance with the above responses and information, OEHHA continues to prefer the proposed chronic REL.

References cited by commentator for benzene

Farris, G. M., Robinson, S. M., Gaido, K. W., Wong, B. A., Wong, V. A., Hahn, W. P., and Shah, R. S. (1997) Fund. and Appl. Toxicol., 36,119-129.

Paustenbach, D. J., Price, P. S., Ollison, W., Blank. C., Jernigan, J. D., Bass, R. D., and Peterson, H. D. (1992) J. Toxicol. Environ. Health, 36, 177-23 1.

Rinsky, R. A., Smith, A. B., Hornung, R., Filoon, T. J., Young R. J., Okun., A. H., and Landrigan, P. J. (1987) N.E.J. M., 316 1044-1050.

Comments on Specific Chemical RELs: Hydrogen Sulfide

[In the draft TSD OEHHA proposed use of the US EPA RfC of 0.7 ppb (0.9 $\mu g/m^3$) as the chronic REL for hydrogen sulfide.]

Comment 25. OEHHA used the 1994 USEPA IRIS database as the basis for their calculation and documentation. On July 1, 1995, USEPA updated the IRIS database record for H_2S. Additional studies on H_2S have been published or initiated since that date. OEHHA should review this new information and revise their recommendation accordingly.

OEHHA recommended that the inhalation reference exposure level for H_2S should be 0.7 parts-per-billion (0.9 $\mu g/m^3$). The calculation and documentation of this value have numerous flaws. WSPA recommends that OEHHA consider a value between 9 and 30 ppb H_2S as the reference exposure level in California. The California ambient air standard for H_2S is 30 ppb to prevent nuisance odor situations. There is no evidence or expectation of toxicity at this concentration. The odor detection level for H_2S is approximately 9 ppb (AIHA, 1989), although this is highly variable. A reference level between 9 and 30 ppb is adequate to prevent adverse health effects as well as community annoyance due to the odor of H_2S (Amoore, 1985). OEHHA should acknowledge that the basis for a reference exposure level in this range is based on aesthetic (odor) rather than toxicity concerns.

Response. All USEPA Reference Concentrations (RfCs), available when the Technical Support Document (TSD) on chronic Reference Exposure Levels was drafted in October 1997, are being proposed as chronic RELs. RfCs are already used by the USEPA and by California's Department of Toxic Substances Control and were earlier incorporated by reference in Appendix F of the Emissions Inventory Criteria and Guidelines for the Air Toxics "Hot Spots" Program for use in screening risk assessments in the Hot Spots Program. These Guidelines were effective July 1, 1997. The Risk Assessment Advisory Committee (RAAC) recommended that CalEPA harmonize where possible with USEPA on risk assessment. Governor's Executive Order W-137-96 concerned the enhancement of consistency and uniformity in risk assessment between Cal EPA and USEPA. Use of RfCs as chronic RELs was one action that OEHHA took to address the RAAC recommendation and to implement the Executive Order. RfCs released after October 1997, including ones that are revisions of those in the October 1997 draft, will be

evaluated for use in the Hot Spots program by reviewing the scientific basis of each RfC when it becomes available and by determining whether the scientific literature cited in the RfC is current. Appropriate RfCs will be submitted to the SRP for review and possible endorsement. OEHHA also noted that IRIS currently lists the RfC as 1 $\mu g/m^3$.

Comment 26. The 1995 USEPA documentation of their inhalation reference concentration (RfC) contains a numerical error. The summary (Section I.B. 1) reports a total uncertainty factor of 1000 and a modifying factor of 1. In Section I.B.3, USEPA explains, "The uncertainty factor of 1000 reflects a factor of 10 to protect sensitive individuals, a factor of 10 to adjust from subchronic studies to a chronic study, and a factor of 10 for both interspecies conversion and database deficiencies." However, this is contradicted in Section I.B.4 when, after a long discussion about the progression of respiratory irritation from repeated exposure, USEPA reports, "On this basis, the standard uncertainty factor of 10 for subchronic-to-chronic extrapolation is reduced by half to a threefold factor." USEPA obviously forgot to use the 3X uncertainty factor in their final calculation. OEHHA also used this subchronic-to-chronic uncertainty factor of 10. This should be reduced to 3.

Response. OEHHA confirms the finding that the text of the IRIS document contains the reference to reducing the subchronic to chronic UF to 3. This comment should also be directed to the USEPA.

Comment 27. A single uncertainty factor of 10 for both interspecies conversion and database deficiencies is unusual. USEPA typically uses separate values for each item. OEHHA followed suit using 3 for interspecies and 3 for a modifying factor because of the lack of reproductive and developmental toxicity data.

We believe that a modification factor of 3 is unwarranted for several reasons. H_2S is a product of bacterial action in the human body. H_2S concentrations exceeding the proposed RfC have been measured in human mouth air, saliva, and periodontal pockets (Blanchette and Cooper, 1976, Rosenberg et al. 1991, Person, S., 1992, Coil and Tonzetich, 1992). Intestinal gas can contain H_2S far in excess of the proposed RfC (Kirk, E. 1949, EPA 1990, Beauchamp et al. 1984). H_2S is emitted from saltwater marshes, animal waste, landfills, rice fields, and by geothermal activity (EPA 1993). Ambient air concentrations of H_2S from natural sources have been estimated to be 0.11 to 0.33 ppb (EPA 1993). Because of our endogenous production of H_2S and its ubiquitous presence in the environment, there is no reason to use a modifying factor to prevent unforeseen effects at low part-per-billion concentrations.

Response. OEHHA staff agree that a single uncertainty factor of 10 for both interspecies conversion and database deficiencies is unusual. In regard to the modifying factor OEHHA staff generally have not used them in the development of RELs. However, there are many substances produced normally in the body that can be hazardous to other parts of the human organism. The hydrochloric acid in the stomach (present in the stomach as

a normal constituent made by the body rather than bacteria) can etch the enamel from teeth when regurgitated and cause serious respiratory problems when aspirated. The body produces carbon monoxide as a result of heme metabolism. Formate is also produced endogenously. Route of exposure must also be considered; for example, hexavalent chromium and beryllium are much less toxic by the oral route compared to the inhalation route. Finally, the modifying factor of 3 was applied by USEPA because of a deficiency of information on the effects of hydrogen sulfide on development, a database deficiency not necessarily related to our endogenous production of H_2S and its ubiquitous presence in the environment. The estimated ambient air concentrations of H_2S from natural sources between 0.11 and 0.33 ppb are below the RfC of 0.7 ppb. (For comparison ozone is another ubiquitous chemical whose background levels of 0.01 to 0.04 ppm are fairly close to the air quality standards of 0.08 to 0.12 ppm.)

Comment 28. Also, by mid-1998, final reports should be available from ongoing studies on the reproductive effects and developmental neurotoxicity of H_2S. Those studies are sponsored by the American Petroleum Institute and can be forwarded to OEHHA if desired. These studies should directly address the reasons that EPA and OEHHA used a modification factor of 3 to calculate their chronic exposure level.

Response. As of January 1999, the only study that OEHHA has been furnished with is an abstract of a 5 consecutive day exposure study on neurobehavioral and neurochemical effects carried out at the CIIT. This study is unlikely to be the basis for a chronic REL.

Comment 29. We agree with the OEHHA interspecies uncertainty factor of 3. Irritant gases like H_2S have a steep dose-response curve for respiratory effects across species. However, data are available that should significantly change the documentation presented by OEHHA. The citation of Bhambhani and Singh (1991) should be deleted as the results reported were not due to H_2S. A larger and more carefully controlled study by Bhambhani et al. (1994) found no effects in human subjects exposed to 5 ppm H_2S**.** Other studies by Bhambhani et al. (1996 and 1997) in human subjects did observe small but statistically significant changes after exposure to 10 ppm H_2S. OEHHA also cites Bhambhani and Singh, 1985, to suggest, "... either that humans are more sensitive to H_2S, or that the measurements in laboratory animals are too crude to detect subtle measures of irritation." OEHHA should note that Bhambhani and Singh (1985) reported only subjective symptoms. No objective measures of respiratory irritation were done. In the subsequent work in Bhambhani's laboratory, no subjective symptoms are reported in their publications as subjects breathed either 5 or 10 ppm H_2S through a mouthpiece. No objective measurements of respiratory irritation were part of the experimental design.

Response. Bhambhani and Singh (1991) conclude that “healthy young male subjects could safely exercise at their maximum metabolic rates while breathing 5.0 ppm H_2S without experiencing a significant reduction in their maximum physical work capacity during short-term incremental exercise.” Bhambhani et al. (1996) found that exposure to H_2S at 5 ppm “might inhibit aerobic metabolism during exercise in healthy men, thereby

increasing their dependency on anaerobic metabolism." Unfortunately the commentator does not indicate what the results are due to, if not due to H_2S.

OEHHA considers that 3 is appropriate as a subchronic to chronic uncertainty factor for the hydrogen sulfide study used (CIIT, 1983). Also OEHHA does not agree with the U.S. EPA position that there is a lack of data on developmental effects of hydrogen sulfide. In addition to the Saillenfait et al. (1989) study in rats in the chronic REL summary, there are several other developmental studies available by S.H. Roth and colleagues on the effects of hydrogen sulfide on the developing nervous system. Also there is now available an epidemiological study by Xu et al. (1998) which is described in our revised summary. Using these changes OEHHA staff calculate a chronic REL of 9 $\mu g/m^3$ for hydrogen sulfide.

