

Appendix D.3 Chronic RELs and toxicity summaries using the previous version of the Hot Spots Risk Assessment guidelines (OEHHA 1999)

Noncancer chronic Reference Exposure Levels determined using previous methodology

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Noncancer chronic Reference Exposure Levels determined using previous methodology

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Table of Chronic RELs determined using the previous Hot Spots Risk Assessment Guidelines (OEHHA 1999)

Substance (CAS #)	Listed in CAPCOA (1993)	Chronic Inhalation REL ($\mu\text{g}/\text{m}^3$)	Oral REL (mg/kg Body Weight)	Hazard Index Target Organs	Human Data
Acrylonitrile (107-13-1)	<input checked="" type="checkbox"/>	5		Respiratory system	
Ammonia (7664-41-7)		200		Respiratory system	
Benzene (71-43-2)		60		Hematopoietic system; development; nervous system	
Beryllium (7440-41-7) and beryllium compounds	<input checked="" type="checkbox"/>	0.007	0.002	Respiratory system; immune system	<input checked="" type="checkbox"/>
Butadiene (106-99-0)		20		Reproductive system	
Cadmium (7440-43-9) & cadmium compounds	<input checked="" type="checkbox"/>	0.02	0.0005	Kidney; respiratory system	<input checked="" type="checkbox"/>
Carbon tetrachloride (56-23-5)	<input checked="" type="checkbox"/>	40		Alimentary system; development; nervous system	
Carbon disulfide (75-15-0)		800		Nervous system; reproductive system	<input checked="" type="checkbox"/>
Chlorinated dioxins (1746-01-6) & dibenzofurans (5120-73-19)		0.00004	1×10^{-8}	Alimentary system (liver); reproductive system; development; endocrine system; respiratory system; hematopoietic system	
Chlorine (7782-50-5)		0.2		Respiratory system	

Table of Chronic RELs determined using the previous Hot Spots Risk Assessment Guidelines (OEHHA 1999)

Substance (CAS #)	Listed in CAPCOA (1993)	Chronic Inhalation REL ($\mu\text{g}/\text{m}^3$)	Oral REL (mg/kg Body Weight)	Hazard Index Target Organs	Human Data
Chlorine dioxide (10049-04-4)		0.6		Respiratory system	
Chlorobenzene (108-90-7)	<input checked="" type="checkbox"/>	1000		Alimentary system; kidney; reproductive system	
Chloroform (67-66-3)	<input checked="" type="checkbox"/>	300		Alimentary system; kidney; development	
Chloropicrin (76-06-2)	<input checked="" type="checkbox"/>	0.4		Respiratory system	
Chromium hexavalent: soluble except chromic trioxide	<input checked="" type="checkbox"/>	0.2	0.02	Respiratory system	
Chromic trioxide (as chromic acid mist)	<input checked="" type="checkbox"/>	0.002		Respiratory system	<input checked="" type="checkbox"/>
Cresol mixtures (1319-77-3)	<input checked="" type="checkbox"/>	600		Nervous system	
Dichlorobenzene (1,4-) (106-46-7)	<input checked="" type="checkbox"/>	800		Nervous system; respiratory system; alimentary system; kidney	
Dichloroethylene (1,1) (75-35-4)	<input checked="" type="checkbox"/>	70		Alimentary system	
Diesel Exhaust*		5		Respiratory system	
Diethanolamine (111-42-2)		3		Cardiovascular system; nervous system	
Dimethylformamide (N,N-) (68-12-2)		80		Alimentary system ; respiratory system	<input checked="" type="checkbox"/>

Table of Chronic RELs determined using the previous Hot Spots Risk Assessment Guidelines (OEHHA 1999)

Substance (CAS #)	Listed in CAPCOA (1993)	Chronic Inhalation REL ($\mu\text{g}/\text{m}^3$)	Oral REL (mg/kg Body Weight)	Hazard Index Target Organs	Human Data
Dioxane (1,4-) (123-91-1)	.	3,000		Alimentary system; kidney; cardiovascular system	
Epichlorohydrin (106-89-8)	<input checked="" type="checkbox"/>	3		Respiratory system; eyes	
Epoxybutane (1,2-) (106-88-7)		20		Respiratory system; cardiovascular system	
Ethylbenzene (100-41-4)		2,000		Development; alimentary system (liver); kidney; endocrine system	
Ethyl chloride (75-00-3)	.	30,000		Development; alimentary system	
Ethylene dibromide (106-93-4)	<input checked="" type="checkbox"/>	0.8		Reproductive system	<input checked="" type="checkbox"/>
Ethylene dichloride (107-06-2)	<input checked="" type="checkbox"/>	400		Alimentary system (liver)	
Ethylene glycol (107-21-1)		400		Respiratory system; kidney; development	<input checked="" type="checkbox"/>
Ethylene glycol monoethyl ether (110-80-5)	.	70		Reproductive system; hematopoietic system	
Ethylene glycol monoethyl ether acetate (111-15-9)	.	300		Development	
Ethylene glycol monomethyl ether (109-86-4)	.	60		Reproductive system	
Ethylene glycol monomethyl ether acetate (110-49-6)	.	90		Reproductive system	

Table of Chronic RELs determined using the previous Hot Spots Risk Assessment Guidelines (OEHHA 1999)

Substance (CAS #)	Listed in CAPCOA (1993)	Chronic Inhalation REL ($\mu\text{g}/\text{m}^3$)	Oral REL (mg/kg Body Weight)	Hazard Index Target Organs	Human Data
Ethylene oxide (75-21-8)	<input checked="" type="checkbox"/>	30		Nervous system	
Fluoride including Hydrogen Fluoride		13 F 14 HF	0.04	Bone and teeth; respiratory system	<input checked="" type="checkbox"/>
Glutaraldehyde (111-30-8)	<input checked="" type="checkbox"/>	0.08		Respiratory system	
Hexane (n-) (110-54-3)		7000		Nervous system	
Hydrazine (302-01-2)	<input checked="" type="checkbox"/>	0.2		Alimentary system; endocrine system	
Hydrogen chloride (7647-01-0)		9		Respiratory system	
Hydrogen cyanide (74-90-8)		9		Nervous system; endocrine system; cardiovascular system	<input checked="" type="checkbox"/>
Hydrogen sulfide (7783-06-4)		10		Respiratory system	
Isophorone (78-59-1)		2000		Development; liver	
Isopropanol (67-63-0)		7,000		Kidney; development	
Maleic anhydride (108-31-6)	<input checked="" type="checkbox"/>	0.7		Respiratory system	
Methanol (67-56-1)		4,000		Development	
Methyl bromide (74-83-9)		5		Respiratory system; nervous system; development	
Methyl chloroform (71-55-6)		1,000		Nervous system	

Table of Chronic RELs determined using the previous Hot Spots Risk Assessment Guidelines (OEHHA 1999)

Substance (CAS #)	Listed in CAPCOA (1993)	Chronic Inhalation REL ($\mu\text{g}/\text{m}^3$)	Oral REL (mg/kg Body Weight)	Hazard Index Target Organs	Human Data
Methyl isocyanate (624-83-9)		1		Respiratory system; reproductive system	
Methyl t-butyl ether (1634-04-4)		8,000		Kidney; eyes; alimentary system (liver)	
Methylene chloride (75-09-2)		400		Cardiovascular system; nervous system	
Methylene dianiline (4,4'-) (101-77-9)	<input checked="" type="checkbox"/>	20		Eyes; alimentary system (hepatotoxicity)	
Methylene Diphenyl Isocyanate (101-68-8)		0.7		Respiratory system	
Naphthalene (91-20-3)		9		Respiratory system	
Nickel & compounds (except nickel oxide)		0.05	0.05	Respiratory system; hematopoietic system	
Nickel oxide (1313-99-1)		0.1	?	Respiratory system; hematopoietic system	
Phenol (108-95-2)		200		Alimentary system; cardiovascular system; kidney; nervous system	
Phosphine (7803-51-2)	<input checked="" type="checkbox"/>	0.8		Respiratory system; alimentary system; nervous system; kidney; hematopoietic system	

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Substance (CAS #)	Listed in CAPCOA (1993)	Chronic Inhalation REL ($\mu\text{g}/\text{m}^3$)	Oral REL (mg/kg Body Weight)	Hazard Index Target Organs	Human Data
Phosphoric acid (7664-38-2)		7		Respiratory system	
Phthalic anhydride (85-44-9)	<input checked="" type="checkbox"/>	20		Respiratory system	<input checked="" type="checkbox"/>
Propylene (115-07-1)		3,000		Respiratory system	
Propylene glycol monomethyl ether (107-98-2)		7,000		Alimentary system (liver)	
Propylene oxide (75-56-9)	<input checked="" type="checkbox"/>	30		Respiratory system	
Selenium and selenium compounds (other than hydrogen selenide)	<input checked="" type="checkbox"/>	20	0.005	Alimentary system; cardiovascular system; nervous system	<input checked="" type="checkbox"/>
Silica (crystalline, respirable)		3		Respiratory system	<input checked="" type="checkbox"/>
Styrene (100-42-5)	<input checked="" type="checkbox"/>	900		Nervous system	<input checked="" type="checkbox"/>
Sulfuric acid (7664-93-9)		1		Respiratory system	
Tetrachloroethylene* (perchloroethylene) (127-18-4)	<input checked="" type="checkbox"/>	35		Kidney; alimentary system (liver)	
Toluene (108-88-3)	<input checked="" type="checkbox"/>	300		Nervous system; respiratory system; development	
Toluene diisocyanates (2,4- & 2,6-)	<input checked="" type="checkbox"/>	0.07		Respiratory system	<input checked="" type="checkbox"/>
Trichloroethylene (79-01-6)	<input checked="" type="checkbox"/>	600		Nervous system; eyes	<input checked="" type="checkbox"/>
Triethylamine (121-44-8)		200		Eyes	
Vinyl acetate (108-05-4)		200		Respiratory system	

Noncancer chronic Reference Exposure Levels determined using previous methodology

Table of Chronic RELs determined using the previous Hot Spots Risk Assessment Guidelines (OEHHA 1999)

Substance (CAS #)	Listed in CAPCOA (1993)	Chronic Inhalation REL ($\mu\text{g}/\text{m}^3$)	Oral REL (mg/kg Body Weight)	Hazard Index Target Organs	Human Data
Xylenes (m-, o-, p-)	<input checked="" type="checkbox"/>	700		Nervous system; respiratory system	<input checked="" type="checkbox"/>

*These peer-reviewed values were developed under the Toxic Air Contaminant (TAC) Program mandated by AB1807.

CHRONIC TOXICITY SUMMARY

ACRYLONITRILE

(Acrylonitrile monomer, cyanoethylene, propenenitrile, 2-propenenitrile, VCN, vinyl cyanide.)

CAS Number: 107-13-1

I. Chronic Toxicity Summary

<i>Inhalation reference exposure level</i>	5 $\mu\text{g}/\text{m}^3$ (2 ppb)
<i>Critical effect(s)</i>	Degeneration and inflammation of nasal epithelium in rats
<i>Hazard index target(s)</i>	Respiratory system

II. Chemical Property Summary (HSDB, 1994)

<i>Description</i>	Clear, colorless to pale yellow liquid (technical grades)
<i>Molecular formula</i>	$\text{C}_3\text{H}_3\text{N}$
<i>Molecular weight</i>	53.1 g/mol
<i>Density</i>	0.81 g/cm ³ @ 25°C
<i>Boiling point</i>	77.3°C
<i>Melting point</i>	-82°C
<i>Vapor pressure</i>	100 torr @ 23°C
<i>Solubility</i>	Soluble in isopropanol, ethanol, ether, acetone, and benzene
<i>Conversion factor</i>	1 ppm = 2.17 mg/m ³ @ 25 °C

III. Major Uses or Sources

Acrylonitrile is produced commercially by propylene ammoxidation, in which propylene, ammonia, and air are reacted by catalyst in a fluidized bed. Acrylonitrile is used primarily as a co-monomer in the production of acrylic and modacrylic fibers. Uses include the production of plastics, surface coatings, nitrile elastomers, barrier resins, and adhesives. It is also a chemical intermediate in the synthesis of various antioxidants, pharmaceuticals, dyes, and surface-active agents. Formerly, acrylonitrile was used as a fumigant for food commodities, flour milling, and bakery food processing equipment (HSDB, 1994). The annual statewide industrial emissions from facilities reporting under the Air Toxics Hot Spots Act in California based on the most recent inventory were estimated to be 3948 pounds of acrylonitrile (CARB, 2000). US EPA (1993) reported a mean ambient air concentration of acrylonitrile at four urban locations in the U.S. of 0.66 $\mu\text{g}/\text{m}^3$.

IV. Effects of Human Exposure

Many occupational epidemiology studies have investigated retrospectively the morbidity and mortality of acrylonitrile exposed workers. An increased incidence of lung cancer was associated with acrylonitrile exposure. No significant excess mortality has been observed for any noncarcinogenic endpoint. One early cross-sectional study (Wilson *et al.*, 1948) observed multiple deleterious effects in synthetic rubber manufacturing workers acutely exposed (20 to 45 minutes) to various concentrations of acrylonitrile (16 to 100 ppm, 34.7 to 217 mg/m³). Mucous membrane irritation, headaches, feelings of apprehension, and nervous irritability were observed in the majority of workers. Other less common symptoms observed included low-grade anemia, leukocytosis, and mild jaundice. These effects were reported to subside with cessation of exposure. Human volunteers exposed for a single 8 hour period to acrylonitrile vapors exhibited no deleterious CNS effects at concentrations ranging from 5.4 to 10.9 mg/m³ (2.4 to 5.0 ppm) (Jakubowski *et al.*, 1987).

A cross-sectional study (Sakurai *et al.*, 1978) found no statistically significant increases in adverse health effects in chronically exposed workers (minimum 5 years) employed at 6 acrylic fiber factories (n = 102 exposed, n = 62 matched controls). Mean acrylonitrile levels ranged from 0.1 to 4.2 ppm (0.2 to 9.1 mg/m³) as determined by personal sampling. Although not statistically significant, slight increases in reddening of the conjunctiva and pharynx were seen in workers from the plant with the highest mean levels (4.2 ppm arithmetic mean). However, this study has limitations, including small sample size and examiner bias, since the medical examiner was not blind to exposure status. The time-weighted average exposure of the group occupationally exposed to 4.2 ppm (9.1 mg/m³) acrylonitrile can be calculated as: $TWA = 9.1 \text{ mg/m}^3 \times (10/20) \text{ m}^3/\text{day} \times 5 \text{ days}/7 \text{ days} = 3 \text{ mg/m}^3$. This level is comparable to the LOAEL (HEC) of 2 mg/m³ derived by the U.S. EPA from the animal study of Quast *et al.* (1980).

Czeizel *et al.* (1999) studied congenital abnormalities in 46,326 infants born between 1980 and 1996 to mothers living within a 25 km radius of an acrylonitrile factory in Nyergesujfalu, Hungary. Ascertainment of cases with congenital abnormalities was based on the Hungarian Congenital Abnormality Registry plus review of pediatric, pathology and cytogenetic records. Particular attention was paid to indicators of germinal mutations (sentinel anomalies, Down's syndrome, and unidentified multiple congenital abnormalities) and to indicators of teratogens (specific pattern of multiple congenital abnormalities). Three congenital abnormalities: pectus excavatum in Tata, 1990-1992 (OR = 78.5, 95%CI = 8.4-729.6), undescended testis in Nyergesujfalu between 1980 and 1983 (8.6, 1.4-54.3) and in Esztergom, 1981-1982 (4.2, 1.3-13.5) and clubfoot in Tata, 1980-1981 (5.5, 1.5-20.3) showed significant time-space clusters in the study area. The risk of undescended testis decreased with increasing distance from the factory. An unusual increase for the combination of oral cleft and cardiac septal defects was seen in multimalformed babies in Tatabanya in 1990. Unfortunately there were no data on levels of acrylonitrile or any other exposure.

V. Effects of Animal Exposure

Quast *et al.* (1980) exposed Sprague-Dawley rats (100/sex/ concentration) 6 hours/day, 5 days/week for 2 years to concentrations of 0, 20, or 80 ppm acrylonitrile vapors (0, 43, or 174 mg/m³). A statistically significant increase in mortality was observed in the first year among 80 ppm exposed rats (male and female). Additionally, the 80 ppm exposed group had a significant decrease in mean body weight. Two tissues, the nasal respiratory epithelium and the brain, exhibited treatment-related adverse effects due to acrylonitrile exposure. Proliferative changes in the brain glial cells (i.e., tumors and early proliferation suggestive of tumors) were significantly increased in the 20 ppm (8/100) and 80 ppm (20/100) females versus female controls (0/100), and in the 80 ppm males (22/99) versus male controls (0/100). Noncarcinogenic, extrarrespiratory effects were observed in the nasal turbinate epithelium at both exposure concentrations, 20 and 80 ppm (see table below). Thus the LOAEL was 20 ppm. No treatment-related effects in the olfactory epithelium, trachea, or lower respiratory epithelium were observed at either concentration.

Effects of acrylonitrile reported by Quast *et al.* (1980)

<i>Effect</i>	<i>Sex</i>	0 ppm	20 ppm	80 ppm
Respiratory epithelium hyperplasia in the nasal turbinates	Male	0/11	4/12	10/10*
Hyperplasia of the mucous secreting cells	Male	0/11	7/12*	8/10*
Focal inflammation in the nasal turbinates	Female	2/11	6/10	7/10*
Flattening of the respiratory epithelium of the nasal turbinates	Female	1/11	7/10*	8/10*
Lung: pneumonia, consolidation, atelectasis, or edema	Male	14/100	27/100*	30/100*
Lung: pneumonia, consolidation, atelectasis, or edema	Female	7/100	2/100	7/100

* statistically significant difference from controls (p<.05)

Maltoni and associates exposed Sprague-Dawley rats (30/sex/concentration) to 0, 5, 10, 20, or 40 ppm acrylonitrile vapor for 5 days/week over 52 weeks, and at 60 ppm for 4 to 7 days, 5 days/week for 104 weeks (Maltoni *et al.*, 1977; Maltoni *et al.*, 1988). Histopathologic examinations were performed, including on lungs, brain, kidney, and liver. No noncarcinogenic effects were reported.

Gagnaire *et al.* (1998) studied motor and sensory conduction velocities (MCV and SCV, respectively) and amplitudes of the sensory and motor action potentials (ASAP and AMAP) of the tail nerve in male Sprague-Dawley rats during chronic treatment with acrylonitrile. (Four other unsaturated aliphatic nitriles were also given orally to other rats.) Rats were given doses of 12.5, 25, and 50 mg/kg of acrylonitrile once a day, 5 days per week for 12 weeks. Rats were also exposed by inhalation to 25, 50, and 100 ppm of acrylonitrile vapors for 6 h/day, 5 days per week, for 24 weeks and neurophysiological examinations were carried out. After oral acrylonitrile, animals developed behavioral sensitization characterized by salivation, locomotor hyperactivity, and moderately intense stereotypies. Rats dosed with 50 mg/kg developed hindlimb weakness associated with decreases in sensory conduction velocity (SCV) and in the amplitude of the sensory action potential (ASAP). Rats exposed to acrylonitrile by inhalation exhibited time- and concentration-dependent decreases in motor conduction velocity (MCV), SCV, and ASAP, which were partially reversible after 8 weeks of recovery. The authors concluded that the nervous system of the rat appears to be a target following either oral or inhalation exposures of acrylonitrile. The NOAEL by inhalation for 24 weeks was 25 ppm.

Changes in electrophysiological parameters after 24 wks of exposure (Gagnaire et al., 1998)

Acrylonitrile	MCV (m/sec)	SCV (m/sec)	AMAP (mvolts)	ASAP (μ vvolts)
0 ppm	42.9 \pm 0.9 ^a	53.3 \pm 1.0	17.8 \pm 1.2	186 \pm 8
25 ppm	41.6 \pm 0.8	50.5 \pm 0.8*	16.1 \pm 0.8	164 \pm 11
50 ppm	38.1 \pm 0.9**	49.1 \pm 0.5***	15.7 \pm 1.0	159 \pm 5*
100 ppm	38.5 \pm 1.2**	48.4 \pm 1.0***	17.4 \pm 0.9	133 \pm 11***

^a Mean \pm SEM; * p<0.05; ** p<0.01; ***p<0.001

In a developmental study, Murray *et al.* (1978) exposed rats to acrylonitrile vapors at 0, 40 ppm (87 mg/m³), or 80 ppm (174 mg/m³) for 6 hours/day during gestational days 6 to 15. In the 80 ppm exposed group, significant increases in fetal malformations were observed including short tail, missing vertebrae, short trunk, omphalocoele, and hemivertebra (Murray *et al.*, 1978). No differences in implantations, live fetuses, or resorptions were seen in the exposed (40 and 80 ppm) versus the control group. Maternal toxicity was observed as decreased body weight at both exposure levels. After adjustment to continuous exposure, this study identified a developmental NOAEL of 10 ppm and a LOAEL of 20 ppm (with maternal toxicity).

Saillenfait *et al.* (1993) studied the developmental toxicity of eight aliphatic mononitriles in Sprague-Dawley rats after inhalation exposure for 6 hr/day during days 6 to 20 of gestation. The range of exposure levels for acrylonitrile was 12, 25, 50, and 100 ppm; group sizes were 20-23 females. Embryoletality was observed after exposure to 25 ppm (54 mg/m³) acrylonitrile in the presence of overt signs of maternal toxicity. Fetal weights were significantly lower at 25 ppm. Thus 12 ppm (26 mg/m³) is a NOAEL for developmental toxicity using this study design.

VI. Derivation of Chronic Reference Exposure Level

<i>Study</i>	Quast <i>et al.</i> , 1980
<i>Study population</i>	Sprague-Dawley rats (100/sex/concentration)
<i>Exposure method</i>	Discontinuous whole-body inhalation exposures (0, 20, or 80 ppm)
<i>Critical effects</i>	Degeneration and inflammation of nasal respiratory epithelium; hyperplasia of mucous secreting cells
<i>LOAEL</i>	20 ppm
<i>NOAEL</i>	Not observed
<i>BMC₀₅</i>	1.5 ppm
<i>Exposure continuity</i>	6 hours/day, 5 days/week
<i>Average experimental exposure</i>	0.27 ppm for BMC ₀₅ (1.5 x 6/24 x 5/7)
<i>Human equivalent concentration</i>	0.067 ppm (gas with extrathoracic respiratory effects; RGDR = 0.25 based on MV = 0.33 m ³ /day, SA(ET) = 11.6 cm ²)
<i>Exposure duration</i>	2 years
<i>LOAEL uncertainty factor</i>	Not needed in the BMC approach
<i>Subchronic uncertainty factor</i>	1
<i>Interspecies uncertainty factor</i>	3
<i>Intraspecies uncertainty factor</i>	10
<i>Cumulative uncertainty factor</i>	30
<i>Inhalation reference exposure level</i>	0.002 ppm (2 ppb; 0.005 mg/m ³ ; 5 µg/m ³)

Sprague-Dawley rats (100/sex/concentration) were exposed 6 hours/day, 5 days/week for 2 years to 0, 20, or 80 ppm acrylonitrile (0, 43, and 174 mg/m³, respectively). Significant degenerative and inflammatory changes were observed in the respiratory epithelium of the nasal turbinates at both exposure concentrations (20 and 80 ppm). This treatment-related irritation of the nasal mucosa appeared in the 20 ppm exposed male rats as either epithelial hyperplasia of the nasal turbinates, or as hyperplasia of the mucous secreting cells. In the 20 ppm exposed females it appeared as either focal inflammation in the nasal turbinates or flattening of the respiratory epithelium of the nasal turbinates. In 80 ppm exposed rats the effects were more severe, including suppurative rhinitis, hyperplasia, focal erosions, and squamous metaplasia of the respiratory epithelium. No treatment-related effects in the olfactory epithelium, trachea, or lower respiratory system were observed at either concentration. This study identified a LOAEL for pathological alterations in the respiratory epithelium of the extrathoracic region of the respiratory tract of 20 ppm (43 mg/m³). The U.S. EPA (1994) based its RfC of 2 µg/m³ on the same study but included a Modifying Factor (MF) of 10 for database deficiencies. The criteria for use of modifying factors are not well specified by U.S. EPA. Such modifying factors were not used by OEHHA.

OEHHA used a benchmark dose approach to determine the chronic REL for acrylonitrile. The cumulative gamma distribution model in the U.S. EPA's BMDS software was individually fit to the data on respiratory epithelium hyperplasia in the nasal turbinates in males, hyperplasia of the

mucous secreting cells in males, focal inflammation in the nasal turbinates in females, and flattening of the respiratory epithelium of the nasal turbinates in females. The resulting BMC₀₅ values (1.27, 1.33, 2.18, 1.35) were averaged to yield a value of 1.5 ppm. The RGDR adjustment and appropriate uncertainty factors were applied as indicated in the above table and resulted in a chronic REL of 5 µg/m³.

For comparison, Gagnaire *et al.* (1998) found a NOAEL for nervous system effects at 24 weeks of 25 ppm, which is equivalent to a continuous exposure of 4.5 ppm. Use of the default RGDR of 1 for systemic effects, a subchronic UF of 3, an interspecies UF of 3, and an intraspecies UF of 10 results in an estimated REL of 45 ppb (100 µg/m³). We were unable to derive a BMC from the neurotoxicity data due partly to the tendency of the animals in the 100 ppm group to yield values for two of the four endpoints measured closer to the controls than those in the 50 ppm group.

As another comparison, Saillenfait *et al.* (1983) found a 12 ppm (26 mg/m³) NOAEL for fetal weight reduction (6 h/d exposure). This is equivalent to a continuous exposure of 3 ppm (on days 6 to 20 of gestation). Use of the default RGDR of 1 for systemic effects, an interspecies UF of 3, and an intraspecies UF of 10 results in an estimated REL of 100 ppb (200 µg/m³).

Finally, after adjustment to continuous exposure, Murray *et al.* (1978) identified a developmental NOAEL, adjusted to continuous exposure, of 10 ppm and a LOAEL of 20 ppm (with maternal toxicity at both levels). Use of the default RGDR of 1 for systemic effects, an interspecies UF of 3, and an intraspecies UF of 10 results in an estimated REL of 30 ppb (70 µg/m³).

VII. Data Strengths and Limitations for Development of the REL

Significant strengths in the chronic REL for acrylonitrile include (1) the availability of chronic inhalation exposure data from a well-conducted study with histopathological analysis and (2) the demonstration of a dose-response relationship. Major uncertainties are (1) the lack of adequate human exposure data, (2) the lack of a NOAEL in the 2 year study, (3) lack of inhalation bioassay in a second species, and (4) lack of reproductive data for inhalation exposures when an oral study showed adverse reproductive effects

When assessing the health effects of acrylonitrile, its carcinogenicity must also be assessed.

VIII. Potential for Differential Impacts on Children's Health

The chronic REL is considerably lower than the comparison estimate based on developmental effects. Although neurotoxicity, an endpoint which is often associated with increased sensitivity of younger animals or humans, was evaluated as one of the alternative endpoints, the comparison reference level for this end point in adults was more than an order of magnitude higher than the REL based on histological changes in the upper respiratory tract. It is therefore considered that the REL is likely to be adequately protective of infants and children.

IX. References

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CHRONIC TOXICITY SUMMARY

AMMONIA*(Anhydrous ammonia; aqueous ammonia)***CAS Registry Number: 7664-41-7****I. Chronic Toxicity Summary**

<i>Inhalation reference exposure level</i>	200 µg/m³ (300 ppb)
<i>Critical effect(s)</i>	Pulmonary function tests or subjective symptomatology in workers
<i>Hazard index target(s)</i>	Respiratory system

II. Physical and Chemical Properties (From HSDB, 1994; 1999)

<i>Description</i>	Colorless gas
<i>Molecular formula</i>	NH ₃
<i>Molecular weight</i>	17.03 g/mol
<i>Density</i>	0.7710 g/L @ 0°C
<i>Boiling point</i>	-33.35° C
<i>Vapor pressure</i>	7510 torr @ 25°C
<i>Solubility</i>	Soluble in water, alcohol, and ether
<i>Conversion factor</i>	1 ppm = 0.71 mg/m ³

III. Major Uses or Sources

This strongly alkaline chemical is widely used in industry as a feed stock for nitrogen-based chemicals such as fertilizers, plastics and explosives (ATSDR, 1990). Ammonia is also used as a refrigerant. The general public is exposed by off-gasing from cleaning solutions containing aqueous ammonia. Household ammonia solutions contain 5-10% ammonia in water while industrial strength can be up to 28%. The annual statewide industrial emissions from facilities reporting under the Air Toxics Hot Spots Act in California based on the most recent inventory were estimated to be 21,832,909 pounds of ammonia (CARB, 1999).

IV. Effects of Human Exposures

Comparisons were made between 52 workers and 31 control subjects in a soda ash plant for pulmonary function and eye, skin and respiratory symptomatology (Holness *et al.*, 1989). The pulmonary function tests included FVC (forced vital capacity – the total amount of air the subject can expel during a forced expiration), FEV₁ (forced expiratory volume in one second), FEF₅₀ (forced expiratory flow rate at 50% of the FVC) and FEF₇₅ (forced expiratory flow rate at

75% of the FVC). Age, height, and pack-years smoked were treated as covariates for the comparisons. The workers were exposed on average for 12.2 years to mean (time-weighted average) ammonia concentrations of 9.2 ppm (6.4 mg/m^3) \pm 1.4 ppm, while controls were exposed to 0.3 ppm (0.21 mg/m^3) \pm 0.1 ppm. No differences in any endpoints (respiratory or cutaneous symptoms, sense of smell, baseline lung function, or change in lung function over a work shift at the beginning and end of a workweek) were reported between the exposed and control groups.

Groups of human volunteers were exposed to 25, 50, or 100 ppm (0, 17.8, 35.5, or 71 mg/m^3) ammonia 5 days/week for 2, 4, or 6 hours/day, respectively, for 6 weeks (Ferguson *et al.*, 1977). Another group of 2 volunteers was exposed to 50 ppm ammonia for 6 hours/day for 6 weeks.

Group	Exposure	Week 1	Week 2	Week 3	Week 4	Week 5	Week 6
A	ppm NH ₃ hours	25 2	50 4	100 6	25 2	50 4	100 6
B	ppm NH ₃ hours	50 6	50 6	50 6	50 6	50 6	50 6
C	ppm NH ₃ hours	100 6	50 4	25 2	25 6	50 4	100 2

Pulmonary function tests (respiration rate, FVC and FEV₁) were measured in addition to subjective complaints of irritation of the eyes and respiratory tract. The difficulty experienced in performing simple cognitive tasks was also measured, as was pulse rate. There were reports of transient irritation of the nose and throat at 50 or 100 ppm. Acclimation to eye, nose, and throat irritation was seen after two to three weeks (in addition to the short-term subjective adaptation). No significant differences between subjects or controls on common biological indicators, in physical exams, or in performance of normal job duties were found. After acclimation, continuous exposure to 100 ppm, with occasional excursions to 200 ppm, was easily tolerated and had no observed effect on general health.

V. Effects of Animal Exposures

Rats were continuously exposed to ammonia at 0, 25, 50, 150, or 250 ppm (0, 18, 36, 107, or 179 mg/m^3) ammonia for 7 days prior to intratracheal inoculation with *Mycoplasma pulmonis*, and from 28 to 42 days following *M. pulmonis* exposure (Broderson *et al.*, 1976). All exposures to ammonia resulted in significantly increased severity of rhinitis, otitis media, tracheitis, and pneumonia characteristic of *M. pulmonis* infection, therefore 25 ppm was a LOAEL in this subchronic study. Exposure to 250 ppm ammonia alone resulted in nasal lesions (epithelial thickening and hyperplasia) which were not like those seen in *M. pulmonis*-infected rats.

The growth of bacteria in the lungs and nasal passages, and the concentration of serum immunoglobulin were significantly increased in rats exposed to 100 ppm (71 mg/m^3) ammonia over that seen in control rats (Schoeb *et al.*, 1982).

Guinea pigs (10/group) and mice (20/group) were continuously exposed to 20 ppm (14.2 mg/m³) ammonia for up to 6 weeks (Anderson *et al.*, 1964). Separate groups of 6 guinea pigs and 21 chickens were exposed to 50 ppm and 20 ppm ammonia for up to 6 and 12 weeks, respectively. All species displayed pulmonary edema, congestion, and hemorrhage after 6 weeks exposure, whereas no effects were seen after only 2 weeks. Guinea pigs exposed to 50 ppm ammonia for 6 weeks exhibited enlarged and congested spleens, congested livers and lungs, and pulmonary edema. Chickens exposed to 200 ppm for 17-21 days showed liver congestion and slight clouding of the cornea. Anderson and associates also showed that a 72-hour exposure to 20 ppm ammonia significantly increased the infection rate of chickens exposed to Newcastle disease virus, while the same effect was observed in chickens exposed to 50 ppm for just 48 hours.

Coon *et al.* (1970) exposed groups of rats (as well as guinea pigs, rabbits, dogs, and monkeys) continuously to ammonia concentrations ranging from 40 to 470 mg/m³. There were no signs of toxicity in 15 rats exposed continuously to 40 mg/m³ for 114 days or in 48 rats exposed continuously to 127 mg/m³ for 90 days. Among 49 rats exposed continuously to 262 mg/m³ for 90 days, 25% had mild nasal discharge. At 455 mg/m³ 50 of 51 rats died. Thus 127 mg/m³ (179 ppm) is a subchronic NOAEL for upper respiratory effects in rats. Coon *et al.* (1970) also found no lung effects in 15 guinea pigs exposed continuously to 40 mg/m³ (28 ppm) ammonia for 114 days.

VI. Derivation of Chronic Reference Exposure Level

<i>Study</i>	Holness <i>et al.</i> , 1989 (supported by Broderon <i>et al.</i> , 1976)
<i>Study population</i>	52 workers; 31 controls
<i>Exposure method</i>	Occupational inhalation
<i>Critical effects</i>	Pulmonary function, eye, skin, and respiratory symptoms of irritation
<i>LOAEL</i>	25 ppm (Broderon <i>et al.</i> , 1976) (rats)
<i>NOAEL</i>	9.2 ppm (Holness <i>et al.</i> , 1989)
<i>Exposure continuity</i>	8 hours/day (10 m ³ /day occupational inhalation rate), 5 days/week
<i>Exposure duration</i>	12.2 years
<i>Average occupational exposure</i>	3 ppm for NOAEL group (9.2 x 10/20 x 5/7)
<i>Human equivalent concentration</i>	3 ppm for NOAEL group
<i>LOAEL uncertainty factor</i>	1
<i>Subchronic uncertainty factor</i>	1
<i>Interspecies uncertainty factor</i>	1
<i>Intraspecies uncertainty factor</i>	10
<i>Cumulative uncertainty factor</i>	10
<i>Inhalation reference exposure level</i>	0.3 ppm (300 ppb; 0.2 mg/m ³ ; 200 µg/m ³)

The Holness *et al.* (1989) study was selected because it was a chronic human study and was published in a respected, peer-reviewed journal. It is also the only chronic study available. The USEPA (1995) based its RfC of 100 µg/m³ on the same study but included a Modifying Factor

(MF) of 3 for database deficiencies. The criteria for use of modifying factors are not well specified by U.S. EPA. Such modifying factors were not used by OEHHA.

For comparison with the proposed REL of 200 $\mu\text{g}/\text{m}^3$ based on human data, we estimated RELs from 2 animal studies. (1) Anderson *et al.* (1964) exposed guinea pigs continuously to 50 ppm (35 mg/m^3) ammonia for 6 weeks and observed pulmonary edema. Use of an RGDR of 0.86 and a cumulative uncertainty factor of 3000 (10 for use of a LOAEL, 10 for subchronic, 3 for interspecies, and 10 for intraspecies) resulted in a REL of 10 $\mu\text{g}/\text{m}^3$. Staff note that the nearly maximal total uncertainty factor of 3000 was used in this estimation. (2) Coon *et al.* (1970) exposed rats continuously to 127 mg/m^3 ammonia for 90 days and saw no signs of toxicity. Use of an RGDR(ET) of 0.16 for nasal effects (observed in rats exposed to higher levels of ammonia in Broderson *et al.* (1976)) and a cumulative uncertainty factor of 100 (3 for subchronic, 3 for interspecies, and 10 for intraspecies) resulted in a REL of 200 $\mu\text{g}/\text{m}^3$.

VII. Data Strengths and Limitations for Development of the REL

Significant strengths in the ammonia REL include (1) the availability of long-term human inhalation exposure data (Holness *et al.*, 1989), (2) the demonstration of consistent effects in experimentally exposed human volunteers following short-term exposures (Ferguson *et al.*, 1977), and (3) reasonable consistency with animal data (Coon *et al.*, 1970).

Major areas of uncertainty are (1) the lack of a NOAEL and LOAEL in a single study, (2) a lack of animal data with chronic exposure and histopathological analyses, and (3) difficulties in estimated human occupational exposures. The overall database for this common chemical is limited.

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*CHRONIC TOXICITY SUMMARY***BENZENE***(Benzol; Benzole; Cyclohexatriene)***CAS Registry Number: 71-43-2****I. Chronic Toxicity Summary**

<i>Inhalation reference exposure level</i>	60 $\mu\text{g}/\text{m}^3$ (20 ppb)
<i>Critical effect(s)</i>	Lowered red and white blood cell counts in occupationally exposed humans
<i>Hazard index target(s)</i>	Hematopoietic system; development; nervous system

II. Physical and Chemical Properties (HSDB, 1994; 1999)

<i>Description</i>	Colorless liquid
<i>Molecular formula</i>	C_6H_6
<i>Molecular weight</i>	78.1 g/mol
<i>Density</i>	0.879 g/cm ³ @ 25° C
<i>Boiling point</i>	80.1°C
<i>Vapor pressure</i>	100 torr @ 26.1°C
<i>Solubility</i>	Soluble in ethanol, chloroform, ether, carbon disulfide, acetone, oils, and glacial acetic acid; slightly soluble in water
<i>Conversion factor</i>	1 ppm = 3.2 mg/m ³ @ 25° C

III. Major Uses or Sources

Benzene has been widely used as a multipurpose organic solvent. This use is now discouraged due to its high toxicity, including carcinogenicity. Present uses include use as a raw material in the synthesis of styrene, phenol, cyclohexane, aniline, and alkyl benzenes in the manufacture of various plastics, resins, and detergents. Syntheses of many pesticides and pharmaceuticals also involve benzene as a chemical intermediate (HSDB, 1994). The tire industry and shoe factories use benzene extensively in their manufacturing processes. Annual demand in the U.S. was estimated to be 6 million tons in 1990 (HSDB, 1994). Benzene exposure also occurs as a result of gasoline and diesel fuel use and combustion (Holmberg and Lundberg, 1985). In 1996, the latest year tabulated, the statewide mean outdoor monitored concentration of benzene was approximately 0.7 ppb (CARB, 1999a). Annual statewide industrial emissions from facilities reporting under the Air Toxics Hot Spots Act in California based on the most recent inventory were estimated to be 750,364 pounds of benzene (CARB, 1999b). (This does not include the large amount of benzene emitted by mobile sources.)

IV. Effects of Human Exposure

The primary toxicological effects of chronic benzene exposure are on the hematopoietic system. Neurological and reproductive/developmental toxic effects are also of concern at slightly higher concentrations. Impairment of immune function and/or various anemias may result from the hematotoxicity. The hematologic lesions in the bone marrow can lead to peripheral lymphocytopenia and/or pancytopenia following chronic exposure. Severe benzene exposures can also lead to life-threatening aplastic anemia. These lesions may lead to the development of leukemia years after apparent recovery from the hematologic damage (DeGowin, 1963).

Kipen *et al.* (1988) performed a retrospective longitudinal study on a cohort of 459 rubber workers, examining the correlation of average benzene exposure with total white blood cell counts taken from the workers. These researchers found a significant ($p < 0.016$) negative correlation between average benzene concentrations in the workplace and white blood cell counts in workers from the years 1940-1948. A reanalysis of these data by Cody *et al.* (1993) showed significant decreases in RBC and WBC counts among a group of 161 workers during the 1946-1949 period compared with their pre-exposure blood cell counts. The decline in blood counts was measured over the course of 12 months following start of exposure. During the course of employment, workers who had low monthly blood cell counts were transferred to other areas with lower benzene exposures, thus potentially creating a bias towards non-significance or removing sensitive subjects from the study population. Since there was a reported 75% rate of job change within the first year of employment, this bias could be highly significant. In addition, there was some indication of blood transfusions used to treat some "anemic" workers, which would cause serious problems in interpreting the RBC data, since RBCs have a long lifespan in the bloodstream. The exposure analysis in this study was performed by Crump and Allen (1984). The range of monthly median exposures was 30-54 ppm throughout the 12-month segment examined. Despite the above-mentioned potential biases, workers exposed above the median concentrations displayed significantly decreased WBC and RBC counts compared with workers exposed to the lower concentrations using a repeated measures analysis of variance.

Tsai *et al.* (1983) examined the mortality from all cancers and leukemia, in addition to hematologic parameters in male workers exposed to benzene for 1-21 years in a refinery from 1952-1978. The cohort of 454 included maintenance workers and utility men and laborers assigned to benzene units on a "regular basis". Exposures to benzene were determined using personal monitors; the median air concentration was 0.53 ppm in the work areas of greatest exposure to benzene. The average length of employment in the cohort was 7.4 years. The analysis of overall mortality in this population revealed no significant excesses. Mortality from all causes and from diseases of the circulatory system was significantly below expected values based on comparable groups of U.S. males. The authors concluded the presence of a healthy worker effect. An internal comparison group of 823 people, including 10% of the workers who were employed in the same plant in operations not related to benzene, showed relative risks for 0.90 and 1.31 for all causes and cancer at all sites, respectively ($p < 0.28$ and 0.23). A subset of 303 workers was followed for medical surveillance. Up to four hematological tests per year were conducted on these workers. Total and differential white blood cell counts, hemoglobin,

hematocrit, red blood cells, platelets and clotting times were found to be within normal (between 5% and 95% percentile) limits in this group.

Collins *et al.* (1997) used routine data from Monsanto's medical/industrial hygiene system to study 387 workers with daily 8-hour time-weighted exposures (TWA) averaging 0.55 ppm benzene (range = 0.01 – 87.69 ppm; based on 4213 personal monitoring samples, less than 5% of which exceeded 2 ppm). Controls were 553 unexposed workers. There was no increase in the prevalence of lymphopenia, an early, sensitive indicator of benzene toxicity, among exposed workers (odds ratio = 0.6; 95% confidence interval = 0.2 to 1.8), taking into account smoking, age, and sex. There also was no increase in risk among workers exposed 5 or more years (odds ratio = 0.6; 95% confidence interval = 0.2 to 1.9). There were no differences between exposed and unexposed workers for other measures of hematotoxicity, including mean corpuscular volume and counts of total white blood cells, red blood cells, hemoglobin, and platelets.

Rothman *et al.* (1996) compared hematologic outcomes in a cross-sectional study of 44 male and female workers heavily exposed to benzene (median = 31 ppm as an 8-hr TWA) and 44 age and gender-matched unexposed controls from China. Hematologic parameters (total WBC, absolute lymphocyte count, platelets, red blood cells, and hematocrit) were decreased among exposed workers compared to controls; an exception was the red blood cell mean corpuscular volume (MCV), which was higher among exposed subjects. In a subgroup of 11 workers with a median 8 hr TWA of 7.6 ppm (range = 1-20 ppm) and not exposed to more than 31 ppm on any of 5 sampling days, only the absolute lymphocyte count was significantly different between exposed workers and controls ($p = 0.03$). Among exposed subjects, a dose response relationship with various measures of current benzene exposure (i.e., personal air monitoring, benzene metabolites in urine) was present only for the total WBC count, the absolute lymphocyte count, and the MCV. Their results support the use of the absolute lymphocyte count as the most sensitive indicator of benzene-induced hematotoxicity.

An examination of 32 patients, who were chronically exposed to benzene vapors ranging from 150 to 650 ppm for 4 months to 15 years, showed that pancytopenia occurred in 28 cases. Bone marrow punctures revealed variable hematopoietic lesions, ranging from acellularity to hypercellularity (Aksoy *et al.*, 1972).

Central nervous system disorders have been reported in individuals with pancytopenia following chronic occupational benzene exposure to unknown concentrations for an average length of time of 6 years (Baslo and Aksoy, 1982).

Runion and Scott (1985) estimated a composite geometric mean benzene concentration in various workplaces containing benzene to be 0.1 ppm (0.32 mg/m^3) (geometric standard deviation = 7.2 ppm, 23.3 mg/m^3). This estimate was based on samples collected by industrial hygienists between the years 1978 and 1983.

V. Effects of Animal Exposure

A number of animal studies have demonstrated that benzene exposure can induce bone marrow damage, changes in circulating blood cells, developmental and reproductive effects, alterations of the immune response, and cancer. With respect to chronic toxicity, hematological changes appear to be the most sensitive indicator.

Wolf *et al.* (1956) studied the effects of repeated exposure to benzene in rabbits (80 ppm, 175 total exposures), rats (88 ppm, 136 total exposures) and guinea pigs (88 ppm, 193 total exposures). The observed effects included leukopenia, increased spleen weight, and histological changes to the bone marrow. Hematologic effects, including leukopenia, were observed in rats exposed to mean concentrations of 44 ppm (143 mg/m³) or greater for 5-8 weeks (Deichmann *et al.*, 1963). Exposure to 31 ppm (100 mg/m³) benzene or less did not result in leukopenia after 3-4 months of exposure. Snyder *et al.* (1978) exposed Sprague-Dawley rats and AKR/J mice to 300 ppm benzene, 6 hours/day, 5 days/week for life. Lymphocytopenia, anemia and decreased survival time were observed in both species. Cronkite *et al.* (1982) exposed male mice to 400 ppm benzene, 6 hours/day, 5 days/week for 9.5 weeks and observed depressed bone marrow cellularity, decreased stem cell count, and altered morphology in spleen colony-forming cells.

Mice have been shown to be more sensitive than rats or rabbits to the hematologic and leukemic effects of benzene (Sabourin *et al.*, 1989; IARC, 1982). Sabourin *et al.* (1988) showed that metabolism of benzene to the toxic hydroquinone, muconic acid, and hydroquinone glucuronide was much more prevalent in the mouse than in rats, whereas the detoxification pathways were approximately equivalent between the two species.

A study on the chronic hematological effects of benzene exposure in C57 Bl/6 male mice (5-6 per group) showed that peripheral lymphocytes, red blood cells and colony-forming units (CFUs) in the bone marrow and spleen were significantly decreased in number after treatment with 10 ppm (32.4 mg/m³) benzene for 6 hours/day, 5 days/week for 178 days (Baarson *et al.*, 1984).

Inhalation of 0, 10, 31, 100, or 301 ppm (0, 32.4, 100.4, 324, or 975 mg/m³) benzene for 6 hours/day for 6 days resulted in a dose-dependent reduction in peripheral lymphocytes, and a reduced proliferative response of B- and T-lymphocytes to mitogenic agents in mice (Rozen *et al.*, 1984). In this study, total peripheral lymphocyte numbers and B-lymphocyte proliferation to lipopolysaccharide were significantly reduced at a concentration of 10 ppm (32.4 mg/m³). The proliferation of T-lymphocytes was significantly reduced at a concentration of 31 ppm (100.4 mg/m³).

Male and female mice (9-10 per group) exposed to 100 ppm (324 mg/m³) benzene or greater for 6 hours/day, 5 days/week for 2 weeks showed decreased bone marrow cellularity and a reduction of pluripotent stem cells in the bone marrow (Cronkite *et al.*, 1985). The decrease in marrow cellularity continued for up to 25 weeks following a 16-week exposure to 300 ppm (972 mg/m³) benzene. Peripheral blood lymphocytes were dose-dependently decreased with benzene exposures of greater than 25 ppm (81 mg/m³) for 16 weeks, but recovered to normal levels following a 16-week recovery period.

Ward *et al.* (1985) exposed 50 Sprague-Dawley rats and 150 CD-1 mice of both sexes to 0, 1, 10, 30, or 300 ppm benzene, 6 hours/day, 5 days/week for 13 weeks. Serial sacrifices were

conducted at 7, 14, 28, 56, and 91 days. No hematological changes were found for mice and rats at 1, 10, or 30 ppm in this study. Significant increases in mean cell volume and mean cell hemoglobin values and decreases in hematocrit, hemoglobin, lymphocyte percentages, and decreases in red cell, leukocyte and platelet counts were observed in male and female mice at 300 ppm. The changes were first observed after 14 days of exposure. Histological changes in mice included myeloid hypoplasia of the bone marrow, lymphoid depletion in the mesenteric lymph node, increased extramedullary hematopoiesis in the spleen, and periarteriolar lymphoid sheath depletion. Effects were less severe in the rats.

Aoyama (1986) showed that a 14-day exposure of mice to 50 ppm (162 mg/m³) benzene resulted in a significantly reduced blood leukocyte count.

The NTP (1986) conducted a bioassay in F344 rats and B6C3F1 mice of benzene by corn oil gavage. Doses were 0, 25, 50, and 100 mg/kg-day for females and 0, 50, 100, and 200 mg/kg-day for males. Dose-related lymphocytopenia and leukocytopenia were observed in both species in all dosed groups but not controls. Mice exhibited lymphoid depletion of the thymus and spleen and hyperplasia of the bone marrow.

Cronkite *et al.* (1989) exposed CBA/Ca mice to 10, 25, 100, 300, 400 and 3000 ppm benzene 6 hours/day, 5 days/week for up to 16 weeks. No effects were observed at the 10 ppm level. Lymphopenia was observed in the 25 ppm exposure group. Higher concentrations of benzene produced dose-dependent decreases in blood lymphocytes, bone marrow cellularity, spleen colony-forming units, and an increased percentage of CFU-S in S-phase synthesis.

Farris *et al.* (1997) exposed B6C3F₁ mice to 1, 5, 10, 100, and 200 ppm benzene for 6 hr/day, 5 days/week, for 1, 2, 4, or 8 weeks. In addition some animals were allowed to recover from the exposure. There were no significant effects on hematopoietic parameters from exposure to 10 ppm benzene or less. Exposure to higher levels reduced the number of total bone marrow cells, progenitor cells, differentiating hematopoietic cells, and most blood parameters. The replication of primitive progenitor cells was increased. The authors suggested that this last effect, in concert with the genotoxicity of benzene, could account for the carcinogenicity of benzene at high concentrations.

Reproductive and developmental effects have been reported following benzene exposure. Coate *et al.* (1984) exposed groups of 40 female rats to 0, 1, 10, 40, and 100 ppm (0, 3.24, 32.4, 129.6, or 324 mg/m³) benzene for 6 hours/day during days 6-15 of gestation. In this study, teratologic evaluations and fetotoxic measurements were done on the fetuses. A significant decrease was noted in the body weights of fetuses from dams exposed to 100 ppm (324 mg/m³). No effects were observed at a concentration of 40 ppm (129.6 mg/m³).

Keller and Snyder (1986) reported that exposure of pregnant mice to concentrations as low as 5 ppm (16 mg/m³) benzene on days 6-15 of gestation (6 hr/day) resulted in bone-marrow hematopoietic changes in the offspring that persisted into adulthood. However, the hematopoietic effects (e.g. bimodal changes in erythroid colony-forming cells) in the above study were of uncertain biological significance. In a similar later study, Keller and Snyder (1988) found that exposure of mice *in utero* to 20 ppm (64 mg/m³) benzene on days 6-15 of gestation resulted in neonatal suppression of erythropoietic precursor cells and persistent,

enhanced granulopoiesis. This effect was considered significant bone-marrow toxicity by the authors. No hematotoxicity was seen in this study at 10 ppm (32 mg/m³).

An exposure of 500 ppm (1,600 mg/m³) benzene through days 6-15 gestation was teratogenic in rats while 50 ppm (160 mg/m³) resulted in reduced fetal weights on day 20 of gestation. No fetal effects were noted at an exposure of 10 ppm (Kuna and Kapp, 1981). An earlier study by Murray *et al.* (1979) showed that inhalation of 500 ppm benzene for 7 hours/day on days 6-15 and days 6-18 of gestation in mice and rabbits, respectively, induced minor skeletal variations in the absence of maternal toxicity. Red and white blood cell counts in the adults of either species were measured by Murray *et al.* (1979) but were not significantly different from control animals. However, fetal mouse hematological effects were not measured.

Tatrai *et al.* (1980) demonstrated decreased fetal body weights and elevated liver weights in rats exposed throughout gestation to 150 mg/m³ (47 ppm).

VI. Derivation of Chronic Reference Exposure Level (REL)

<i>Study</i>	Tsai <i>et al.</i> (1983)
<i>Study population</i>	303 Male refinery workers
<i>Exposure method</i>	Occupational exposures for 1-21 years
<i>Critical effects</i>	Hematological effects
<i>LOAEL</i>	Not observed
<i>NOAEL</i>	0.53 ppm
<i>Exposure continuity</i>	8 hr/day (10 m ³ per 20 m ³ day), 5 days/week
<i>Exposure duration</i>	7.4 years average (for the full cohort of 454); 32% of the workers were exposed for more than 10 years
<i>Average occupational exposure</i>	0.19 ppm
<i>Human equivalent concentration</i>	0.19 ppm
<i>LOAEL uncertainty factor</i>	1
<i>Subchronic uncertainty factor</i>	1
<i>Interspecies uncertainty factor</i>	1
<i>Intraspecies uncertainty factor</i>	10
<i>Cumulative uncertainty factor</i>	10
<i>Inhalation reference exposure level</i>	0.02 ppm (20 ppb; 0.06 mg/m ³ ; 60 µg/m ³)

Staff identified Tsai *et al.* (1983) as the most appropriate study for a chronic REL derivation. The authors examined hematologic parameters in 303 male workers exposed to benzene for 1-21 years in a refinery from 1952-1978. Follow-up success was 99.3% in the entire cohort of 359. A total of approximately 1400 samples for hematological tests and 900 for blood chemistry tests were taken between 1959 and 1979. Exposures to benzene were determined using personal monitors. Data consisting of 1394 personal samples indicated that 84% of all benzene samples were less than 1 ppm; the median air concentration of benzene was 0.53 ppm in the work areas of greatest exposure to benzene ("benzene related areas", for example, production of benzene

and cyclohexane and also of cumene). The average length of employment in the cohort was 7.4 years. Mortality from all causes and from diseases of the circulatory system was significantly below expected values based on comparable groups of U.S. males. The authors concluded the presence of a healthy worker effect. An analysis using an internal comparison group of 823 people, including 10% of the workers who were employed in the same plant in operations not related to benzene, showed relative risks for 0.90 and 1.31 for all causes and cancer at all sites, respectively ($p < 0.28$ and 0.23). Total and differential white blood cell counts, hemoglobin, hematocrit, red blood cells, platelets and clotting times were found to be within normal (between 5% and 95% percentile) limits in this group. Although the exposure duration averaged only 7.4 years, the study was considered to be chronic since 32% of the workers had been exposed for more than 10 years.

VII. Data Strengths and Limitations for Development of the REL

Both the animal and human databases for benzene are excellent. Although the study by Tsai *et al.* (1983) is a free-standing NOAEL, the endpoint examined is a known sensitive measure of benzene toxicity in humans. In addition, the LOAEL for the same endpoint in workers reported by Cody *et al.* (1993) help form a dose-response relationship and also yield an REL which is consistent with that derived from Tsai *et al.* (1983). The study by Cody *et al.* (1993), since it failed to identify a NOAEL and was only for a period of 1 year, contained a greater degree of uncertainty in extrapolation to a chronic community Reference Exposure Level. The recent results of Collins *et al.* (1997) that included a NOAEL of 0.55 ppm and of Rothman *et al.* (1996) that included a LOAEL of 7.6 ppm are consistent with those of Tsai *et al.* Therefore the study by Tsai *et al.* (1983) was used as the basis for the chronic REL for benzene.

In the Cody *et al.* (1993) study, significant hematological effects, including reduced RBC and WBC counts, were observed in 161 male rubber workers exposed to median peak concentrations (i.e., only the peak concentrations for any given exposure time were reported) of 30-54 ppm or more for a 12-month period during 1948. The 30 ppm value was considered a 1-year LOAEL for hematological effects. In this rubber plant, workers who had blood dyscrasias were excluded from working in the high benzene units. Furthermore, individual workers having more than a 25% decrease in WBC counts from their pre-employment background count were removed from the high benzene units and placed in other units with lower benzene concentrations. Sensitive individuals therefore could have been excluded from the analysis. The 30 ppm value is the low end of the range of median values (30-54 ppm) reported by Crump and used in the Kipen *et al.* (1988) and Cody *et al.* (1993) studies. An equivalent continuous exposure of 10.7 ppm can be calculated by assuming that workers inhaled 10 m^3 of their total 20 m^3 of air per day during their work-shift, and by adjusting for a normal 5 day work week. Application of uncertainty factors for subchronic exposures, estimation of a NOAEL, and for protection of sensitive subpopulations (10 for each) results in an REL of 0.01 ppm (10 ppb; $30 \mu\text{g}/\text{m}^3$). This is comparable to the REL based on Tsai *et al.* (1983).

Ward *et al.* (1996) determined a relationship between occupational exposures to benzene and decreased red and white cell counts. A modeled dose-response relationship indicated a possibility for hematologic effects at concentrations below 5 ppm. However, no specific measures of the actual effects at concentrations below 2 ppm were taken, and the Tsai *et al.* (1983) data were not considered in their analysis. The purpose of this study was to characterize

the trend for effects at low concentrations of benzene. A NOAEL or LOAEL was not identified in the study. The selection of a NOAEL of 0.53 ppm is therefore not inconsistent with the results of the Ward *et al.* (1996) study.

The human data presented by Tsai and associates were selected over animal studies because the collective human data were considered adequate in terms of sample size, exposure duration, and health effects evaluation.

For comparison with the REL of 20 ppb based on human data, we estimated a REL based on the chronic inhalation study in mice by Baarson *et al.* (1984), which showed that bone-marrow progenitor cells were markedly suppressed after intermittent exposures (6 hr/day, 5 days/week) to 10 ppm benzene for 6 months. An extrapolation of this value to an equivalent continuous exposure resulted in a concentration of 1.8 ppm. Application of an RGDR of 1 for a systemic effect and uncertainty factors of 3 and 10 for inter- and intraspecies variability, and 10 for estimation of a NOAEL from the LOAEL would result in an REL of 6 ppb (20 $\mu\text{g}/\text{m}^3$). The Farris *et al.* (1997) 8 week study indicated a LOAEL of 100 ppm and a NOAEL of 10 ppm for hematological effects. Application of an RGDR of 1 and UFs of 10 for subchronic, 3 for interspecies and 10 for intraspecies extrapolation (total UF = 300) also resulted in an estimated REL of 6 ppb, in reasonable agreement with the proposed REL of 20 ppb. One could also crudely approximate an inhalation REL from the oral NTP bioassay where a dose of 25 mg/kg-day was associated with hematological effects. The concentration approximately equivalent to a 25 mg/kg dose for a 70 kg human breathing 20 cubic meters per day is 27 ppm. Assuming this is a LOAEL and applying an RGDR of 1 for systemic effects, a 3 fold UF for extrapolation to humans, a 10-fold UF for LOAEL to NOAEL extrapolation and a 10-fold UF for intraspecies extrapolation yields a REL of 90 ppb. There are a number of uncertainties to this approach, yet it comes within a factor of 5 of the proposed REL based on human studies.

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CHRONIC TOXICITY SUMMARY

BERYLLIUM AND BERYLLIUM COMPOUNDS*(beryllium-9; glucinium; glucinum; beryllium metallic)***CAS Registry Number: 7440-41-7***(beryllium oxide; beryllia; beryllium monoxide)***CAS Registry Number: 1304-56-9***(beryllium hydroxide; beryllium hydrate; beryllium dihydroxide)***CAS Registry Number: 13327-32-7***(beryllium sulfate; sulfuric acid; beryllium salt)***CAS Registry Number: 13510-49-1****I. Chronic Toxicity Summary**

<i>Inhalation reference exposure level</i>	0.007 $\mu\text{g Be/m}^3$
<i>Critical effect(s)</i>	Beryllium sensitization and chronic beryllium disease in occupationally exposed humans
<i>Hazard index target(s)</i>	Respiratory system; immune system
<i>Oral reference exposure level</i>	0.002 mg/kg-day
<i>Critical effect</i>	Small intestinal lesions in dogs
<i>Hazard index target(s)</i>	Gastrointestinal tract/liver

II. Physical and Chemical Properties Summary (ATSDR, 1993)

	<i>Metallic beryllium</i>	<i>Beryllium oxide</i>	<i>Beryllium hydroxide</i>	<i>Beryllium sulfate</i>
<i>Description</i>	Solid gray, hexagonal structure	White light, amorphous powder	White amorphous powder or crystalline	Colorless tetragonal crystals
<i>Molecular formula</i>	Be	BeO	Be(OH) ₂	BeSO ₄
<i>Molecular weight</i>	9.012 g/mol	25.01 g/mol	43.03 g/mol	105.07 g/mol
<i>Solubility</i>	Insoluble in water			Soluble
<i>Conversion factor</i>	Not applicable			

III. Major Uses and Sources

Beryllium is a metallic element mined as bertrandite and beryl mineral ores. As the lightest structural metal, beryllium is used in the space, aircraft, and nuclear industries in a variety of components including aircraft disc brakes, x-ray transmission windows, vehicle optics, nuclear reactor neutron reflectors, fuel containers, precision instruments, rocket propellants, navigational systems, heat shields, and mirrors. In addition to the four species listed, there are many other beryllium-containing compounds, including other salts, ores, and alloys (see, e.g., CRC, 1994). The annual statewide industrial emissions from facilities reporting under the Air Toxics Hot Spots Act in California based on the most recent inventory were estimated to be 2279 pounds of beryllium (CARB, 2000).

Beryllium alloys, especially the hardest alloy beryllium copper, are used in electrical equipment, precision instruments, springs, valves, non-sparking tools, and in molds for injection-molded plastics for automotive, industrial, and consumer applications. Beryllium oxide is used in high-technology ceramics, electronic heat sinks, electrical insulators, crucibles, thermocouple tubing, and laser structural components. Other beryllium compounds, including the chloride, nitrate, fluoride, and sulfate, are utilized as chemical reagents or generated from the refining of beryllium-containing ores.

Beryllium is naturally emitted into the atmosphere by windblown dusts and volcanic particles. However, the major emission source is the combustion of coal and fuel oil, which releases beryllium-containing particulates and ash. Other beryllium-releasing industrial processes include ore processing, metal fabrication, beryllium oxide production, and municipal waste incineration (ATSDR, 1993). Beryllium also occurs in tobacco smoke (0-0.0005 $\mu\text{g}/\text{cigarette}$) (Smith *et al.*, 1997).

IV. Effects of Human Exposure

The respiratory tract is the major target organ system in humans following the inhalation of beryllium. The common symptoms of chronic beryllium disease (CBD) include shortness of breath upon exertion, weight loss, cough, fatigue, chest pain, anorexia, and overall weakness. Most studies reporting adverse respiratory effects in humans involve occupational exposure to beryllium. Exposure to soluble beryllium compounds is associated with acute beryllium pneumonitis (Eisenbud *et al.*, 1948). Exposure to either soluble or insoluble beryllium compounds may result in obstructive and restrictive diseases of the lung, called chronic beryllium disease (berylliosis) (Cotes *et al.*, 1983; Johnson, 1983; Infante *et al.*, 1980; Kriebel *et al.*, 1988a; Metzner and Lieben, 1961). The total number of beryllium-related disease cases has declined since the adoption of industrial standards (Eisenbud and Lisson, 1983; ATSDR, 1993).

Historically, beryllium pneumonitis has been associated with occupational concentrations over $0.1 \text{ mg Be}/\text{m}^3$, primarily as beryllium sulfate or beryllium fluoride (Eisenbud *et al.*, 1948). The atmospheric concentrations related to chronic beryllium disease have been more difficult to define, in part due to the lack of individual exposure estimates, especially in the studies derived

from the berylliosis case registries (Infante *et al.*, 1980; Lieben and Metzner, 1959). However, Infante and associates (1980) reported significantly increased mortality due to non-neoplastic respiratory disease in beryllium-exposed workers, and noted one case of chronic berylliosis in a worker following seven years exposure to $\leq 2 \mu\text{g Be/m}^3$. In a 30-year follow-up study of 146 beryllium-exposed workers, Cotes *et al.* (1983) identified seven cases of chronic beryllium related disease. All the cases were exposed to beryllium oxide or hydroxide, but in a wide range of retrospectively estimated doses (over 3000 samples from 1952 to 1960). The estimated average daily exposure did not exceed $2 \mu\text{g/m}^3$ for the ten site/process classifications, but 318 samples did exceed $2 \mu\text{g Be/m}^3$ (and 20 samples were greater than $25 \mu\text{g Be/m}^3$). No atmospheric samples were available after 1963, even though the exposure occurred through 1973. The LOAEL for occupationally induced berylliosis observed in this study was estimated from uncertain exposure data to be less than $2 \mu\text{g Be/m}^3$.

One cross-sectional study (Kriebel *et al.*, 1988a; Kriebel *et al.*, 1988b) estimated beryllium exposure levels for 309 workers originally surveyed in 1977, with a median duration of exposure of 17 years (range 2 to 39 years). Historic plant levels were estimated to be as high as $100 \mu\text{g Be/m}^3$, and, even as late as 1975, some job classifications exceeded $10 \mu\text{g Be/m}^3$. The workers' median cumulative exposure was $65 \mu\text{g Be/m}^3\text{-year}$ (range 0.1 to $4400 \mu\text{g Be/m}^3\text{-years}$); the median lifetime exposure estimate was $4.3 \mu\text{g/m}^3$ (range 0.01 to $150 \mu\text{g/m}^3$). Spirometric measurement of pulmonary function, chest x-rays, and arterial blood gas measurements were collected. Decrements in lung function, as defined by forced vital capacity (FVC) and forced expiratory volume in one second (FEV_1), were associated with cumulative exposure up to 20 years prior to the health survey, even in workers with no radiographic abnormalities. Differences in alveolar-arterial oxygen gradient were associated with cumulative exposure in the 10 years prior to the study. These endpoints give a LOAEL of $39 \mu\text{g/m}^3\text{-years}$ (geometric mean cumulative exposure) for decrements in pulmonary function and changes in arterial blood gases.

Non-occupational beryllium-related chronic disease has been reported in individuals residing in the vicinity of beryllium manufacturing industries (Eisenbud *et al.*, 1949; Metzner and Lieben, 1961). An early cross-sectional study (Eisenbud *et al.*, 1949) described 11 cases of non-occupational berylliosis after x-ray and clinical examination of approximately 10,000 residents near a beryllium fabrication facility in Lorain, Ohio. Ten of the cases resided within 3/4 mile of the plant (up to 7 years duration), and five cases resided within 1/4 mile. The authors estimated a 1% disease incidence within 1/4 mile (500 individuals). Atmospheric sampling in 1947 identified an average level of $0.2 \mu\text{g Be/m}^3$ at 1/4 mile decreasing to $0 \mu\text{g Be/m}^3$ at 10 miles, but samples varied up to 100 fold over the 10 week sampling period. Utilizing current and historical exposure estimates based on discharge, process, inventory, and building design changes, this study estimated a chronic LOAEL in the range of 0.01 to $0.1 \mu\text{g Be/m}^3$ for continuous exposure to beryllium compounds, based on the development of chronic berylliosis.

Metzner and Lieben (1961) also reported 26 cases of chronic berylliosis in a population of approximately 100,000, living within 7 miles of a refining and alloy fabrication plant (duration 6 to 19 years). Neighborhood exposure assessment conducted over 14 months during 1958 and 1959 identified a mean level of $0.0155 \mu\text{g Be/m}^3$, with 10% of the samples registering over $0.03 \mu\text{g Be/m}^3$. Limited measurements conducted earlier at the site were higher (1.0 to $1.8 \mu\text{g Be/m}^3$ in 1953 and 0.91 to $1.4 \mu\text{g Be/m}^3$ in 1954).

Chronic beryllium disease appears to involve a cell-mediated immune response, especially granulomatous reactions found in the lungs of sensitive individuals. Humans exposed to beryllium compounds have demonstrated increased T-cell activity (*in vitro*) and histological abnormalities of the lymph nodes (Cullen *et al.*, 1987; Johnson, 1983). Johnson (1983) described granuloma of lymph nodes and chronic interstitial pneumonitis in a small number of beryllium metal handling machinists (LOAEL = 4.6 $\mu\text{g Be}/\text{m}^3$). A second study identified granulomatous lung lesions, scarred lung tissue, and breathing difficulties in workers from a precious metal refining facility exposed to a mixture of beryllium and other metals (Cullen *et al.*, 1987). Also, altered proliferative responses of lymphocytes obtained by bronchoalveolar lavage indicated increased T-cell activity *in vitro*. Cullen *et al.* (1987) reported a mean exposure level of 1.2 $\mu\text{g Be}/\text{m}^3$ (range = 0.22 – 43 $\mu\text{g}/\text{m}^3$). USEPA (1998) and ATSDR (2000) considered 0.52 $\mu\text{g Be}/\text{m}^3$ to be the LOAEL for CBD from this study since this was the average concentration in the furnace area where 4 of the 5 CBD cases worked.

Sensitization to beryllium, as measured by the beryllium lymphocyte proliferation test (BeLPT), can occur in the absence of chronic beryllium disease (Kreiss *et al.*, 1989). The authors hoped that the identification of sensitized individuals without disease might prevent clinical disease, presumably by removing the individuals from exposure to beryllium. Some beryllium-sensitized individuals progress to having clinical disease (Newman *et al.*, 1992). Data obtained from a four-year survey conducted at beryllium-copper alloy manufacturing factories in Japan (Yoshida *et al.*, 1997) indicated that the T cells of workers continuously exposed to more than 0.01 $\mu\text{g Be}/\text{m}^3$ were activated and that the cell-mediated immune (CMI) response was promoted. The BeLPT in workers exposed to less than 0.01 $\mu\text{g Be}/\text{m}^3$ was unaffected.

Genetic influences on development of CBD have been identified. CBD is associated with the allelic substitution of glutamic acid for lysine at position 69 in the HLA-DPB1 protein (Richiardi *et al.*, 1993). Up to 97% of CBD patients may have the Glu69 marker, but only 30-45% of beryllium-exposed, unaffected individuals carry the same marker. Because CBD occurs in only 1-6% of exposed workers, Glu69 is not likely to be the only genetic factor influencing the development of CBD. Changes in other sequences of the HLA-DPB1 gene and in the copy number of Glu69 are also involved (Wang *et al.*, 1999).

The Rocky Flats Environmental Technology Site in Colorado is part of the U.S. Department of Energy nuclear weapons complex. Operations using Be began in 1953, Be production operations began in 1957, and the first case of CBD was diagnosed in a machinist in 1984. Exposures could have occurred during foundry operations, casting, shearing, rolling, cutting, welding, machining, sanding, polishing, assembly, and chemical analysis operations. Since 1991, 29 cases of CBD and 76-78 cases of beryllium sensitization have been identified (Stange *et al.*, 1996). Several cases appear to have had only minimal Be exposure, since the employees were in administrative functions, not primary beryllium operations. Personal air monitoring devices used over a period of 4 years showed a breathing zone level of 1.04 $\mu\text{g Be}/\text{m}^3$. ATSDR (2000) considered 1.04 $\mu\text{g Be}/\text{m}^3$ to be the LOAEL for this study. A recent case-control study of workers at Rocky Flats (Viet *et al.*, 2000) suggested that exposures of workers to lower Be levels might lower the future incidence of CBD, but not necessarily the incidence of sensitivity to Be.