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Nickel Development Institute, NiPERA, and INCO United States, Inc.

Comments on the chronic REL proposed for nickel were made by the Nickel Development Institute, the Nickel Producers Environmental Research Association (NiPERA), and INCO United States, Inc. in a letter dated January 27, 1998. OEHHA proposed a chronic inhalation REL of 0.05 $\mu g/m^3$ for nickel for respiratory system and immune system toxicity.

Introductory comment. The Comments focus on OEHHA's derivation of a noncancer chronic Reference Exposure Level (REL) for nickel and nickel compounds based on the results of a two-year bioassay of nickel sulfate hexahydrate. As explained in the Comments, it is scientifically inappropriate to establish a single REL to be applied to metallic nickel and all nickel compounds. Such an approach ignores the well-documented differences in toxicity among the various forms (or species) of nickel. Accordingly, using OEHHA's methodology, we have calculated separate RELs for nickel sulfate hexahydrate (and other soluble nickel species), nickel oxide (and other insoluble nickel species), and nickel subsulfide. Because they are far and away the predominant nickel species in the ambient air of California, the RELs for nickel sulfate and nickel oxide are the most relevant of the RELs for purposes of the Air Toxics "Hot Spots" Program.

Nickel subsulfide constitutes a negligible fraction of total nickel in the ambient air. Consequently, the REL for nickel subsulfide has no practical relevance under the Air Toxics "Hot Spots" Program. Metallic/elemental nickel also constitutes a negligible percentage of the total nickel to which California residents may be exposed via inhalation, so there is no need to calculate a REL for metallic nickel. Furthermore, there are no inhalation data from which such a REL could be calculated directly. It is clear, however, that metallic nickel is less toxic than the nickel compounds for which RELs can be calculated. Hence, if a REL is set for metallic nickel, it would have to be at least as high or higher than the highest REL for any of these nickel compounds.

Response. OEHHA responds to the substantive points on each issue below.

Comment 1. Establishing a single REL for elemental nickel and all inorganic nickel compounds ignores the importance of speciation in evaluating the toxicity of the different forms of nickel. Each compound or species of a metal has its own physico-chemical properties that dictate how it behaves under a given set of conditions, including interactions with biological organisms. This point holds even if the free metal ion is assumed to be the toxic species, because the different physico-chemical properties of various forms of the metal will largely determine the extent to which the free metal ion can be made bioavailable and delivered to a relevant biological site within an organism.[a]

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[a] See Conard, B., "Is Nickel Safe? A Toxicology Primer," in Pyrometallurgical Operations Environment Vessel Integrity in High-intensity Smelting and Converting

As U.S. EPA has emphasized: "Speciation and associated solubility of metal species ... are key factors that influence the bioavailability of metals" and their "fate, transport, and uptake in various media (e.g., plant tissue, animal tissue) and receptors."[b]

It is not surprising, then, that different forms of nickel exhibit different toxicities. For example, the oral LD50 value for nickel carbonyl is 50 mg/kg body weight; for nickel sulfate hexahydrate, it is 300 mg/kg - while for green nickel oxide and nickel subsulfide, it is >5000 mg/kg.[c] While the LD50 values relate to acute toxicity, speciation also is important in evaluating chronic toxicity and potential carcinogenicity of the various forms of nickel. U.S. EPA, for example, has distinguished among different nickel species for purposes of cancer classifications.[d] So has the American Conference of Governmental Industrial Hygienists ("ACGIH"), which recently adopted three different carcinogen classifications for different nickel species as part of its Threshold Limit Value (TLV) program.[e] Similarly, in the two-year bioassay studies referenced by OEHHA in the TSD, the U.S. National Toxicology Program (NTP) found markedly different results with regard to the potential carcinogenicity of nickel subsulfide, nickel oxide, and nickel sulfate hexahydrate in rats and mice.[f]

Processes. C. Diaz, et al., Editors. Proceedings of the Nickel-Cobalt 97 International Symposium-Vol. 111, August 1997, Sudbury, Ontario.

[b] Hazardous Waste Identification Rule, 60 Fed. Reg. 66344, 66363/1-2 (December 21, 1995).

[c] See Reagan, E.L. Acute oral toxicity study in rats with green high temperature nickel oxide. Journal of the American College of Toxicology 11(6):689, 1992; Reagan, E.L. Acute oral LD(50) study in rats with nickel sulfate hexahydrate. Journal of the American College of Toxicology11(6):685, 1992; Reagan, E.L. Acute oral toxicity study in rats with nickel subsulfide. Journal of the American College of Toxicology 11 (6):691, 1992.

[d] See 51 Fed. Reg. 34135 (September 25, 1986); see also 59 Fed. Reg. 15504 (April 1, 1994) (EPA divides nickel species into four subsets for purposes of setting proposed de minimis emissions levels and establishing toxicity rankings) under a Clean Air Act hazardous air pollutant program.

[e] Insoluble nickel compound and nickel subsulfide were designated Category Al - Confirmed Human Carcinogens. Soluble nickel compounds were placed in Category A4 – Not Classifiable as a Human Carcinogen. Elemental/metallic nickel was placed in Category A5 – Not Suspected as a human Carcinogen. See ACGIH, 1997 TLVs ® and BEIs® at 42-43 (Notice of Intended Changes). These proposed TLV recommendations and carcinogen classifications were ratified as "adopted" values by the ACGIH Board of Directors on November 1, 1997.

[f] Nickel subsulfide showed clear evidence of carcinogenic activity in male and female rats, and no evidence of carcinogenicity in mice of either sex. Nickel oxide showed some evidence of carcinogenic activity in male and female rats but no dose-response between the mid- and high-dose groups, no evidence of carcinogenic activity in male mice, and only equivocal evidence in female mice. Nickel sulfate hexahydrate showed no evidence

Speciation has long been recognized as a critical factor in evaluating the noncancer chronic toxicity of nickel as well. Thus, in setting time-weighted average TLVs for long-term occupational exposure, ACGIH historically differentiated between metallic nickel and insoluble nickel compounds on the one hand (TLV = 1.0 mg Ni/m^3) and soluble nickel compounds on the other (TLV = 0. 1 mg Ni/m^3).[g] The most recent update of the TLVs draws additional distinctions among the different forms of nickel.[h]

A large body of research has been conducted to elucidate the relative respiratory toxicities of the primary nickel compounds in animals. Much of the most relevant recent work in this area has been performed by the same researchers who conducted the NTP bioassays of nickel compounds referenced by OEHHA in the TSD. (Benson et al. 1985; 1986a; 1986b; 1987; 1988; 1989; 1990; and Dunnick, 1989; 1995). These studies have related the toxicity of nickel compounds to their water solubility and subsequent clearance from the respiratory tract, which is most rapid for water soluble nickel (and nickel chloride) followed by nickel subsulfide and nickel oxide.[i]

The observed toxicity for soluble nickel compounds is due to a direct cytotoxic action of the nickel ion, released in the fluid of the alveolus, on the stromal cells in the lung.[j] Insoluble nickel compounds, by contrast, do not readily release nickel ions in the alveolar fluid. Instead, they elicit a large macrophage "hyperplasia," and their toxicity is related to the phagocytosis of the insoluble particles and the subsequent release of nickel

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of carcinogenic activity in male or female rats and no evidence in male or female mice. See 61 Fed. Reg. 66054-66057 (December 16, 1996).

[g] See ACGIH, 1997 TLVs® and BEIs® at 30. The U.S. Department of Labor's Occupational Safety and Health Administration (OSHA) adopted this same distinction for its permissible exposure limits (PELs) in 1989. See 54 Fed. Reg. 2332, 2946 (January 19, 1989). However, the PELs for nickel and nickel compounds, along with hundreds of other updated PELs, were set aside by the U.S. Court of Appeals for the Eleventh Circuit in 1992 for reasons unrelated to the specific values that OSHA had adopted for the different forms of nickel. See AFL-CIO v. OSHA 965 F.2d 962 (11th Cir. 1992).

[h] See ACGIH, 1997 TLVs® and BEIs® at 42 (setting different numerical TLV values for elemental/metallic nickel, insoluble nickel compounds, and soluble nickel compounds/nickel subsulfide). As noted above, these proposed TLVs were ratified as adopted values on November 1, 1997. See note 6, supra.

[i] See Dunnick, J. K., *et al.* Lung toxicity after 13-week inhalation exposure to nickel oxide, nickel subsulfide, or nickel sulfate hexahydrate in F344/N rats and B6C3F1 mice. Fundamental and Applied Toxicol 12: 584-594, (1989)

[j] See Benson, J.M., *et al.* Biochemical responses of rat and mouse lung to inhaled nickel compounds. Toxicology 57(3):255-266 (1989).

ions inside the cell.[k] Nickel subsulfide has been shown to be appreciably more cytotoxic in vivo than nickel oxide.[l] In fact, the toxicity of nickel oxide approximates the particle overload effects seen with compounds such as carbon black rather than the direct stromal or phagocytic cell cytotoxicities seen with soluble nickel compounds and nickel subsulfide, respectively.[m]

Against this background, it is difficult to understand how OEHHA can justify establishing a single REL for elemental nickel and all nickel compounds based on toxicological results for nickel sulfate hexahydrate. OEHHA's apparent rationale for doing so is that in the NTP studies, nickel sulfate hexahydrate, nickel subsulfide, and nickel oxide "all showed similar non-carcinogenic effects in rats and mice.[n] That may well be, but that does not mean they cause the effects by the same mechanism or at the same dose level. In fact, as indicated above, soluble and insoluble nickel compounds exert their toxicity by different mechanisms, and the dose levels for the various compounds in the NTP studies were not the same.[o] Moreover, elemental/metallic nickel was not administered in the NTP studies at all, so there is no basis for concluding that it would produce the same toxic effects and at the same dose as nickel sulfate hexahydrate.

In sum, it is not appropriate to apply a REL derived from rodent studies of nickel sulfate hexahydrate to all other forms of nickel - including insoluble nickel species and metallic nickel. To do so would be like calculating a REL for methanol and applying it to ethanol, ethylene glycol, and all other "alcohols." Just as the various forms of nickel have different acute toxicities and carcinogenic potentials, they have different noncancer chronic toxicity profiles. By assigning a single nickel sulfate-derived REL to elemental nickel and all inorganic nickel compounds, OEHHA has ignored the fundamental importance of speciation in evaluating the potential toxicity of the different forms of nickel. We urge OEHHA to reconsider this overly simplistic approach and to establish species-specific RELs instead.

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[k] See Dunnick, J. K., *et al.* Comparative carcinogenic effects of nickel subsulfide, nickel oxide, or nickel sulfate hexahydrate chronic exposures in the lung. Cancer Res. 55:5251-5256 (1995).

[l] See Benson J.M., *et al.* Comparative acute toxicity of four nickel compounds to F-344 rat lung. Fundamental and Applied Toxicology 7(2):340-347 (1986).

[m] See Dunnick, J. K. *et al.* Comparative carcinogenic effects of nickel subsulfide, nickel oxide, or nickel sulfate hexahydrate chronic exposures in the lung. Cancer Res.55:5251-5256 (1995); Oller A.R., M. Costa, and G. Oberdorster. Carcinogenicity assessment of selected nickel compounds. Toxicol. Appl. Pharmacol. 143(l):152-66 (1997).

[n] See TSD Appendix A at A-538.

[o] In the two-year NTP studies, the LOAEL for nickel sulfate hexahydrate (0.06 mg Ni/m^3) was roughly one-half of the LOAEL for nickel subsulfide (0. 11 mg Ni/m^3) and an order of magnitude lower than the LOAEL for nickel oxide (0. 5 mg Ni/m^3).

Response. The risk assessment for nickel compounds proceeded under a presumption that compounds comprised of the same inorganic element will have somewhat similar health effects and potencies. The presumption does not require that the toxicities be identical and, in fact, acknowledges the existence of differences by selecting the most sensitive effect of the most potent chemical species in order to assure protection over a broad chemical class. All results, including null results, should be evaluated to determine whether they do establish a substantial difference amongst the species in the class. The evidence sufficient to sustain speciation of a substance would correspondingly also sustain the development of any needed, alternative, health-risk guidance values.

In addition, the question of speciation needs to be addressed, not in general, but with respect to the particular health endpoints of concern and the agents to be speciated. Chemical species differences which are material with respect to one mechanism of action or health effect may not be similarly material for another. For example, with respect to inhalation exposures, speciation of a particular form of nickel may be warranted for one effect of concern (e.g., lung irritation) or route of exposure (oral, inhalation) but not another (e.g., lung cancer).

The comment offers several lines of evidence which support the speciation of nickel compounds with respect to the derivation of a noncancer, chronic REL based upon respiratory toxicity. The comment cites several kinds of indirect and direct toxicological evidence (differences amongst nickel compounds with respect to physico-chemical properties, acute oral toxicities, inhalation carcinogenic potential, and respiratory system effects and mechanisms) to support the speciation of nickel compounds in general and with respect to the proposed REL.

With respect to differences in physico-chemical properties, OEHHA agrees that the different nickel species vary greatly (from soluble to insoluble) with respect to their physico-chemical properties. OEHHA also agrees, as discussed in our 1991 risk assessment document[5], that "the different physico-chemical properties of various forms of the metal will largely determine the extent to which the free metal can be made bioavailable and delivered to a relevant biological site within an organism." OEHHA also agrees with the comment that the different nickel species present different acute *oral* toxicities which may relate to their solubility. For instance, insoluble compounds are more likely to simply pass through the digestive tract without absorption.

With respect to inhalation exposures, it is less clear how solubility will affect the lung response. After inhalation, both insoluble and soluble compounds are directly deposited into the lung. It might be presumed that more soluble species will be more quickly removed from the lung and that such compounds would in general be less harmful than the insoluble forms of nickel thought to be retained by the lung. However,

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[5] Office of Environmental Health Hazard Identification, Identification of Nickel and Nickel Compounds as Toxic Air Contaminants. 1991

the NTP non-cancer, chronic inhalation toxicity findings for (insoluble) nickel subsulfide, (insoluble) nickel oxide, and (soluble) nickel sulfate suggest otherwise and do not support such easy interpretations. In fact, much of the discussion of solubility/bioavailability related differences in mechanisms cited by the comment were offered by the original investigators as possible reasons to explain the apparently greater toxicity (carcinogenicity) of the insoluble nickel species in the rat. Here, the same data would be offered to argue for a lesser non-cancer, chronic respiratory toxicity. Clearly, such indirect evidence needs to be particularly interpreted with caution.

The comment cites, as indirect evidence of the merits of its position, that the American Conference of Governmental Industrial Hygienists (ACGIH), the Occupational Safety and Health Administration (OSHA), and the United States Environmental Protection Agency (USEPA) have speciated nickel compounds. With respect to the ACGIH, the ACGIH is a non-governmental body that develops exposure guidelines, which are not regulatory standards, to limit occupational exposures, not environmental exposures. The ACGIH holds that its limits are not to be used for any other purpose including the "evaluation and control of community air pollution nuisances." Not withstanding this distinction, OEHHA in responding below to the major substantive issues has indicated where and why OEHHA differed with the ACGIH approaches.

With respect to OSHA, the current OSHA position reflects the statutorily mandated blanket adoption, upon its creation over 25 years ago, of the ACGIH guidelines existing at that time. The OSHA position on nickel speciation is therefore not independent of the ACGIH.

OSHA in the cited (and overturned) 1989 rulemaking proposed to adopt as its own standards the updated ACGIH guideline values for the various nickel compounds. That effort was a small part of a much larger effort to adopt, with correspondingly minimal critical review, as governmental standards over 400 other occupational exposure guidelines developed by the ACGIH. While that ruling was overturned on general principles which were not "specifically" related to nickel, the findings of the court bar any reasonable inference that the OSHA rulemaking adequately reached the scientific merits as to whether or how nickel compounds might be speciated. The federal court stated in its conclusion "It is clear that the analytical approach used by OSHA in promulgating its revised Air Contaminants Standard is so flawed that it cannot stand. OSHA not only mislabeled this a "generic" rulemaking, but it inappropriately treated it as such. The result of this approach is a set of 428 inadequately supported standards. OSHA has lumped together substances and affected industries and provided such inadequate explanation that it is virtually impossible for a reviewing court to determine if sufficient evidence supports the agency's conclusions. The individual substances discussed in this opinion are merely examples of what is endemic in the Air Contaminants Standard as a whole." [AFL-CIO v. OSHA, 965 F.2d 962]. OSHA simply did not reach the merits of nickel speciation in the overturned effort. Nor, in that effort, did OSHA propose to adopt the recommendations of the National Institute for Occupational Safety and Health (NIOSH) for an occupational Reference Exposure Level of 15 $\mu g/m^3$ for nickel compounds.

The USEPA quotation [60 Fed. Reg. 66344, December 21, 1995] cited by the comment, ”Speciation and associated solubility of metal species … are key factors that influence the bioavailability of metals” and their “fate and uptake in various media (e.g., plant tissue, animal tissue) and receptors,” is uncontroversial as a general proposition (see above). Here, we are concerned with inhalation exposures to nickel compounds and their effects at the site of exposure, the lung. Issues related to environmental fate and transport that often are greatly affected by solubility considerations and that are important to the context of the proffered quotation (hazardous waste regulations) are clearly not involved.

By contrast, the Agency for Toxic Substances Disease Research (ATSDR), in its 1997 document Toxicological Profile for Nickel (updated), recommended a Minimal Risk Level of 0.2 $\mu g/m^3$ for nickel compounds. The ATSDR, in stating that the MRL value (derived from the same NTP nickel sulfate study used by OEHHA in this proposed rulemaking) was most appropriate for use in evaluating the health risks associated with soluble nickel compounds, did not limit its application to only soluble compounds.