Kreiss *et al.* (1996) investigated the prevalence of beryllium sensitization in relation to work process and beryllium exposure measurements in a beryllia ceramics plant that had operated since 1980. In 1992 they interviewed 136 employees (97.8% of the workforce), ascertained beryllium sensitization with the beryllium lymphocyte proliferation blood test (BeLPT), and reviewed industrial hygiene measurements. Eight employees were beryllium-sensitized (5.9%); six of the eight had granulomatous disease based on transbronchial lung biopsy. Machinists had a Be sensitization rate of 14.3% compared to 1.2% among other employees. Machining operations (drilling, dicing, centerless grinding, and/or surface grinding) had significantly higher general area and breathing zone measurements than other work processes during the time in which most beryllium-sensitized cases had started machining. Daily weighted average estimates of exposure for machining processes also exceeded estimates for other work processes in that time period (median daily weighted average = $0.9 \mu\text{g}/\text{m}^3$). Daily weighted averages for the machining process accounted for the majority of exceedances of the $2.0 \mu\text{g}/\text{m}^3$ OSHA Permissible Exposure Limit (PEL); 8.1% of machining daily weighted averages were above the PEL. The LOAEL from this study was $0.55 \mu\text{g}/\text{m}^3$, the median exposure of the sensitized workers.

The facility was again surveyed in 1998 after some attempts were made to lower exposure to beryllium (Henneberger *et al.*, 2001). The investigators separated the workers into 77 long-term workers hired before the 1992 screening and 74 short-term workers hired after 1992. Among 20 short-term workers exposed to the lowest mean Be level (0.05 to $0.19 \mu\text{g}/\text{m}^3$), two showed Be sensitivity by the BeLPT test. Thus a fraction of workers appears to be exquisitely sensitive to beryllium.

Based on a review of this and other occupational studies Wambach and Tuggle (2000) have suggested that the workplace standard of $2 \mu\text{g}/\text{m}^3$ be lowered to $0.1 \mu\text{g}/\text{m}^3$. Some workers might still be sensitized to beryllium at this level (Yoshida *et al.*, 1997).

V. Effects of Animal Exposure

Three chronic studies, two in rats (Vorwald and Reeves, 1959; Reeves *et al.*, 1967) and one in guinea pigs (Reeves *et al.*, 1970), observed adverse inflammatory and proliferative respiratory changes following inhalation exposure to beryllium compounds. Vorwald and Reeves (1959) observed inflamed lungs and fibrosis in rats exposed to $0.006 \text{ mg Be}/\text{m}^3$ (as BeO) for an unspecified duration. A later study exposed Sprague-Dawley CD rats for 72 weeks (7 hr/d, 5 d/wk) to $34.25 \mu\text{g Be}/\text{m}^3$ from BeSO₄ (Reeves *et al.*, 1967). Gross and histological changes observed in exposed versus unexposed rats included increased lung weight, inflamed lungs, emphysema, arteriolar wall thickening, granulomas, fibrosis, and proliferative responses within the alveoli (LOAEL = $34.25 \mu\text{g Be}/\text{m}^3$). Guinea pigs were exposed to 0, 3.7, 15.4, or $29.3 \mu\text{g Be}/\text{m}^3$ (from the sulfate) for 6 hours/day, 5 days/week for up to 1 year (Reeves *et al.*, 1970). Respiratory alterations observed in the beryllium-exposed groups included increased tracheobronchial lymph node and lung wet weights, interstitial pneumonitis, and granulomatous lesions. These adverse respiratory effects were observed in all the beryllium dosed groups and indicated a chronic inhalation LOAEL of $3.7 \mu\text{g Be}/\text{m}^3$.

Wagner *et al.* (1969) exposed monkeys, rats, and hamsters to 0.21 and 0.62 mg Be/m³ as fumes from bertrandite or beryl ore, respectively, for 6 hours/day, 5 days/week for up to 17 months. Exposed animals displayed severe effects, including (1) bronchial lymphocytic infiltrates, abscesses, consolidated lobes, and granulomatous lesions after exposure to 0.21 mg Be/m³ from bertrandite ore, and (2) inflamed lungs, fibrosis, and granuloma after exposure to 0.62 mg Be/m³ from beryl ore. Lung inflammation was observed in the exposed monkeys, and a few granulomatous lung lesions were observed in the hamsters after similar exposure conditions (up to 23 months).

Immunological effects have been observed in a few subchronic studies (Schepers, 1964; Schepers *et al.*, 1957; Stiefel *et al.*, 1980). Schepers (1964) exposed monkeys (*Macacus mullata*) to three soluble forms of beryllium (BeF₂, BeSO₄, BeHPO₄) daily for 6 hours/day over 7 to 30 days. Increased lung weight, inflammation, emphysema, and fibrosis of the lung were observed after 17 days at 0.198 mg Be/m³ (as BeSO₄). Histological examination found pleuritis, congestion, emphysema, consolidation, and edema of the lung. Immunological effects were seen as hyperplasia of the lymph nodes typical of immune activation after 7 to 18 days exposure to either 0.198 or 0.184 mg Be/m³ as the sulfate or fluoride. A subchronic inhalation study reported immunological effects as increased, beryllium-specific stimulation of T-lymphocytes *in vitro* from Wistar rats and guinea pigs exposed daily (6 hours/day) over 10 weeks (LOAEL = 0.5 mg/m³) (Stiefel *et al.*, 1980). However, a subchronic inhalation study in Wistar and Sherman rats (Schepers *et al.*, 1957) observed multiple lung alterations including granulomas (LOAEL = 35 µg Be/m³) but did not find any accompanying immunological effects after 30 days discontinuous exposure (5-6 d/wk, 4-8 hr/d) to beryllium fumes from BeSO₄.

VI. Derivation of Chronic Reference Exposure Levels

Derivation of Inhalation Reference Exposure Level

<i>Key study</i>	Kreiss <i>et al.</i> , 1996
<i>Study population</i>	8 beryllium-sensitized workers among 136 employees in a beryllia ceramics plant
<i>Exposure method</i>	Workplace
<i>Critical effects</i>	Beryllium sensitization (chronic beryllium disease)
<i>LOAEL</i>	0.55 $\mu\text{g}/\text{m}^3$ (median exposure of sensitized workers)
<i>NOAEL</i>	Not observed
<i>Exposure continuity</i>	Workplace
<i>Average experimental exposure</i>	0.2 $\mu\text{g}/\text{m}^3$ for LOAEL group (0.55 x 10/20 x 5/7)
<i>Human equivalent concentration</i>	0.2 $\mu\text{g}/\text{m}^3$
<i>Exposure duration</i>	6.1 years (5 mo – 10 yr)
<i>LOAEL uncertainty factor</i>	10 (low incidence but serious, irreversible chronic disease)
<i>Subchronic uncertainty factor</i>	1
<i>Interspecies uncertainty factor</i>	1
<i>Intraspecies uncertainty factor</i>	3 (sensitized may not be only sensitive subpopulation) (see below)
<i>Cumulative uncertainty factor</i>	30
<i>Inhalation chronic REL</i>	0.007 $\mu\text{g}/\text{m}^3$
<i>Supportive study</i>	Eisenbud <i>et al.</i> (1949)
<i>Study population</i>	Approximately 10,000 individuals within 2 miles of a beryllium manufacturing plant
<i>Exposure method</i>	Environmental exposure
<i>Critical effects</i>	Pulmonary berylliosis in 11 residents
<i>LOAEL</i>	0.03 $\mu\text{g}/\text{m}^3$ (geometric mean of range of measured exposures associated with berylliosis of 0.01 to 0.1 $\mu\text{g}/\text{m}^3$)
<i>NOAEL</i>	Not observed
<i>Exposure continuity</i>	Continuous
<i>Average exposure</i>	Estimated to be approximately 0.3 $\mu\text{g}/\text{m}^3$ (historical exposures estimated to be 10-fold higher than measured values) for LOAEL group
<i>Human equivalent concentration</i>	0.3 $\mu\text{g}/\text{m}^3$ for LOAEL group
<i>Exposure duration</i>	Up to 7 years
<i>LOAEL uncertainty factor</i>	10
<i>Subchronic uncertainty factor</i>	3
<i>Interspecies uncertainty factor</i>	1
<i>Intraspecies uncertainty factor</i>	3
<i>Cumulative uncertainty factor</i>	100
<i>Inhalation chronic REL</i>	0.003 $\mu\text{g}/\text{m}^3$

U.S. EPA (1998) developed an RfC of $0.02 \mu\text{g}/\text{m}^3$ based on beryllium sensitization and progression to chronic beryllium disease (CBD) identified by Kreiss *et al.* (1996). The Kreiss *et al.* (1996) occupational exposure study identified a LOAEL for beryllium sensitization in workers of $0.55 \mu\text{g}/\text{m}^3$ (median of average exposure concentrations of the 8 Be sensitized workers). The Eisenbud *et al.* (1949) study, which U.S. EPA used as a co-principal study and which in U.S. EPA's opinion used relatively insensitive screening methods, suggested a NOAEL of $0.01\text{-}0.1 \mu\text{g}/\text{m}^3$ in community residents living near a beryllium plant. U.S. EPA used the LOAEL from the Kreiss *et al.* (1996) study for the operational derivation of the RfC, because the screening method used in the Eisenbud *et al.* (1949) study was considered to be less sensitive than the method used in the Kreiss *et al.* (1996) study. The LOAEL was time adjusted to $0.2 \mu\text{g}/\text{m}^3$, then a total UF of 10 was used to obtain the RfC of $0.02 \mu\text{g}/\text{m}^3$. The UF of 10 was comprised of a UF of 3 to account for the sensitive nature of the subclinical endpoint (beryllium sensitization) and a database UF of 3 to account for the poor quality of exposure monitoring in the Kreiss *et al.* and Eisenbud *et al.* studies. Poor exposure monitoring was also a problem in other epidemiology studies that assessed the incidence of beryllium sensitization. The U.S. EPA did not explicitly apply a LOAEL to NOAEL uncertainty factor. Thus implicitly the factor is 1.

OEHHA prefers to use the methodology for assignment of UFs, which is described in OEHHA (2000) and used in our derivation of the REL for beryllium, including use of a LOAEL to NOAEL Uncertainty Factor of 10. Since chronic beryllium disease (CBD) is serious, chronic, disabling, usually irreversible, and often fatal (Newman *et al.*, 1997), it is difficult to justify use of a LOAEL to NOAEL factor of only 3. OEHHA has not used database deficiency UFs since the criteria for use of such factors are not well specified by U.S. EPA. The people who get CBD are likely that part of the population who are by nature more sensitive to beryllium, for example those with the human leukocyte antigen (HLA) class II marker HLA-DP Glu69 (Richeldi *et al.*, 1993; Saltini *et al.*, 1998). Although it is likely that the effects are seen in a "sensitive subpopulation," OEHHA applied an intraspecies uncertainty factor (UF_H). OEHHA used an intermediate UF_H of 3, since 1) there may be other population factors involved in being sensitive, such as immature lungs, and 2) all the diseased were initially healthy adult workers.

For comparison the LOAEL from guinea pigs of $3.7 \mu\text{g Be}/\text{m}^3$ (Reeves *et al.*, 1970) is equivalent to a continuous exposure of $0.66 \mu\text{g}/\text{m}^3$. Division by UFs of 10 for intraspecies, 10 for interspecies (since HEC adjustments are not available yet for guinea pigs), and 10 for use of a LOAEL results in a REL of $0.0007 \mu\text{g}/\text{m}^3$

VII. Data Strengths and Limitations for Development of the REL

The major strength of the inhalation chronic REL for beryllium is the use of human data from persons occupationally exposed. The major uncertainties are the lack of a NOAEL observation in the key study, the lack of long-term exposure data, the difficulty of estimating exposures, and the lack of chronic exposure data.

VIII. Potential for Differential Impacts on Children's Health

No evidence to support a differential effect of beryllium on infants or children was found in the literature. However, children have developed beryllium disease from metal brought home on the parents' work clothes and by living near a facility using beryllium. Unfortunately the number of children and their ages were not published (Eisenbud *et al.*, 1948).

Derivation of Chronic Oral Reference Exposure Level

In addition to being inhaled, airborne beryllium can settle onto crops and soil and enter the body by ingestion. Thus an oral chronic reference exposure level for beryllium is also required for conducting Air Toxics Hot Spots risk assessments.

<i>Study</i>	Morgareidge <i>et al.</i> , 1976
<i>Study population</i>	Male and female dogs (5/sex/group)
<i>Exposure method</i>	Diet containing 0, 1, 5, 50 or 500 ppm Be as beryllium sulfate tetrahydrate
<i>Critical effects</i>	Small intestinal lesions
<i>LOAEL</i>	500 ppm
<i>NOAEL</i>	50 ppm (1.2 mg/kg bw-day)
<i>Exposure continuity</i>	Continuous
<i>Exposure duration</i>	Up to 3 years, 4 months
<i>Average experimental exposure</i>	1.2 mg/kg bw-day (males, 1.1; females, 1.3)
<i>BMD₀₅</i>	0.244 mg/kg-day
<i>LOAEL uncertainty factor</i>	Not needed in BMD approach
<i>Subchronic uncertainty factor</i>	1
<i>Interspecies uncertainty factor</i>	10
<i>Intraspecies factor</i>	10
<i>Cumulative uncertainty factor</i>	100
<i>Oral reference exposure level</i>	0.002 mg/kg-day

Morgareidge *et al.* (1976) conducted a long-term feeding study in which beagle dogs (aged 8 to 12 mo) were fed diets (for 1 h per day) containing 0, 5, 50, or 500 ppm Be for 172 weeks. The 500 ppm group was terminated at 33 weeks because of overt signs of toxicity, and an additional group was added to the study and fed a diet containing 1 ppm Be (for 143 weeks). The 1, 5, 50, and 500 ppm concentrations corresponded to doses of 0.023, 0.12, 1.1, and 12.2 mg/kg-day for males and 0.029, 0.15, 1.3, and 17.4 mg/kg-day for females. All animals in the 500 ppm group showed fairly extensive erosive (ulcerative) and inflammatory lesions in the gastrointestinal tract. These occurred predominantly in the small intestine and to a lesser extent in the stomach and large intestine, and were considered treatment related. All animals with stomach or large intestinal lesions also had lesions in the small intestine, except for one animal (whose stomach lesions were very localized and not very severe). Lesions in the small intestine (4/5 males and 5/5 females) were considered to be treatment-related and included desquamation of the epithelium, edema, fibrin thrombi, acute inflammation, subacute/chronic inflammation, necrosis and thinning/atrophy of the epithelium, and ulceration. High-dose animals also showed

moderate to marked erythroid hypoplasia of the bone marrow, which the authors also considered treatment related. (Bile stasis and vasculitis in the liver, acute inflammation in the lymph nodes, and kidney occurring in these animals was attributed to a likely systemic bacterial invasion through the damaged intestinal mucosa.) In the 50 ppm group, one female dog, which died after 70 weeks of treatment, showed gastrointestinal lesions, which were less severe, but occurred in the same locations and appeared to be the same types of lesions as those in dogs administered 500 ppm. The observation that beryllium is poorly absorbed by the gastrointestinal tract (Owen, 1990; ATSDR, 2000) probably explains why lesions were not seen outside the gastrointestinal tract. In addition the predominance of lesions in the small intestine may have been partly due to precipitation of beryllium phosphate there due to the slightly alkaline pH (Reeves, 1965). Thus 500 ppm was a LOAEL and 50 ppm was a NOAEL (statistically) for gastrointestinal lesions.

USEPA used the same study to derive its RfD of 0.002 mg/kg-day. The U.S. EPA stated its confidence in the RfD as: study - medium; database - low to medium, and RfD - low to medium. USEPA used a BD₁₀ approach and included a database UF of 3. OEHHA used a BD₀₅ approach (specifically a Weibull model in the USEPA's BMDS software) and did not include a database UF since the criteria for use of modifying factors such as this are not well specified by U.S. EPA. However, the final value for the oral chronic REL was the same as the USEPA's RfD.

This RfD and the oral REL are limited to soluble beryllium salts. Data on the teratogenicity or reproductive effects of beryllium are limited. Beryllium has been reported to produce terata and increased mortality in chick embryos.

When assessing the health effects of beryllium, its carcinogenicity must also be assessed.

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CHRONIC TOXICITY SUMMARY

1,3-BUTADIENE*(butadiene; buta-1,3-diene; biethylene; bivinyl; divinyl; vinylethylene)***CAS Registry Number: 106-99-0****I. Chronic Toxicity Summary**

<i>Inhalation reference exposure level</i>	20 µg/m³ (8 ppb)
<i>Critical effect(s)</i>	Increased incidence of ovarian atrophy in mice
<i>Hazard index target(s)</i>	Female reproductive system

II. Physical and Chemical Properties Summary (HSDB, 2000; CRC, 1995)

<i>Description</i>	Colorless gas
<i>Molecular formula</i>	C ₄ H ₆
<i>Molecular weight</i>	54.09 g/mol
<i>Boiling point</i>	-4.4°C
<i>Melting point</i>	-108.9°C
<i>Vapor pressure</i>	910 torr at 20°C
<i>Solubility</i>	Very slightly soluble in water (735 mg/L); soluble in ethanol, ether, acetone, benzene and organic solvents
<i>Conversion factor</i>	1 ppm = 2.21 mg/m ³ at 25°C

III. Major Uses and Sources

1,3-Butadiene is a major commodity product of the petrochemical industry, usually produced as a by-product of ethylene. The majority of 1,3-butadiene is used in the production of styrene-butadiene rubber copolymers (SBR). Other applications include use as a polymer component for polybutadiene, hexamethylene diamine, styrene-butadiene latex, acrylonitrile-butadiene-styrene (ABS) resins, chloroprene and nitrile rubbers. A variety of industrial syntheses use 1,3-butadiene resins (AB as a chemical intermediate, such as in the production of adiponitrile (a nylon precursor), captan and captofol fungicides, ethylidene norbornene and sulfolane, boron alkyls, and hexachlorobutadiene. Additionally, 1,3-butadiene is found in automobile exhaust, gasoline vapor, fossil fuel incineration products, and cigarette smoke (HSDB, 2000). In 1996, the latest year tabulated, the statewide mean outdoor monitored concentration of 1,3-butadiene was approximately 0.2 ppb (CARB, 1999). The South Coast Air Quality Management District (SCAQMD, 2000) detected ambient levels of 1,3-butadiene ranging from 0.1 to 0.8 ppb at 10 stationary monitors placed throughout the South Coast Air Basin. The annual statewide industrial emissions from facilities reporting under the Air Toxics Hot Spots Act in California

based on the most recent inventory were estimated to be 20,846 pounds of 1,3-butadiene (CARB, 2000).

IV. Effects of Human Exposure

An early occupational study reported complaints of irritation of eyes, nasal passages, throat, and lungs in rubber manufacturing workers following acute exposure to unknown levels of 1,3-butadiene (Wilson, 1944). Additional symptoms reported included coughing, fatigue, and drowsiness; however, all symptoms ceased on removal from the exposure.

Studies on the chronic effects of 1,3-butadiene have been centered in the styrene-butadiene rubber manufacturing industry, which uses large quantities of 1,3-butadiene, and in the 1,3-butadiene monomer industry. One retrospective epidemiological study reported an increase in overall mortality, emphysema, and cardiovascular diseases (chronic rheumatic and arteriosclerotic heart disease) among rubber workers (McMichael *et al.*, 1976). Two other occupational studies (Divine and Hartman, 1996; Matanoski *et al.*, 1990) indicated that the standardized mortality ratio for deaths from arteriosclerotic heart disease was elevated (~1.4-1.8) among black workers in the 1,3-butadiene rubber industry. Other occupational studies have described the potential for adverse hematological effects due to butadiene exposure (Checkoway and Williams, 1982; McMichael *et al.*, 1975). A survey of workers at a styrene-butadiene rubber plant revealed slightly lower levels (but within normal range) of red blood cells, hemoglobin, platelets, and neutrophils in exposed (mean = 20 ppm) versus unexposed workers (Checkoway and Williams, 1982). And 1,3-butadiene has been implicated in hematopoietic malignancies among styrene-butadiene rubber workers at levels lower than 20 ppm (McMichael *et al.*, 1975). Since the workers in these studies were exposed to mixtures of chemicals, the specific contribution of butadiene to the adverse respiratory and hematopoietic effects remains unclear.

V. Effects of Animal Exposure

The few available chronic animal inhalation studies have focused on the potential carcinogenicity of 1,3-butadiene. The National Toxicology Program (NTP) has sponsored two chronic inhalation studies in B6C3F₁ mice (NTP, 1984; Melnick *et al.*, 1990; NTP, 1993), while Hazelton Laboratories Europe (HLE) Ltd. conducted a chronic inhalation study in Sprague-Dawley rats (HLE, 1981; Owen *et al.*, 1987; Owen and Glaister, 1990).

The two B6C3F₁ mice inhalation studies sponsored by NTP (Huff *et al.*, 1985; Melnick *et al.*, 1990; NTP, 1984; NTP, 1993), although focused on carcinogenicity, identified other adverse chronic effects. The earlier NTP (1984) study in mice administered 0, 625 or 1250 ppm 1,3-butadiene for 6 hours/day, 5 days/week for up to 61 weeks. Nonneoplastic changes observed were elevated testicular and ovarian atrophy at both doses (625 and 1250 ppm); liver necrosis in male mice at both doses and in female mice at 1250 ppm; and nonneoplastic lesions in the nasal cavity at 1250 ppm. At the highest dose, adverse changes in the nasal cavity included chronic inflammation, fibrosis, cartilaginous metaplasia, osseous metaplasia, and atrophy of the sensory epithelium. No nasal or respiratory lesions were seen in the controls. This study identified a chronic LOAEL of 625 ppm for gonadal atrophy in both sexes.

The later NTP study (Melnick *et al.*, 1990; NTP, 1993) used lower exposure concentrations of 1,3-butadiene (0, 6.25, 20, 62.5, 200 or 625 ppm) administered 6 hours/day, 5 days/week for up to 2 years. Two-year survival was significantly decreased in mice exposed to 20 ppm and greater, primarily due to chemical-related malignant neoplasms. Increased incidences of non-neoplastic lesions in exposed mice included bone marrow atrophy, gonadal atrophy (testicular, ovarian and uterine), angiectasis, alveolar epithelial hyperplasia, forestomach epithelial hyperplasia, and cardiac endothelial hyperplasia. Gonadal atrophy was observed at 200 ppm and 625 ppm for males and at 6.25 ppm and higher for females. Bone marrow toxicity (regenerative anemia) was seen at 62.5 ppm and higher. This study identified a chronic LOAEL of 6.25 ppm for reproductive toxicity, and a NOAEL of 200 ppm and a LOAEL of 625 for non-neoplastic hematotoxic effects.

Table 1. Reproductive system atrophy and 2 year survival (NTP, 1993)

	<i>Butadiene (ppm)</i>	<i>Female survival</i>	<i>Atrophy of ovary</i>	<i>Atrophy of uterus</i>	<i>Male survival</i>	<i>Atrophy of testicle</i>
	0	37/50	4/49	1/50	35/50	1/50
	6.25	33/50	19/49	0/49	39/50	3/50
	20	24/50	32/48	1/50	24/50	4/50
	62.5	11/50	42/50	1/49	22/50	2/48
	200	0/50	43/50	8/50	4/50	6/49
	625	0/80	69/79	41/78	0/70	53/72

The U.S. EPA (1985) reviewed data from a 2-year chronic inhalation toxicity study sponsored by the International Institute of Synthetic Rubber Producers (IISRP) at Hazelton Laboratories Europe, Ltd (1981) on Sprague-Dawley rats exposed to 0, 1000 or 8000 ppm 1,3-butadiene. Results from the study were also reported later by Owen *et al.* (1987; 1990). Minor clinical effects, including excessive eye and nose secretions plus slight ataxia, were observed between 2 and 5 months in rats exposed to 8000 ppm 1,3-butadiene. Alterations in organ weight were also observed in this high exposure group. A dose-related increase in liver weights was observed at both the 52-week interim kill and at study termination. Absolute and relative kidney weight was also significantly increased and associated with nephrosis. No reproductive organ atrophy was reported in this rat study; however, tumors were found in reproductive tissues (Owen *et al.*, 1987).

Penn and Snyder (1996a,b) exposed cockerels (young male chickens) to 0 or 20 ppm 1,3-butadiene 6 hr/day, 5 days/week for 16 weeks to study arteriosclerotic plaque development. The cockerel is a sensitive animal model for studying the effects of environmental arteriosclerotic plaque-promoting agents. Plaque frequency and location were not affected. However, plaque sizes were significantly larger in 1,3-butadiene-treated cockerels than in controls.

The U.S. EPA (1985) described another secondary report, that of Miller (1978), which reviewed a group of Russian studies of subchronic 1,3-butadiene exposure in rats. One study (reported by Ripp in 1967) continuously exposed rats to relatively lower concentrations of 0.45, 1.4 or 13.5 ppm. At 13.5 ppm, blood cholinesterase was elevated, blood pressure was lowered, and motor

activity was decreased. Histopathological changes reported at 0.45 ppm were congestion in the spleen and hyperemia and leukocyte infiltration of cardiac tissue. Alterations in lung tissue noted at 1.4 and 13.5 ppm included atelectasis, interstitial pneumonia, and emphysema. No other studies used such low exposure levels or measured such endpoints. Unfortunately, the specific research methods and results for this study are unavailable for direct review and comparison.

A series of reproductive and developmental toxicity studies undertaken by U.S. EPA was summarized by Morrissey *et al.* (1990). In developmental toxicity studies, pregnant female rats and mice were exposed to 0, 40, 200, or 1000 ppm 1,3-butadiene for 6 hrs/day on days 6-15 of gestation. In rats, maternal body weight gain and extra-gestational body weight gain was reduced at the highest exposure. However, no evidence of developmental toxicity was observed. In mice, maternal body weight gain and extra-gestational body weight gain were reduced at 200 and 1000 ppm. Gravid uterine weight was reduced at 1000 ppm. Fetal and placental weights were reduced in an exposure-dependent manner with reduced male fetal body weight reaching statistical significance at 40 ppm and above. In the sperm head morphology assay and the dominant lethality study, groups of male mice were exposed to 200, 1000, and 5000 ppm 1,3-butadiene for 5 consecutive days. Concentration-related small increases in the percentages of abnormal sperm heads were observed, but were statistically significant only at the two highest exposures. Dominant lethal effects were observed only in the first two weeks following exposure. At week 1, the percentage of dead implants/total implants was increased only at 1000 ppm, and the percentage of females with ≥ 2 dead implants was increased at 200 and 1000 ppm. The number of dead implants/pregnancy was increased beginning at 1000 ppm at week 1, and 200 and 1000 ppm at week 2. While not strongly concentration dependent, the dominant lethality results are consistent with an adverse effect of 1,3-butadiene on more mature cells (spermatozoa and spermatids).

An acute and subchronic (10 week) study identified male-mediated F_1 effects in mice exposed to 12.5 or 1250 ppm 1,3-butadiene for 6 hours/day, 5 days/week (Anderson *et al.*, 1996). An additional group of mice were also exposed to 6250 ppm 1,3-butadiene in the acute study. Meaningful toxic effects were not observed in the acute study and no reproductive parameters were affected in either study. In the 10-week study, 1250 ppm (2762.4 mg/m³) resulted in a statistically significant reduction in the number of implantations, an induction of dominant lethal mutations, an increased incidence of early and late deaths, and an increase in abnormalities. The lower level of 12.5 ppm (27.63 mg/m³) resulted in an increase of late deaths and fetal abnormalities.

A follow-up of the Anderson *et al.* (1996) dominant lethality study exposed male mice to 12.5 or 125 ppm 1,3-butadiene under the same subchronic exposure conditions (Brinkworth *et al.*, 1998). A statistically significant increase in early deaths was observed at 125 ppm. The incidences of late deaths, dead fetuses, and abnormalities were elevated at 125 ppm but were not statistically significant. Testicular DNA damage, as detected by the Comet assay, was observed at 125 ppm.

Further dominant lethality studies in rodents by the same research group exposed male mice to 12.5, 65, and 130 ppm 1,3-butadiene 6 hr/day, 5 days/week for four weeks (Anderson *et al.*, 1998). Groups of male rats were also exposed to 65, 400, and 1250 ppm 1,3-butadiene 6 hr/day, 5 days/week for 10 weeks. In mice, a statistically significant increase in early deaths was

observed at 65 and 130 ppm but was not dose-related. Male-mediated effects in rats were not observed at any exposure level.

Pacchierotti *et al.* (1998) investigated 1,3-butadiene-induced toxic effects on spermatogenic cell stages and first-cleavage embryos. Exposure of male mice to 130, 500, and 1300 ppm 1,3-butadiene 6 hr/day for 5 days did not result in an increase of unfertilized oocytes after pairing with untreated females. However, statistically significant increases of cytogenetic aberrations in first-cleavage embryos were observed in the first mating week in mice exposed to 500 and 1300 ppm, and in the second mating week in mice treated with 1300 ppm. Treatment-related effects on differentiating spermatogonia were shown by a concentration-dependent decrease of round spermatids occurring 21 days after exposure, and confirmed 7 days later by a similar decrease of elongated spermatids. Testis weight was significantly reduced at all doses tested, 21 days after the end of exposure. A dose-dependent increase of variant sperm with single-stranded DNA content was observed 28 days after exposure, and attained statistical significance at 1300 ppm.

VI. Derivation of Chronic Reference Exposure Level (REL)

<i>Study</i>	NTP (1993)
<i>Study population</i>	B6C3F ₁ mice (70/sex/group)
<i>Exposure method</i>	Discontinuous inhalation (0, 6.25, 20, 62.5, 200, 625 ppm) over 2 years
<i>Critical effects</i>	Increased incidence of ovarian atrophy
<i>LOAEL</i>	6.25 ppm
<i>NOAEL</i>	Not observed
<i>BMC₀₅</i>	1.40 ppm
<i>Exposure continuity</i>	6 hr/d, 5 d/wk
<i>Exposure duration</i>	103 weeks
<i>Average experimental exposure</i>	0.25 ppm for BMC ₀₅ (1.40 ppm x 6/24 hr/day x 5/7 days/week)
<i>Human equivalent concentration</i>	0.25 ppm (gas with systemic effects, based on RGDR = 1.0 using default assumption that lambda (a) = lambda (h))
<i>LOAEL uncertainty factor</i>	Not needed in the BMC approach
<i>Subchronic uncertainty factor</i>	1
<i>Interspecies uncertainty factor</i>	3
<i>Intraspecies uncertainty factor</i>	10
<i>Cumulative uncertainty factor</i>	30
<i>Inhalation reference exposure level</i>	8 ppb (0.008 ppm; 0.02 mg/m ³ ; 20 µg/m ³)

The chronic REL for butadiene is based on an increased incidence of ovarian atrophy in mice. Characteristically, affected females had no evidence of oocytes, follicles, or corpora lutea. Significant reproductive toxicity was observed in both sexes of mice at the interim 9-month, interim 15-month, and 2-year study termination as gonadal atrophy (NTP, 1993). Testicular atrophy was induced in male B6C3F₁ mice at 625 ppm or above in this principal study and in a previous study (NTP, 1984). In female mice exposed for 9-months, ovarian atrophy was

observed at 200 and 625 ppm (442 or 1381 mg/m³, respectively). After 15 months, ovarian atrophy was observed at exposure levels of 20 ppm (44.2 mg/m³) and above. In mice exposed for up to 2 years (103 weeks), the incidence of ovarian atrophy increased at all exposure concentrations relative to controls, which establishes a chronic LOAEL of 6.25 ppm (13.81 mg/m³) for reproductive toxicity.

Presentation of the ovarian atrophy data in quantal form (see Table 1) allows the use of the benchmark concentration (BMC) approach to determine the REL. A log-normal probit analysis (U.S. EPA, National Center for Environmental Assessment, benchmark dose software, version 1.20) using only the control group and the log-dose of the three lowest butadiene exposure groups provided the lowest chi-square value (i.e., the best line fit to the data points). The proportion of mice developing ovarian atrophy in the two highest exposure groups did not increase appreciably with increasing exposure concentration, and therefore, deviated from the log-normal probit plot. The significantly shortened survival rate in these two groups may be one reason for this deviation. Another possible cause is that a relatively resistant subgroup of mice (to ovarian atrophy) is revealed at the two highest doses following 2-year exposure to 1,3-butadiene. Thus, it may be biologically plausible to remove these resistant subgroups when using a BMC approach. The maximum likelihood estimate (MLE) for a 5% response was 1.53 ppm. The resulting 95% lower confidence limit at the MLE provided a BMC₀₅ of 1.40 ppm. A BMC₀₅ is considered to be similar to a NOAEL in estimating a concentration associated with a low level of risk.

The mouse ovary is more sensitive to butadiene's epoxide metabolites than the rat ovary. Doerr *et al.* (1996) administered butadiene monoepoxide (BMO) or butadiene diepoxide (BDE) intraperitoneally to female B6C3F1 mice and Sprague-Dawley rats for 30 days and found that BMO and BDE exhibited a greater ovotoxic potential in the mice compared to the rats. Dahl *et al.* (1991) reported that, for equivalent inhalation exposures, the concentrations of total butadiene metabolites in blood were 5-50 times lower in the monkeys than in the mice and 4-14 times lower than in the rats. People may be more like the monkey than the mouse or the rat in their formation of epoxides from butadiene. In vitro metabolism studies with human liver tissue present conflicting results regarding whether humans would be more like rats or mice in forming epoxide metabolites (Bond *et al.*, 1996; Duescher and Elfarra, 1994). The considerable degree of interindividual variability in human samples was a reason given for the inconsistencies. Several pharmacokinetic models (Sweeney *et al.*, 1997; reviewed by Himmelstein *et al.*, 1997) have been developed to adjust for species differences in pharmacokinetics. However, an interspecies pharmacodynamic adjustment for this ovarian atrophy endpoint with butadiene is still needed. Therefore OEHHA staff use an interspecies uncertainty factor of 3 to account for pharmacodynamic differences between mice and women.

Christian (1996) has postulated that it may be inappropriate to develop health-protective values for 1,3-butadiene based on 2-year ovarian atrophy in mice because the mice are beyond their normal reproductive age. It was suggested that the 15-month evaluation of ovarian atrophy conducted by the NTP (1993) would be a better indicator of reproductive risk. However, OEHHA staff believes that butadiene-induced ovarian atrophy represents a toxic manifestation in an organ system. The fact that it occurs in a reproductive organ is immaterial for the development of a chronic REL. Nonetheless, a comparison REL based on the 15-month interim

evaluation for ovarian atrophy can be estimated. Quantal data at the 15-month interim evaluation shows that no mice developed ovarian atrophy (0/10) in the control group or at the lowest exposure. Ovarian atrophy was observed in 1/10, 9/10, 7/10, and 2/2 mice at the 20, 62.5, 200, and 625 ppm exposure groups, respectively. A log-normal probit analysis (U.S. EPA, National Center for Environmental Assessment, benchmark dose software draft, beta version 1.1b) based on the 15-month ovarian atrophy data provided an MLE of 8.12 ppm and a BMC_{05} of 3.08 ppm. Following adjustment for exposure continuity (6/24 hr/day, 5/7 days/wk) to 0.55 ppm and dividing by a total UF of 30 (3 for interspecies variability and 10 for intraspecies variability), a REL of 20 ppb ($40 \mu\text{g}/\text{m}^3$) was attained.