Footnote d of the comment states that the USEPA speciated nickel compounds in a Clean Air Act rulemaking [51 Federal Registrar 34135]. However, in that rulemaking, the USEPA relied upon exposure information to exclude nickel carbonyl and nickel subsulfide from its regulatory efforts. It did not address nickel speciation according to relative solubilities.

The question of speciation needs to be addressed, not in general, but with respect to the particular health endpoints of concern, the agents to be speciated, and the route of exposure. With respect to the differential carcinogenicity of nickel compounds by inhalation, OEHHA believes that this evidence is not closely on point as to the non-cancer health effects of nickel compounds. OEHHA also believes that the comment overstates what is known about the relative carcinogenic potential of various nickel species either in animals:

NTP Cancer Bioassay Results in $B6C3F_1$ Mice.

Nickel subsulfide and nickel sulfate each gave null results in the mouse inhalation carcinogenicity bioassays. Nickel oxide provided equivocal results. These results make the mouse results moot with respect to the speciation of these compounds as to their carcinogenic potential.

NTP Lung Cancer Observations in F344/N Rats.

As noted in the comment, nickel sulfate hexahydrate did not cause lung cancers in the F344/N rat by inhalation. Nickel subsulfide did cause lung cancers in rats of both sexes and nickel oxide provided some evidence of lung cancers in rats of both sexes. In accord with the comments, OEHHA accepts these experimental results. However, in stating that the "(NTP) found markedly different results with regard to the potential carcinogenicity of nickel subsulfide, nickel oxide, and nickel sulfate hexahydrate in rats

and mice," the comment gives undue weight to the null results obtained for nickel sulfate given the assay conditions and results. The question as to whether or not the NTP bioassays do reliably distinguish the carcinogenic potency of soluble nickel compounds from the insoluble nickel compounds deserves further examination.

In order to either qualitatively or quantitatively distinguish the carcinogenic potential of nickel sulfate from that of nickel subsulfide and nickel oxide, not only the results, but the conditions, of the NTP bioassays for these compounds need to be compared. The issue turns on the relative meaning of the null result for nickel sulfate. This difference in outcomes for the three nickel species may reflect any of three general possibilities: 1. qualitative differences in effect among the three compounds, 2. a lower potency for nickel sulfate, or 3. the relative power of the different bioassays to detect a carcinogenic effect under their test conditions.

The conditions of the rat bioassay make it difficult to distinguish whether the difference for nickel sulfate represents a qualitative difference in carcinogenic potential, a small quantitative difference in potency, or no difference in potency. The highest exposure concentration for nickel sulfate was equivalent to the lowest exposure concentration for nickel subsulfide (0.11 mg nickel/m^3). The differences in incidence for lung adenomas and carcinomas combined among the various groups at these exposure levels were small: For nickel sulfate, the incidences were 1/54 female rats and 3/55 male rats. For nickel subsulfide the incidences were 6/53 and 6/53. The incidences of lung cancer in the rats exposed to nickel oxide at a level equivalent to 0.5 mg nickel/m^3 were even lower: 0/53 and 1/53 for females and males respectively. Such small differences do not well distinguish the carcinogenic potential of nickel sulfate from nickel subsulfide and, especially, nickel oxide. It is not possible to reliably distinguish between the possibility that the null result for nickel sulfate reflects no carcinogenic potential, a slightly lesser potency than that of nickel subsulfide, or the possibility that the nickel sulfate bioassays lacked the experimental power to detect an effect of nickel sulfate which is of equal magnitude to that of nickel subsulfide.

OEHHA does not fault the NTP bioassay procedures. Nickel sulfate possessed greater pulmonary toxicity than the other two nickel species in the 13-week range finding studies. This toxicity limited the range of exposure levels at which nickel sulfate could be tested and reduced the sensitivity of the bioassay to detect a carcinogenic effect at concentrations of interest. In addition, in the two year bioassay, the toxic responses in the lung of rats and mice exposed by inhalation to nickel sulfate were less severe than those in the lungs of rats and mice exposed to either nickel oxide or nickel subsulfide. Nickel sulfate may not have been tested as close to the maximally tolerated exposures as were the other two compounds.

Nor does analysis of the available lung burden information help with interpreting the differences in results among the three nickel species. Exposure to nickel sulfate (0.11 mg nickel/m^3) was associated with a lung burden of 1-2 μg Ni/g lung at 15 months. Exposure to the insoluble nickel subsulfide at (0.11 mg nickel/m^3) was associated with a lung burden of 4 μg nickel/g lung at 15 months. However, nickel oxide did substantially

accumulate in the rat lung over the course of the two-year bioassay. Therefore, nickel oxide exposure (0.5 mg nickel/m^3) gave a much higher lung burden of about 300 μg nickel/g lung at 15 months. This result, coupled with the equivocal effects found for nickel oxide, suggests that the toxicokinetics of the nickel species can not reliably guide interpretations of the observed differences in response amongst these three species of nickel compounds.

Based upon these uncertainties in the available information, the null result in the nickel sulfate bioassays, when contrasted to the results obtained for nickel subsulfide and nickel oxide, is not sufficient to reliably distinguish, even with respect to the rat lung, the carcinogenic potential of nickel sulfate from either nickel subsulfide or nickel oxide.

OEHHA also believes that the comment overstates what is known about the relative carcinogenic potential of the various nickel species in humans[6,7,8]. OEHHA[9] has similarly determined that the available epidemiological studies do not demonstrate material and substantial differences amongst the nickel species studied as to their potential carcinogenicity for the respiratory tract.

The proposed chronic REL is based upon the 1994 NTP rat inhalation study using nickel sulfate hexahydrate. It is with respect to the respiratory effects observed in that study that the speciation of nickel compounds needs to be addressed. To that end, the

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[6] Report of the International Committee on Nickel Carcinogenesis in Man. Scand. J. Work Environ. Health 16(1):1-82 (1990).

The ICNCM summary position with respect to soluble nickel compounds is found on p.70 of their report: Their conclusion begins "There was strong evidence, primarily based upon the large excesses observed for electrolysis workers of the Kristiansand, Norway refinery that exposure to soluble nickel was associated with increased respiratory cancer risk." In their overall conclusion to the document, the ICNCM report states (p.74, Concluding Remarks, first paragraph) "There was also evidence that soluble nickel exposure increased the risk of these cancers (lung and nasal cancers) and that it may enhance risks associated with exposure to less soluble forms of nickel.

[7] International Agency for Research on Cancer, Nickel and Nickel Compounds, IARC Monographs 49, p. 410.

IARC also evaluated the carcinogenicity of nickel and nickel compounds, and largely based upon the epidemiological evidence assembled by the ICNCM, concluded that there was "sufficient evidence in humans for the carcinogenicity of nickel sulfate, and of the combinations of nickel sulfides and oxides encountered in the nickel refining industry."

[8] This point also speaks to the conclusions drawn by the American Conference of Governmental Industrial Hygienists, and cited by the comment, with respect to speciation of nickel compounds and carcinogenicity. Based upon the NTP bioassay results, the ACGIH concluded, "It is clear that carcinogenicity varies with the form of nickel used."(TLV Recommendation, p.8). Therefore, as the ACGIH sought to address cancer and non-cancer hazards, this conclusion of the ACGIH implicitly influenced their decision to speciate.

[9]Office of Environmental Health Hazard Identification, Identification of Nickel and Nickel Compounds as Toxic Air Contaminants. 1991

comment marshaled the above indirect evidence. This indirect evidence is not determinative to the extent that it is not known how any differences in physico-chemical properties, acute toxicity, or carcinogenicity relate to the chronic respiratory system health effects upon which the REL is based and their particular mechanisms.

B. Direct Evidence Regarding Speciation

OEHHA concurs that the NTP chronic inhalation bioassays provide the most reliable information with respect to the speciation of nickel compounds as to their chronic inhalation health effects. The results to be compared were obtained under very similar laboratory conditions and protocols and also under the auspices of the NTP, which provides assurances that the results for the different species are readily comparable and highly reliable. This information bears directly on dose-response differences amongst nickel species with respect to chronic inhalation injury to the rat lung. Indeed, the experiments were designed with speciation in mind. However, the dose response analyses of the comment (discussed below) gave alternative chronic RELs for (insoluble) nickel oxide (0.33 $\mu g/m^3$) and (soluble) nickel sulfate (0.29 $\mu g/m^3$) that are within 10% of each other. Within the range of scientific uncertainty, the values are not distinguishable and, at the relevant level of one significant digit, in fact, they are the same (0.3 $\mu g/m^3$). Thus, by the comment's analyses, there would be no practical consequences to speciation. Any material differences would also be insubstantial.

However, OEHHA disagrees (see response to Comment 2 below) with the comment's approach to dose response assessment for soluble nickel and continues to believe that OEHHA's proposed value of 0.05 $\mu g/m^3$ is more appropriate. The difference between the value for nickel oxide of 0.33 $\mu g/m^3$ and the value for nickel sulfate of 0.05 $\mu g/m^3$ is over 6-fold and would be clearly substantial regardless of the extent to which the similar respiratory effects of nickel oxide and nickel sulfate are mediated by the same or different mechanisms.[10] Therefore, if the comment's dose response assessment satisfies the OEHHA chronic REL guidelines, it would be appropriate to provide a separate REL for nickel oxide which reflects this difference. OEHHA responds to the comment's derivation of proposed alternative RELs for nickel oxide, nickel subsulfide, and metallic nickel below (see response to comment 3).