Another comparison to the proposed REL can be made using the dominant lethality study of Anderson *et al.* (1998). Early fetal deaths were observed at 65 and 125 ppm, but not 12.5 ppm. An earlier dominant lethality study (Anderson *et al.*, 1996) indicated that early deaths may occur at 12.5 ppm but the toxicological effect could not be repeated at this concentration in subsequent studies. The average exposure duration at the NOAEL is 3.125 ppm (12.5 ppm x 6 hr/24 hr). Use of an RGDR of 1 and a cumulative uncertainty factor of 30 (3 for interspecies and 10 for intraspecies) resulted in a REL of 0.1 ppm ($0.2 \text{ mg}/\text{m}^3$). Since the endpoint is a function of exposure during sperm maturation, no subchronic UF was used. The U.S. EPA had observed developmental toxicity in fetal rats (reduced male fetal body weight) at 40 ppm (Morrissey *et al.*, 1990). However, unlike the Anderson *et al.* (1998) study, a NOAEL was not determined.

Recent studies have implicated 1,3-butadiene in accelerating arteriosclerotic plaque development in cockerels (Penn and Snyder, 1996a,b), although no animal studies in mammals have implicated 1,3-butadiene in this disease. The worker study by McMichael *et al.* (1976) observed a slight increase in mortality from arteriosclerosis among all rubber workers. But more recent mortality studies in the rubber industry found no association or found an actual mortality decrement from arteriosclerosis and other circulatory diseases when compared to a reference population, suggesting a 'healthy worker' effect (Divine and Hartman, 1996; Matanoski *et al.*, 1990; Sathiakumar *et al.*, 1998).

When mortality among rubber workers was adjusted for race, two studies found that black rubber workers had a small, although statistically significant, increased mortality from arteriosclerosis compared to the black male U.S. population (Divine and Hartman, 1996; Matanoski *et al.*, 1990). But a larger study of black workers in the rubber industry found no association between circulatory diseases, which includes arteriosclerosis, and mortality (Sathiakumar *et al.*, 1998). Weaknesses in these worker analyses include relatively small cohort sizes, the bias of having racial information on all deaths and not on all living workers, the lack of racial data on some workers (up to 15% of cohort), and the lack of complete or specific work histories of the subjects. Also, black men of certain age groups are known to have an increased standardized mortality ratio for arteriosclerotic (ischemic) heart disease compared to white men (CDC, 2000). Limited data, conflicting worker mortality results, and lack of underlying mechanisms of action prevent the use of these findings in 1,3-butadiene REL development. However, there clearly is a need for further animal and epidemiological studies to determine if there is a true association between 1,3-butadiene exposure and arteriosclerotic diseases.

VII. Data Strengths and Limitations for Development of the REL

The major strength of the 1,3-butadiene REL is the observation of a dose-response effect in a well-conducted lifetime inhalation exposure study. The major weaknesses are the lack of adequate human health effects and metabolism data and the lack of a NOAEL observation in the key study.

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CHRONIC TOXICITY SUMMARY

CADMIUM AND CADMIUM COMPOUNDS

CAS Registry Number: 7440-43-9

I. Chronic Toxicity Summary

<i>Inhalation reference exposure level</i>	0.02 µg/m³ (respirable)
<i>Critical effect(s)</i>	Kidney effects (proteinuria) and respiratory effects (reduction in forced vital capacity and reduction in peak expiratory flow rate) in occupationally exposed humans
<i>Hazard index target(s)</i>	Kidney; respiratory system

II. Physical and Chemical Properties (ATSDR, 1993)

<i>Description</i>	Blue-white solid
<i>Molecular formula</i>	Cd
<i>Molecular weight</i>	112.41 g/mol
<i>Density</i>	8.642 g/cm ³ @ 20°C
<i>Boiling point</i>	765°C (CRC, 1994)
<i>Melting point</i>	320.9°C
<i>Vapor pressure</i>	1 torr @ 394°C
<i>Conversion factor</i>	Not applicable

III. Major Uses or Sources

The production of nickel-cadmium batteries is currently the primary use of cadmium (ATSDR, 1993). Cadmium, a by-product of zinc- and sulfide-ore processing, is also used for metal plating and in pigments and plastics. The annual statewide industrial emissions from facilities reporting under the Air Toxics Hot Spots Act in California based on the most recent inventory were estimated to be 3672 pounds of cadmium (CARB, 2000).

IV. Effects of Human Exposure

Pulmonary and renal function were examined in three worker groups: women with less than 20 years of exposure [group E1]; men with less than 20 years of exposure [group E2], and men with more than 20 years of exposure [group E3] (Lauwerys *et al.*, 1974). Exposed groups were matched to control groups in terms of age, body size, cigarettes smoked per day, duration of smoking, and duration of employment. Although urine cadmium concentrations were significantly elevated, the subjects in E1 did not exhibit pulmonary function changes or

proteinuria indicative of renal impairment. The workers in E1 had been exposed for a mean of 4.08 years to $31 \mu\text{g}/\text{m}^3$ total cadmium ($1.4 \mu\text{g}/\text{m}^3$ respirable cadmium). The 27 workers in E2 had been exposed for a mean of 8.6 years to $134 \mu\text{g}/\text{m}^3$ total cadmium ($88 \mu\text{g}/\text{m}^3$ respirable cadmium). The blood and urinary cadmium levels of these workers were also significantly elevated compared to matched controls. Glomerular proteinuria was observed in 15% of the workers in E2 and in 68% of workers in E3. The 22 workers of E3 had been exposed for a mean of 27.8 years to $66 \mu\text{g}/\text{m}^3$ total cadmium ($21 \mu\text{g}/\text{m}^3$ respirable cadmium). Significantly increased levels of cadmium were observed in the blood and urine, and workers in E3 also exhibited significant decreases in some measures of pulmonary function (forced vital capacity, forced expiratory volume in one second, and peak expiratory flow rate). This study identifies the kidney as the key target organ of chronic cadmium exposure. For respirable cadmium, this study indicates a LOAEL of $21 \mu\text{g}/\text{m}^3$ for workers exposed for 28 years and a NOAEL of $1.4 \mu\text{g}/\text{m}^3$ for workers exposed for 4 years.

A study of 82 cadmium exposed workers reports the time-weighted cumulative exposure index (TWE) and cadmium body burden determined in vivo (Ellis *et al.*, 1985). Evidence of renal dysfunction (usually elevated urinary β_2 -microglobulin) was consistently observed when the worker's liver cadmium burden exceeded 40 ppm and the time-weighted cumulative exposure index exceeded 400-500 $\mu\text{g years}/\text{m}^3$.

A detailed investigation of renal function in 75 male cadmium-exposed workers identified significant increases in urinary excretion of several low- and high molecular weight proteins, including β_2 -microglobulin, and significant decreases in renal reabsorption of calcium, urate, and phosphate compared to controls (Mason *et al.*, 1988). Exposures, which ranged from 36 to $600 \mu\text{g}/\text{m}^3$, were determined from background or personal exposure measurements made between 1964 and 1983, or were estimated. A time-weighted cumulative exposure index (TWE) was determined for each subject. A two phase linear regression model was applied to the data to identify inflection points for each biochemical parameter. The biochemical indicators most highly correlated to exposure were urinary retinol binding protein and urinary β_2 -microglobulin. Of these, the most sensitive parameter, urinary β_2 -microglobulin, demonstrated an inflection point at $1108 \mu\text{g years}/\text{m}^3$ with a 95% lower confidence limit of $509 \mu\text{g years}/\text{m}^3$. The endpoint selected is indicative of defects in tubular reabsorption of proteins.

Diminished sensitivity of smell has also been observed in cadmium exposed workers (Rose *et al.*, 1992). Cadmium body burden, β_2 -microglobulin levels, and olfactory function were measured in a group of 55 male workers exposed to cadmium fumes in a brazing operation. A group of 15 control workers was also tested. Exposed workers exhibited high urinary cadmium levels, tubular proteinuria, and a significant, selective defect in odor detection threshold.

V. Effects of Animal Exposure

Interstitial infiltration of lymphocytes and leukocytes and hyaline casts were observed in the kidneys of rabbits following exposure to $6.5 \text{ mg}/\text{m}^3$ cadmium-iron dust for 3 hours per day, 21 days per month for 9 months (Friberg, 1950). Proteinuria was observed in the majority of exposed rabbits by the fourth month of exposure. Increased lung weights and emphysema were

also observed. The trachea and nasal mucous membranes exhibited chronic inflammatory changes (not specified) and lymphocyte infiltration. The kidney contained the greatest concentration of cadmium. This study also exposed a group of rabbits to 9.1 mg/m^3 cadmium-iron dust for 3 hours per day, 23 days per month, for 7 months. Two rabbits in this group died from acute pneumonia at one month, and one rabbit was terminated at 3 months of exposure. Findings at necropsy were similar, although more severe than those observed in rabbits exposed to 6.5 mg/m^3 . Chronic bronchitis and hyperplasia of the bronchiolar epithelium were observed in the higher dose group in addition to the findings previously noted.

Male and female rats were exposed to 0, 0.3, 1.0, or 2.0 mg Cd/m^3 (as CdCl_2) 6 hours per day, 5 days per week for a total of 62 exposures (Kutzman *et al.*, 1986). Rapid, shallow breathing and marked weight loss were observed in the highest dose group; all animals in this group died within the first 45 days of exposure. A dose-dependent increase in lung weight was observed in the remaining dose groups and a statistically significant increase in lung collagen and elastin was observed in rats exposed to 1.0 mg/m^3 . Pathological changes noted in the terminal bronchioles include flattening and hyperplasia of type II cells, and infiltration of macrophages, mononuclear cells, and polymorphonuclear leukocytes. Proliferation of fibroblasts with deposition of collagen was also noted.

Male rats were exposed continuously to 0, 30, or $90 \text{ } \mu\text{g Cd/m}^3$ cadmium oxide (CdO) dust for up to 18 months (Takenaka *et al.*, 1990). Animals exposed to $30 \text{ } \mu\text{g/m}^3$ were sacrificed at 6 and 18 months of exposure. Although some rats in the high dose group were terminated after 6 months of exposure, the remaining rats were terminated after 7 months due to increased mortality and were not included in the study. Inflammation and hyperplasia of the alveolar epithelium occurred in animals of both groups after 6 months of exposure with more marked changes observed in the high dose group. Abnormal proliferation of the epithelium was observed in the low dose group following 18 months of exposure. Lung tumors observed in both dose groups were characterized as being duration dependent.

VI. Derivation of Chronic Reference Exposure Levels (REL)

Derivation of Chronic Inhalation Reference Exposure Level

<i>Study</i>	Lauwerys <i>et al.</i> , 1974
<i>Study population</i>	Humans (22 exposed men and 22 unexposed men in LOAEL group; 31 exposed women and 31 non-exposed women in NOAEL group)
<i>Exposure method</i>	Occupational exposures
<i>Critical effects</i>	Kidney effects - proteinuria in 68% of LOAEL group Respiratory effects – reduction in forced vital capacity (FVC), forced expiratory flow in 1 second (FEV ₁); reduction in peak expiratory flow rate
<i>LOAEL</i>	21 µg/m ³ respirable cadmium
<i>NOAEL</i>	1.4 µg/m ³ respirable cadmium
<i>Exposure continuity</i>	Assumed to be 5 days/week for 8 hours/day during which 10 m ³ air is breathed
<i>Average occupational exposure</i>	0.5 µg/m ³ for NOAEL group (1.4 x 10/20 x 5/7)
<i>Human equivalent concentration</i>	0.5 µg/m ³ for NOAEL group
<i>Exposure duration</i>	Average of 4.1 years (1 to 12 years) for NOAEL group
<i>LOAEL uncertainty factor</i>	1
<i>Subchronic uncertainty factor</i>	3
<i>Interspecies uncertainty factor</i>	1
<i>Intraspecies uncertainty factor</i>	10
<i>Cumulative uncertainty factor</i>	30
<i>Inhalation reference exposure level</i>	0.02 µg/m ³

VII. Data Strengths and Limitations for Development of the REL

This evaluation of a chronic REL for cadmium is strengthened by being based on a human exposure study of workers exposed to cadmium for periods of 1 to over 20 years. The exposed group was matched to a control group in terms of age, body size, cigarettes smoked per day, duration of smoking, and duration of employment. The factory process was unchanged over the study period suggesting that exposures may have remained relatively constant over time. Significant areas of uncertainty include an incomplete knowledge of the past exposures over the full study interval and the relatively small number of subjects in the study.

A similar evaluation of the LOAEL group led to an alternate estimate for an inhalation reference exposure level of 0.05 µg/m³. The LOAEL group had an average occupational exposure of 5.0 µg/m³ and an average exposure duration of 27.8 years (21 to 40 years). Default uncertainty

factors included a 10-fold LOAEL uncertainty factor and a 10-fold intraspecies uncertainty factor (UF).

For comparison, using data presented by Ellis and associates (1985) and Mason and associates (1993) correlating human cumulative exposures (in terms of $\mu\text{g}\cdot\text{years}/\text{m}^3$) and renal tubular protein reabsorption, a LOAEL of $500 \mu\text{g}\cdot\text{years}/\text{m}^3$ was predicted. This correlates to $7 \mu\text{g}/\text{m}^3$ over 70 years. A time-weighted exposure to account for continuous exposure rather than 40 hour per week occupational exposure is $1.7 \mu\text{g}/\text{m}^3$. Applying a 10-fold LOAEL uncertainty factor and a 10-fold intraspecies uncertainty factor results in a REL value of $0.02 \mu\text{g}/\text{m}^3$, the same value obtained using the Lauwerys *et al.* data. U.S. EPA has not published an RfC for cadmium.

In addition to being inhaled, airborne cadmium can settle onto crops and soil and enter the body by ingestion. Thus an oral chronic reference exposure level for cadmium is also required. We propose adopting the U.S. EPA RfD as the chronic oral REL.

Derivation of Chronic Oral Reference Exposure Level (U.S. EPA RfD)

<i>Study</i>	U.S. EPA, 1985
<i>Study population</i>	Humans
<i>Exposure method</i>	Food and drinking water
<i>Critical effects</i>	Significant proteinuria
<i>LOAEL</i>	Not observed
<i>NOAEL</i>	0.005 mg/kg bw-day
<i>Exposure continuity</i>	Chronic
<i>Exposure duration</i>	Up to lifetime
<i>Average exposure</i>	0.005 mg/kg bw-day
<i>LOAEL uncertainty factor</i>	1
<i>Subchronic uncertainty factor</i>	1
<i>Interspecies uncertainty factor</i>	1
<i>Intraspecies factor</i>	10
<i>Cumulative uncertainty factor</i>	10
<i>Oral reference exposure level</i>	0.0005 mg/kg bw-day

The oral REL is the U.S. EPA's Reference Dose (RfD) (U.S. EPA, 1996). A concentration of $200 \mu\text{g}$ cadmium (Cd)/gm wet human renal cortex is the highest renal level not associated with significant proteinuria (U.S. EPA, 1985). A toxicokinetic model is available to determine the level of chronic human oral exposure (NOAEL) which results in $200 \mu\text{g}$ Cd/gm wet weight human renal cortex. The model assumes that 0.01% of the Cd body burden is eliminated per day (U.S. EPA, 1985). Assuming 2.5% absorption of Cd from food or 5% from water, the toxicokinetic model predicts that the NOAEL for chronic Cd exposure is 0.005 and 0.01 mg Cd/kg/day from water and food, respectively (i.e., levels which would result in $200 \mu\text{g}$ Cd/gm wet weight human renal cortex). Thus, based on an estimated NOAEL of 0.005 mg Cd/kg/day for Cd in drinking water and an UF of 10, an RfD of 0.0005 mg Cd/kg/day (water) was calculated; an equivalent RfD for Cd in food is 0.001 mg Cd/kg/day.

Cd is unusual in relation to most, if not all, of the substances for which an oral RfD has been determined in that a vast quantity of both human and animal toxicity data are available. The RfD is based on the highest level of Cd in the human renal cortex (i.e., the critical level) not associated with significant proteinuria (i.e., the critical effect). A toxicokinetic model has been used to determine the highest level of exposure associated with the lack of a critical effect. Since the fraction of ingested Cd that is absorbed appears to vary with the source (e.g., food vs. drinking water), it is necessary to allow for this difference in absorption when using the toxicokinetic model to determine an RfD.

The uncertainty factor of 10 is used to account for intrahuman variability to the toxicity of this chemical in the absence of specific data on sensitive individuals. No modifying factor was used.

U.S. EPA stated its confidence in the RfD as: Study - Not applicable; Data Base - High; and RfD - High. The choice of NOAEL does not reflect the information from any single study. Rather, it reflects the data obtained from many studies on the toxicity of cadmium in both humans and animals. These data also permit calculation of pharmacokinetic parameters of cadmium including absorption, distribution, metabolism, and elimination. All this information considered together gives high confidence in the data base. High confidence in the RfD follows as well.

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CHRONIC TOXICITY SUMMARY

CARBON DISULFIDE*(carbon bisulfide; carbon sulfide; dithiocarbonic anhydride)***CAS Registry Number: 75-15-0****I. Chronic Toxicity Summary**

<i>Inhalation reference exposure level</i>	800 $\mu\text{g}/\text{m}^3$ (300 ppb)
<i>Critical effect(s)</i>	CNS/PNS (reduction in motor nerve conduction velocities in occupationally-exposed humans)
<i>Hazard index target(s)</i>	Nervous system; reproductive system

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

<i>Description</i>	Clear, colorless or faintly yellow liquid
<i>Molecular formula</i>	CS ₂
<i>Molecular weight</i>	76.14 g/mol
<i>Boiling point</i>	46.5°C
<i>Melting point</i>	-111.5°C
<i>Vapor pressure</i>	297 torr @ 20°C
<i>Solubility</i>	Slightly soluble in water (2.94 g/L); miscible in anhydrous methanol, ethanol, ether, benzene, chloroform, and carbon tetrachloride
<i>Conversion factor</i>	3.1 mg/m ³ per ppm at 25°C

III. Major Uses and Sources

The most prominent industrial use of carbon disulfide is in the production of viscose rayon fibers. Carbon disulfide is also used in the production of carbon tetrachloride and cellophane, and, as a solvent for rubber, sulfur, oils, resins, and waxes. In the past, carbon disulfide was used in soil fumigation and insect control in stored grain. Industrial processes that produce carbon disulfide as a by-product include coal blast furnaces and oil refining (HSDB, 1995). Carbon disulfide is also a breakdown product of metam sodium. The annual statewide industrial emissions from facilities reporting under the Air Toxics Hot Spots Act in California based on the most recent inventory were estimated to be 1562 pounds of carbon disulfide (CARB, 2000).

IV. Effects of Human Exposure

A primary target of carbon disulfide (CS₂) toxicity is the nervous system. The major neurotoxic action of carbon disulfide is the development of mental disturbances. These include change of personality, irritability, and forgetfulness, often with accompanying neurophysiological and neuropathological changes after prolonged exposure. Such changes include decreased peripheral nerve impulse conduction, motor and/or sensory neuropathies, cerebral or cerebellar atrophy, and neuropsychological organic changes (Aaserud *et al.* 1988, 1990, 1992; Foa *et al.*, 1976; Hirata *et al.* 1992; Ruijten *et al.* 1990, 1993). Alterations in behavioral indices have historically been associated with high levels of CS₂, often in excess of 20 ppm (Foa *et al.* 1976; Hanninen *et al.*, 1978).

Studies have identified alterations in the nerve conduction of workers chronically exposed to lower CS₂ levels (Hirata *et al.*, 1992a; Johnson *et al.*, 1983; Ruijten *et al.*, 1990; Ruijten *et al.*, 1993). A cross-sectional study of Japanese spinning workers identified alterations in the central nervous system as measured by brain stem auditory evoked potential (BAEP) (Hirata *et al.*, 1992). The latencies of the three main BAEP components increased significantly in workersexposed to CS₂ for more than 20 years when compared to controls. CS₂ exposures ranged from 3.3 to 8.2 ppm (mean = 4.76 ppm). Ruijten *et al.* (1993) identified mild presymptomatic nerve impairment (decreased conduction velocities and response amplitudes) in 44 CS₂-exposed workers with an average cumulative exposure range from 192 to 213 ppm-year (mean duration = 26.1 years).

A NIOSH occupational study evaluated the effects of CS₂ on the peripheral nervous system. Johnson *et al.* (1983) identified a significant dose related reduction in the maximum motor nerve conduction velocities (MCV) in the calves and ankles of male viscose rayon workers exposed to high (median = 7.6 ppm) CS₂ levels versus a comparison group exposed to low concentrations (median = 0.2 ppm). The workers were all employed in artificial fiber production in the same plant. Since these reduced MCVs were still within the normal range, the authors considered the measured difference an indication of minimal neurotoxicity. The mean exposure concentration for all exposed workers (n = 145) ranged from 0.6 to 16 ppm (mean = 7.6 ppm) with a mean duration of 12.1 years. This study identified a chronic LOAEL of 7.6 ppm for minor neurological effects (decreased peroneal nerve MCV and sural nerve conduction velocity).

Another epidemiological study evaluated a group of 111 Belgian viscose rayon factory workers exposed to 4 to 112 mg/m³ CS₂ (time-weighted average 1 to 40 mg/m³) (Vanhoorne *et al.*, 1995). Among four categories of cumulative exposure (0, 1 to 300, 301 to 600, and greater than 600 mg/m³-years), a clear dose-response effect was observed for reduced mean peroneal motor nerve conduction velocities in both fast and slow fibers. Unfortunately, the data are incompletely reported, and the mean duration of exposure is not given. Subgroups of workers whose exposures ever exceeded 10 ppm (n=64) and never exceeded 10 ppm (n=30) each showed significantly reduced fibular nerve motor conduction velocities compared with non-exposed workers.

Vascular atherosclerotic changes are also considered a major effect of chronic carbon disulfide exposure. Several occupational studies have demonstrated an increase in the mortality due to

ischemic heart disease in CS₂ exposed workers (Hernberg *et al.*, 1970; MacMahon and Monson, 1988; Tiller *et al.*, 1968; Tolonen *et al.*, 1979). A 2.5-fold excess in mortality from coronary heart disease in workers exposed to CS₂ was first reported by Tiller *et al.* (1968). A subsequent prospective study by Hernberg *et al.* (1970) found a 5.6-fold increased risk in coronary heart disease mortality and a 3-fold increased risk of a first nonfatal myocardial infarction in CS₂ exposed workers.

Male workers (n=177) in a Polish fiber plant were exposed to CS₂ for an average of 14 years (range of 5 to 38 years). Controls were 93 healthy male workers from other factories that did not use carbon disulfide. Carbon disulfide exposed workers had higher rates (42%) of 24-hour electrocardiographic abnormalities than non-exposed workers (24%, p=0.006) (Bortkiewicz *et al.*, 2001). The most common abnormalities were ventricular extrasystoles and repolarization disturbances, the latter occurring most often in workers with the longest CS₂ exposures. Long-term blood pressure monitoring did not reveal any differences between exposed and control groups.

Male workers in a Belgian viscose rayon factory (n=85) were estimated by personal active sampling to have exposures of 2 to 32 mg/m³ CS₂. Controls were 37 non-exposed workers from factories that did not use CS₂. Exposed workers had reduced common carotid artery distensibility as measured with ultrasound sonography, while the carotid artery compliance coefficient was not significantly affected. Also, blood pressures and cholesterol levels were not significantly different than observed among control workers (Kotseva *et al.*, 2001a). Differences in carotid artery distensibility remained significant after adjustment for age, smoking, alcohol consumption, ethnicity, body mass index, heart rate, and systolic blood pressure.

Egeland *et al.* (1992) and Vanhoorne *et al.* (1992) have reported that human exposure to CS₂ for more than one year causes increases in biochemical changes often associated with cardiovascular disease - diastolic blood pressure, low density lipoprotein cholesterol, and apolipoproteins A1 and B. Egeland *et al.* (1992) used cross sectional data on 165 CS₂-exposed workers (245 controls) collected in 1979 by Fajen *et al.* (1981). The affected workers were exposed for at least 1 year in a viscose rayon factory to an estimated median TWA (8-hour) of 7.6 ppm. The Egeland *et al.* (1992) study indicated that modest CS₂ exposure (range = 3.4 to 5.1 ppm, median = 4.1 ppm) was associated with increased low density lipoprotein cholesterol (LDLc), the type of increase associated with atherosclerotic heart disease. No significant differences were seen between controls and the low CS₂ exposed group (range = 0.04 to 1.02 ppm, median = 1.00 ppm). Study NOAEL and LOAEL for increased LDLc and diastolic blood pressure were thus 1.0 ppm and 4.1 ppm, respectively. Vanhoorne *et al.* (1992) identified increased LDL-cholesterol, apolipoprotein B, systolic and diastolic blood pressure as indicative of an increased coronary risk in workers from a Belgian viscose rayon factory (115 exposed and 76 controls). CS₂ concentrations ranged from 1 to 36 ppm. Duration of exposure was not indicated. Even though these biochemical changes were observed, no significant increases in cardiovascular disease, such as angina, myocardial infarction, or ischemia, were determined by ECG changes.

Workers (n=141) with a minimum of 1 year employment in viscose rayons factories were compared with 141 age and gender-matched plastic industry workers. Current exposures were estimated as 1 to 30 mg/m³ (03 to 10 ppm). Exposed workers were categorized as group 1 or

group 2, with cumulative exposures of less than or greater than 100 mg/m³-years, respectively. Group 2 (p<0.001) but not group 1 workers had increased mean total cholesterol (5.3 and 4.5 mmol/l) compared with controls (4.6 mmol/l) (Kotseva, 2001b).

CS₂ causes reproductive toxicity in both males and females. Lancranjan *et al.* (1969), Lancranjan (1972), Cirla *et al.* (1978), and Wagar *et al.* (1983) studied male reproductive effects of occupational exposure to CS₂ and showed significant adverse effects on spermatogenesis, levels of serum FSH and LH, and libido; these effects persisted in 66% of the workers subject to follow-up. Zhou *et al.* (1988) investigated pregnancy outcomes and menstrual disturbances in 265 women occupationally exposed to CS₂ in five facilities and 291 controls. The CS₂-exposed women had a significantly higher incidence of menstrual disturbances versus the control group (overall 34.9% vs. 18.2%). CS₂ levels varied between the five facilities (exposure category means of low = 3.1 mg/m³, intermediate = 6.5 mg/m³, and high = 14.8 mg/m³), but all workers from these CS₂ facilities had significantly higher incidences of menstrual disturbance. Irregularity of menstruation was the most common disturbance, followed by abnormal bleeding. No evidence was observed to indicate an adverse effect on the term and outcome of pregnancy.

An abstract of an epidemiological study of birth defects among female workers occupationally exposed to CS₂, was reported by Bao *et al.* (1991). Exposures were at rayon factories in four Chinese provinces and began at least 6 months prior to pregnancy and continued during pregnancy. An increased rate of birth defects (2.6% vs. 1.3%) among 682 exposed women was noted compared to 745 women in the control group. The most common defects were congenital heart defects, inguinal hernia, and CNS defects. However, there was no significant difference in birth defects between those with estimated exposures greater than 10 mg/m³ compared to those with lower exposures. There were no differences in rates of stillbirth, low birth weight, or neonatal or perinatal deaths among any of the groups.

The possibility of determining LOAEL and/or NOAEL values for the major CS₂-related adverse effects from epidemiology studies, which predominately use workers from the viscose rayon industry, is limited. The limitations include incomplete historical exposure measurements, concurrent exposure to other chemicals (including hydrogen sulfide or methylene chloride), lack of personal exposure determinations, and a high variability of individual exposures due to decreases of plant CS₂ concentrations over time.

V. Effects of Animal Exposure

Studies investigating the potential for CS₂ toxicity in animals have usually been limited by intermediate or subchronic duration (less than 1 year) and a lack of multiple dose or exposure groups. The neuropathologic changes consistently observed in rodents following CS₂ exposure include axonal swelling, demyelination, swelling at neuromuscular junctions, muscle atrophy and degeneration, damage to terminal axons, and nerve fiber breakdown (Clerici and Fechter, 1991; Colombi *et al.* 1981; Eskin *et al.*, 1988; Jirmanova and Lukas, 1984; Maroni *et al.*, 1979; Szendzikowski *et al.*, 1973). These adverse effects have been observed over a range of

exposures (250 to 800 ppm), but few studies have attempted to establish a dose response for this CS₂-induced neurotoxicity.

In a 90 day subchronic inhalation study, Sprague-Dawley and Fischer 344 rats exposed discontinuously (6 hours/day, 5 days/week) to CS₂ developed morphological alterations in nerves including axonal swelling and myelin degradation (Gottfried *et al.*, 1985). This study established a subchronic NOAEL of 50 ppm and a LOAEL of 300 ppm for morphological changes in nerves. A longer inhalation study in Wistar rats observed impairment in the conduction velocity of the sciatic and tibial nerves after 6 and 12 months of intermittent exposure to 289 ppm CS₂ (LOAEL of 289 ppm) (Knobloch *et al.*, 1979).

In a 13-week subchronic study, male and female F344 rats inhaled 0, 50, 500, or 800 ppm CS₂ discontinuously (6 h/day, 5 days per week) (Sills *et al.*, 1998). Development of distal axonopathy in the muscular branch of the posterior tibial nerve (MBPTN) and spinal cord was examined. After 13 weeks, giant swollen axons were observed with thin myelin sheaths as well as some degenerated and regenerated axons. Axonal swelling was noted in the spinal cords of rats exposed to 500 or 800 ppm CS₂. In the 800 ppm group, additional axonal swelling was observed in the muscular branch of the posterior tibial nerve. Neurofilament deposits were found in swollen axons in the spinal cord and MBPTN. The NOAEL for axonal swelling was 50 ppm.

Wronska-Nofer (1973) showed a positive relationship between the level of triglycerides, the rate of cholesterol synthesis, and CS₂ exposure in Wistar rats exposed to 0, 73.8, 160, 321, or 546 ppm CS₂ for 5 hours/day, 6 days/week over 8 months. This study found a subchronic LOAEL of 73.8 ppm for disturbances in lipid metabolism (increase in serum cholesterol and serum triglycerides).

Lewis *et al.* (1999) investigated the capacity of CS₂ to induce arterial fatty deposits by itself, and its ability to enhance the rate of fatty deposit formation induced by a high fat diet. Groups of 20 female C57BL/6 mice were exposed to 0, 50, 500, or 800 ppm CS₂ by inhalation. Half the animals in each group were placed on an atherogenic high fat diet and half on a control diet. Mice were necropsied after 1, 4, 8, 12, 16, or 20 weeks of exposure, and the rates of fatty deposit formation under the aortic valve leaflets were evaluated. Exposure of mice on the control diet to 500 and 800 ppm CS₂ induced a small but significant increase in the rate of fatty deposit formation over non-exposed controls. In the animals on the high fat diet there was marked enhancement of the rate of fatty deposit formation in mice exposed to 500 and 800 ppm over the animals on the high fat diet alone. In addition, there was a small but significant enhancement in mice exposed to 50 ppm over the rate of fatty deposit formation induced by the high fat diet alone. Thus CS₂ is atherogenic at high concentrations and in conjunction with other risk factors, CS₂ at relatively low concentrations can enhance atherogenesis in mice. Fifty ppm is thus the study LOAEL.

Hepatic toxicity has also been induced in rats exposed to relatively high doses of CS₂, usually following pretreatment with liver inducers such as phenobarbital. Bond *et al.* (1969) showed that high doses of CS₂ to rats produced an increase in periportal liver fat, and decreases in hepatic cytochrome P450 content and in microsomal mixed function oxidase (MFO) activity. After phenobarbital induction, exposed rats exhibited more severe hepatotoxicity characterized by hydropic degeneration and necrosis. Other hepatotoxic effects seen after CS₂ exposures greater

than 400 ppm include increases in relative liver weight (Sokal, 1973), stimulation of liver microsomal lipid peroxidation (Wronska-Nofer *et al.*, 1986), and decreases in hepatic cholesterol synthesis (Simmons *et al.*, 1988).

The 24-hr lethal ip LD₅₀ values for CS₂ were estimated in 1-, 5-, 10-, 20-, 30- and 40-day-old rats (sample size not specified) (Green and Hunter, 1985). 1-day-old rats (LD₅₀ 583 mg/kg, ip) were about 3-times more susceptible than 20-day-old rats (LD₅₀ 1545 mg/kg, ip).

¹⁴C- and ³⁵S-labelled CS₂ was given ip to 1-, 5-, 10-, 20-, 30-, and 40-day-old rats (Snyderwine and Hunter, 1987). Thirty- and forty-day-old rats (sample size not reported) metabolized significantly more CS₂ to CO₂ and expired significantly less CS₂ than 1- to 20-day-old rats. Twenty-four hr after administration, up to 13 times more ³⁵S -label (radioactivity per g of tissue) were present in organs from 1-day-old rats than in similar organs from 40-day-old rats. The study does not specifically address the toxicological implications of the metabolic differences, and did not include fully mature animals. However, inability to detoxify CS₂ would lead to higher tissue concentrations and thus, potentially, increased toxicity.

The metabolite responsible for CS₂ hepatotoxicity is believed to be reactive sulfur atoms that covalently bind to cellular macromolecules (Dalvi, 1988). Similarly, the correlation between increased lethality (Green and Hunter, 1985) and increasing binding of ³⁵S -label (Snyderwine and Hunter, 1987) in younger CS₂-exposed animals is consistent with a role for reactive sulfur. Neurotoxicity of CS₂ results from the formation of thiourea lysine cross-links between neurofilament proteins (DeCaprio *et al.*, 1992; Valentine *et al.*, 1997; Erve *et al.*, 1998).

New Zealand white rabbits (24 per group) inhaled 0, 60, 100, 300, 600 or 1200 ppm CS₂ for 6 h/d on gestation days 6 to 18 (Pathology Associates, 1991). Developmental toxicity (NOAEL = 300 ppm; 930 mg/m³) was noted at concentrations lower than those associated with significant maternal toxicity (NOAEL = 600 ppm; 1860 mg/m³) (Pathology Associates, 1991). The adults did have some slight hematological changes at the 600 ppm level, but the authors questioned the biological significance of these marginal findings. Reduced fetal body weights were noted at 600 and 1200 ppm. Cumulative malformations were increased in the 1200 (3720 mg/m³) but not 600 ppm group, though there were no significant increases in any specific malformation in any group. Maternal effects at 1200 ppm included decreased body weight, ataxia, wheezing, and tremors. In an initial range-finding study, exposure to 3000 ppm was associated with significant lethality.

Rats were exposed to 100 mg/m³ (32 ppm) for 4 hr/d on gestation days 7 and 8, and the embryos explanted to culture medium at day 9.5. Growth of explants of 10 treated and 17 control embryos was monitored for 44 hours. CS₂ at this concentration induced growth retardation in treated embryos relative to controls (Zhao *et al.*, 1997).

In a two-generation study, Tabacova *et al.* (1983) exposed pregnant Albino rats (30-32 pregnant females per group) to 0.03, 10, 100, or 200 mg/m³ (0.01, 3, 32, or 64 ppm) CS₂. The two highest dose levels were both teratogenic and maternally neurotoxic. There were no significant adverse effects in the F1 generation at the 2 low dose levels. However, significant increases in teratogenicity were found in the F2 generation at 10 mg/m³, as well as increased postnatal

neurological effects including hypoactivity, mild ataxia and gait disturbances, hind-limb weakness, spinning and tremor (Tabacova et al., 1983). While the overall rate of malformations (club foot, hydrocephalus, microcephalus, generalized edema) exhibited a dose-response trend, with increased effects in the F2 generation, the specific malformations exhibited a less-consistent pattern. For example, while club foot was the predominant malformation in the F1 fetuses (occurring at 100 and 200 mg/m³); much lower rates of club foot were noted in the F2 generation (including none in the 200 mg/m³ group). Limitations of the study include a lack of information on chemical purity and exposure methods, lack of concurrent controls, lack of clear dose-response trend, and incomplete reporting on the statistical significance of reported behavioral effects.