Comment 2. In calculating the REL for nickel sulfate hexahydrate, OEHHA made an unwarranted dose adjustment. In calculating the REL for nickel sulfate hexahydrate, OEHHA made an adjustment to translate the 6 hours/day/week intermittent exposure regimen of the rats in the NTP study to a continuous exposure scenario. Such an adjustment is justified for substances whose toxic effects are mainly duration-dependent, rather than concentration-dependent, and that are slowly excreted from the body. A dose

[10] Regardless of expected differences in the efficacy of soluble and insoluble forms of nickel as vehicles for the Ni ion, the *Benson* and *Dunnick* references relied upon by the comment each found that both soluble and insoluble nickel species caused macrophage hyperplasia and chronic active inflammation of the lung in rats and mice.

adjustment of this sort is not appropriate, however, for a substance like nickel sulfate hexahydrate whose effects are mainly concentration-dependent and that is rapidly metabolized and excreted. Thus, as stated by the Agency for Toxic Substances and Disease Registry (ATSDR) in its Guidance for Derivation of Minimal Risk Levels (which correspond in terms of purpose and basic derivation technique to OEHHA's RELs): "When the critical effects are mainly dependent on the exposure concentrations and the substance being tested is rapidly metabolized and/or excreted, dose adjustment is inappropriate."

Response. Many studies have shown that the effects of nickel on the lung are concentration-dependent. Effects such as fibrosis, which are observed only upon long duration exposure, suggest the accumulation of harm over time. These studies do not examine how the duration of any assumed concentration-dependent mediating effects, such as active inflammation, relate to the chronic outcomes.

With respect to inflammation, the data also indicate duration-dependence:

Comparison of the 13-week inhalation study results for nickel sulfate hexahydrate to the 2 year study results with respect to chronic inflammation of the rat lung[11]:

Study duration:		13 weeks		2 years	
Nickel sulfate air concentration (mg/m^3):		0.25	0.5	0.25	0.5
Lung Chronic inflammation					
	Males	0/10	0/10	42/53	46/53
	Females	0/10	0/10	49/52	52/53

These results show an effect of duration of exposure.

Furthermore, given a lung half-life of from one to three days in the rat, the potential for nickel sulfate to concentrate in the rat lung over the course of several days of administration seems clear. Most of the time-adjustment factor relates to the 6-hour/day exposure regimen which entailed a four-fold adjustment factor to reflect that for only one-fourth of the time were the animals exposed each day. The remainder of the adjustment factor represents a $7/5^{ths}$ term to account for the lack of study exposures during weekends. Even if the effects of nickel sulfate on the lung were related solely to the concentration of nickel sulfate in the lung, a time adjustment factor of at least four-fold for partial exposures each day would be appropriate. In addition, in the instance of a lung half-life of one to three days, the smaller seven-fifths adjustment for two day gaps in exposure during the weekends would be appropriate.

This analysis is consistent, in fact, with the ATSDR's own treatment of the nickel sulfate data in the document, Toxicological Profile for Nickel,[12] cited by the comment. There,

[11] National Toxicology Program, Toxicology and Carcinogenesis Studies of Nickel Sulfate Hexahydrate in F333/N Rats and B6C3f1 Mice.

[12] ATSDR, Toxicological Profile for Nickel (September 1997 update), p. 32.

in developing a Minimal Risk Level for nickel compounds, the ATSDR adjusted these nickel exposures by factors of four and seven-fifths to account for the exposure gaps.

Comment 3. Setting RELs for Nickel Oxide, Nickel Subsulfide, and Metallic Nickel. The NTP two-year bioassay results for green nickel oxide and nickel subsulfide can be used to derive RELs for those substances as well. In contrast to nickel sulfate, excretion of these compounds is moderate to slow; hence, a dose adjustment to translate intermittent exposure concentrations into continuous exposure concentrations may be appropriate. In addition, because NOAELs were not identified in those studies, LOAELs must be used to derive the RELs. Consequently, a LOAEL uncertainty factor must be applied as part of the calculation. Under OEHHA's methodology, a LOAEL uncertainty factor of 10 is applied where the adverse effects are severe, and a LOAEL uncertainty factor of 3 is applied when the adverse effects are mild. In the NTP studies, lesions were scored on the following severity scale: 1 = minimal; 2 = mild; 3 = moderate; 4 = marked. Bearing these factors in mind, RELs for nickel oxide and nickel subsulfide may be derived as follows.

A. Nickel Oxide.

In the two-year NTP study, the LOAEL for green nickel oxide based on chronic inflammation in the lung was 0.5 mg Ni/m^3, and the lesions were graded as 1.6 in male rats and 1.7 in female rats, indicating that the inflammation was in the minimal to mild range.[p] Accordingly, under OEHHA's methodology, a LOAEL uncertainty factor of 3 should be applied in calculating the REL for nickel oxide. The resulting value is 0.33 mg Ni/m^3 calculated as follows:

LOAEL:	500 μg Ni/m^3
Exposure continuity:	6 hours/day, 5 days/week
Exposure duration:	104 weeks
Average experimental exposure:	89.5 μg Ni/m^3 (using dose adjustment factor of 0.179)
Human equivalent concentration:	30 μg Ni/m^3 (using RDDR = 0.29)
LOAEL uncertainty factor:	3
Interspecies uncertainty factor:	3
Intraspecies uncertainty factor:	10
Cumulative uncertainty factor:	90
Inhalation REL:	0.29 μg Ni/m^3

[p] See NTP, Toxicology and Carcinogenesis Studies of Nickel Oxide in F344/N Rats and B6C3F1 Mice, Technical Report Series No. 451 (NIH Publication No. 96-3367, July 1996) at 63-64, Table 14. The nickel oxide concentration of 0.62 mg/m^3 was equivalent to 0.5 mg Ni/m^3. See id. at 40.

$$REL = \frac{500\ \mu g\ Ni/m^3 \times 0.179 \times 0.29}{3 \times 3 \times 10} = \frac{30\ \mu g\ Ni/m^3}{90} = 0.33\ \mu g\ Ni/m^3$$

In the absence of additional compound-specific data, it would be reasonable to apply the REL for nickel oxide as a surrogate for other insoluble nickel compounds (e.g., nickel carbonate) and sparingly soluble nickel compounds (e.g., nickel hydroxide) as well.

B. Nickel Subsulfide.

In the two-year NTP study, the LOAEL for nickel subsulfide based on chronic inflammation in the lung was 0. 11 mg Ni/m', and the lesions were graded as 1.8 in male rats and 1. 7 in female rats, indicating that the inflammation was in the minimal to mild range.[q] Accordingly, under OEHHA`s methodology, a LOAEL uncertainty factor of 3 should be applied in calculating the REL for nickel subsulfide. The resulting value is 0.06 μg Ni/m3, calculated as follows:

LOAEL	110 μg Ni/m^3
Exposure continuity:	6 hours/day, 5 days/week
Exposure duration:	104 weeks
Average experimental exposure:	19.7 μg Ni/m^3 (using dose adjustment factor of 0.179)
Human equivalent concentration:	5.7 μg Ni/m^3 (using RDDR = 0.29)
LOAEL uncertainty factor:	3
Interspecies uncertainty factor:	3
Intraspecies uncertainty factor:	10
Cumulative uncertainty factor:	90
Inhalation REL:	0.06 μg Ni/m^3

$$REL = \frac{110\ \mu g\ Ni/m^3 \times 0.179 \times 0.29}{3 \times 3 \times 10} = \frac{5.7\ \mu g\ Ni/m^3}{90} = 0.06\ \mu g\ Ni/m^3$$

Response: OEHHA disagrees with the comment's use of a LOAEL uncertainty factor of three in deriving the nickel oxide REL. In order to use a LOAEL uncertainty factor of three rather than ten, OEHHA requires first that the effect of concern be of slight severity and second that less than half the animals exposed at the LOAEL be affected.[13] These criteria help to insure that the LOAEL is likely to be closer to the NOAEL than otherwise.

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[q] See NTP, Toxicology and Carcinogenesis Studies of Nickel Subsulfide in F344/N Rats and B6C3F, Mice, Technical Report Series No. 453 (NIH Publication No. 96-3369, July 1996) at 63-64, Table 14. The nickel subsulfide concentration of 0. 15 mg/m^3 was equivalent to 0.11 $mgNi/m^3$. See id. at 40.

[13] OEHHA, Cal/EPA. Determination of Chronic Noncancer Reference Exposure Levels, p. 20, October 1997 Draft for Public Comment.

OEHHA agrees with the comment that the pulmonary effects at issue were classified by the NTP as being in the minimal to mild range (1.6 - 1.7) at the LOAEL dose. However, the OEHHA guidance also requires that the effect be observed in less than half the subjects at the LOAEL dose. In the NTP study, for male and female rats combined, the frequencies of chronic lung inflammation were greatly increased for the LOAEL group (105/106) and as compared to the controls (46/105). It could be argued that about one-half the LOAEL incidence reflects a contribution from the background rate and that the background rate should be excluded. If this were done, it would still be found that nearly all the remaining animals (constituting more than half of the original groups) were affected. In addition, if the expected control/background were excluded, it would also be appropriate to correct for their influence on the overall severity score. If this correction were made, an adjusted effect severity score of slightly greater than 2.0 (a mild effect) would result.