Wistar albino rats (32 animals per group) were exposed to 50, 100, or 200 mg/m³ (16, 32, or 64 ppm) CS₂ for 8 hours per day throughout gestation. There were no statistically significant results in the 50 mg/m³ group. In the 100 and 200 mg/m³ groups, there were statistically significant increases in reduced fetal body weights, and reduced post natal body weights for 21 days, which subsequently disappeared. There was an increase in external malformations (hydrocephalus, club foot, and tail deformations) at the two higher doses (Tabacova et al., 1978).

Behavioral effects were examined in the offspring of Lati:CFY rats (8 per group) exposed to 0, 10, 700, or 2000 mg/m³ CS₂ (3, 230, or 640 ppm) for 6 hours per days over days 7 to 15 of gestation. The two high doses caused significant perinatal mortality. Avoidance conditioning was tested using a bell as a conditional stimulus prior to an electric shock. The animals learned to avoid the shock by jumping onto a pole at the sound of the bell. The latency to jump onto the pole and errors were measured as a means to evaluate avoidance conditioning in the treated versus control animals. The authors reported that there was a dose-related change in avoidance conditioning among male pups over the first 15 days (Lehotsky et al., 1985). While the magnitude of the effect on avoidance conditioning was greater at all doses relative to controls, and at 2000 mg/m³ compared with 700 mg/m³, the effect was virtually identical between the 10 and 700 mg/m³. This lack of dose-response effect raises some question about the significance of this finding.

Effects of low (0.03 and 10 mg/m³; 0.01 and 3 ppm) prenatal exposures (8 hours per day throughout gestation) of CS₂ were studied in Wistar albino rats. No congenital malformations or significant prenatal effects were found in the 9-11 litters evaluated at each dose. Mortality during postnatal days 10 through 21 was increased in the 10 mg/m³ group. Delays in the development of visual and auditory function were reported in the higher dose group (Tabacova and Balabaeva, 1980). There was no mention of maternal toxicity in this study.

Several other studies yielded either no teratogenic effects or effects only at maternally toxic exposures. Saillenfait et al. (1989) exposed rats via inhalation to 0, 100, 200, 400, or 800 ppm CS₂ for 6h/d during days 6-20 of gestation. Lower exposures (100 or 200 ppm; 310 or 620 mg/m³) were not associated with maternal toxicity or adverse effects on the developing embryo or fetus. Higher concentrations (400 or 800 ppm; 1240 or 2480 mg/m³) yielded a significant reduction of maternal weight gain as well as reductions of fetal body weight and a low incidence of club foot. Significant increases in unossified sternbrae were reported following 800 ppm (2480 mg/m³) exposures. Nemeč et al. (1993) reported no teratogenicity or maternal,

developmental, or reproductive toxicity among pregnant CD rats and their offspring following exposure to 125 or 250 ppm (388 or 775 mg/m³) from 2 weeks prior to mating through gestation day 19. At 500 ppm, dams had decreased body weight gain and food consumption; decreased litter viability but no teratogenic effects were noted. CS₂ was not found to be teratogenic or embryotoxic following intraperitoneal administration to rats on days 1-15 of gestation (Beliles et al., 1980; Hardin et al., 1981). No significant effects were noted in animal inhalation exposures (20 to 40 ppm; 62 to 125 mg/m³ CS₂) with either rats on days 1-19 of gestation or rabbits on days 1-24 of gestation.

VI. Derivation of Chronic Reference Exposure Level

<i>Study</i>	Johnson <i>et al.</i> (1983)
<i>Study population</i>	145 occupationally exposed workers and 212 comparison workers
<i>Exposure method</i>	Discontinuous occupational inhalation exposures (mean of 7.6 ppm and range of 0.6 to 16 ppm)
<i>Critical effects</i>	Reduction in motor nerve conduction velocities (decreased peroneal nerve MCV and sural nerve SVC)
<i>LOAEL</i>	7.6 ppm
<i>NOAEL</i>	Not observed
<i>Exposure continuity</i>	8 hr/day, 5 days/week
<i>Average occupational exposure</i>	2.7 ppm for LOAEL group (7.6 x 10/20 x 5/7)
<i>Benchmark concentration (BMC₀₅)</i>	6.86 ppm (continuity-weighted exposure of 2.54 ppm)
<i>Human equivalent concentration</i>	2.54 ppm for BMC ₀₅ (6.86 x 10/20 x 5/7)
<i>Exposure duration</i>	Mean of 12.1 years (SD 6.9 years)
<i>Subchronic uncertainty factor</i>	1
<i>LOAEL uncertainty factor</i>	Not needed in BMC approach
<i>Interspecies uncertainty factor</i>	1
<i>Intraspecies uncertainty factor</i>	10
<i>Cumulative uncertainty factor</i>	10
<i>Inhalation reference exposure level</i>	0.3 ppm (300 ppb; 0.8 mg/m ³ ; 800 µg/m ³)

A benchmark dose analysis was performed on the peroneal MCV data. The NIOSH exposure data were regrouped into 8 geometrically spaced dose groups (Table 1).

Table 1. Peroneal MCV data used for benchmark dose modeling

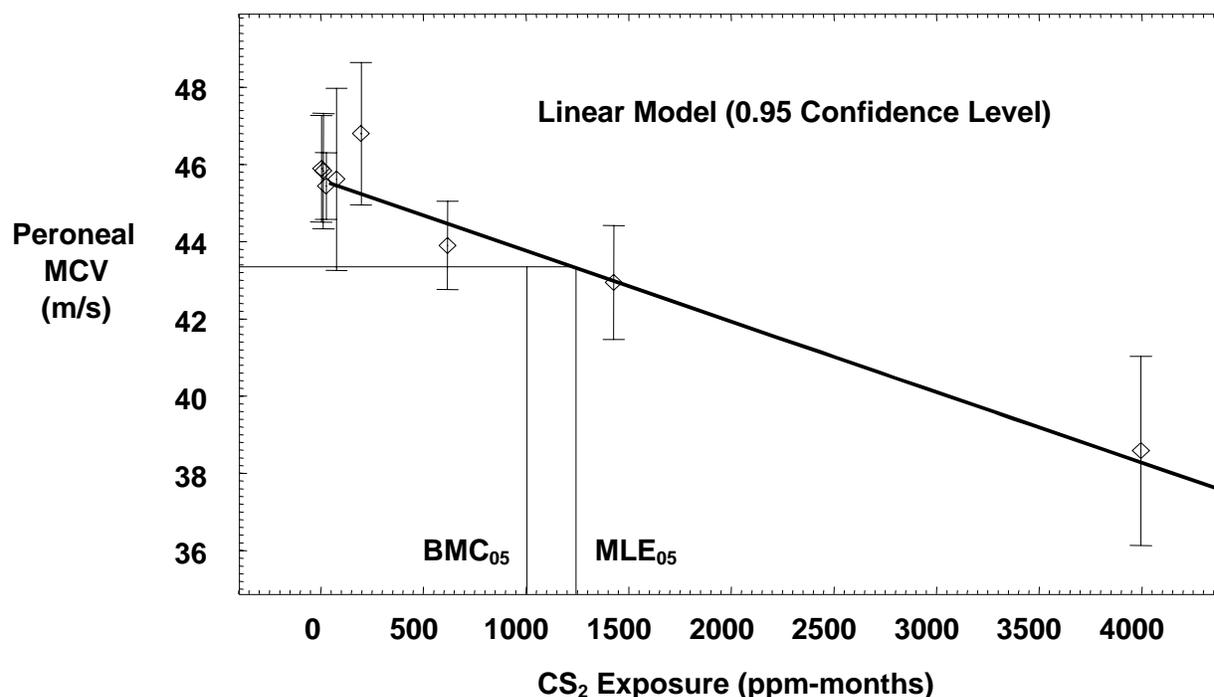
<i>Exposure (ppm-months)</i>	<i>Subjects</i>	<i>Peroneal MCV (m/s)</i>	
		<i>Mean</i>	<i>Std. Dev.</i>
3.8 (2 - 6)	32	45.9	3.8
13.1 (6 - 16)	61	45.8	5.8
26.5 (16-44)	140	45.4	5.2
77.3 (44-122)	17	45.6	4.6
197 (122 – 336)	17	46.8	3.6
619 (336 – 929)	54	43.9	4.2
1428 (929-2563)	61	42.9	5.8
3997 (2563 – 7075)	19	38.6	5.1

Model fitting was conducted with U.S. Environmental Protection Agency BMDS Benchmark Dose Software, Version 1.3. Four continuous data models were compared: linear, polynomial (v. 2.1), power (v. 2.1) and hill (v. 2.1) models. All four models adequately fit the data set (Table 2).

Table 2. Benchmark dose modeling results

Model	<i>MLE₀₅ (ppm-mo)</i>	<i>BMC₀₅ (ppm-mo)</i>	<i>p value</i>
Linear	1245	1005	0.84
Polynomial	1100	736	0.78
Hill	1092	670	0.65
Power	1245	1005	0.58

The BMC₀₅ from the best-fitting linear model was used. An occupational BMC₀₅ of 6.9 ppm was derived by dividing the 1005 ppm-month value by the average exposure duration of 145 months (12.1 years). The time-weighted average value was thus 2.5 ppm (6.9 ppm x 10/20 x 5/7).



The U.S. EPA (1995) based its RfC of $700 \mu\text{g}/\text{m}^3$ on the same study but used a BMC_{10} and included a Modifying Factor (MF) of 3 for database deficiencies. The criteria for use of modifying factors are not well specified by U.S. EPA. Such modifying factors are not used by OEHHA. In addition OEHHA prefers use of a BMC_{05} since in practice it tends to be closer to the NOAEL while the BMC_{10} is often closer to the LOAEL (OEHHA, 2000).

For comparison, 50 ppm was a 13 week NOAEL in rats for axonal swelling (Sills *et al.*, 1998). The equivalent continuous exposure is 8.9 ppm. Use of an RGDR of 1, an interspecies UF of 3, a subchronic UF of 3, and an intraspecies UF of 10 results in a REL of 90 ppb.

VII. Data Strengths and Limitations for Development of the REL

The major strengths of the REL for carbon disulfide are the use of human data, the observation of a dose-response effect, and the duration of exposures. The major uncertainties are the poor quantitation of actual exposure magnitude over time and the limited nature of the health effects studies which have been conducted.

VIII. Potential for Differential Impacts on Children's Health

The data available on the developmental toxicity of carbon disulfide are equivocal. Several studies reported that adverse developmental effects are only noted with exposures exceeding 100 ppm, while Tabacova and Balabaeva (1980) and Lehotsky *et al.* (1985) reported transient effects

at levels as low as 10 mg/m³ (3 ppm). The results of these two studies are not consistent with the database as a whole. While further research into behavioral effects of low concentrations of CS₂ would better clarify the risks associated with such exposures, no adverse effects have been reported at concentrations below the REL of 800 µg/m³ (300 ppb).

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CHRONIC TOXICITY SUMMARY

CARBON TETRACHLORIDE

(carbon chloride; carbon tet; freon 10; halon-104; methane tetrachloride; necatrine; tetrachlorocarbon; tetrachloromethane; tetraform; tetrasol; univerm)

CAS Registry Number: 56-23-5

I. Chronic Toxicity Summary

<i>Inhalation reference exposure level</i>	40 $\mu\text{g}/\text{m}^3$ (6 ppb)
<i>Critical effect(s)</i>	Increased liver weight and hepatic fatty infiltration in guinea pigs
<i>Hazard index target(s)</i>	Alimentary system; development (teratogenicity); nervous system

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

<i>Description</i>	Colorless liquid
<i>Molecular formula</i>	CCl_4
<i>Molecular weight</i>	153.8 g/mol
<i>Density</i>	1.59 g/cm ³ @ 20°C
<i>Boiling point</i>	76.7°C
<i>Melting point</i>	-23°C
<i>Vapor pressure</i>	91.3 torr @ 20°C
<i>Solubility</i>	Soluble in acetone, ethanol, benzene, carbon disulfide, slightly soluble in water
<i>Conversion factor</i>	1 ppm = 6.3 mg/m ³ @ 25°C

III. Major Uses or Sources

Carbon tetrachloride was formerly used for metal degreasing and as a dry-cleaning fluid, fabric-spotting fluid, fire-extinguisher fluid, grain fumigant and reaction medium (DeShon, 1979). Carbon tetrachloride is used as a solvent for the recovery of tin in tin-plating waste and in the manufacture of semiconductors. It is used in petrol additives, refrigerants, metal degreasing, and as a catalyst in the production of polymers. Carbon tetrachloride is also used as a chemical intermediate in the production of fluorocarbons and some pesticides (HSDB, 1995). In 1996, the latest year tabulated, the statewide mean outdoor monitored concentration of carbon tetrachloride was approximately 0.08 ppb (CARB, 1999a). The annual statewide industrial emissions from facilities reporting under the Air Toxics Hot Spots Act in California based on the most recent inventory were estimated to be 8781 pounds of carbon tetrachloride (CARB, 2000).

IV. Effects of Human Exposure

Kazantzis *et al.* (1960) evaluated 17 employees of a quartz processing factory who were occupationally exposed to 45-100 ppm (284-630 mg/m³) carbon tetrachloride (CCl₄) vapor. Fifteen of the 17 workers complained of symptoms including nausea, anorexia, vomiting, flatulence, epigastric discomfort or distention, depressive symptoms, headache or giddiness for up to 4 months prior to the evaluation. A week after CCl₄ concentrations were reduced to 0-9 ppm with control measures, workers were symptom-free.

V. Effects of Animal Exposure

Adams *et al.* (1952) chronically exposed albino Wistar rats, guinea pigs, albino rabbits and rhesus monkeys to 0, 5, 10, 25, 50, 100, 200 and 400 ppm CCl₄ for varying duration. For each exposure group, two control groups were devised (unexposed and air-exposed controls) consisting of animals similar in age, sex, weight and number. The 2 control groups responded similarly to the experimental protocol.

In the 100, 200 and 400 ppm exposure groups (Adams *et al.*, 1952), mortality was excessive with moderate to severe liver cirrhosis and other various pathological changes in all the species tested. Fifteen male and 15 female rats were exposed to 50 ppm CCl₄ 134 times for 187 days. They experienced decreased body weight gain and liver weight increase as well as moderate fatty degeneration and slight to moderate liver cirrhosis. Females showed kidney weight increase and four rats showed slight to moderate swelling of the kidney tubular epithelium. Guinea pigs (8 males and 8 females; 143 exposures in 200 days) showed depressed growth in the first two weeks, enlarged livers, moderate fatty degeneration and liver cirrhosis, and increased levels of liver total lipids, neutral fat, esterified cholesterol and plasma prothrombin clotting time.

The rabbit group of 2 males and 2 females, which underwent 155 exposures to 50 ppm in 216 days, showed slightly depressed growth and increased kidney weights, prolonged plasma prothrombin clotting time, and moderate fatty degeneration and cirrhosis of the liver.

No change was seen in the group of 2 male monkeys exposed 198 times to 50 ppm in 277 days (Adams *et al.*, 1952). One monkey experienced depressed weight gain compared to the other monkey and the controls, but no other adverse effects were seen with respect to organ weights, tissue examination, total liver lipid, blood urea nitrogen, blood non-protein nitrogen, serum phosphatase, plasma prothrombin clotting time, phospholipid, neutral fat, and free esterified cholesterol.

At 25 ppm CCl₄, 15 male and 15 female rats were exposed 137 times for 191 days. Early growth depression in males was observed, although final body weights did not significantly differ from the controls. Significant liver weight increase and slight to moderate fatty degeneration occurred. Liver lipid content was nearly twice the level of the controls and esterified cholesterol was five times that of the controls. For this exposure, phospholipid and neutral fat were not measured. Five male guinea pigs were exposed 133 times over 185 days and 5 female guinea pigs were exposed 93 times over 126 days. Symptoms included growth depression, liver weight

increase, increased plasma prothrombin clotting time, slight to moderate fatty degeneration, twice the level of the control total liver lipid, and five times the control level of esterified cholesterol. After 178 exposures to 25 ppm over 248 days, rabbits (2 per sex) showed increased liver weights and slight to moderate liver cirrhosis and fatty degeneration.

Twenty male and 20 female rats were exposed 136 times over a period of 192 days to 10 ppm CCl_4 . These rats exhibited increase in liver weight, slight to moderate fatty degeneration and total lipid, neutral fat and esterified cholesterol levels that were twice the control levels. Guinea pigs (8 male and 8 female), which were exposed 139 times over 197 days, experienced liver weight increase, slight to moderate fatty degeneration without cirrhosis, and increased levels of total lipid, neutral fat, and esterified cholesterol. In an additional group of 18 male rats exposed 13 times to 10 ppm, slight fatty degeneration was seen as early as 17 days. Two male and two female rabbits tolerated the same regimen as the guinea pigs and showed no symptoms as a result of the exposure. Sixteen additional guinea pigs developed hepatic changes after 12 exposures in 16 days.

Twenty-five male and 23 female rats, exposed 145 times over 205 days to 5 ppm CCl_4 , had no adverse effects. Nine male and nine female guinea pigs exposed 143 times over 203 days showed a statistically significant increase in the liver weights (females only), but only slightly higher liver lipid content. No additional histopathological effects were seen at this level of exposure.

In a more recent study, Prendergast *et al.* (1967) exposed 15 Long-Evans or Sprague-Dawley rats, 15 guinea pigs, 3 rabbits, 2 dogs, and 3 monkeys 30 times to a concentration of $515 \pm 39 \text{ mg/m}^3$ (81.7 ppm) carbon tetrachloride (CCl_4) 8 hours a day, 5 days a week, for 6 weeks. (This intermittent exposure is equivalent to a continuous exposure to 123 mg/m^3 .) Additionally, two 90 day continuous exposure studies were conducted. One study exposed 15 rats, 15 guinea pigs, 2 rabbits, 2 dogs and 3 monkeys to $61 \pm 5.2 \text{ mg/m}^3$ CCl_4 and the other exposed 15 rats, 3 rabbits, 2 dogs and 3 monkeys continuously to $6.1 \pm 0.3 \text{ mg/m}^3$ CCl_4 in inhalation chambers. Control groups consisted of 304 rats, 314 guinea pigs, 34 dogs, 48 rabbits and 57 monkeys. All the animals' weights were recorded prior to the study, at monthly intervals throughout the study, and at the conclusion of the study.

During the 6 week study, one monkey died following the 7th exposure, and 3 guinea pigs died following the 20th, 22nd, and 30th exposures, respectively. Monkeys, guinea pigs, dogs and rabbits all exhibited weight loss. A high percentage of mottled livers was seen in all species except dogs. Histopathologic examination of the lungs and livers showed morphological changes in all the animals exposed to CCl_4 (most prominently the guinea pigs). The guinea pigs were the most sensitive species displaying discolored lungs, fatty livers, bile duct proliferation, fibrosis, focal inflammatory cell infiltration, hepatic cell degeneration and regeneration, early portal cirrhosis, and alteration of lobular structure. Hepatic lipid content in the guinea pigs was $35.4 \pm 10.7\%$ compared to the control value of $11.0 \pm 3.6\%$. Alterations of liver lipid content were also observed, to a lesser extent, in the other four species; the most severe alteration occurred in the rats, less severe alteration in rabbits and dogs, and the least severe in the monkeys.

During the 61 mg/m³ (9.7 ppm) CCl₄ continuous exposure study, 3 guinea pigs died (one each after 47, 63, and 71 days). All the monkeys were emaciated and experienced hair loss. Depressed body weight increases were seen in all exposed animals compared to the controls. Autopsies showed enlarged and/or discolored livers in a high percentage (not given) of monkeys, guinea pigs, rabbits, and rats. Rats and guinea pigs showed hepatic fatty acid changes, and a moderate reduction in succinic dehydrogenase activity was also evident in guinea pigs. Varying but lesser degrees of these changes were also seen in the other species tested.

The low concentration of 6.1 mg/m³ (1 ppm) CCl₄ was attained by diluting the CCl₄ to 10% of the above concentration with *n*-octane, resulting in a solution of 6.1 mg/m³ CCl₄ in 61 mg/m³ of *n*-octane (Prendergast *et al.*, 1967)). The level of *n*-octane used was shown to be nontoxic by an *n*-octane control, which yielded no effects. (The current TLV for *n*-octane is 1400 mg/m³ (300 ppm) (ACGIH, 1992).) No animals died during this study, and no signs of toxicity were noted. All exposed animals except the rats showed reduced weight gain when compared to the controls, and all species exhibited nonspecific inflammatory lung changes. Guinea pig liver lipid contents and serum urea nitrogen concentrations were similar to the control values. In several animals there were some nonspecific inflammatory changes in the liver, kidney and heart, but the authors did not attribute these to the chemical exposure. There was no other observed hematologic or histopathologic toxicity at this level.

Shimizu *et al.* (1973) exposed groups of 4 female Sprague-Dawley rats to 10, 50 and 100 ppm of CCl₄ vapor for 3 hours a day, 6 days a week for up to 6-8 weeks. The rats were terminated two days after the last inhalation. Accumulation of CCl₄ occurred in the adipose tissue and was measured after 1 and 3 weeks of exposure. For the 10 ppm group, accumulation was gradual, reaching a level of 1/3 the amount found in the 50 ppm group after 6 weeks. A slight increase of triglycerides in the liver (6.2-6.4 mg/g) was observed in the 10 ppm group, but no control group was used for comparison.

The intermittent exposure caused a more pronounced and higher number of change indices to occur (34 as opposed to the 17 change indices of the monotonous regimen), indicating a greater intensity of liver damage. Changes included a significant decrease in hippuric acid synthesis, presence of mitochondrial enzymes (glutamate dehydrogenase and ornithine carbonyl transferase) in the blood (indicating severe damage to hepatocytes), significant increase in cytoplasmic enzyme activity, and a decrease in the level of cytochrome P-450 in liver tissue. The effects seen in the monotonous group were the same variety as those in the intermittent group, but were less intense. The content of CCl₄ in the blood was similar for both the intermittent and monotonous exposure groups. Another test was performed over a period of 27 days varying the regimen, and therefore the concentration, of intermittent exposure while keeping the TWA level of CCl₄ stable. Increasing the concentration threefold or fivefold with five 10 minute peaks did not potentiate the toxic effects. Varying the regimen tenfold to five 5-minute peaks (peak exposure 402 mg/m³ (63.8 ppm)) with a time weighted average exposure of 6.5 ppm (41±1 mg/m³) did, however, result in more severe liver damage.

Sakata *et al.* (1987) exposed 10-15 male Sprague-Dawley rats to <10 ppm CCl₄ vapor for 15 minutes a day, twice a week for 8 weeks. All the rats had chronic liver damage involving

nodular liver surfaces and extensive fibrosis. Researchers also found similar results in rats after 8 weeks of subcutaneous injections of 0.1 mL of 50% CCl₄ solution in olive oil twice a week.

Ideura *et al.* (1993) exposed male Wistar rats to CCl₄ vapor for 7 minutes, 3 times a week for 6-10 weeks (concentration unspecified). Six experimental groups of 4-5 rats were used, two of which were exposed for 10 weeks, another two for 6 weeks, and two unexposed control groups. Following the last exposures, rats were injected with varying amounts of endotoxin (1.0 mL lipopolysaccharide (LPS)). The rats were sacrificed 24 hours after the injection and processed for histological examination. Examination of the rats' left kidneys and livers revealed liver cirrhosis with destruction of normal structure and massive ascites retention after 10 weeks of exposure as compared to the controls. Those exposed for 6 weeks exhibited an increase in fibrous tissue. The control groups displayed normal liver structure. Researchers found that rats previously resistant to endotoxin became susceptible following CCl₄ exposure, which was manifested as induced acute renal tubular necrosis in cirrhotic rats.

Yoshimura *et al.* (1993) performed a similar experiment to that of Ideura *et al.* (1992) by exposing male Wistar rats for 6 (5 rats) and 10 weeks (5 rats) to 99% CCl₄ vapor for 3 minutes a day. A control group of 5 rats was given phenobarbitone for 10 weeks. After 24 hours following the final exposure, rats were injected with endotoxin. Six weeks of CCl₄ exposure caused liver fibrosis with bridging fibrosis, while 10 weeks of exposure to CCl₄ caused liver cirrhosis and destruction of the normal liver architecture.

Pregnant rats were exposed to 0, 300, or 1000 ppm (0, 1938, or 6460 mg/m³) carbon tetrachloride for 7 hours/day on days 6-15 of gestation (Schwetz *et al.*, 1974). Significant fetal growth retardation, measured by decreased crown-rump length and body weight, was observed in the offspring of the exposed groups (n = 22 litters) compared with controls (n = 43 litters). Subcutaneous edema was observed in the 300 ppm group but not in the 1000 ppm group. Sternebral anomalies were observed in the 1000 ppm group.

Effects of Chronic CCl₄ Exposure (Adams *et al.*, 1952)

<i>Species</i>	<i>Concentration (ppm)</i>	<i>Group size</i>	<i>Endpoint</i>	<i>Exposure scenario (days exposed/ experiment length)</i>
Rats (male)	50 ppm	15	liver damage: fatty degeneration and cirrhosis; growth depression	134/187
Rats (female)	50 ppm	15	same effects as males with the addition of increased kidney weight	134/187
Guinea pigs	50 ppm	16	liver damage: fatty degeneration and cirrhosis; growth depression	143/200
Rabbits	50 ppm	4	enlarged kidney; liver damage: fatty degeneration and cirrhosis; growth depression	155/216
Monkeys	50 ppm	2	one experienced growth depression	198/277
Rats	25 ppm	30	liver damage; early growth depression	137/191
Guinea pigs (male)	25 ppm	5	liver damage: fatty degeneration; growth depression	133/185
Guinea pigs (female)	25 ppm	5	liver damage: fatty degeneration; growth depression	93/126
Rabbits	25 ppm	4	liver damage: fatty degeneration; and cirrhosis	178/248
Rats	10 ppm	40	liver damage: fatty degeneration	136/192
Guinea pigs	10 ppm	16	liver damage: fatty degeneration	139/197
Rats	5 ppm	48	no adverse effects	145/205
Guinea pigs (male)	5 ppm	9	no adverse effects	143/203
Guinea pigs (female)	5 ppm	9	liver damage	143/203

Data from Guinea Pigs and Rats Exposed to 5 ppm CCl₄ for 7 Months (Adams *et al.*, 1952)

←----- g organ weight/g body weight---→

Group	<i>n</i>	<i>BW</i> (g)	<i>Lung</i>	<i>Heart</i>	<i>Liver</i>	<i>Kidneys</i>
Rats, male						
Unexposed controls	11	336	0.65	0.32	2.38	0.65
Air-exposed controls	16	322	0.62	0.31	2.25	0.66
5 ppm CCl ₄	13	336	0.62	0.31	2.23	0.65
Rats, female						
Unexposed controls	14	204	0.86	0.38	2.41	0.73
Air-exposed controls	17	209	0.76	0.37	2.76	0.76
5 ppm CCl ₄	18	214	0.81	0.38	2.58	0.73
Guinea pigs, male						
Air-exposed controls	7	695	0.79	0.27	3.07	0.63
5 ppm CCl ₄	8	669	0.82	0.27	3.14	0.65
Guinea pigs, female						
Air-exposed controls	9	611	0.81	0.27	2.58	0.59
5 ppm CCl ₄	6	636	0.78	0.26	2.82*	0.57

* p =0.004

VI. Derivation of Chronic Reference Exposure Level (REL)

<i>Study</i>	Adams <i>et al.</i> (1952)
<i>Study population</i>	9 male and 9 female guinea pigs
<i>Exposure method</i>	Discontinuous whole-body inhalation
<i>Critical effects</i>	Increase in liver weight and liver lipid content in females
<i>LOAEL</i>	5 ppm
<i>NOAEL</i>	Not observed
<i>Exposure continuity</i>	7 hours/day, 5 days/week
<i>Average experimental exposure</i>	1.0 ppm
<i>Human equivalent concentration</i>	1.7 ppm (gas with systemic effects, based on RGDR = 1.7 for lambda (a) : lambda (h) (Gargas <i>et al.</i> 1989))
<i>Exposure duration</i>	143 exposures over 203 days (7.3 months)
<i>LOAEL uncertainty factor</i>	3 (mild effect; only in one sex of one species)
<i>Subchronic uncertainty factor</i>	3 (7.3 mo/6 yr guinea pig life-span = 10.1%)
<i>Interspecies uncertainty factor</i>	3
<i>Intraspecies uncertainty factor</i>	10
<i>Cumulative uncertainty factor</i>	300
<i>Inhalation reference exposure level</i>	0.006 ppm (6 ppb; 40 µg/m ³ ; 0.04 mg/m ³)

Of the 2 adequate chronic inhalation studies available on CCl₄, the Adams *et al.* (1952) study was chosen over the Prendergast *et al.* (1967) study as the key reference for the carbon tetrachloride chronic REL. The Adams *et al.* (1952) experiment was conducted over a longer

duration. In addition, the Adams study contained more specific endpoints of liver damage that were consistent with the mechanism of carbon tetrachloride toxicity. Both studies resulted in hepatic effects with exposed rats appearing less sensitive than the affected monkeys or guinea pigs.

For comparison, conversion of the oral U.S. EPA RfD value of 0.7 $\mu\text{g}/\text{kg}/\text{day}$ to an equivalent inhalation value by route-to-route extrapolation yields an inhalation REL estimate of 2.5 $\mu\text{g}/\text{m}^3$. As another comparison, if the 6.1 mg/m^3 continuous exposure in Prendergast *et al.* (1967) is a NOAEL (for rats), the resulting REL estimate would be 60 $\mu\text{g}/\text{m}^3$. If the 6.1 mg/m^3 continuous exposure is a mild LOAEL, the resulting REL estimate would be 20 $\mu\text{g}/\text{m}^3$.

VII. Data Strengths and Limitations for Development of the REL

The major strengths of the REL for carbon tetrachloride are the chronic exposure study used and the target tissue affected. The major uncertainties are the lack of human data, the lack of a NOAEL observation, the small sample sizes used, and the lack of comprehensive multiple dose studies.

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CHRONIC TOXICITY SUMMARY

CHLORINATED DIBENZO-*p*-DIOXINS and CHLORINATED DIBENZOFURANS (INCLUDING 2,3,7,8-TETRACHLORODIBENZO-P-DIOXIN)

(Polychlorinated dibenzo-*p*-dioxins (PCDDs) and polychlorinated dibenzofurans (PCDFs) including 2,3,7,8-Tetrachlorodibenzo-*p*-dioxin (2,3,7,8-TCDD) which is the principal congener of concern based on toxicity)

CAS Registry Number: 1746-01-6 (TCDD); 5120-73-19 (TCDF)

I. Chronic Toxicity Summary

<i>Inhalation reference exposure level</i>	0.00004 µg/m³ (40 pg/m³)
<i>Oral reference exposure level</i>	1 x 10⁻⁸ mg/kg/day (10 pg/kg/day)
<i>Critical effect(s)</i>	Increased mortality, decreased weight gain, depression of erythroid parameters, increased urinary excretion of porphyrins and delta-aminolevulinic acid, increased serum activities of alkaline phosphatase, gamma-glutamyl transferase and glutamic-pyruvic transaminase, gross and histopathological changes in the liver, lymphoid tissue, lung and vascular tissues in rats.
<i>Hazard index target(s)</i>	Alimentary system (liver); reproductive system; development; endocrine system; respiratory system; hematopoietic system

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

<i>Description</i>	All are white crystalline powders at 25° C.
<i>Molecular Formula</i>	C ₁₂ H ₄ C ₁₄ O ₂ (TCDD)
<i>Molecular Weight</i>	321.97 g/mol (TCDD)
<i>Density</i>	1.827 g/ml (estimated for TCDD)
<i>Boiling Point</i>	412.2°C (estimated for TCDD)
<i>Melting Point</i>	305-306°C (TCDD)
<i>Vapor Pressure</i>	1.52 x 10 ⁻⁹ torr at 25°C (TCDD)
<i>Solubility</i>	In water: 19.3 ng/L at 22°C (TCDD)
<i>Log K_{ow}</i>	6.15-7.28 (6.8 for TCDD)
<i>(octanol/water partition coefficient)</i>	
<i>Log K_{oc}</i>	6.0-7.39
<i>(organic-carbon distribution coefficient)</i>	
<i>Henry's Law Constant</i>	8.1 x 10 ⁻⁵ ATM-m ³ /mol

III. Major Uses and Sources

The chlorinated dioxins and furans are generated as by-products from various combustion and chemical processes. PCDDs are produced during incomplete combustion of chlorine containing wastes like municipal solid waste, sewage sludge, and hospital and hazardous wastes. Various metallurgical processes involving heat, and burning of coal, wood, petroleum products and used tires for energy generation also generate PCDDs. Chemical manufacturing of chlorinated phenols (e.g., pentachlorophenol), polychlorinated biphenyls (PCBs), the phenoxy herbicides (e.g., 2,4,5 T), chlorinated benzenes, chlorinated aliphatic compounds, chlorinated catalysts and halogenated diphenyl ethers are known to generate PCDDs as a by-product under certain conditions. While manufacture of many of these compounds and formulations has been discontinued in the United States, continued manufacture elsewhere in the world combined with use and disposal of products containing PCDD by-products results in the inadvertent release of PCDDs into the environment. Industrial and municipal processes in which naturally occurring phenolic compounds are chlorinated can produce PCDDs; the best example is chlorine bleaching of wood pulp in the manufacture of paper products. Additionally, municipal sewage sludge has been documented to occasionally contain PCDDs and PCDFs. Annual statewide industrial emissions from facilities reporting under the Air Toxics Hot Spots Act in California based on the most recent inventory were estimated to be 0.123 pounds of 2,3,7,8-TCDD, 0.244 pounds of 1,2,3,4,7,8-hexachlorodibenzodioxin and lesser amounts of other polychlorinated dibenzodioxins and dibenzofurans (CARB, 1999).

IIIa. 2,3,7,8 Tetrachlorodibenzo-p-dioxin Toxic Equivalent

2,3,7,8-Tetrachlorodibenzo-p-dioxin is considered the most potent congener of the polychlorinated dibenzo-p-dioxins (PCDDs) and polychlorinated dibenzofurans (PCDFs) families of compounds. Potency of PCDD and PCDF congeners correlates with the binding affinity to the cytosolic Ah receptor. Structure activity studies have demonstrated that optimal biological activity and Ah-receptor binding requires congeners with a planar conformation and chlorines at the corners of the molecule at the 2,3,7,8 positions (Poland and Knutson, 1982; Safe, 1986). Chlorines at both ortho positions in these molecules (i.e., positions 1 and 9) sterically hinder a planar conformation that lessens the congeners' biological activity. Thus only 15 of 210 different PCDDs and PCDFs congeners possess significant biological activity based on chlorines in the 2,3,7,8 positions and some degree of planar conformation (Safe, 1986; U.S. EPA 1989). These include two tetrachloro-congeners: 2,3,7,8-tetrachlorodibenzo-p-dioxin and 2,3,7,8-tetrachlorodibenzofuran; three pentachloro congeners: 1,2,3,7,8-pentachlorodibenzo-p-dioxin, 1,2,3,7,8-pentachlorodibenzofuran, and 2,3,4,7,8-pentachlorodibenzofuran; seven hexachloro congeners: 1,2,3,4,7,8 or 1,2,3,6,7,8 or 1,2,3,7,8,9-hexachlorodibenzo-p-dioxins and hexachlorodibenzofurans and 2,3,4,6,7,8-hexachlorodibenzofuran; and three heptachloro congeners: 1,2,3,4,6,7,8-heptachlorodibenzo-p-dioxin, 1,2,3,4,6,7,8-heptachlorodibenzofuran and 1,2,3,4,7,8,9-heptachlorodibenzofuran (U.S. EPA, 1989). The structures of the dibenzo-p-dioxins and dibenzofurans along with their numbering schemes are shown in Figure 1. Toxic equivalents are calculated relative to the most potent congener, 2,3,7,8-tetrachlorodibenzo-p-dioxin, and are determined based on structure activity studies examining relative affinity for the

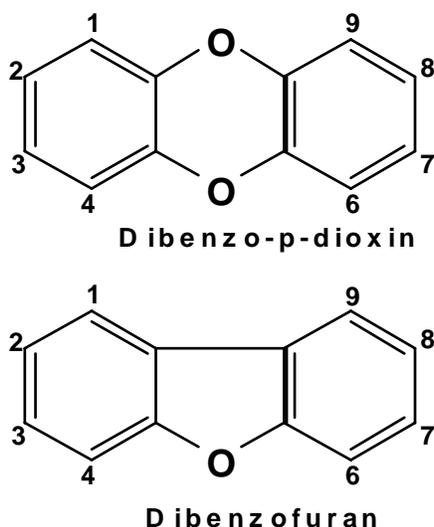
Ah receptor as well as on relative toxicity of different congeners. Values for the international system of toxic equivalents are provided in Table 1 (U.S. EPA, 1989).

Table 1. International Toxic Equivalency Factors (I-TEFs) for PCDDs and PCDFs Chlorinated in the 2,3,7, and 8 Positions. (U.S. EPA 1989.)

Compound ^{1,2}	I-TEF
Mono-, Di-, and Tri-CDDs and CDFs	0
<u>TetraCDD</u>	
2,3,7,8-substituted	1.0
Others	0
<u>PentaCDD</u>	
2,3,7,8-substituted	0.5
Others	0
<u>HexaCDD</u>	
2,3,7,8-substituted	0.1
Others	0
<u>HeptaCDD</u>	
2,3,7,8-substituted	0.01
Others	0
<u>OctaCDD</u>	0.001
<u>TetraCDF</u>	
<u>2,3,7,8</u>	0.1
<u>Others</u>	0
<u>PentaCDF</u>	
1,2,3,7,8-PentaCDF	0.05
2,3,4,7,8-PentaCDF	0.5
others	0
<u>HexaCDF</u>	
2,3,7,8-substituted	0.1
Others	0
<u>HeptaCDF</u>	
2,3,7,8-substituted	0.01
Others	0
<u>OctaCDF</u>	0.001

¹ CDD designates chlorinated dibenzo-p-dioxin

² CDF designates chlorinated dibenzofuran

Figure 1. Structures of the Dibenzo-p-dioxins and Dibenzofurans

IV. Effects of Human Exposure

The information available on possible chronic toxic effects in humans is complicated by the relative insensitivity of epidemiological studies, the limited ability of case studies of exposed individuals to establish cause and effect relationships, the heterogeneous nature of human populations, the broad spectrum of exposures to other toxic agents in the human environment, and the episodic exposure of many of the exposed human populations which have been studied (e.g., Seveso, Italy). As a result, a limited number of effects have been associated with exposure to dioxins in humans. The meaning of these effects in terms of toxicity in most cases remains to be clarified. The majority of information comes from cross-sectional medical studies.

Chloracne is the most widely recognized effect of exposure to 2,3,7,8-TCDD and TCDD-like PCDDs and PCDFs. Chloracne is a persistent condition, which is characterized by comedones, keratin cysts and inflamed papules and is seen after acute and chronic exposure to various chlorinated aromatic compounds (Moses and Prioleau, 1985). Other dermal effects include hyperpigmentation and hirsutism or hypertrichosis (Jirasek *et al.*, 1974; Goldman, 1972; Suskind *et al.*, 1953; Ashe and Suskind, 1950); both appear to resolve themselves more quickly over time than chloracne, making them more of an acute response rather than a chronic response (U.S. EPA, 1994a). Epidemiological data available for 2,3,7,8-TCDD have not allowed a determination of the threshold dose required for production of chloracne (U.S. EPA, 1994b). Case studies suggest that there may be a relationship between 2,3,7,8-TCDD exposure and hepatomegaly (Reggiani, 1980; Jirasek *et al.*, 1974; Suskind *et al.*, 1953; Ashe and Suskind, 1950) and hepatic enzyme changes (Mocarelli *et al.*, 1986; May, 1982; Martin 1984; Moses *et al.*, 1984). Nevertheless, cross sectional epidemiological studies of trichlorophenol (TCP) production workers (Suskind and Hertzberg., 1984; Bond *et al.*, 1983; Moses *et al.*, 1984; Calvert *et al.* 1992), Vietnam veterans (Centers for Disease Control Vietnam Experience Study, 1988; Roegner *et al.*, 1991) and Missouri residents (Webb *et al.*, 1989; Hoffman *et al.*, 1986)

found little evidence for an association between exposure and hepatomegaly suggesting that this is not a chronic response. There is a consistent pattern of increased levels of serum gamma glutamyl transferase in populations exposed to 2,3,7,8-TCDD which is presumably of hepatic origin (Mocarelli, 1986; Caramaschi *et al.*, 1981, May, 1982; Martin, 1984; Moses *et al.*, 1984; Calvert *et al.*, 1992; Centers For Disease Control Vietnam Experience Study, 1988). Two cross sectional studies have associated diabetes and elevated fasting serum glucose levels with relatively high serum 2,3,7,8-TCDD levels (Sweeney *et al.*, 1992; Roegner *et al.*, 1991). However other studies provided mixed results (Moses *et al.*, 1984; Centers for Disease Control Vietnam Experience Study, 1988; Ott *et al.*, 1993). TCDD has been associated with effects on reproductive hormonal status in males. The likelihood of abnormally low testosterone levels was 2 to 4 times greater in individuals with serum 2,3,7,8-TCDD levels above 20 pg/ml (Egeland *et al.* 1994) and increased serum levels of luteinizing hormone and follicle stimulating hormone have been documented (Egeland *et al.*, 1994). A number of other effects have been reported that were either not seen as chronic effects or effects seen long term in only one population of exposed persons. These include elevated liver enzymes (aspartate aminotransferase and alanine aminotransferase), pulmonary disorders, neurologic disorders, and changes in porphyrin metabolism and kidney disorders (U.S. EPA, 1994c). Areas in which there is presently insufficient information to draw solid conclusions include effects on the circulatory system, reproductive effects, immunological effects, effects on metabolism and handling of lipids, and on thyroid function (U.S. EPA, 1994c). Recent findings in Rhesus monkeys have shown 2,3,7,8-TCDD to cause endometriosis (Reier *et al.*, 1993) and epidemiological studies are currently underway to determine if there is an association between TCDD exposure and endometriosis in human populations exposed by the Seveso accident.

Potential effects of a toxicant on normal fetal development include fetal death, growth retardation, structural malformations and organ system dysfunction. Evidence for all four of these responses has been seen in human populations exposed to dioxin-like compounds. In these poisoning episodes populations were exposed to a complex mixture of halogenated aromatic hydrocarbons contained within PCBs, PCDFs and PCDDs mixtures thus limiting the conclusions that could be drawn from the data. In the Yusho and Yu-Cheng poisoning episodes, human populations consumed rice oil contaminated with PCBs, PCDFs and PCDDs. Yu-Cheng women experienced high perinatal mortality in hyperpigmented infants born to affected mothers (Hsu *et al.* 1985). This occurred in women with overt signs of toxicity (chloracne) (Rogan, 1982) and Rogan notes that, when there is no sign of toxicity in the mother, the likelihood of fetotoxicity appears to lessen considerably in the infants. Signs of toxicity from dioxin like compounds were absent in infants born to mothers apparently not affected in the Seveso, Italy and Times Beach, Missouri, incidents (Reggiani, 1989; Hoffman and Stehr-Green, 1989), which supports Rogan's conclusion. There was an increased incidence of decreased birth weight in infants born to affected mothers in the Yusho and Yu-Cheng incidents suggesting fetal growth retardation (Wong and Huang, 1981; Law *et al.*, 1981; Lan *et al.*, 1989; Rogan *et al.*, 1988). The structural malformation, rocker bottom heel, was observed in Yusho infants (Yamashita and Hayashi, 1985) making this malformation a possible result of exposure to dioxin-like compounds. Nevertheless, it is unknown if these compounds produce malformations in humans. Evidence for possible organ system dysfunction in humans comes from a study of Yu-Cheng children which found that children exposed in utero experienced delays in attaining developmental milestones, and exhibited neurobehavioral abnormalities (Rogan *et al.*, 1988)

suggesting involvement of CNS function. Dysfunction of dermal tissues is noted in exposed infants of the Yusho and Yu-Cheng incidents and is characterized by hyperpigmentation of the skin, fingernails, and toenails, hypersecretion of the meibomian glands, and premature tooth eruption (Taki *et al.*, 1969; Yamaguchi *et al.*, 1971; Funatsu *et al.*, 1971; Wong and Huang, 1981; Hsu *et al.*, 1985; Yamashita and Hayashi, 1985; Rogan *et al.*, 1988; Rogan, 1989; Lan *et al.*, 1989).

V. Effects of Animal Exposure

The toxicity to laboratory animals encompasses a number of areas including changes in energy metabolism manifested as wasting syndrome, hepatotoxicity, effects on tissue of epithelial origin, various endocrine effects, effects on vitamin A storage and use, immune system effects and reproductive and developmental toxicity. The limited number of chronic studies available do not examine all these endpoints. Therefore subchronic exposures are included here in order to provide a more complete coverage of potential chronic toxic effects of these compounds.

Wasting syndrome is one of the most broadly occurring toxic effects. The wasting syndrome is characterized by loss of adipose tissue and lean muscle mass and is produced in all species and strains tested, but there are difference in sensitivity (U.S. EPA 1994d; Peterson *et al.*, 1984; Max and Silbergeld, 1987). Numerous studies have not yet established the mechanism of wasting syndrome (U.S. EPA, 1994e). Hepatotoxicity is also seen in all species tested, but there is considerable variation in species sensitivity (U.S. EPA, 1994d). TCDD induces hyperplasia and hypertrophy of liver parenchymal cells. Morphological and biochemical changes in the liver include increased SGOT and SGPT, induction of microsomal monooxygenases and proliferation of the smooth endoplasmic reticulum, porphyria, increased regenerative DNA synthesis, hyperlipidemia, hyperbilirubinemia, hypercholesterolemia, hyperproteinemia, degenerative and necrotic changes, mononuclear cell infiltration, multinucleated giant hepatocytes, increased numbers of mitotic figures, and parenchymal cell necrosis (U.S. EPA, 1994d; WHO/IPCS, 1989). Epithelial effects seen include chloracne (rabbit ear and the hairless mouse) (Jones and Krizek, 1962; Schwetz *et al.*, 1973) and hyperplasia and/or metaplasia of gastric mucosa, intestinal mucosa, the urinary tract, the bile duct and the gall bladder (U.S. EPA 1994f). TCDD exposure results in endocrine like effects including epidermal growth factor like effects such as early eye opening and incisor eruption in the mouse neonate (Madhukar *et al.*, 1984), glucocorticoid like effects such as involution of lymphoid tissues (U.S. EPA, 1994g; Sunahara *et al.*, 1989), alteration in thyroid hormone levels and in some cases thyroid hormone like effects (WHO/IPCS, 1989; Rozman *et al.*, 1984), decreases in serum testosterone and dihydrotestosterone (Mittler *et al.*, 1984; Keys *et al.*, 1985; Moore and Peterson, 1985), and changes in arachidonic acid metabolism and prostaglandin synthesis (Quilley and Rifkind, 1986; Rifkind *et al.*, 1990). TCDD is known to decrease hepatic vitamin A storage (Thunberg *et al.*, 1979). TCDD and other dioxin like PCDDs and PCDFs are potent suppressors of both cellular and humoral immune system function, characteristically producing thymic involution at low doses and involution of other lymphoid tissues at higher doses (U.S. EPA 1994h).

In animal studies there is a large body of information available documenting both developmental and reproductive toxicity of 2,3,7,8-TCDD and other PCDDs and PCDFs. These compounds are

acutely toxic to early life stages of fish and birds with fish being most sensitive (LD₅₀ of 0.4 µg/kg for rainbow trout sac fry eggs and LD₅₀ of 34 ng/kg for lake trout eggs); some species of birds are also relatively sensitive (LD₅₀ of 0.25 µg/kg for chicken eggs) (Peterson *et al.*, 1993). 2,3,7,8-TCDD has been documented to increase the incidence of prenatal mortality in a number of species of laboratory animals including the Rhesus monkey, Guinea pig, rabbit, rat, hamster, and mouse (Peterson *et al.*, 1993). Exposure to 2,3,7,8-TCDD during gestation produces a characteristic set of fetotoxic responses in most laboratory animals which includes: thymic hypoplasia, subcutaneous edema, and decreased growth (Peterson *et al.*, 1993). More species specific responses include cleft palate formation in the mouse at doses below maternal toxicity (Moore *et al.*, 1973; Smith *et al.*, 1976; Couture *et al.*, 1990), intestinal hemorrhage in the rat (Sparschu *et al.*, 1971), hydronephrosis in the mouse and hamster (Moore *et al.*, 1973; Smith *et al.*, 1976; Couture *et al.*, 1990; Birnbaum *et al.*, 1989; Olson *et al.*, 1990), and extra ribs in the rabbit (Giavini *et al.*, 1982). Female rats have also been found to be affected by perinatal exposure to 2,3,7,8-TCDD with clefting of the clitoris, incomplete or absent vaginal opening and a smaller vaginal orifice after a dose of 1 µg/kg to the mother on day 15 of gestation (Gray *et al.*, 1993).

A number of effects on adult reproductive function are seen in male animals exposed in utero to 2,3,7,8-TCDD. TCDD reduces plasma androgen levels in the adult male rat and perinatal exposure decreases spermatogenesis, spermatogenic function and reproductive capability, feminizes male sexual behavior, and feminizes male gonadotrophic function (LH secretion) (Mably *et al.*, 1991; Mably *et al.*, 1992a,b,c). Evidence suggests that these effects are the result of impaired sexual differentiation of the CNS, which in male rats is dependent on exposure of the developing brain to testosterone.

There are numerous studies detailing the effects of the PCDDs, PCDFs and other dioxin like compounds, however a large number of these studies were conducted as either acute or subchronic exposures, studies in which it is unlikely that body burdens had reached steady state levels. Detailed below are three chronic studies that were considered in the setting of a chronic toxicity exposure level.

The most definitive study of chronic toxicity in rats is that of Kociba *et al.* (1978). This study involved the administration of 2,3,7,8-TCDD in the diet at doses of 1 ng/kg/day, 10 ng/kg/day, and 100 ng/kg/day to groups of 50 male and 50 female Sprague Dawley rats for two years. A group of 86 male and 86 female rats received diet with solvent vehicle alone and served as controls. The following observations (excluding carcinogenic effects) were seen at the 100 ng/kg/day dose: increased mortality, decreased weight gain, depressed erythroid values, increased urinary excretion of porphyrins and delta-aminolevulinic acid, and increased serum activities of alkaline phosphatase, gamma-glutamyl transferase, and glutamic-pyruvic transaminase. Histopathologic changes were noted in the liver, lymphoid tissue, respiratory and vascular tissues. The primary ultrastructural change in the liver was proliferation of the rough endoplasmic reticulum. At the 10 ng/kg/day dose the severity of toxic symptoms was less than that of the 100 ng/kg/day dose and included increased urinary excretion of porphyrins in females as well as liver and lung lesions. The 1 ng/kg/day dose produced no discernible significant toxic effects. Interpretation of this study by the authors was that the 1 ng/kg/day dose was a NOAEL.

Two chronic toxicity studies are available in the mouse. The first is a one year study conducted by Toth *et al.* (1979) using male Swiss mice administered weekly oral doses of 7, 700, and 7000 ng/kg/day. In this study 2,3,7,8-TCDD administration resulted in amyloidosis and dermatitis in 0 of 38 control animals, 5 of 44 animals receiving 7 ng/kg/day, 10 of 44 animals receiving 700 ng/kg/day and 17 of 43 animals receiving 7,000 ng/kg/day. The other study was from the NTP 1982 gavage study (NTP, 1982) in B6C3F1 mice. This study employed groups of 50 male and 50 female mice. The males received doses of 0, 10, 50, and 500 ng/kg/week by gavage for two years while female mice received doses of 0, 40, 200, and 2000 ng/kg/week by gavage for two years. No adverse effects were seen at the lowest doses tested in each sex, which correspond to NOAELs of approximately 1.4 and 6 ng/kg/day for males and females, respectively. Neither chronic toxicity study in mice reported data on enzyme activity.

VI. Derivation of Chronic Reference Exposure Level (REL)

<i>Study</i>	Kociba <i>et al.</i> (1978)
<i>Study population</i>	Sprague-Dawley rats of both sexes (50/treatment group/sex)
<i>Exposure method</i>	Continuous dietary exposure starting at seven weeks of age for 2 years
<i>Critical effects</i>	Increased mortality, decreased weight gain, depression of hematologic measures, increased urinary excretion of porphyrins and delta-aminolevulinic acid, increased serum activities of alkaline phosphatase, gamma-glutamyl transferase and glutamic-pyruvic transaminase, gross and histopathological changes in the liver, lymphoid tissue, lung and vascular tissues
<i>Observed LOAEL</i>	210 ppt in diet (0.01 µg/kg/day)
<i>Observed NOAEL</i>	22 ppt in diet (0.001 µg/kg/day)
<i>Exposure continuity</i>	Continuous exposure via the diet
<i>Exposure duration</i>	2 years
<i>Subchronic uncertainty factor</i>	1
<i>LOAEL uncertainty factor</i>	1
<i>Interspecies uncertainty factor</i>	10
<i>Intraspecies uncertainty factor</i>	10
<i>Cumulative uncertainty factor</i>	100
<i>Oral reference exposure level</i>	10 pg/kg/day
<i>Route-to-route extrapolation</i>	3,500 µg/m ³ per mg/kg/day
<i>Inhalation reference exposure level</i>	40 pg/m ³ (0.00004 µg/m ³)

The data available for chronic toxic effects in humans have a number of limitations. Some studies did not determine the body burden of compounds necessary to estimate dose.; The Yusho and Yu-Cheng poisoning episodes have uncertainty because exposure was to complex mixtures of halogenated aromatic hydrocarbons rather than to individual congeners. And epidemiological

studies and case studies have limitations in determining cause and effect relationships. Therefore, an animal study was chosen for determination of a NOAEL/LOAEL. The study chosen for use was that of Kociba *et al.* (1978), based on the duration of the study (2 years), the number of animals employed (50 per treatment group per sex), testing of both sexes, a dose range, which spanned from an apparent NOAEL to severe hepatic effects including carcinogenic effects, a complete histopathological examination of all organ systems, examination of urinary excretion of porphyrins and delta-aminolevulinic acid, and determination of serum activities of alkaline phosphatase, gamma-glutamyl transferase, and glutamic-pyruvic transaminase. The elevation of human serum values for gamma-glutamyl transferase is one of the consistently seen chronic responses in exposed human populations and reflects changes in liver biochemistry. Thus the examination of markers of liver toxicity also altered in animal models of chronic toxicity make the Kociba study an appropriate choice for detecting potential chronic toxic effects of 2,3,7,8-TCDD in humans. The NOAEL in the Kociba *et al.* (1978) study was determined to be 1 ng/kg body weight/day. For the purposes of determining the REL the 1 ng/kg/day dose was considered to be a NOAEL based upon the observations of Kociba *et al.* (1978).

VII. Data Strengths and Limitations for Development of the REL

NOAELs from a number of other studies compare favorably with the 1 ng/kg/day NOAEL. These include the NOAEL from the NTP (1982) study in B6C3F1 mice and the NOEL for enzyme induction in rats and marmosets calculated by Neubert (1991) of 1 ng/kg. Furthermore the 1 ng/kg/day NOAEL is lower than the LOAELs observed by Toth *et al.* (1979) of 7 ng/kg/day in mice and by Schantz *et al.* (1978) of 2.3 ng/kg/day in rhesus monkeys. Current exposure assessments for 2,3,7,8-TCDD and other dioxin-like compounds including the PCBs, PCDDs, and PCDFs estimate that the average daily background dose in the U.S. is 3-6 pg TEQ/kg/day (U.S. EPA 1994i) also placing the REL close to background exposures. The REL of 10 pg/kg/day should be protective of chronic effects on liver function and avoid significant increases in exposure over the background level of human exposure.

The strengths of the inhalation REL include the availability of chronic exposure data from a well-conducted study with histopathological analysis, the observation of a NOAEL, and the demonstration of a dose-response relationship. Major areas of uncertainty are the lack of adequate human exposure data and the lack of chronic inhalation exposure studies.

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CHRONIC TOXICITY SUMMARY

CHLORINE

CAS Registry Number: 7782-50-5

I. Chronic Toxicity Summary

<i>Inhalation reference exposure level</i>	0.2 $\mu\text{g}/\text{m}^3$ (0.08 ppb)
<i>Critical effect(s)</i>	Hyperplasia in respiratory epithelium in female rats
<i>Hazard index target(s)</i>	Respiratory system

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

<i>Description</i>	Yellow/green gas
<i>Molecular formula</i>	Cl_2
<i>Molecular weight</i>	70.906 (Weast, 1989)
<i>Density</i>	2.9 g/L @ 25°C and 1 ATM
<i>Boiling point</i>	-34.04° C
<i>Vapor pressure</i>	5 atm @ 10.3°C; 5830 torr @ 25°C
<i>Solubility</i>	Slightly soluble in water (310 mL per 100 mL water at 10° C; 1.46 g per 100 mL water at 0° C)
<i>Conversion factor</i>	1 ppm = 2.9 mg/m^3 @ 25° C

III. Major Uses and Sources

In an industrial setting, chlorine is widely used as an oxidizing agent in water treatment and chemical processes. Chlorine is also used to disinfect swimming pool water. Chlorine gas is sometimes used at large public pools while household pools typically use hypochlorite solutions. Chlorine is an integral part of the bleaching process of wood pulp in pulpmills, although chlorine dioxide is replacing this use of chlorine. Chlorine as sodium hypochlorite is commonly used as a household cleaner and disinfectant (HSDB, 1995). The annual statewide industrial emissions from facilities reporting under the Air Toxics Hot Spots Act in California based on the most recent inventory were estimated to be 244,955 pounds of chlorine (CARB, 1999).

IV. Effects of Human Exposure

Shi and associates (1990) evaluated 353 workers from a diaphragm cell chlorine chemical plant. The workers ranged in age from 23-52 years with an average of 42.4 years. Two groups were compiled with respect to the workers' length of exposure in years. Group A consisted of 220 workers who were employed/ exposed for 10-25 years. Group B consisted of 133 workers

employed for less than 10 years. Both groups of workers were exposed to a range of 2.60-11.0 mg/m³ (0.37-1.75 ppm) chlorine. The control group's average age was 39.7 years (ranging from 26-55 years), and it consisted of 192 workers not exposed to chlorine, but working within the same plant. For all the groups, respiratory symptoms and smoking habits were evaluated as well as clinical examinations, ENT examinations, chest x-rays and pulmonary function tests. Groups A and B showed 3-8 times higher incidence of upper airway complaints than the control workers. Current smokers in groups A and B experienced the highest incidence of pulmonary symptoms and group A workers had a higher prevalence of rhino-pharyngeal signs than the control workers. Abnormalities in chest x-rays were seen in 8.6% of group A workers and in 2.8% of group B workers, compared to 2.3% of the control workers. Groups A and B showed significantly impaired pulmonary function in tests of V50/H and FEF₂₅₋₇₅ (forced expiratory flow between 25 and 75% of forced vital capacity (FVC), the total amount of air the subject can expel during a forced expiration) - compared with the control group, and group A showed reduced FEV₁ (forced expiratory volume in 1 second) results compared to the control group.

Kennedy *et al.* (1991) compared 321 pulpmill workers (189 of whom were exposed to chlorine or chlorine dioxide "gassings") to a control group of 237 rail yard workers in similar working conditions but not exposed to chlorine (79% and 84% respective participation rates). The workers had been employed for an average of 13 years at the pulpmill and 12.7 years at the rail yard. Chlorine gas and chlorine dioxide levels were measured together over a 4 week period during mainly a 12 hour shift. Time weighted averages (TWA) were <0.1 ppm, with the highest of <0.1-0.3 ppm. A significantly higher prevalence of wheezing was seen in pulpmill workers (both smokers and nonsmokers) who had reported more than one episode of chlorine "gassing" as compared to the rail yard workers and pulpmill workers with no chlorine gas exposure. More airflow obstruction was observed in exposed workers in spite of their nonsmoking and ex-smoking status, correlating to significantly lower average values for MMF (maximal mid-expiratory flow) and for the FEV₁ to FVC ratio. Comparison of pulpmill workers exposed to chlorine and /or chlorine dioxide with those pulpmill workers not exposed, suggests that chronic respiratory health impairment is associated with exposure to chlorine and/or chlorine dioxide. These researchers hypothesized that after the first high exposure incident, an inflammatory response occurred in small airways and that this reaction did not resolve in those workers who were continuously or repeatedly exposed to the irritant. It was also suggested that chronic airflow obstruction caused by repeated minor exposures led to chronic respiratory disability in some of the workers.

Patil *et al.* (1970) evaluated the exposure of 332 male diaphragm cell workers to 0.006-1.42 ppm chlorine gas (a range with a time-weighted average of 0.146 ±0.287; most workers were exposed to less than 1 ppm). A control group consisting of 382 workers from 25 representative chlorine manufacturing plants was also studied. Both groups were comprised of men between the ages of 19-69 with a mean age of 31.2 ±11.0 years. Physical examinations (blood and urine analysis, chest x-rays and electrocardiograms) were conducted, in most cases, within the first six months of the study year. At two month intervals, each plant was surveyed and chlorine levels were determined. Exposed employees were grouped according to job classification. Researchers found the average number of exposure years for the study group to be 10.9 ± 2.8 years and concluded that the exposure level had no correlation to the number of years exposure. Ninety-eight of the 332 workers were found to have abnormal teeth and gums, but no dose-response

relationship was concluded. Similarly, no dose-response relationships were shown with the symptoms of sputum production, cough, dyspnea, history of frequent colds, palpitation, chest pain, vital capacity, maximum breathing capacity and forced expiratory volume. Any deterioration in pulmonary function was shown to be age related. Of the 332 exposed workers, 9.4% experienced abnormal EKGs. 8.5% of the control group showed the same abnormalities, but this difference was not significant. Above 0.5 ppm, an increase appeared in the incidence of fatigue. No neurological defects developed and there was no noted prolonged anoxia as a result of the chlorine exposure. Also, no consistent gastrointestinal trouble or abnormal incidence of dermatitis was found. Exposed workers showed elevated white blood cell counts and decreased hematocrit values compared to the control group.

Bherer *et al.* (1994) conducted a follow up study of the Quebec pulp mill research done by Courteau and associates over a time interval of 18-24 months after the incidents of repeated exposures. Fifty-eight of the original 289 exposed workers from the moderate to high risk group were studied for developing reactive airways dysfunction syndrome (RADS). Workers at a moderate risk were defined as having shortness of breath after their most significant exposure, but not at the time of the initial study by Courteau *et al.* Moderate risk workers also had a record of other significant medical conditions and/or were 50 years of age or older. High risk workers were defined as those experiencing shortness of breath that continued one month after the exposure and/or abnormal lung sounds. Ninety percent of the follow up group completed questionnaires which revealed a 91% incidence of respiratory symptoms. Spirometry assessments and methacholine inhalation tests were conducted on 51 of the 58 workers. Twenty-three percent of the 58 workers still experienced bronchial obstruction and 41% continued to have bronchial hyper-responsiveness. Lower baseline FEV₁ was seen in those with a lower PC₂₀, and 52% of these workers showed an FEV₁ < 80% predicted.

Enarson *et al.* (1984) compared 392 pulpmill workers exposed to chlorine (unspecified duration) to a comparable group of 310 rail yard workers living in the same community, but not exposed to chlorine. In the pulpmill areas surveyed that predominantly had significant chlorine gas levels (machine room and bleach plant), workers were exposed to either an average of 0.02 ppm or 0.18 ppm Cl₂ respectively. Of the machine room workers, 23.2% experienced a cough as did 32.8% of those in the bleach plant, compared to 22.3% of the control rail yard workers. Chest tightness occurred in 31.5% of the machine room workers and 39.6% of the bleach plant workers as compared to 21.3% of the control. Only data from Caucasian subjects were reported.

Chester *et al.* (1969) evaluated 139 workers occupationally exposed to <1 ppm chlorine for an unspecified duration. Fifty-five of the 139 workers were exposed to additional accidental high concentrations of chlorine, which were severe enough to require oxygen therapy. Ventilation was affected by chlorine inhalation, with a decrease in the maximal midexpiratory flow (MMF). Smokers in this group had significantly reduced FVC, FEV₁ and MMF compared to nonsmokers. Fifty-six of the 139 subjects showed abnormal posteroanterior chest films, 49 of which had parenchyma and/or hilar calcifications consistent with old granulomatous disease and 11 of which had multiple, bilateral and diffuse calcifications. Researchers suggest that the first ventilation function affected in obstructive airway disease is MMF.

V. Effects of Exposure to Animals

Wolf *et al.* (1995) exposed male and female B6C3F1 mice and F344 rats to chlorine gas concentrations of 0 ppm, 0.4 ppm, 1.0 ppm and 2.5 ppm. The exposures were carried out for 104 weeks at 6 hr/day 3 days/week for female rats and 6 hr/day 5 days/week for mice and male rats. Based on previous studies, the authors determined that female rats could not tolerate 5 days/week exposure to chlorine. Each treatment group contained 320 male and 320 female mice. The rats were studied in groups of 70, yielding 280 per gender per species. For the first 13 weeks of observation, body weights and clinical observations were noted weekly, and for the remainder of the study, they were recorded once every two weeks. After 52 weeks, 10 rats were euthanized and autopsied. Organ weights were recorded, and hematologic and clinical chemistry parameters were determined. These same measurements were performed on all of the surviving mice and rats at the conclusion of the 104 weeks. Male mice exposed to 1.0 and 2.5 ppm Cl₂ showed decreased weight gain compared to controls while only female mice exposed to 2.5 ppm Cl₂ showed decreased weight gain. Male rats showed decreased weight gain at all levels of exposure while female rats showed the same result at only 1.0 and 2.5 ppm Cl₂ exposures. Various nonneoplastic nasal lesions were seen in all the airway epithelial types in the nose and at all levels of exposures for both species. These lesions were evaluated against background lesions found in the control animals. A statistically significant incidence of fenestration was seen in all three exposure concentrations of Cl₂. Statistically significant responses were seen in the traditional and respiratory epithelial regions of all exposed rats and mice. Statistically significant damage to olfactory epithelium occurred in all exposed rats and female mice and also in the 1.0 and 2.5 ppm exposed groups of male mice.

Klonne *et al.* (1987) exposed 32 male and female rhesus monkeys to chlorine gas for one year to measured concentrations of 0, 0.1, 0.5, and 2.3 ppm Cl₂. These monkeys were exposed to chlorine for 6 hours/day, 5 days/week. The monkeys were evaluated periodically on the basis of body weight, electrocardiograms, neurologic examinations, pulmonary function, hematologic parameters, serum chemistry, urinalysis, and blood gas and pH levels. Results were compared to the same test measurements recorded prior to the study. No significant difference was seen in body weight at any point in the experiment. Ocular irritation (tearing, rubbing of the eyes, reddened eyes) was observed after 6 weeks of exposure in the 2.3 ppm group. No exposure-related differences were seen in neurologic examinations, electrocardiograms, clinical chemistry, urinalysis, hematology or blood gas levels. Also, no exposure-related changes were observed in the parameters of ventilation distribution. Pulmonary function evaluations yielded a statistically significant trend for increasing pulmonary diffusing capacity and distribution of ventilation values for males and females in the 2.3 ppm exposure group. Both males and females of the 2.3 ppm group exhibited statistically significant increased incidence of respiratory epithelial hyperplasia. A mild form of the lesions was also seen in the 0.5 ppm group, 0.1 ppm group (females only) and one male in the control group. Two parasitic infections occurred, affecting the respiratory tract and resulting in 11 monkeys housing parasites and/or ova. Additionally, 16 monkeys displayed histologic changes characteristic of the presence of the parasites. However, the parasitic induced lesions were not associated with lesions in the respiratory epithelium.

VI. Derivation of Chronic Reference Exposure Level (REL)

<i>Study</i>	Wolf <i>et al.</i> , 1995
<i>Study population</i>	Female F344 rats (70 per group)
<i>Exposure method</i>	Discontinuous whole-body inhalation exposure (0, 0.4, 1.0 or 2.5 ppm)
<i>Critical effects</i>	Upper respiratory epithelial lesions (see following table)
<i>LOAEL</i>	0.4 ppm
<i>NOAEL</i>	Not established
<i>BMC₀₅</i>	0.14 ppm
<i>Exposure continuity</i>	6 hours/day, 3 days/week (MWF)
<i>Average experimental exposure</i>	0.015 ppm
<i>Human equivalent concentration</i>	0.0024 ppm (gas with extrathoracic respiratory effects, RGDR = 0.16 based on BW = 229 g, MV = 0.17 L/min, SA(ET) = 15 cm ²)
<i>Exposure duration</i>	2 years
<i>LOAEL uncertainty factor</i>	(not needed in the BMC approach)
<i>Subchronic uncertainty factor</i>	1
<i>Interspecies uncertainty factor</i>	3
<i>Intraspecies uncertainty factor</i>	10
<i>Cumulative uncertainty factor</i>	30
<i>Inhalation reference exposure level</i>	0.08 ppb (0.20 µg/m ³)

A benchmark dose analysis was performed using a log-normal probit analysis (Tox-Risk, version 3.5; ICF-Kaiser Inc., Ruston, LA) of the female rat data. Using the data for glandular epithelial eosinophilic proteinaceous accumulation (see Table 1 below) to derive the BMC₀₅ resulted in a 3-fold lower value than the LOAEL of 0.4 ppm, or BMC₀₅ = 0.14 ppm. (Adequate benchmark dose estimates could not be obtained for the other nasal lesions due to high background rates and shallow dose-response relationships.) A BMC₀₅ is considered to be similar to a NOAEL in estimating a concentration associated with a low level of risk.

The Wolf *et al.* (1995) study of mice and rats was chosen as the key reference for the chlorine chronic REL for several reasons. First, the duration of the experiment was for a full lifetime of two years. Second, the sample sizes were large (280 per sex per species). Finally, appropriate sensitive endpoints of respiratory epithelial damage were examined. The mice and male rats were exposed to chlorine for 6 hours/day, 5 days/week, but the female rats were only exposed for 3 days/week as the authors observed the females to be more sensitive than the males. Table 1 shows the histological findings of the female rats. Statistically significant results ($p < 0.05$) were seen for all the tissues at 0.4 ppm chlorine exposure and above.

Table 1. Female Rat Epithelial Lesions following Chronic Chlorine Exposure
(based on Table 5 of Wolf *et al.*, 1995)

Tissues	0 ppm	0.4 ppm	1.0 ppm	2.5 ppm
Goblet cell hyperplasia	3/70 (4%)	50/70 (71%)	63/70 (90%)	64/70 (91%)
Respiratory epithelium eosinophilic proteinaceous accumulation	49/70 (70%)	60/70 (85%)	59/70 (84%)	65/70 (93%)
Glandular epithelium eosinophilic proteinaceous accumulation	16/70 (23%)	28/70 (40%)	52/70 (75%)	53/70 (76%)
Olfactory epithelium eosinophilic proteinaceous accumulation	36/70 (52%)	64/70 (91%)	69/70 (99%)	69/70 (99%)

The Wolf *et al.* (1995) study was chosen over the Klonne *et al.* (1987) monkey study for the following reasons: the monkeys were exposed for only one year of their total 35 year lifetime, and the sample sizes were considerably smaller (4 monkeys per sex per group) than the mouse and rat groups (280 per sex per species). Although the exposure durations differed between the two studies, the histological results were similar, differing only slightly in the region of occurrence. The monkeys displayed both tracheal and nasal lesions. Both the rodents and the monkeys showed upper respiratory epithelial lesions, thus suggesting that the rodents may be an appropriate model for humans.