Applying the LOAEL uncertainty factor of 10 would result in a proposed REL for nickel oxide of 0.1 μg Ni/m^3. This value is twice the value (0.05 μg Ni/m^3) developed by OEHHA.

The comment stated that its nickel oxide alternative REL of 0.33 μg Ni/m^3 should also be used to regulate other insoluble and partially soluble forms of nickel: "In the absence of additional compound specific data, it would be reasonable to apply the REL for nickel oxide as a surrogate for other insoluble nickel compounds (e.g., nickel carbonate) and sparingly soluble nickel compounds (e.g., nickel hydroxide) as well". OEHHA disagrees. In setting inhalation exposure RELs for groups of compounds, OEHHA uses the most sensitive strain, species, sex, chronic endpoint, and agent for each group of substances. The comment's proposed alternative REL of 0.33 μg/m^3 for nickel oxide is also substantially different from the comment's REL of 0.06 μg/m^3 for (insoluble) nickel subsulfide. Therefore, it would be inappropriate to apply the nickel oxide value to all other "insoluble and sparingly soluble" nickel compounds. The comment's alternative value for nickel subsulfide of 0.06 μg/m^3 would be preferred for all other forms of insoluble nickel compounds[14].

Furthermore, as the value proposed for (insoluble) nickel subsulfide is not substantially different from that proposed by OEHHA for (soluble) nickel sulfate, regardless of any extent to which there are any differences in the mechanisms of action or

[14] This result would not be changed even if the ARB were to exclude nickel subsulfide from regulation on the strength of the comment's assertion that no one was exposed to nickel subsulfide in California. Nickel subsulfide's membership in the set of "insoluble and poorly soluble" nickel compounds is simply a matter of physico-chemical fact, not regulatory fiat. The relevance of the health findings for nickel subsulfide to other members of the "insoluble and poorly soluble" set of nickel compounds stands regardless of where ever else nickel subsulfide might be consigned in a regulatory scheme. The comment also requests that if a REL is to be provided for metallic nickel that the REL be at least as high as that of nickel oxide (0.33 ug/m^3). However, for these reasons, OEHHA also believes that the proposed REL value of 0.05 ug/m^3 should be applied to metallic nickel as well.

effect, there would also be no reason to even further speciate nickel compounds with respect to the chronic REL.[15]

However, for similar reasons to the nickel oxide case, OEHHA also disagrees with the comment's use of a partial LOAEL factor of three in the dose response assessment for nickel subsulfide. OEHHA considers a value of ten to have been more appropriate. The combined incidence of adverse effects for male and female rats in the nickel subsulfide LOAEL group was much greater than that seen in controls with respect to lung fibrosis (98/106 vs. 2/106) and chronic inflammation (104/106 v. 16/106); the average severity of the chronic inflammation in the LOAEL group (2.5) indicated mild to moderate effects. If a LOAEL factor of ten were applied to the nickel subsulfide dose-response assessment, the resulting REL value would be 0.02 ug Ni/m^3.

In developing its response to these comments, OEHHA has identified the following possible REL values:

Form of Nickel	REL Value	Based Upon	Cumulative Safety Factor
Nickel oxide:	0.1 μg Ni/m^3	LOAEL	300
Nickel sulfate:	0.05 μg Ni/m^3	NOAEL	30
Nickel subsulfide	0.02 μg Ni/m^3	LOAEL	300

The above results, which span a four-fold range, are generally consistent with the presumption that compounds comprised of the same inorganic elements will have somewhat similar health effects and potencies. In providing a REL for "Nickel and Nickel Compounds," OEHHA prefers to use the REL value derived from the soluble nickel data, and not the value derived from the nickel subsulfide data. Nickel sulfate and nickel subsulfide produced similar chronic, noncancer pulmonary effects of similar severity. The NOAEL in the nickel sulfate study was below the LOAEL in the nickel subsulfide study. This preference for the REL value derived from the nickel sulfate study data eliminates the additional uncertainty inherent in the use of the nickel subsulfide study data with its higher LOAEL.

However, the results of the NTP studies and these dose response analyses do support the speciation of nickel oxide. The health effects data for nickel oxide indicate that its adverse pulmonary effects were less severe (absence of fibrosis, lower chronic lung inflammation severity scores) at higher doses than the pulmonary effects observed for nickel sulfate and nickel subsulfide. The higher chronic REL value for nickel oxide of 0.1 μg/m^3 reflects these dose response differences. Furthermore, while it is based upon a LOAEL, the lower severity of the adverse health effects at the LOAEL mitigates some of the uncertainty associated with use of a LOAEL rather than a NOAEL. OEHHA therefore concludes that 0.1 μg/m^3 is an appropriate REL for nickel oxide.

[15] This result speaks to the ACGIH's assertion that speciation of nickel is warranted as soluble nickel compounds pose a greater risk of pulmonary inflammation than insoluble compounds (TLV documentation, page 9).

Comment 4. Having calculated a REL for nickel subsulfide, we would like to add an important caveat. The vast preponderance of nickel emitted to the ambient air in California is in the form of nickel sulfate and oxidic nickel.[r] Nickel subsulfide constitutes a negligible fraction of total nickel in the ambient air; thus, the general population in California will have virtually no exposure to nickel subsulfide. Consequently, the REL for nickel subsulfide has no practical relevance under the Air Toxics "Hot Spots" program.

Response. To the extent that the predominant exposures to nickel in California are to oxidic nickel and nickel sulfate, the inclusion and provision of RELs for other nickel compounds in the regulatory scheme is without practical consequences. However, to the extent that the inclusion of other nickel compounds in the regulatory scheme dissuades the substitution of one regulated nickel species by another unregulated species (e.g. nickel sulfate by nickel chloride), the inclusion of these other nickel compounds protects the public health. In addition, to the extent to which there are or may develop exposures to these other forms of nickel, the inclusion of other nickel compounds serves to directly protect the public health.

Comment 5. Metallic nickel has not been the subject of an inhalation toxicology study, so there are no data from which a REL for metallic nickel can be calculated directly. And there really is no reason to establish a REL for metallic nickel because members of the general population (for whom RELs are established) are not exposed to metallic nickel via inhalation. Furthermore, because of its limited bioavailability[s] and the largely negative findings of epidemiological investigations, it is generally accepted that metallic nickel is less toxic than nickel compounds. Thus, ACGIH's recently adopted TLV for elemental/metallic nickel is 15 times higher than the TLVs for soluble nickel compounds and nickel subsulfide and 7 ½ times higher than the TLV for insoluble nickel compounds.[t] Similarly, while the International Committee on Nickel Carcinogenesis in Man (ICNCM) concluded that workplace exposures to certain nickel compounds have been associated with increased risks of lung and nasal cancers, it found no evidence that metallic nickel was associated with increased lung and nasal cancer risks."[u]

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[r] See CARB, Proposed Identification of Nickel As A Toxic Air Contaminant: Technical Support Document Part A (June 199 1) at A-6 through A- 12 and Table 11- 1.

[s] Since elemental/metallic nickel is not soluble in water, it cannot become bioavailable without first undergoing an oxidizing chemical reaction - referred to as corrosion - that produces a different nickel species.

[t] See ACGIH, 1997 TLVs© and BEIs© at 42. As noted above, these proposed TLVs were ratified as adopted values on November 1, 1997. See note 6, supra.

[u] See Report of the ICNCM, 16 Scandinavian J. Work, Environ. & Health, February 1990 at 74. The

In these circumstances, there is no need to set a REL for metallic nickel, and we suggest that none be set. If OEHHA is determined to identify a REL for metallic nickel, however, it seems clear that, even using the most conservative assumptions, the REL should be at least as high - indeed, higher - than the REL for nickel oxide, i.e., equal to or greater than 0.33 μg Ni/m^3. This REL clearly would be far lower than is necessary to protect against any chronic health hazards that might be associated with inhalation exposure to metallic nickel. But, in the absence of relevant studies from which a REL for metallic nickel can be calculated directly, applying the REL for insoluble nickel oxide to insoluble metallic nickel may be viewed as the most logical, if overly conservative, approach - particularly since metallic nickel, as noted above, must undergo an oxidizing reaction before it can become bioavailable.

Response. With respect to speciation of elemental nickel, the comment also states: "Moreover, elemental/metallic nickel was not administered in the NTP studies at all, so there is no basis for concluding that it would produce the same toxic effects and at the same dose as nickel sulfate hexahydrate." The comment is correct, but applies the wrong test. The risk assessment for nickel compounds proceeded under a presumption that compounds comprised of the same inorganic elements will have somewhat similar health effects and potencies. As elemental/metallic nickel was not administered in the NTP studies at all, those studies provide no basis for concluding that elemental/metallic nickel would not produce similar harmful effects.