For comparison with the proposed REL of 0.08 ppb ($0.2 \mu\text{g}/\text{m}^3$) using the BMC approach, we estimated a REL of 0.02 ppb ($0.06 \mu\text{g}/\text{m}^3$) based on the same rat study but using the NOAEL/UF approach with a LOAEL of 0.4 ppm divided by a total UF of 300 (10 for LOAEL, 3 for interspecies, and 10 for intraspecies) and the RGDR of 0.16. As another comparison, using 0.1 ppm as a LOAEL for respiratory epithelial lesions in female monkeys, the LOAEL can be time-adjusted to an equivalent continuous value of 24 ppb. Applying a UF_L of 3 for a mild effect, a UF_S of 10 since it was only a 6 month study, an interspecies UF of 3 for monkeys, and an intraspecies UF of 10 results in an estimated REL of 0.02 ppb ($0.06 \mu\text{g}/\text{m}^3$).

The human studies were examined for possible use in the calculation of a REL. The studies were limited by very variable exposures (e.g., Patil *et al.* (1970)), the presence of serious adverse health effects in some workers (chest x-ray abnormalities in Shi (1990), abnormal teeth and gums in 98 of 332 workers in Paril *et al.* 1970)), exposure to other compounds such as chlorine dioxide (Kennedy *et al.* (1991)), multiple acute “gassings” with chlorine (Kennedy *et al.* (1991)), and absence of data on cigarette smoking, also a respiratory system irritant. As an illustration of what would be estimated, the study of Shi (1990) had a mean workplace exposure of $4.82 \text{ mg}/\text{m}^3$ (1.7 ppm). This LOAEL was time adjusted to an equivalent continuous exposure of $1.72 \text{ mg}/\text{m}^3$,

then divided by an uncertainty factor of 100 (10 for use of a LOAEL and 10 for intraspecies variability) to yield a REL of 20 $\mu\text{g}/\text{m}^3$ (7 ppb). However, the use of a LOAEL default uncertainty factor of 10 does not seem adequate for frank, possibly irreversible effects such as the chest x-ray abnormalities reported. There is currently no methodology to deal with such effects in REL development.

Adequate benchmark dose estimates could not be obtained for the other nasal lesions due to high background rates and shallow dose-response relationships.

VII. Data Strengths and Limitations for Development of the REL

The strengths of the inhalation REL for chlorine include the availability of chronic multiple-dose inhalation exposure data from a recent (1995), well-conducted animal study with histopathological analysis. Major areas of uncertainty are the lack of adequate human exposure data, the lack of observation of a NOAEL, and limited reproductive toxicity data.

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*CHRONIC TOXICITY SUMMARY***CHLORINE DIOXIDE**

*(anthium dioxide; alclide; chlorine oxide; chlorine peroxide;
chloryl radical; doxide 50)*

CAS Registry Number: 10049-04-4

I. Chronic Toxicity Summary

<i>Inhalation reference exposure level</i>	0.6 µg/m³ (0.2 ppb)
<i>Critical effect(s)</i>	Vascular congestion and peribronchiolar edema; hemorrhagic alveoli and congested capillaries in the lung in rats
<i>Hazard index target(s)</i>	Respiratory system

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

<i>Description</i>	Yellow to red liquid or gas
<i>Molecular formula</i>	ClO ₂
<i>Molecular weight</i>	67.45 g/mol
<i>Density</i>	1.642 g/cm ³ @ 0°C (liquid)
<i>Boiling point</i>	9.9-11°C
<i>Melting point</i>	-59.5°C
<i>Solubility</i>	Soluble in water, alkaline and sulfuric acid solutions
<i>Conversion factor</i>	1 ppm = 2.76 mg/m ³

III. Major Uses or Sources

Chlorine dioxide is used directly as a bleaching agent for cellulose, textiles, flour, leather, oils, and beeswax. It is also used in the purification of water and as a bactericide and antiseptic (HSDB, 1994). The annual statewide industrial emissions from facilities reporting under the Air Toxics Hot Spots Act in California based on the most recent inventory were estimated to be 1136 pounds of chlorine dioxide (CARB, 2000).

IV. Effects of Human Exposures

Case reports of human occupational exposure to chlorine dioxide have shown that 19 ppm was fatal to one worker and 5 ppm was definitely irritating (Elkins, 1959). Seven out of 12 workers exposed regularly to chlorine dioxide at levels generally below 0.1 ppm (0.28 mg/m³) reported symptoms of ocular and respiratory irritation leading to slight bronchitis (Gloemme and

Lundgren, 1957). However, the authors ascribed the bronchitis to occasional acute excursions of chlorine dioxide levels above 0.1 ppm due to technical problems such as equipment leakage. Concurrent exposure to chlorine and chlorine dioxide in pulp mill workers resulted in an increase in the reporting of subjective symptoms of irritation (Ferris *et al.*, 1967). In this study, the chlorine dioxide concentrations ranged from trace levels to 0.25 ppm (0.69 mg/m³). No differences were found between these workers and controls by pulmonary function tests.

V. Effects of Animal Exposures

Eight rats (sex unspecified) were exposed for 5 hours/day, 5 days/week, for 2 months to 0 or 1 ppm (2.8 mg/m³) chlorine dioxide (Paulet and Debrousses, 1972). The number of control animals was not specified. Microscopic evaluation of the lungs revealed vascular congestion and peribronchiolar edema in all animals exposed to chlorine dioxide. The subchronic LOAEL for respiratory effects was therefore 1 ppm (2.8 mg/m³).

An earlier study by these researchers (Paulet and Debrousses, 1970) examined the effects of exposure to 2.5, 5, or 10 ppm chlorine dioxide for several hours/day for 30 days in rats and rabbits (n = 4-10 animals per group). Body weights, blood cell counts, and histopathological examination of the liver, lungs, and other tissues were measured in each group. At 10 ppm, nasal discharge, localized bronchopneumonia, and desquamated alveolar epithelium were observed. White and red blood cell counts were also increased with this exposure. Rats and rabbits exposed to 2.5 ppm for 7 hours/day for 30 days or for 4 hours/day for 45 days, respectively, showed significant respiratory effects, including hemorrhagic alveoli and inflammatory infiltration of the alveolar spaces.

Rats exposed to 5, 10, or 15 ppm (13.8, 27.6, or 41.4 mg/m³) chlorine dioxide for 15 minutes, 2 or 4 times/day, for 1 month showed an increase in congested lungs, nasal discharge, and catarrhus lesions of the alveoli beginning at 10 ppm (Paulet and Debrousses, 1974). No significant changes in these parameters were seen at 5 ppm.

Dalhamn (1957) found that acute exposure to 260 ppm chlorine dioxide for 2 hours resulted in the death of 1 out of 4 rats. Five out of 5 rats died during exposures of 4 hours/day for 14 days. All exposed animals exhibited signs of respiratory distress and ocular discharge. No effects were seen in 5 rats exposed to 0.1 ppm for 5 hours/day, 7 days/week, for 10 weeks. Thus 0.1 ppm was a subchronic NOAEL.

VI. Derivation of Chronic Reference Exposure Level (REL)

<i>Study</i>	Paulet and Debrousses (1970, 1972)
<i>Study population</i>	Wistar rats (8 per exposure concentration)
<i>Exposure method</i>	Discontinuous whole-body inhalation (0 or 1 ppm)
<i>Critical effects</i>	Vascular congestion; peribronchial edema in all animals; lung alveolar damage
<i>LOAEL</i>	1 ppm (2.8 mg/m ³)
<i>NOAEL</i>	Not observed
<i>Exposure continuity</i>	5 hours/day, 5 days/week
<i>Exposure duration</i>	2 months (2/24 = 8.3% of lifetime)
<i>Average experimental exposure</i>	0.15 ppm for LOAEL group (1 x 5/24 x 5/7)
<i>Human equivalent concentration</i>	0.23 ppm for LOAEL group (gas with thoracic respiratory effects, RGDR = 1.57 based on MV = 0.17 m ³ , SA(Th) = 3,460 cm ²)
<i>LOAEL uncertainty factor</i>	10
<i>Subchronic uncertainty factor</i>	3
<i>Interspecies uncertainty factor</i>	3
<i>Intraspecies uncertainty factor</i>	10
<i>Cumulative uncertainty factor</i>	1,000
<i>Inhalation reference exposure level</i>	0.0002 ppm (0.2 ppb, 0.0006 mg/m ³ , 0.6 µg/m ³)

The U.S. EPA (1995) based its RfC of 0.2 µg/m³ on the same study but included a Modifying Factor (MF) of 3 for database deficiencies. The criteria for use of modifying factors are not well specified by U.S. EPA. Such modifying factors were not used by OEHHA. In addition OEHHA assigned uncertainty factors according to its peer-reviewed, approved methodology (OEHHA, 2000).

OEHHA earlier developed a chronic REL for chlorine of 0.2 µg/m³ (0.08 ppb) based on hyperplasia in respiratory epithelium in female rats. Based on chemical reactivity, the REL for chlorine dioxide might be expected to be lower than that for chlorine. However, there are much less toxicologic data available for chlorine dioxide than for chlorine.

VII. Data Strengths and Limitations for Development of the REL

The REL for chlorine dioxide had uncertainties in all areas of concern. Thus the best available study was still limited by lack of multiple exposure concentrations, by the relatively short duration of exposures, and by the small number of animals examined. Adequate human health effects information is lacking, although it appears likely that the proposed REL would be protective of the effects reported in the single limited human study available. Other limitations were the lack of dose-response information and the lack of comprehensive data on multi-organ effects.

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CHRONIC TOXICITY SUMMARY

CHLOROBENZENE

(monochlorobenzene; benzene chloride; benzene monochloride; chlorobenzene; chlorbenzol; phenyl chloride)

CAS Registry Number: 108-90-7

I. Chronic Toxicity Summary

<i>Inhalation reference exposure level</i>	1000 µg/m³ (300 ppb)
<i>Critical effect(s)</i>	Increased liver weights, hepatocellular hypertrophy, renal degeneration and inflammation, and testicular degeneration in rats
<i>Hazard index target(s)</i>	Alimentary system; kidney; reproductive system

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

<i>Description</i>	Colorless, neutral liquid
<i>Molecular formula</i>	C ₆ H ₅ Cl
<i>Molecular weight</i>	112.56 g/mol
<i>Boiling point</i>	132°C
<i>Melting point</i>	-45.2°C
<i>Vapor pressure</i>	11.8 torr at 25°C
<i>Solubility</i>	Practically insoluble in water (0.049 g/100 ml); soluble in alcohol, benzene, chloroform, diethyl ether
<i>Conversion factor</i>	1 ppm = 4.60 mg/m ³ at 25 °C

III. Major Uses and Sources

As one of the most widely used chlorinated benzenes, mono-chlorobenzene has been a major chemical for at least 50 years. It was historically important in the manufacture of chlorinated pesticides, especially DDT, and in the production of phenol and aniline. Monochlorobenzene's principal current use is as a chemical intermediate in the production of chemicals such as nitrochlorobenzenes and diphenyl oxide. These chemicals are subsequently used in the production of herbicides, dyestuffs, and rubber chemicals. Additionally, monochlorobenzene is used as a solvent in degreasing processes (e.g., in metal cleaning operations), paints, adhesives, waxes and polishes (HSDB, 1995; NIOSH, 1993). The annual statewide industrial emissions from facilities reporting under the Air Toxics Hot Spots Act in California based on the most recent inventory were estimated to be 29,451 pounds of chlorobenzene (CARB, 2000).

IV. Effects of Human Exposure

Even though monochlorobenzene has been used industrially for many years, few epidemiologic and/or occupational studies have addressed the potential health status of workers chronically exposed to monochlorobenzene (NIOSH, 1993). A Russian occupational study (Rozenbaum *et al.*, 1947, as reported by the U.S. EPA, 1988) describes multiple central nervous system effects, including headache, numbness, dizziness, cyanosis, hyperesthesia, and muscle spasms, after intermittent exposure over 2 years to monochlorobenzene in a mixed chemical environment. No specific exposure levels or histopathologic data were reported.

Two small studies utilizing volunteers exposed to single doses of monochlorobenzene have reported central nervous system effects (Ogata *et al.*, 1991; Tarkhova, 1965). An exposure chamber study of five volunteers exposed up to 60 ppm monochlorobenzene (276 mg/m³) for a single 7 hour exposure described acute subjective symptoms such as drowsiness, headache, eye irritation, and sore throat (Ogata *et al.*, 1991). One other human volunteer study described altered electrical activity of the cerebral cortex in four individuals exposed to 43.4 ppm monochlorobenzene vapors for 2.5 minutes (Tarkhova, 1965).

V. Effects of Animal Exposure

No chronic inhalation studies have evaluated the toxicity of monochlorobenzene. Only a single, oral chronic carcinogenicity study (NTP, 1985) has evaluated the long-term adverse effects of monochlorobenzene administration. However, a few subchronic inhalation studies have demonstrated adverse effects on the liver, the kidney, and, to a lesser extent, blood parameters following monochlorobenzene exposure over a period of weeks or months (Dilley, 1977; John *et al.*, 1984; Nair *et al.*, 1987).

One subchronic study evaluated Sprague-Dawley male rats and rabbits exposed to 0, 75, or 200 ppm of monochlorobenzene for 7 hr/day, 5 days/week, for up to 24 weeks (Dilley, 1977). In rats, monochlorobenzene-related toxicity included increased absolute and relative (to brain- or body-weight) organ weights (especially the liver) after 11 and 24 weeks of exposure (LOAEL 75 ppm). Male rabbits also demonstrated increases in liver weight after 24 weeks of exposure (LOAEL = 75 ppm). Some hematological changes were reported in rats including differences in platelet and reticulocyte counts between control and exposed animals; however, some changes observed at 11 weeks were variable and comparable to controls at 24 weeks (red blood cell count, hemoglobin, hematocrit, and white blood cell count). Pathological changes were observed in rats, with occasional focal lesions in the adrenal cortex, tubular lesions in the kidneys, and congestion in the liver and kidneys.

Two other subchronic inhalation studies reported adverse organ effects following monochlorobenzene exposure in rats and rabbits (John *et al.*, 1984; Nair *et al.*, 1987). In the first study, John *et al.* (1984) reported increased liver weights in rats and rabbits following short-term (10 or 13 day, 6 hours/day) monochlorobenzene exposure (LOAEL = 590 ppm in rats and 210 ppm in rabbits). Nair *et al.* (1987) exposed male and female Sprague-Dawley rats to 0, 50, 150, or 450 ppm monochlorobenzene vapors daily for 6 hours over 10-11 weeks prior to mating, and

up to day 20 of gestation for 2 generations. Nair *et al.* found dose-related changes in the livers, kidneys, and testes in both generations of males (F₀ and F₁). Hepatotoxicity occurred as hepatocellular hypertrophy and increased liver weights (mean and absolute) at concentrations greater than 50 ppm (LOAEL = 150 ppm). At this concentration (150 ppm), renal changes included tubular dilation, interstitial nephritis, and foci of regenerative epithelium. Testicular degeneration of the germinal epithelium occurred in both generations of exposed males, but no chlorobenzene-induced adverse effects on reproductive performance or fertility were seen.

VI. Derivation of Chronic Reference Exposure Level (REL)

<i>Study</i>	Nair <i>et al.</i> (1987)
<i>Study population</i>	Sprague-Dawley rats (30/sex/group)
<i>Exposure method</i>	Discontinuous inhalation exposures (0, 50, 150, and 450 ppm)
<i>Critical Effects</i>	Increases in absolute and relative liver weights (F ₀ and F ₁ both sexes), hepatocellular hypertrophy (F ₀ and F ₁ males), renal degeneration and inflammation (F ₀ and F ₁ both sexes), testicular degeneration (F ₀ and F ₁ males).
<i>LOAEL</i>	150 ppm
<i>NOAEL</i>	50 ppm
<i>Exposure continuity</i>	6 hours/day, 7 days/week
<i>Exposure duration</i>	11 weeks
<i>Average experimental exposure</i>	13 ppm for NOAEL group (50 x 6/24)
<i>Human equivalent concentration</i>	26 ppm (gas with systemic effects, based on RGDR = 2.0 for lambda (a) : lambda (h)) (Gargas <i>et al.</i> , 1989)
<i>LOAEL uncertainty factor</i>	1
<i>Subchronic uncertainty factor</i>	3
<i>Interspecies uncertainty factor</i>	3
<i>Intraspecies uncertainty factor</i>	10
<i>Cumulative uncertainty factor</i>	100
<i>Inhalation reference exposure level</i>	0.3 ppm (300 ppb; 1.0 mg/m ³ , 1000 µg/m ³)

Of the three inhalation studies available (Dilley, 1977; John *et al.*, 1984; Nair *et al.*, 1987), the Nair *et al.* (1987) two generational developmental study was selected for identifying a NOAEL and LOAEL. It best presented the histopathology of the adverse effects, and demonstrated a dose response relationship for these effects (statistically significant increases in mean liver weights, incidence of renal changes, and testicular degeneration).

Another subchronic inhalation study (Dilley, 1977) also observed increases in organ weights, including the liver, in rats after 11 and 24 weeks exposure to 75 and 250 ppm monochlorobenzene (LOAEL = 75 ppm), and in rabbits at 24 weeks. Similar adverse liver and kidney effects were found in subchronic oral bioassays (Kluwe *et al.*, 1985; NTP, 1985). These

include increases in liver weight and hepatocellular degeneration in rats (LOAEL = 125 mg/kg/day) and mice (LOAEL = 250 mg/kg/day), and renal necrosis and degeneration in rats (LOAEL = 500 mg/kg/day) and mice (LOAEL = 250 mg/kg/day) after 13 weeks oral exposure to chlorobenzene.

Uncertainty factors are appropriate due to the lack of chronic studies, both animal bioassay and human, and the limited number of subchronic inhalation studies, thereby requiring estimation of the chronic REL from this shorter term, single species study. The magnitude of interspecies variation remains unknown, as few species have been tested and human data for comparison are lacking. However, metabolic studies have demonstrated species variation in the urinary elimination of chlorobenzene metabolites (Ogata and Shimada 1983; Ogata *et al.*, 1991; Yoshida *et al.*, 1986). Humans metabolize and excrete chlorobenzene predominately as free and conjugated forms of 4-chlorocatechol and chlorophenols, while the main rodent urinary metabolite, p-chlorophenylmercapturic acid, is found in minor amounts (<0.5%). No information exists which identifies human subpopulations possibly susceptible to monochlorobenzene exposure.

For comparison with the proposed REL, a REL can be derived from the 24 week LOAEL of 75 ppm for liver effects (Dilley, 1977). The LOAEL is equivalent to a continuous exposure LOAEL of 15.6 ppm. Multiplying by the RGDR of 2 and dividing by a cumulative UF of 100 (3 for LOAEL, 3 for interspecies and 10 for intraspecies) also yields an estimate of 300 ppb.

VII. Data Strengths and Limitations for Development of the REL

The strengths of the inhalation REL for chlorobenzene include the observation of a NOAEL, the availability of subchronic inhalation exposure data from a well-conducted study with histopathological analysis, and the demonstration of a dose-response relationship. Major areas of uncertainty are the lack of adequate human exposure data and limited reproductive toxicity data.

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CHRONIC TOXICITY SUMMARY

CHLOROFORM*(trichloromethane; formyl trichloride; methenyl trichloride; methyl trichloride)***CAS Registry Number: 67-66-3****I. Chronic Toxicity Summary**

<i>Inhalation reference exposure level</i>	300 µg/m³ (50 ppb)
<i>Critical effect(s)</i>	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
<i>Hazard index target(s)</i>	Alimentary system; kidney; teratogenicity

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

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

III. Major Uses and Sources

Chloroform (CHCl₃) is used in industry and laboratory settings as a solvent for adhesives, pesticides, fats, oils and rubbers. It is also used as a chemical intermediate in the synthesis of fluorocarbon 22, dyes, pesticides, and tribromomethane. Chloroform is produced as a byproduct of water, sewage, and wood pulp chlorination (HSDB, 1995). In 1996, the latest year tabulated, the statewide mean outdoor monitored concentration of chloroform was approximately 0.037 ppb (CARB 1999a). The annual statewide industrial emissions from facilities reporting under the Air Toxics Hot Spots Act in California based on the most recent inventory were estimated to be 79,949 pounds of chloroform (CARB 1999b).

IV. Effects of Human Exposure

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

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

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

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

More recently, Li *et al.* (1993) reported on 61 chloroform exposed workers from a variety of production factories. Exposure levels at 3 representative worksites varied widely, from 4.27 to 147.91 mg/m³ (0.9 to 30 ppm) (119 samples), with 45% of the samples below 20 mg/m³. The exposed workers were subclassified for some studies according to exposure levels into group 1 (mean level = 13.49 mg/m³ or 2.8 ppm) and group 2 (mean level = 29.51 mg/m³ or 6 ppm). Workers exposed to chloroform had slight liver damage indicated by higher (abnormal) levels of serum prealbumin (in group 2) and transferrin (in both groups) than those of control workers. Neurobehavioral functions were also affected, manifested as increases in scores of passive mood states and dose-related, negative changes in neurobehavioral testing.

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

V. Effects of Animal Exposure

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

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

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

At 90 days there was a generalized atrophy of the ethmoid turbinates at concentrations of 2 ppm (the lowest concentration tested) and above.

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

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

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

* $p < 0.05$

VI. Derivation of Chronic Reference Exposure Level (REL)

<i>Study</i>	Torkelson <i>et al.</i> (1976)
<i>Study population</i>	Rats, unspecified strain (12/sex/group)
<i>Exposure method</i>	Discontinuous whole-body inhalation exposures (0, 25, 50, 85 ppm)
<i>Critical effects</i>	Pathological changes in liver (degenerative), and kidneys (cloudy swelling)
<i>LOAEL</i>	25 ppm
<i>NOAEL</i>	Not observed
<i>Exposure continuity</i>	7 hr/day for 5 days/week for 6 months
<i>Average experimental exposure</i>	5.3 ppm for LOAEL group (25 x 7/24 x 5/7)
<i>Human equivalent concentration</i>	15.9 ppm for LOAEL group (gas with systemic effects, based on RGDR = 3.0 for lambda (a) : lambda (h) (Gargas <i>et al.</i> , 1989))
<i>Exposure duration</i>	6 months
<i>LOAEL uncertainty factor</i>	10
<i>Subchronic uncertainty factor</i>	1
<i>Interspecies uncertainty factor</i>	3
<i>Intraspecies uncertainty factor</i>	10
<i>Cumulative uncertainty factor</i>	300
<i>Inhalation reference exposure level</i>	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/m³) and in male rats at 25 ppm (122 mg/m³) 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/m³) 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/m³) after at least 1 year (Bomski *et al.*, 1967) and 14 to 400 ppm chloroform (68 to 1952 mg/m³) 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/m³ with a geometric average of 20.46 mg/m³. 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/m³) 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/m³) 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

CHLOROPICRIN*(trichloronitromethane; nitrochloroform; nitrochloromethane)***CAS Registry Number: 76-06-2****I. Chronic Toxicity Summary**

<i>Inhalation reference exposure level</i>	0.4 µg/m³ (0.05 ppb)
<i>Critical effect(s)</i>	Nasal rhinitis and bronchiectasis in mice
<i>Hazard index target(s)</i>	Respiratory system

II. Chemical Property Summary (from HSDB (1996) except as noted)

<i>Description</i>	Colorless to faint yellow liquid
<i>Molecular formula</i>	CCl ₃ NO ₂
<i>Molecular weight</i>	164.4 g/mol
<i>Boiling point</i>	112°C
<i>Melting point</i>	-64°C (CRC, 1994)
<i>Vapor pressure</i>	5.7 torr @ 0°C (Fries and West, 1921); 3.2 kPa (24 torr) @ 25°C (Tomlin, 1994)
<i>Solubility</i>	1.6 g/L water @ 25°C; 2.272 g/L water @ 0°C 1.9 g/L water @ 20°C; miscible with benzene, ethanol, carbon disulfide, ether, carbon tetrachloride, acetone, methanol, acetic acid
<i>Conversion factor</i>	6.72 µg/m ³ per ppb at 25°C

III. Major Uses and Sources

Chloropicrin is used primarily as a preplant soil fumigant against insects and fungi; it also kills weed and grass seeds when applied to soil. Chloropicrin is occasionally used as a fumigant in grain elevators and storage bins (HSDB, 1996). Chloropicrin is used as an indicator chemical in other fumigants such as methyl bromide because of its potent irritant properties. Chloropicrin was used in World War I as a chemical warfare agent because of its potent activity as a lachrymator. Chloropicrin has a minor use in the chemical synthesis of methyl violet. Chloropicrin can also form in drinking water as a result of chlorination processes (Duguet *et al.*, 1985; Merlet *et al.*, 1985). The annual statewide industrial emissions from facilities reporting under the Air Toxics Hot Spots Act in California based on the most recent inventory were estimated to be 1507 pounds of chloropicrin (CARB, 2000). This does not include emissions from its major use as a preplant soil fumigant, either alone or in combination with other

fumigants, because agricultural field applications are not covered under the Air Toxics Hot Spots program. Approximately 3,630,000 lbs. of chloropicrin were used in agriculture in California in 1999 (DPR, 2000).

IV. Effects of Human Exposure

No studies are available which describe toxic effects to humans from chronic exposure to chloropicrin. Human exposures to concentrations less than 1 ppm for very short periods of time are extremely irritating (ACGIH, 1992; Fries and West, 1921). The threshold of odor detection in humans is approximately 1 ppm (ACGIH, 1992).

V. Effects of Animal Exposure

Burleigh-Flayer and Benson (1995) conducted a chronic inhalation bioassay with CD rats (50-60 per sex per dose) exposed discontinuously to 0 (air), 0.1, 0.5, or 1.0 ppm 99.6% pure chloropicrin vapor 6 hours/day for 5 consecutive days/week over 107 weeks. Clinical signs (such as hypoactivity and decreased startle response) were increased in both sexes, primarily at 1.0 ppm. Increased mortality was noted in males at 0.5 and 1 ppm and in females at 1 ppm. Absolute and relative increased lung and liver weights and increased nasal rhinitis were reported in both sexes at the 1 ppm level. However, no effects were seen at 0.1 ppm. Thus this study yielded a NOAEL of 0.1 ppm (0.67 mg/m³) for chronic non-cancer effects in rats.

Results from chronic inhalation of chloropicrin in rats (Burleigh-Flayer and Benson, 1995)

Chloropicrin	Lung wt., m	Lung wt., f	Rhinitis, m	Rhinitis, f	Mean survival, m
0	2.086 g	1.574 g	20/50	18/50	696 d
0.1 ppm	2.089 g	1.464 g	24/50	17/50	669 d
0.5 ppm	2.202 g	1.460 g	21/50	26/50	672 d*
1.0 ppm	2.448 g	1.633 g	35/50**	23/50	647 d**

*p<0.05; **p<0.01

A similar study in mice (Burleigh-Flayer *et al.*, 1995) resulted in the same NOAEL. CD-1 mice (50/sex/dose) were exposed to chloropicrin (99.6% pure) vapor at 0 (air), 0.1, 0.5, or 1.0 ppm for 6 hours/day, 5 days/week for at least 78 weeks. Body weights and body weight gains were significantly decreased in both sexes at ≥ 0.5 ppm. Food consumption was decreased in males at 1.0 ppm and in females at ≥ 0.5 ppm. Absolute and relative lung weights were increased in a dose-related manner in both sexes at ≥ 0.5 ppm. Changes in pathology observed macroscopically in the 1.0 ppm males included increased numbers of lung nodules and increased numbers of kidney cysts. In females lung masses and kidney cysts were seen at 0.5 ppm. Microscopic pathology changes included increased nasal cavity lesions (including serous exudate, hyaline epithelial inclusions, rhinitis, olfactory and epithelial atrophy) and lung lesions (including alveolar protein deposits, alveolar histiocytosis, hemorrhage, peribronchiolar lymphocytic infiltrate, bronchiectasis, bronchial submucosal fibrosis, peribronchiolar smooth muscle hyperplasia), in addition to kidney cysts at ≥ 0.5 ppm (CDPR, 2000).

Results from chronic inhalation of chloropicrin in mice (Burleigh-Flayer *et al.*, 1995)

Chloropicrin	Rhinitis, m	Rhinitis, f	Bronchiectasis, m	Bronchiectasis, f	
0	6/50	3/50	0/50	0/50	
0.1 ppm	7/50	6/50	3/50	5/50	
0.5 ppm	17/50**	18/50**	28/50**	28/50**	
1.0 ppm	35/50**	32/50**	41/50**	44/50**	

**p<0.01

Yoshida *et al.* (1987) exposed groups of 12 male Fischer 344 rats intermittently to 0, 0.37, 0.67, 1.58, or 2.93 ppm chloropicrin vapor 6 h/day, 5 days/week for 13 weeks. Mean body weights were reduced in the highest 2 exposure groups, and red blood cell count, hematocrit, and hemoglobin concentration were significantly increased in the 2.93 ppm group. The treatment-related histological lesions reported were degeneration and necrosis of the bronchial and bronchiolar epithelia at 2.93 ppm and hypertrophy of these epithelia at 1.58 ppm. Thus the primary target organ was the respiratory tract and the subchronic NOAEL was 0.67 ppm (4.5 mg/m³). (Eyelid closure and decrease in motor activity were seen in all exposure groups only during exposure. No morphological changes were seen at 0.67 ppm, so the authors deemed the behavior changes minor and not toxicologically important.)

Male Swiss-Webster mice (group numbers ranging from 16-24) were exposed by inhalation to a single level of different sensory irritants including chloropicrin for 6 hours/day for 5 days; unexposed control groups had 8-10 mice (Buckley *et al.*, 1984). The exposure level for chloropicrin was 7.9 ppm, which approximated the level sufficient to cause a 50% decrease in respiratory rate in mice (RD₅₀) (Kane *et al.*, 1979). Half the exposed mice and half the control animals were terminated immediately after the exposures and the other half 72 hours after the last exposure. All were examined for respiratory tract lesions. Body weights of chloropicrin exposed animals were reduced 10-25% below controls, but increased to normal levels during the recovery period. Nasal exudate and distention of the abdomen were observed. "Moderate" lesions, characterized by exfoliation, erosion, ulceration, or necrosis, were observed in the respiratory and olfactory epithelium, and minimal inflammation and squamous metaplasia were observed in the respiratory epithelium alone. Moderate to severe damage to the lower respiratory tract was described as "fibrosing peribronchitis and peribronchiolitis". Exfoliation, hyperplasia, and squamous metaplasia were also noted.

Condie *et al.* (1994) conducted a study of the toxicity of chloropicrin by oral exposure in Sprague-Dawley rats. Ten and ninety-day studies were conducted by dosing animals daily with chloropicrin in vehicle (corn oil) at a volume of 1 ml/kg. Groups of 10 rats/sex/group were dosed with 0, 10, 20, 40, and 80 mg/kg for the 10-day study and with 0, 2, 8, and 32 mg/kg for the 90-day study. Parameters examined included mortality, body weight, food and water consumption, hematology, serum clinical chemistry, and gross pathology and histology of organs. Only the high-dose group and the control group animals from the 90-day study were examined histopathologically. In the 90-day study, 6 males and 2 females in the 32 mg/kg dose group and 1 male and 3 females in the 8 mg/kg dose group died before the scheduled termination time. The authors noted signs of pulmonary complications (inflammation and congestion) in the dead animals. Previously, the animals had shown signs of respiratory distress, including

wheezing and dyspnea. The deaths were considered to be exposure related and most likely due to aspiration of chloropicrin. Among the survivors, mean body weight, hemoglobin levels, and hematocrits were significantly reduced in males in the 32 mg/kg dose group. Absolute thymus weights were reduced in female rats at 32 mg/kg, and female rats in the 8 mg/kg dose group showed decreased white blood cell count. Most animals in the 32 mg/kg dose group (>60%) showed histopathological changes in the forestomach including chronic inflammation, acantholysis, and hyperkeratosis. The authors considered the NOAEL to be 8 mg/kg/day.

VI. Derivation of Chronic Reference Exposure Level (REL)

<i>Study</i>	Burleigh-Flayer and Benson (1995)
<i>Study population</i>	CD-1 mice (60 per sex per dose)
<i>Exposure method</i>	Discontinuous inhalation (0, 0.1, 0.5 or 1.0 ppm)
<i>Critical effects</i>	Nasal rhinitis; bronchiectasis
<i>LOAEL</i>	0.5 ppm
<i>NOAEL</i>	0.1 ppm
<i>Exposure continuity</i>	6 hours/day, 5 days/week
<i>Exposure duration</i>	107 weeks
<i>BMC₀₅</i>	0.042 ppm
<i>Average experimental exposure</i>	0.0075 ppm at the BMC ₀₅ (0.042 x 6/24 x 5/7)
<i>Human equivalent concentration</i>	0.0016 ppm at the BMC ₀₅ (gas with extrathoracic respiratory effects, RGDR = 0.21 based on MV = 0.044 L/min and SA(ET) = 3 cm ²)
<i>LOAEL uncertainty factor</i>	not needed in the BMC approach
<i>Subchronic uncertainty factor</i>	1
<i>Interspecies uncertainty factor</i>	3 (since RGDR adjustment was made)
<i>Intraspecies uncertainty factor</i>	10
<i>Cumulative uncertainty factor</i>	30
<i>Inhalation reference exposure level</i>	0.05 ppb (0.4 µg/m ³)

The data on bronchiectasis incidence in male and female mice were combined and the chronic REL for chloropicrin was developed using the BMC approach. Of the several models tested, the Gamma MultiHit Model gave the best fit to the combined bronchiectasis data (p = 0.9750). The MLE₀₅ was 0.070 ppm and the BMC₀₅ was 0.042 ppm. Use of time extrapolation to equivalent continuous exposure, an RGDR adjustment for the area of the respiratory tract affected, and a total uncertainty factor of 30 resulted in a chronic REL of 0.05 ppb (0.4 µg/m³).

The chronic study in mice (Burleigh-Flayer *et al.*, 1995) yielded the same NOAEL of 0.1 ppm as the chronic study in rats (Burleigh-Flayer and Benson, 1995). Use of the mouse data with the NOAEL/UF approach led to a cREL estimate of 0.1 ppb. Use of the rat data yielded a chronic REL estimate of 0.2 ppb by the NOAEL/UF approach.

As another comparison, the study of Yoshida *et al.* (1987) found a NOAEL in rats of 0.67 ppm for intermittent exposure for 13 weeks. This is equivalent to a continuous exposure of 120 ppb. Use of an RGDR of 0.25 for rats and a total uncertainty factor of 100 (3 for subchronic, 3 for interspecies, and 10 for intraspecies) results in a REL estimate of 0.03 ppb (0.2 $\mu\text{g}/\text{m}^3$).

VII. Data Strengths and Limitations for Development of the REL

Significant strengths in the REL for chloropicrin include the duration of exposure (lifetime) in the key study, the multiple dose study design with adequate sample sizes, and the demonstration of a NOAEL in rats and mice. Major areas of uncertainty are the lack of adequate human exposure data, limited reproductive toxicity data, and the appropriateness of time extrapolation of concentrations that cause irritative effects such as rhinitis.

VIII. Potential for Differential Impacts on Children's Health

Chloropicrin is a respiratory irritant. Respiratory irritants often have steep dose-response curves. Thus use of the human intraspecies factor of 10 should result in a REL that adequately protects children. Exacerbation of asthma, which has a more severe impact on children than on adults, is a known response to some respiratory irritants. However, there is no direct evidence in the literature to quantify such a response to chloropicrin, or to quantify a differential effect of chloropicrin on infants or children. We are currently evaluating our risk assessment methodologies, in particular the intraspecies uncertainty factor (UF_H), for adequacy in protecting infants and children. While we have not so far identified any indications that the currently used UF_H of 10 might be less than adequate to protect infants and children, this possibility should be considered in evaluating any exposure situation involving chronic exposures of infants or children to chloropicrin.