The comment also correctly points out that the ICNCM study found "no evidence that metallic nickel was associated with increased lung and nasal cancer risks". However, OEHHA disagrees with the comment that this is a reason to not set a chronic REL for metallic nickel. The California chronic RELs are not set to protect against cancer. They are meant to protect against non-cancer, chronic health effects. The lack of carcinogenicity findings therefore is not determinative. Given the absence of comparable inhalation toxicity studies for metallic nickel, this presumption is not overcome as to respiratory effects from inhalation exposures.

With respect to whether to apply a nickel oxide REL value to metallic nickel, while there are physico-chemical differences between metallic nickel and soluble nickel, there are also physico-chemical differences between metallic nickel and insoluble forms of nickel. OEHHA selects the most sensitive effect of the most potent chemical species in order to assure protection over a broad chemical class. Nickel sulfate and nickel subsulfide, and not nickel oxide, are the most potent forms of nickel with respect to inhalation exposures. The nickel sulfate REL value therefore applies to metallic nickel.

International Agency for Research on Cancer (IARC) also has distinguished between metallic nickel and nickel compounds in assigning cancer classifications, with metallic nickel being classified as having a much lower carcinogenic potential than nickel compounds. See IARC Monographs on the Evaluation of Carcinogenic Risks to Humans, Vol. 49 (1990) at 411.

As indicated above, to the extent that the predominant exposures to nickel in California are to oxidic nickel and nickel sulfate, the inclusion and provision of RELs for other nickel compounds in the regulatory scheme is without practical consequences. However, to the extent that the inclusion of other nickel compounds in the regulatory scheme dissuades the substitution of one regulated nickel species by another unregulated species (e.g., nickel sulfate by nickel chloride), the inclusion of these other nickel compounds protects the public health. In addition, to the extent to which there are, or may develop, exposures to these other forms of nickel, the inclusion of other nickel compounds serves to directly protect the public health.

Finally, facilities subject to the Hot Spots program do not speciate their emissions of metals. Without such speciation, different RELs for different forms of nickel would be of toxicological interest but not of practical use in the Hot Spots Program.

SIRC

December 31, 1997

RECEIVED
DEC 31 1997

Dr. William A. Vance
Chronic RELs
Office of Environmental Health Hazard Assessment
301 Capitol Mall, 2nd Floor, Room 205
Sacramento, CA 95814-4327

Re: COMMENTS OF THE STYRENE INFORMATION AND RESEARCH CENTER[1] ON THE OFFICE OF ENVIRONMENTAL HEALTH HAZARD ASSESSMENT TECHNICAL SUPPORT DOCUMENT FOR THE DETERMINATION OF NONCANCER CHRONIC REFERENCE EXPOSURE LEVELS

Dear Dr. Vance:

The Styrene Information and Research Center (SIRC) is a special purpose group of The Society of the Plastics Industry, Inc.

The California Office of Environmental Health Hazard Assessment (OEHHA) is presenting a method for deriving inhalation exposure levels to protect the public from exposures from potentially hazardous substances. The chronic exposure levels are primarily for risk characterization or industrial emissions and incorporate many recommendations of the U.S. Environmental Protection Agency (USEPA) and the National Academy of Sciences (NAS). The purpose of these comments is to provide perspective on the chronic toxicity summary for styrene.

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[1] SIRC is a non-profit organization formed in 1987 to explore the health effects of styrene and act as a liaison between the styrene industry and U.S. and international regulatory agencies in disseminating the results of state-of-the-art research. SIRC's membership includes styrene manufacturers and users representing more than 95% of the industry. SIRC member companies are either directly involved in the manufacturing or processing of styrene monomer or the fabrication of consumer products from derivatives of styrene. SIRC is a special purpose group of The Society of the Plastics Industry, Inc. (SPI).

SPI is a trade association of nearly 2,000 members representing all segments of the plastics industry in the United States. SPI's business units and committees are composed of plastics processors, raw materials suppliers, machinery manufacturers, moldmakers and other industry-related groups and individuals. Founded in 1937, SPI serves as the "voice" of the plastics industry.

The plastics industry is heavily represented in the State of California and contributes significantly to the state economy. There are 2,320 plastics industry facilities which employ over 111,000 people in California. In all, the plastics industry contributed $17.4 billion to the State of California's economy in 1994, the last year data is available.

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SIRC does not contest OEHHA's recommended chronic REL of 0.3 parts per million (ppm) (1mg/m^3) for styrene. Additionally, SIRC does not contest OEHHA's use of USEPA's Reference Concentration (RfC) as the basis for developing styrene chronic REL. SIRC is pleased to have the opportunity to comment on three sections of the chronic toxicity summary for styrene in the Chronic REL technical document.

As OEHHA finalizes the *Noncancer Chronic Reference Exposure Levels* technical document these comments will clarify misstated information on Section VI (*Derivation of the U.S. EPA Reference Concentration*). Also in Section VII (*Analysis of the U.S. EPA RfC for Styrene*), these comments will address the two aspects of the USEPA RfC for styrene for which OEHHA states additional analysis is necessary.

I. Derivation of the US EPA RfC (US EPA, 1996)

The beginning of Section VI on page A – 659 of *OEHHA's Noncancer Chronic Reference Exposure Levels* document contains a table identifying the information used by USEPA to develop the federal reference concentration (RfC) for styrene. Two changes need to be made to the table in order for the table to be accurate. First and foremost, the *Inhalation reference exposure level* should be **0.3 ppm**, not 0.03 ppm. The federal REL is stated correctly throughout the remainder of the document.

Secondly, according to the USEPA's Integrated Risk Information System (IRIS) and based on the study of Mutti *et al* (1984), 22 ppm (94 mg/cu.m.) was judged by USEPA to be a No Observable Exposure Level (NOAEL).[2]

Historically, SIRC has had reservations regarding the adequacy of the Mutti study and believes the USEPA's RfC is biased low, however, because the USEPA uses the Mutti study as the basis for its RfC, SIRC does not object to OEHHA's attempt to be consistent with USEPA.

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[2] USEPA, *Styrene on Integrated Risk Information System*, 1993, page 4.

II. Analysis of the USEPA RfC for Styrene

OEHHA states in the *Noncancer Chronic Reference Exposure Level* technical document that although they recommend USEPA's RfC of 0.3 ppm for styrene, there are two aspects of the RfC which requires additional analysis. The two aspects needing additional analysis are (1) the choice of an effective air styrene level (i.e., LOAEL or NOAEL and the assigned exposure level) and (2) the application of Uncertainty Factors (UF).[3]

A. Effective Exposure Level

Based on the study Mutti *et al* (1984), SIRC supports the evaluation of the effective exposure level the USEPA included in determining the RfC for styrene in its IRIS review. According to USEPA in IRIS,

> "When the results were analyzed using duration of exposure as a covariate, increases in reaction times and a decrease in digit symbol (memory, concentration) were apparent. The only test showing results in the lowest exposure group, short-term verbal memory loss exhibited no concentration-response relationship. The neuropsychological results from this study are from established tests for CNS dysfunction, are present when compared against a stringently matched control population, and show concentration-response relationships. Also, the deficiencies noted in the reaction times corroborate the results presented by Moller *et al.* (1990) and others discussed below.
>
> The concentration-response relationship between urinary metabolite concentration (mandelic acid and phenylglyoxylic acid levels normalized to creatinine in "morning-after" urine) and test results indicated a significant effect level in the subgroup whose urine contained 150-299 mmole urinary metabolites/mole creatinine. Workers with metabolite concentrations of up to150 mmoles/mole appeared to have no significant effects, and this level is therefore designated as the NOAEL in this study. The authors state that this level of urinary metabolites corresponds to a mean daily 8-hour exposure to air styrene of 25 ppm (106 mg/cu.m). Derivation of this air level is from the creatinine-normalized, combined concentration of the styrene metabolites, MA and PGA, in urine collected from the workers on

[3] Office of Environmental Health Hazard Assessment, *Draft Technical Support Document for the Determination of Noncancer Chronic Reference Exposure Levels*, October 1997, page A – 660.

> Saturday mornings. Guillemin *et al.* (1982) demonstrated a logarithmic relationship (r = 0.871) between the summation of urinary metabolites (MA + PGA, next morning) and air concentrations of styrene (ppm x hours). Guillemin calculated the mean combined urinary metabolite concentration (next morning) for an 8-hour exposure to 100 ppm. This relationship was used by both Mutti *et al.* (1984) and Guillemin and Berode (1988) in a proportional manner to obtain styrene air levels at lower urinary metabolite concentrations. The 95% confidence interval was also calculated for an 8-hour exposure at 100 ppm, the lower limit of the confidence calculation being 88% of the mean styrene exposure. This factor was applied directly to the NOAEL of 25 ppm [25 ppm x 0.88 = 22 ppm (94 mg/cu.m)]. Due to the construction of the subgroups, designation of a LOAEL was the lower limit of the subgroup in which adverse effects were observed [i.e., greater than the NOAEL of 22 ppm (94 mg/cu.m)]."[4]

Mutti and colleagues conclusions stated that the workers with metabolite concentrations of up to 150 mmoles/mole appeared to have no significant effects.

B. Uncertainty Factors (UF)

SIRC understands the difficulty in assessing the Mutti study, however, USEPA's interpretation of the study by imposing a cumulative uncertainty factor of 30 is appropriate. USEPA addresses the reasoning for the UF in IRIS:

> "A partial UF of 3 was used for data base inadequacy, including the lack of concentration-response information on respiratory tract effects. A partial UF of 3 instead of 10 was used for intraspecies variability since the lower 95% confidence limit of the exposure extrapolation was used and because Perbellini *et al.* (1988) demonstrated that this biological exposure index (i.e., urinary metabolites) accounts for differences in pharmacokinetic/ physiologic parameters such as alveolar ventilation rate. A partial UF of 3 instead of 10 was also evoked for lack of information on chronic studies as the average exposure duration of the principal study of Mutti *et al.* (1984) was not long enough (8.6 years) to be considered chronic. The total uncertainty is therefore 30 (three times the one-half logarithm of 10)."[5]

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[4] See footnote 2.
[5] Ibid., page 5.

SIRC disagrees with a UF of 10 for intraspecies variability that OEHHA mentions in its document largely on the points that are discussed in the above excerpt from IRIS. Additionally, OEHHA suggests a potential increase in the UF (from 3 to 10) for intraspecies variability based on synergistic action of styrene on the central nervous system of a population that is malnourished. OEHHA basis the argument on a study that suggests such effects of ingested styrene on rats fed low protein diets (Khanna *et al.* 1994). In linking the two potential effects, OEHHA is comparing apples and oranges. Ingested styrene entails an entirely different mode of action into the body and is addressed by the federal reference dose (RfD). USEPA used the Mutti study because it is a reasonably good study that focuses on worker exposures, that has consistent results to other worker exposure studies, and because it is a human study, there is no need to impose interspecies variability and subchronic uncertainty factors. By linking the Khanna study with the Mutti work, OEHHA is indirectly imposing an interspecies variability uncertainty factor to reflect a potential decrease in the RfC to 0.035 ppm. SIRC does not support OEHHA's interpretation of 0.035 ppm by incorporating the Khanna *et al* (1994) study into the state document, and because OEHHA is not recommending 0.035 ppm as the California chronic REL for styrene, it should be taken out of the document.

III. Conclusion

SIRC does not contest OEHHA's recommended chronic REL of 0.3 parts per million (ppm) (1mg/m^3) for styrene. Additionally, SIRC does not contest OEHHA's use of USEPA's Reference Concentration (RfC) as the basis for developing styrene chronic REL. It is recommended that OEHHA make the appropriate changes to the Section VI table on page A-659 of its *Technical Support Document for the Determination of Noncancer Chronic Reference Exposure Levels*, as identified above in section II.A of these comments.

SIRC does, however, recommend that because OEHHA is not recommending a chronic REL for styrene based on the analysis illustrated on page A-662 of its technical document, therefore the analysis regarding its discrepancy with the USEPA's RfC should be taken out of the document.

SIRC will be more than happy to supply additional comments or schedule discussions with OEHHA in the future.

Respectfully submitted,

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Comments on the chronic REL for **styrene** were made by Jeffrey C. Terry, Manager for State Government Relations of the Styrene Information and Research Center (SIRC). OEHHA recommended use of the US EPA Reference Concentration of 1,000 $\mu g/m^3$ based on neurotoxicity in humans as the chronic REL for styrene.

Comment 1. Effective Exposure Level. SIRC supports the evaluation of the effective exposure level the USEPA included in determining the RfC for styrene in its IRIS review. Mutti et al. concluded that the workers with metabolites of up to 150 mmoles/mole appeared to have no significant effects. SIRC recommends, as OEHHA is adopting the USEPA RfC, that OEHHA not include any discrepancy between its analysis of the effective exposure level and that of the USEPA.

Response: While OEHHA has recommended that the USEPA RfC be adopted as the California chronic inhalation REL, OEHHA is also charged under Health and Safety Code Section 39660(c)1 with providing information on the completeness and quality of the available data. This "discrepancy" relates to determination of a LOAEL or NOAEL for use in the dose response assessment. The "discrepancy" has been disclosed as it reflects an important issue associated with California's adoption of the RfC as a chronic REL.

In the Mutti et al (1984b) study, tests for some individuals in the lowest exposure group did provide abnormal results; and, conversely, tests on some of the individuals in the highest exposed groups did not provide abnormal results. The statement that "workers with metabolites of up to 150 mmoles/mole appeared to have no *significant* effects" has meaning in so far as it pertains to statistical comparisons that bear on the experience of groups, not individuals. The mean exposure for the lowest exposure group was 75 mmoles/mole. The value of 150 mmoles/mole represents the designated upper limit of the exposures for this group. It is the mean value of 75 mmoles/mole, and not the designated upper exposure level of the lowest exposure group, which most accurately represents the exposure history of that group. Therefore, 75 mmoles/mole is the appropriate starting point for dose response assessment.

Comment 2. Uncertainty Factors. SIRC understands the difficulty in assessing the Mutti study. However, USEPA's interpretation of the study by imposing a cumulative uncertainty factor of 30 is appropriate. SIRC disagrees with an UF of 10 for intraspecies variability that OEHHA mentions. SIRC quoted the USEPA's IRIS:

> " A partial UF of 3 was used for database inadequacy, including the lack of concentration-response information on respiratory tract effects. A partial UF of 3 instead of 10 was used for intraspecies variability since the lower confidence limit of the exposure extrapolation was used and because Perbellini et al. (1988) demonstrated that this biological exposure index (i.e. urinary metabolites) accounts for differences in pharmacokinetic/physiologic parameters such as alveolar ventilation rate. A partial UF of 3 instead of 10 was also evoked for lack of information on chronic studies as the

average exposure duration of the principal study of Mutti et al. (1984) was not long enough (8.6 years) to be considered chronic. The total uncertainty is therefore 30."

SIRC also disagreed with the OEHHA speculation that potential nutritional differences also supported use of the full intraspecies uncertainty factor of 10.

SIRC requested that the inconsistency with the USEPA RfC analysis be taken out of the OEHHA document.

Response: The USEPA based its rationale for a partial intraspecies uncertainty of 3 on its use of the lower 95 percent confidence limit of its estimate of the central tendency value for the air styrene concentrations predicted from the 150 mmole/mole urinary styrene metabolite concentrations observed in the Mutti et al. (1984) study. The USEPA opined that use of this partial uncertainty factor was justified since the urinary metabolites' biological index took into account differences in pharmacokinetic/physiologic parameters and also because use of the lower 95 per cent confidence limit takes into account some of the intraindividual variation in the toxicokinetics of styrene.

OEHHA does not dispute that the urinary metabolites' biological index takes into account differences in pharmacokinetic/physiologic parameters. If a chronic REL were to be expressed in terms of urinary metabolite levels, a partial uncertainty factor of 3 would be appropriate to the extent that the toxicokinetic contribution to intraindividual variance was substantially eliminated by use of a standard based upon urine metabolite levels. However, here, the chronic REL is expressed in terms of a styrene air concentration, not the concentration of styrene metabolites in the urine. The contribution of toxicokinetics to the overall variance is no longer taken into account when the standard is expressed in units of air styrene concentrations, and not urinary styrene metabolite levels.

The USEPA did, however, use the lower 95 per cent level confidence limit of the airborne styrene concentration associated with 150 mmoles/mole (mmoles styrene metabolite per mole of urinary creatinine) in its dose response assessment. The USEPA stated that the choice of this value took into account some of the population variance due to toxicokinetic differences. This value, which was 88% of the central value, is based upon the *standard error* of a mean value. Therefore, this 95% lower confidence limit is an inadequate measure of the range of individual response characteristics, which relate more reliably to the *standard deviation* of the study population. When sample sizes are large, standard errors especially convey very little information about the standard deviation of the population. The Guillemin et al. (1982) study employed a large study population (N = 90). The USEPA methodology could not capture the variability that it sought to take into account when it selected the 95% lower confidence limit of the air styrene concentration associated with the 150 mmoles/mole styrene metabolite level. Therefore, this approach may not have warranted use of a partial uncertainty factor.

While nutritional factors are known to alter the human response to other chemical species, OEHHA's opinion that an intraspecies uncertainty factor of 10 was preferable did not turn on the issue of malnutrition as a potential contributor to the variability of the human response to styrene. The study on which the RfC was based addressed the effects of styrene

on an occupational cohort. Clearly, since the USEPA study is based upon a worker population, the issue of malnutrition was a secondary consideration.

Worker studies, as discussed in the OEHHA draft document, do not capture the variability of the general population, which is to be protected by the chronic REL. Working populations are typically healthier than the general population and also do not share its age distribution. Furthermore, the eligibility criteria of the Mutti et al. (1984) study excluded workers with a variety of diseases. Thus, even if the USEPA methodology had captured the magnitude of the intraindividual toxicokinetic variability of the worker population in Mutti et al. (1984), it could not have adequately captured the toxicokinetic variability of a general population comprised of the elderly and children as well as those with medical conditions.

OEHHA disagrees that mention of the Khanna et al. (1994) study indirectly imposes an interspecies uncertainty factor. No such factor was applied. The Khanna et al. (1994) study raises the potential for a greater than usual intraspecies variability due to the effects of malnutrition. Similar human data are not available. The Khanna et al. (1994) study was discussed in the context of what was the most appropriate *intraspecies* uncertainty factor to use in the risk assessment.