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CHRONIC TOXICITY SUMMARY

**CHROMIUM, HEXA VALENT
(SOLUBLE COMPOUNDS)**

<i>Molecular Formula</i>	<i>Molecular Weight</i>	<i>Synonyms</i>	<i>CAS Registry Number</i>
CrO ₃	99.99 g/mol	Chromic trioxide, chromium oxide, chromium trioxide, chromium (VI) oxide. (In acid aqueous solutions, exists as H ₂ CrO ₄ – “chromic acid”)	1333-82-0
K ₂ CrO ₄	194.20 g/mol	Potassium chromate, dipotassium chromate, potassium (VI) chromate, dipotassium monochromate, chromate of potash	7789-00-6
Li ₂ CrO ₄	129.87 g/mol	Lithium chromate, chromium lithium oxide, chromic acid dilithium salt, lithium chromate (VI)	14307-35-8
Na ₂ CrO ₄	161.97 g/mol	Sodium chromate, chromic acid disodium salt, chromium disodium oxide, sodium chromate (VI), chromate of soda	7775-11-3
K ₂ Cr ₂ O ₇	294.20 g/mol	Potassium dichromate, dichromic acid dipotassium salt, bichromate of potash	7778-50-9
Na ₂ Cr ₂ O ₇	261.96 g/mol	Sodium dichromate, bichromate of sodium, dichromic acid disodium salt, chromium sodium oxide	10588-01-9

I. Chronic Toxicity Summary**A. Soluble Hexavalent Chromium Compounds (except chromic trioxide)***Inhalation reference exposure level***0.2 µg Cr(VI)/m³***Critical effect(s)*

Bronchoalveolar hyperplasia in lungs of rats

Hazard index target(s)

Respiratory system

*Oral reference exposure level***0.02 mg Cr(VI)/kg/day***Critical effect(s)*

Red blood cell effects (decreased mean corpuscular volume (MCV) and mean corpuscular hemoglobin (MCH)) in mice

Hazard index target(s)

Hematopoietic system

B. Chromic Trioxide (as chromic acid mist)

<i>Inhalation reference exposure level</i>	0.002 µg Cr(VI)/m³
<i>Critical effect(s)</i>	Respiratory effects (nasal atrophy, nasal mucosal ulcerations, nasal septal perforations, transient pulmonary function changes) in human occupational study
<i>Hazard index target(s)</i>	Respiratory system

II. Physical and Chemical Properties (HSDB, 2000; CRC, 1994)

<i>Description</i>	CrO ₃ : dark red or brown crystals, flakes, or powder, exists as chromic acid (H ₂ CrO ₄) in solution; K ₂ CrO ₄ , Na ₂ CrO ₄ : yellow crystals; K ₂ Cr ₂ O ₇ , Na ₂ Cr ₂ O ₇ : orange-red crystals; Li ₂ CrO ₄ : yellow crystalline powder
<i>Molecular formula</i>	See above
<i>Molecular weight</i>	See above
<i>Density</i>	CrO ₃ : 2.70 g/cm ³ @ 25°C
<i>Boiling point</i>	CrO ₃ : decomposes (temperature not available); K ₂ Cr ₂ O ₇ : 500 °C with decomposition; Na ₂ Cr ₂ O ₇ : 400 °C
<i>Melting point</i>	CrO ₃ : 197 °C; K ₂ CrO ₄ : 975 °C; Na ₂ CrO ₄ : 792 °C; K ₂ Cr ₂ O ₇ : 398 °C; Na ₂ Cr ₂ O ₇ : 356.7 °C
<i>Vapor pressure</i>	Not applicable
<i>Solubility</i>	CrO ₃ : soluble in water, ethyl alcohol, ethyl ether, sulfuric and nitric acid; K ₂ CrO ₄ , K ₂ Cr ₂ O ₇ , Na ₂ Cr ₂ O ₇ : soluble in water, insoluble in ethyl alcohol; Na ₂ CrO ₄ : soluble in water, slightly soluble in ethyl alcohol; Li ₂ CrO ₄ : soluble in water and ethyl alcohol
<i>Conversion factor</i>	Not applicable for particulates and mists

III. Major Uses or Sources

Hexavalent chromium (Cr(VI)) is considerably more toxic than trivalent chromium (Cr(III)), the form most commonly found naturally (ATSDR, 1993). Cr(VI) is generally produced by industrial processes. While more information is available on the toxicity of soluble Cr(VI)

compounds, information on poorly soluble Cr(VI) compounds has been included where applicable. In California, the major emission source of Cr(VI) results from the chrome plating industry (CARB, 1997). Chromic acid, used to electroplate metal parts, is the most common Cr(VI) compound produced in the U.S. (ATSDR, 1998). Chromic acid is also registered as a fungicide and pesticide in California for use in wood and lumber protection treatments (CDPR, 1998). Chromic acid solutions used for this purpose in the most recent year of reporting (1998) was 71,109 lbs. Minute emissions of Cr(VI) may result from lead chromate in paint used for road striping and from coatings in the aerospace and auto refinishing industries, although uses of Cr(IV)-containing coatings by these industries in California are decreasing (CARB, 1997 and 1988). Use of Cr(VI) as a corrosion inhibitor in cooling tower water is prohibited in California, and recently, in the remainder of the U.S. as well. Fuel combustion releases trace amounts of chromium (CARB, 1988). Most, if not all, of this emitted chromium is in the Cr(III) state. In the chromium ferroalloy industry, sodium chromate and dichromate can be produced from imported chromite (Cr(III)) ore. However, no such facilities in California have reported production or emission of these Cr(VI) compounds.

Primary routes of potential human exposure to chromium compounds are inhalation, ingestion, and dermal contact. Exposure to chromic acid is most often in the form of a mist; exposure to other soluble forms of Cr(VI) is as components of aerosols or particulate matter. The physical, chemical, and potency differences between Cr(VI) dusts and chromic acid mists necessitated the development of separate RELs for each. Environmental exposures would most likely occur through exposure to Cr(VI) dusts (U.S. EPA, 1998). Cr(VI) may persist in water as water-soluble complex anions. However, any Cr(VI) settling in the soil or water is expected to be eventually reduced to Cr(III) by organic matter. The South Coast Air Quality Management District (SCAQMD, 2000) detected ambient levels of hexavalent chromium ranging from 0.0001 to 0.0003 $\mu\text{g}/\text{m}^3$ at 10 stationary monitors placed throughout the South Coast Air Basin. The annual statewide industrial emissions from facilities reporting under the Air Toxics Hot Spots Act in California based on the most recent inventory were estimated to be 2311 pounds of hexavalent chromium (CARB, 2000).

IV. Effects of Human Exposure

Cr(VI) forms oxyanions at physiological pH (CrO_4^{-2}), which are quite similar to sulfate (SO_4^{-2}) and phosphate (HPO_4^{-3}) anions. Therefore, it is able to penetrate virtually every cell in the body because all cells transport sulfate and phosphate (Costa, 1997). Harmful effects are speculated to be related to the reduction of Cr(VI) to Cr(III) intracellularly when it crosses the cell membrane and forms complexes with intracellular macromolecules. Thus, Cr(VI) compounds have the potential to injure numerous organ systems. Toxicity following chronic Cr(VI) exposure has been reported in the respiratory tract, gastrointestinal system, eyes and conjunctiva, kidney, and hematopoietic system. Cr(VI) is corrosive and exposure to chromic acid mists may cause chronic skin ulcerations and upper respiratory lesions (U.S. EPA, 1998). In addition, allergic skin and respiratory reactions can occur with no relation to dose.

Nasal tissue damage has been frequently observed in chromium plating workers exposed chronically to chromic acid mists (Bloomfield and Blum, 1928; Vigliani and Zurlo, 1955;

Kleinfeld and Rosso, 1965; Gomes, 1972; Sorahan *et al.*, 1998). However, workers in the chromate extraction and ferrochromium industry, exposed to particulates containing soluble Cr(VI) compounds, have also reported nasal lesions (Mancuso, 1951; Federal Security Agency, 1953; Machle and Gregorius, 1948; Wang *et al.*, 1994; Walsh, 1953). Other less frequent mucous membrane injuries have been reported in workers exposed to chromate dust and chromic acid including sinusitis, laryngitis, conjunctivitis, and oral ulcerations (Mancuso, 1951; Federal Security Agency, 1953; Johansen *et al.*, 1994). Nasal lesions include perforated septum, ulcerated septum, nasal atrophy, nosebleed, and inflamed mucosa following exposure to air chromium levels of about 0.1 to 5.6 mg/m³. Exposure duration, when reported, ranged from 2 weeks to 25 years. However, there were problems in quantifying the effect for the above studies. The difficulties were primarily lack of adequate methods or data for determining exposure duration and/or exposure levels. The occupational studies summarized below provide the most reliable estimates of inhalation durations and concentrations resulting in chronic toxicity.

Workers exposed to $\geq 2 \mu\text{g}/\text{m}^3$ Cr(VI) as chromic acid exhibited an increased incidence of nasal atrophy, nasal mucosal ulcerations, and nasal septal perforations as compared to controls (Lindberg and Hedenstierna, 1983). Workers exposed to less than 2 $\mu\text{g}/\text{m}^3$ (expressed as $\leq 1.9 \mu\text{g}/\text{m}^3$) exhibited an increased incidence of irritated nasal mucosa and nasal atrophy compared to controls. The median exposure time of exposed workers was 2.5 years (range = 0.2-23.6 years). Frequency of throat and chest symptoms was similar to that of controls. The same study reported statistically significant decreases in forced expiratory volume in 1 second (FEV₁), forced vital capacity (FVC), and mean forced expiratory flow during the middle of the FVC in 1 second (FEF₂₅₋₇₅) measurements taken on a Thursday afternoon as compared to those taken on a Monday morning in nonsmoking workers exposed to 2 $\mu\text{g}/\text{m}^3$ Cr(VI) or more. Similar changes were observed in the smokers although only the difference in the FVC measured on a Thursday was statistically significant. No significant differences were observed between pulmonary function measurements of exposed and unexposed workers taken on a Monday morning (prior to a work week of exposure). Thus the authors infer that the observed pulmonary function changes are transient.

Nasal lesions were observed in 35 of 37 chrome platers exposed to a mean breathing zone concentration of 7.1 $\mu\text{g}/\text{m}^3$ (range = 1.4-49.3 $\mu\text{g}/\text{m}^3$) total chromium for an average of 2.2 years (range = 1.2 weeks-11 years) (Cohen *et al.*, 1974). Actual exposure to Cr(VI) averaged 2.9 $\mu\text{g}/\text{m}^3$ (range = 0.09-9.1 $\mu\text{g}/\text{m}^3$). Workers employed more than one year had significantly greater nasal pathology than workers employed one year or less. Due to poor personal hygiene habits of the exposed workers, a 'direct contact' etiology may explain some of the nasal lesions.

Urinary levels of β_2 -microglobulin in 24 chrome platers increased in dose-dependent fashion with increasing intensity of exposure to Cr(VI), indicating a nephrotoxic effect resulting from inhalation of Cr(VI) (Lindberg and Vesterberg, 1983). The 8-hr mean Cr(VI) levels ranged from 2 to 20 $\mu\text{g}/\text{m}^3$ and averaged 6 $\mu\text{g}/\text{m}^3$. Total exposure times ranged from 0.1 to 26 years and averaged 5.3 years. Most of the 24 chrome workers had irritation symptoms of the airways. As a group, the chrome platers had significantly higher levels of urinary β_2 -microglobulin compared to a group of 27 referents. Comparison of 27 referents to a group of 27 ex-chrome-platers found no difference in urinary β_2 -microglobulin levels, even though seven of the ex-chrome-platers had a permanent perforation of their nasal septum (indicating past exposure to high levels of Cr(VI)).

There was no correlation between total exposure time and urinary β_2 -microglobulin levels. Urinary albumin levels remained unchanged in the Cr(VI)-exposed group. The results suggest that the nephrotoxic effects are reversible at the exposure levels studied.

Gastritis and duodenal ulcers, in addition to ulceration and perforation of the nasal septum, were observed in chrome platers exposed to a mean breathing zone concentration of $4 \mu\text{g}/\text{m}^3$ chromic acid for an average of 7.5 years (Lucas and Kramkowski, 1975).

Male workers in the chromate and dichromate production industry, whose occupational exposures were 0.05 - $1.0 \text{ mg Cr(VI)}/\text{m}^3$ as chromium trioxide for a mean of 7 years, were reported to have elevated levels of low molecular weight proteins (retinol binding protein and tubular antigens) in the urine (Franchini and Mutti, 1988). The authors suggest that the presence of such proteins in the urine is an early indicator of kidney damage.

The respiratory health of workers exposed to low levels of dusts containing Cr(VI) was investigated at a stainless steel production plant (Huvinen *et al.*, 1996). The data were presented as total chromium exposure and Cr(VI) exposure. A combined total of 109 exposed workers in the furnace department (median Cr(VI) exposure approximately 0.075 - $0.45 \mu\text{g}/\text{m}^3$) and the steel smelting shop (average Cr(VI) exposure $0.5 \mu\text{g}/\text{m}^3$) was compared to a control group of 95 workers that worked in the cold rolling mill. Total work exposure duration was 16.0 years (range: 8-26 years). No significant differences in lung function tests and radiological findings were observed between exposed and control workers. After controlling for age and smoking, no differences were observed for the prevalence of rhinitis, eye irritation, or respiratory symptoms between the two groups.

In a study summarized by U.S. EPA (1998), oral ulcers, diarrhea, stomach ache, indigestion, leukocytosis and vomiting were reported among a group of 155 Chinese villagers exposed to contaminated well-water containing $20 \text{ mg}/\text{L}$ Cr(VI) in 1965 (Zhang and XiLin, 1987). However, precise exposure concentrations, exposure durations, and confounding factors were not provided. A follow-up study to assess cancer mortality reported that the average Cr(VI) concentration in 1965 from 170 wells of the most impacted village was only 2.6 ppm, and maximum levels did not exceed 5 ppm (Zhang and Li, 1997). Non-cancer effects were not presented and the apparent discrepancy in water levels of Cr(VI) with the earlier study was not discussed.

V. Effects of Animal Exposure

Exposure of C57BL/6 mice to 0 or $13 \text{ mg}/\text{m}^3$ CaCrO_4 dust (about 136 animals/sex/group) 5 hr/day, 5 days/wk for life resulted in emphysema-like changes of the lung, 'bronchiolarization' of the alveoli, and epithelial necrosis, marked hyperplasia, and atrophy of the bronchi in treated mice (Nettesheim *et al.*, 1971). Other non-cancer histopathological findings in exposed mice included atrophy of the lymph nodes, spleen, and liver, and occasional small ulcerations of the stomach and intestinal mucosa. Cessation of body weight gain in both sexes was observed following the sixth month of exposure to the chromate dust.

Glaser *et al.* (1986) exposed 20 male Wistar rats/group to 25, 50, and 100 $\mu\text{g}/\text{m}^3$ aerosolized sodium dichromate solution and to 100 $\mu\text{g}/\text{m}^3$ of a pyrolyzed Cr(VI)/Cr(III) (3:2) oxide dust mixture 22-23 hr/day for 18 months. Observation in filtered air continued for another 12 months thereafter. A control group consisted of 40 rats. Mortality and body weights were unaffected by treatment. Lung chromium retention at the end of the study was 10-fold greater in rats exposed to the slightly water soluble chromium oxide mixture compared to high dose rats exposed to water-soluble sodium dichromate. No clinical signs of irritation were observed in any group. No hematological effects were noted in rats exposed to sodium dichromate. Rats exposed to the chromium oxide mixture had a significantly elevated white blood cell count at the 17th and 18th month, and significantly elevated red blood cells, hematocrits, and hemoglobin levels at the 27th month. Mean serum content of total immunoglobulin was significantly reduced in this group at 6 months exposure. Significantly increased lung weights were observed in chromium oxide-exposed rats, and for livers of sodium dichromate-exposed rats at the highest dose. Pigment-loaded macrophages were found in the sodium dichromate-exposed rats in a dose dependent manner and also in the chromium oxide group. Chromium oxide-exposed rats also developed focal thickened septa, partially combined with interstitial fibrosis and accumulation of eosinophilic substance in the alveolar lumens. The authors concluded that the hematological and pulmonary effects may be due to Cr-accumulation in the lungs and to depressed lung clearance function.

Rats exposed to 200 $\mu\text{g}/\text{m}^3$ Cr(VI) as aerosolized sodium dichromate by inhalation for 22 hours per day for 42 days exhibited decreased alveolar macrophage phagocytic activity; the lung clearance of inert iron oxide was significantly reduced in exposed rats compared to controls (Glaser *et al.*, 1985). Increased alveolar macrophage activity and a significantly elevated antibody response to injected sheep red blood cells were observed in rats exposed to 25 or 50 $\mu\text{g}/\text{m}^3$ Cr(VI) for 22 hours per day for 28 days. Ninety day exposure under the same exposure protocol resulted in increased rat lung and spleen weights at 50, 100 and 200 $\mu\text{g}/\text{m}^3$, but not 25 $\mu\text{g}/\text{m}^3$ (Glaser *et al.*, 1985). Histopathology of major organs was similar among all groups. Bronchoalveolar lavage fluid contained decreased macrophage cell counts above 25 $\mu\text{g}/\text{m}^3$. Increased antibody response to injected sheep red blood cells was observed in all treatment groups, while alveolar macrophage activity was elevated at 25 and 50 $\mu\text{g}/\text{m}^3$, but was significantly reduced at 200 $\mu\text{g}/\text{m}^3$.

A later experiment exposed male rats to 0, 50, 100, 200, or 400 $\mu\text{g Cr}/\text{m}^3$ 22 hours per day, 7 days per week for 90 days (Glaser *et al.*, 1990). Average measured concentrations were 0, 54, 109, 204, and 403 $\mu\text{g Cr}/\text{m}^3$, respectively. Subacute respiratory dyspnea and reduction in body weight gain were observed at the two highest exposures. Mean white blood cell count increased in a dose-dependent manner among treated rats, but returned to normal 30 days following cessation of exposure. Histopathological examination revealed histiocytosis (macrophage accumulation) in all treatment groups (Table 1). Bronchoalveolar lavage fluid (BALF) contained elevated levels of albumin, lactate dehydrogenase (LDH), and total protein in all exposed groups. Statistically significant elevations in these parameters were observed mainly in the 200 and 400 $\mu\text{g}/\text{m}^3$ exposure groups. At necropsy, a statistically significant increase in lung weight (g dry wt/kg body wt) was observed in rats exposed to 100, 200, and 400 $\mu\text{g}/\text{m}^3$ as compared to controls. Lung weights were still significantly elevated in the three highest exposure groups 30 days following cessation of exposure. An analysis of the data (Malsch *et al.*, 1994) determined a

benchmark dose (95% confidence interval with dose associated with a 10% elevation in the parameter) for each of these endpoints. The analysis also examined changes in lung and spleen weight reported in Glaser *et al.* (1985). The most sensitive endpoint was LDH in BALF.

Table 1. Key bronchoalveolar lavage fluid (BALF) and histopathological findings after 90 days exposure to sodium dichromate (Glaser *et al.*, 1990).

$\mu\text{g Cr/m}^3$	Total Protein in BALF ^a (mg/L)	Albumin in BALF (mg/L)	LDH in BALF (U/L)	Bronchoalveolar Hyperplasia	Lung Histiocytosis	Right lung dry weight (g/kg BW)
0	226±30	77±13	29±5	0/10	2/10	0.44±0.03
50	396±79**	115±23**	34±3*	3/10	9/10	0.48±0.05
100	326±35**	86±13	31±4	2/10	10/10	0.50±0.06*
200	703±178**	117±20**	63±11**	3/10	9/10	0.55±0.04**
400	975±246**	184±59**	83±17**	7/10	10/10	0.65±0.05**

a All BALF parameters are mean + SD, n = 10/group

* p < 0.05; ** p < 0.001: comparison of exposed groups vs. controls

Cohen *et al.* (1998) investigated the immunotoxicologic effects of inhaled chromium by exposing F-344 rats (10/group/exposure duration) nose-only to 0 and 360 $\mu\text{g/m}^3$ potassium chromate 5 hr/day, 5 days/week for 2 or 4 weeks. Exposed rats had greater levels of total recoverable cells, neutrophils, and monocytes in bronchopulmonary lavage compared to controls at 2 and/or 4 weeks. Pulmonary macrophages (PM) were reduced, although total PM levels remained unaffected. Four-week exposure to potassium chromate also resulted in modulated PM-inducible interleukins-1 and -6, and tumor necrosis factor- α , and increased PM basal nitric oxide production and interferon- γ -primed/zymosan-stimulated reactive oxygen intermediate production.

Nasal septal perforation, hyperplastic and metaplastic changes in the larynx, trachea, and bronchus, and emphysema were observed in mice exposed two days per week for 12 months to CrO₃ mist (Adachi, 1987; Adachi *et al.*, 1986). Chromic acid concentrations were either 3.63 mg/m³ for 30 minutes per day or 1.81 mg/m³ for 120 minutes per day. An additional 20 mice exposed to 1.81 mg/m³ were necropsied 6 months after the last exposure. Lesions of the nasal septum, trachea, and lungs were still evident in some mice.

The investigators of the toxicity studies summarized below administered soluble Cr(VI) compounds to experimental animals by the oral route.

Groups of eight male and eight female Sprague-Dawley rats were supplied with drinking water containing 0-11 ppm (0-11 mg/L) Cr(VI), as K₂CrO₄, for 1 year (Mackenzie *et al.*, 1958). The control group (10/sex) received distilled water. A second experiment involved three groups of 12 male and 9 female rats. One group was given 25 ppm (25 mg/L) Cr(VI); a second received 25 ppm chromium in the form of chromic chloride; and the controls received distilled water. For rats treated with 0-11 ppm (in the diet), hematological determinations (red and white blood cell counts, differential white cell counts, and hemoglobin) were performed monthly, and tissues (livers, kidneys and femurs) were examined at 6 months and 1 year. Spleens were also examined

at 1 year. The 25 ppm groups (and corresponding controls) were examined similarly, except that no animals were killed at 6 months. No significant adverse effects were seen in appearance, weight gain, or food consumption, and there were no treatment-related effects regarding hematological parameters or other tissues in any treatment group. The rats receiving 25 ppm Cr(VI) showed an approximate 20% reduction in water consumption. This dose corresponds to 2.4 mg Cr(VI)/kg/day based on actual body weight and water consumption data. An abrupt rise in tissue chromium concentrations was noted in rats treated with greater than 5 ppm. The authors stated that “apparently, tissues can accumulate considerable quantities of chromium before pathological changes result.” In the 25 ppm treatment groups, tissue concentrations of chromium were approximately 9 times higher for those treated with hexavalent chromium than for the trivalent group.

Anwar *et al.* (1961) observed no significant effects in groups of female dogs (2/dose group) given 0, 0.45, 2.25, 4.5, 6.75, or 11.2 ppm Cr(VI) (as K_2CrO_4) in drinking water for 4 years. The calculated doses ranged from 0.012-0.30 mg/kg of Cr(VI).

Numerous rodent studies have been recently undertaken to investigate the reproductive and developmental effects of Cr(VI) exposure via the drinking water (Trivedi *et al.*, 1989; Junaid *et al.*, 1995; Murthy *et al.*, 1996; Junaid *et al.*, 1996a; Junaid *et al.*, 1996b; Kanojia *et al.*, 1996; Elbetieha and Al-Hamood, 1997; Al-Hamood *et al.*, 1998; Kanojia *et al.*, 1998). Exposure concentrations ranged from 250 to 5000 ppm for durations as short as five days during gestation to as long as 3 months pre-gestational exposure. In general, the longer exposures resulted in more serious reproductive and developmental effects.

Kanojia *et al.* (1998) administered 0, 250, 500, and 750 ppm potassium dichromate via drinking water to female Druckrey strain rats for 90 days prior to gestation. Based on daily water intake and final body weights, the estimated daily Cr(VI) intake was 33, 68, and 98 mg/kg-day, respectively. Ten to 15% mortality, hair loss, lethargy, aggressiveness and a significant reduction in body weight gain were observed in rats at the two highest doses. While not statistically significant, weight of the low dose rats were 32% lower than controls. All treated rats were acyclic at the end of the 90 day exposure period and an additional 15-20 days without Cr(VI) exposure were needed for the estrus cycle to start. Mating and fertility indexes decreased with increasing Cr(VI) intake. Ten rats/group were sacrificed on day 19 of gestation for fetotoxicity assessment. Significantly reduced fetal weight and increased pre- and post-implantation loss occurred at all dose levels. Gross and skeletal abnormalities in low dose fetuses included subdermal hemorrhagic patches, drooping wrists, and reduced caudal bone ossification. No gross visceral abnormalities were seen in treated groups.

Administration of potassium dichromate to rats (Kanojia *et al.*, 1996) and mice (Junaid *et al.*, 1996a) in drinking water at concentrations of 250, 500, and 750 ppm for 20 days prior to gestation resulted in increased post-implantation loss and decreased placental weight in both species at the lowest dose. Also at this dose level, decreased fetal weight and crown-rump length were observed in mice, and increased resorptions and decreased number of live fetuses were observed in rats. Gross and skeletal abnormalities were observed in both species beginning at the 500 ppm dose level.

Groups of Sprague-Dawley rats (NTP, 1996a) and BALB/C mice (NTP, 1996b) were administered potassium dichromate in their diet at 0, 15, 50, 100, or 400 ppm for 9 weeks (24 males and 48 females/species/group) followed by a recovery period of 8 weeks. Average Cr(VI) consumption for male/female rats were 1/1, 3/3, 6/7, and 24/28 mg/kg-day, respectively. Average Cr(VI) consumption for male/female mice were 3/5, 10/16, 21/34, and 92/137 mg/kg-day, respectively. Six males and 12 females of both species were necropsied after 3, 6, or 9 weeks of treatment or after the full recovery period. There was no treatment-related histopathology observed in kidneys, ovaries, and testes in either species. Hematological analysis revealed slight decreases in mean corpuscular volume (MCV) and mean corpuscular hemoglobin (MCH) at the highest dose in both species, which is indicative of iron deficiency. MCV and MCH were normal in these groups following the 8-week recovery period. Microscopic evaluation of the livers of mice noted cytoplasmic vacuolization of hepatocytes in treated animals beginning at 50 ppm. Also in mice, there was a slight decrease in mean body weights in the 400 ppm males (5-9%) and females (4%) and the 100 ppm females (2-4%) during the dosing periods. Feed consumption by mice was generally increased in all treated groups, particularly the 400 ppm males and females. During the recovery period, feed consumption was comparable across groups.

The NTP (1997) investigated the potential reproductive toxicity of Cr(VI) in mice using the Reproductive Assessment by Continuous Breeding protocol. Groups of 20 male and female pairs of BALB/c mice (F_0) were exposed to 0, 100, 200, and 400 ppm potassium dichromate in their diet during the continuous breeding phase (approximately 12 weeks). F_1 generation litters received the same concentration of Cr(VI) in their diet as their F_0 parents and were used for assessment of second generation reproductive toxicity at sexual maturity. There were no treatment-related changes in any of the reproductive parameters in this study. In F_1 mice, the MCV was slightly decreased in males at the two highest doses, and slightly decreased in females in all dose groups. MCH and hemoglobin were slightly reduced in high dose males and high dose females, respectively. Mean body weights of the high dose F_0 and F_1 animals were slightly decreased, and mean food consumption in the F_1 mice was elevated. Reduced mean absolute liver weights were observed in 400 ppm F_0 mice of both sexes. The mean calculated doses were 19.4, 38.6, and 85.7 mg/kg-day for F_0 males and females and 22.4, 45.5, and 104.9 mg/kg-day for F_1 males and females in the 100, 200, and 400 ppm dose groups, respectively.

In an investigation of the spermatogenic and steroidogenic effects of Cr(VI), Chowdhury and Mitra (1995) administered 0, 20, 40, and 60 mg/kg-day sodium dichromate by oral gavage to male rats for 90 days. Reduced Leydig cell population, reduced body and testicular weight, and degeneration of testicular tissue was observed at the two highest doses. Biochemical measures of spermatogenic and steroidogenic impairment, including decreased testicular DNA, RNA, protein, serum testosterone, and $3\beta\text{-}\Delta^5$ -hydroxy steroid dehydrogenase ($3\beta\text{-}\Delta^5\text{-HCH}$), were also reduced at the two highest doses. Only relatively small reductions in testicular protein, $3\beta\text{-}\Delta^5\text{-HCH}$, and serum testosterone were seen in the 20 mg/kg rats.

VI. Derivation of Chronic Reference Exposure Levels (RELs)

A. Derivation of Chronic Inhalation Reference Exposure Level for Soluble Hexavalent Chromium Compounds other than Chromic Trioxide

<i>Study</i>	Glaser <i>et al.</i> , 1990
<i>Study population</i>	Male Wistar rats (30 per group)
<i>Exposure method</i>	Discontinuous whole-body inhalation (0, 54, 109, 204, or 403 $\mu\text{g Cr(VI)/m}^3$ as sodium dichromate aerosol)
<i>Critical effects</i>	Bronchoalveolar hyperplasia
<i>LOAEL</i>	50 $\mu\text{g/m}^3$
<i>NOAEL</i>	Not observed
<i>BMC₀₅</i>	12.50 $\mu\text{g/m}^3$
<i>Exposure continuity</i>	22 hr/day, 7 days/week
<i>Exposure duration</i>	90 days
<i>Average exposure</i>	11.46 $\mu\text{g/m}^3$ Cr(VI) (12.50 x 22/24)
<i>Human equivalent concentration</i>	24.47 $\mu\text{g/m}^3$ Cr(VI) (2.1355 [RDDR] x 11.46)
<i>LOAEL uncertainty factor</i>	Not needed in the BMC approach
<i>Subchronic uncertainty factor</i>	3
<i>Interspecies uncertainty factor</i>	3
<i>Intraspecies uncertainty factor</i>	10
<i>Cumulative uncertainty factor</i>	100
<i>Inhalation reference exposure level</i>	0.2 $\mu\text{g/m}^3$ (0.0002 mg/m^3)

The study by Glaser *et al.* (1990) provides the best available inhalation data that demonstrate a dose-response relationship for various pulmonary toxicity endpoints. The BMC₀₅ of 12.50 $\mu\text{g/m}^3$ was derived from quantal data for bronchoalveolar hyperplasia. The presence of bronchoalveolar hyperplasia in exposed rats is supported by other indicators of lung inflammation, including increased total protein, LDH, and albumin in BALF (see Table 1). A quantal-linear model analysis (U.S. EPA, National Center for Environmental Assessment, benchmark dose software, version 1.20) of the quantal data provided the most reasonable line fit and resulted in the lowest BMC₀₅. A BMC₀₅ is considered to be similar to a NOAEL in estimating a concentration associated with a low level of risk. Lung histiocytosis (macrophage accumulation) was present in nearly all exposed animals, but this quantal data set was only suitable for a NOAEL/LOAEL approach and was not considered as direct an indicator of lung injury as bronchoalveolar hyperplasia.

Based on OEHHA methodology, a comparison REL developed using the NOAEL/LOAEL approach would yield 0.3 $\mu\text{g/m}^3$. Adjustment of the LOAEL of 50 $\mu\text{g/m}^3$ (a NOAEL was not observed) to the human equivalent concentration uses the same parameters as shown in the REL derivation above. However, a LOAEL UF of 3 is added to the existing UFs to result in a cumulative UF of 300.

The U.S. EPA (1998) RfC of 0.1 $\mu\text{g/m}^3$ is also based on data from Glaser *et al.* (1990), but derived a BMC₁₀ (16 $\mu\text{g/m}^3$), as developed by Malsch *et al.* (1994), from continuous data of

LDH in BALF. Using a polynomial model provided by a different benchmark software package (*THC*, Clement International Corp., Ruston LA), increasing LDH concentration in BALF with increasing dose provided the lowest BMC_{10} among the various BALF endpoints. OEHHA is currently not developing BMCs for RELs based on continuous data. A BMC_{05} derived from quantal data and a BMC_{05} derived from continuous data may not have the same meaning. Conceivably, depending on the standard deviations of the data points, the BMC_{05} based on continuous data could still be above the statistically significant effect level. OEHHA believes that further evaluation of BMC's based on continuous data is needed prior to their application to RELs.

OEHHA and U.S. EPA also diverge on the assignment of the Subchronic UF. The Glaser *et al.* (1990) study indicated that chromium was still accumulating in lung tissue at the end of 90 days. This evidence and the fact that the study did not investigate upper airway effects and other extrapulmonary effects led U.S. EPA to assign a subchronic UF of 10 (U.S. EPA, 1998). Based on OEHHA methodology, OEHHA used a subchronic UF of 3. In support of a UF of 3, the 18-month sodium dichromate exposure study performed by Glaser *et al.* (1986), under similar exposure conditions used in the key 90-day study, did not find histopathological evidence of lung inflammation or major organ effects, or suggest severe chromium accumulation in exposed rats. However, BALF analysis was not performed in the chronic study.

For comparison with the proposed REL, the occupational study by Huvinen *et al.* (1996) established a NOAEL of $0.5 \mu\text{g}/\text{m}^3$ for lack of pulmonary findings. However, this study is deficient for REL purposes due to the lack of a LOAEL. Unfortunately, other occupational studies suffered from lack of adequate methods or data for determining exposure duration and/or exposure levels. Use of an occupational time adjustment (10/20 m^3 inhaled/day, 5/7 days/week) and an interspecies UF of 10 for the Huvinen *et al.* (1996) study would result in an estimated REL of $0.02 \mu\text{g}/\text{m}^3$. Average exposure duration was 16 years, so a subchronic UF of 1 was sufficient.

B. Derivation of Chronic Inhalation Reference Exposure Level for CrO_3 as Chromic Acid

<i>Study</i>	Lindberg and Hedenstierna, 1983
<i>Study population</i>	Human workers (100 exposed workers, 119 unexposed controls)
<i>Exposure method</i>	Occupational exposure to chromic acid mist
<i>Critical effects</i>	Nasal atrophy, nasal mucosal ulcerations, nasal septal perforations, transient pulmonary function changes
<i>LOAEL</i>	1.9 $\mu\text{g}/\text{m}^3$ established as “low exposure” group (8-hr mean $\leq 1.9 \mu\text{g}/\text{m}^3$)
<i>NOAEL</i>	Not observed
<i>Exposure continuity</i>	8 hr/day (10 m^3 per 20 m^3 day), 5 days/week
<i>Exposure duration</i>	Mean of 2.5 years (range = 0.2 - 23.6 years)
<i>Average exposure</i>	0.68 $\mu\text{g}/\text{m}^3$ Cr(VI) (1.9 x 10/20 x 5/7)
<i>Human equivalent concentration</i>	0.68 $\mu\text{g}/\text{m}^3$ Cr(VI)
<i>LOAEL uncertainty factor</i>	3
<i>Subchronic uncertainty factor</i>	10
<i>Interspecies uncertainty factor</i>	1
<i>Intraspecies uncertainty factor</i>	10
<i>Cumulative uncertainty factor</i>	300
<i>Inhalation reference exposure level</i>	0.002 $\mu\text{g}/\text{m}^3$ (0.000002 mg/m^3)

The occupational exposure study of Lindberg and Hedenstierna (1983) was selected as the best available human study. A 3-fold LOAEL to NOAEL uncertainty factor (UF) was applied due to the low incidence of nasal atrophy at the LOAEL (4 out of 19) and the apparent reversibility of the lesion at this exposure level. While Lindberg and Hedenstierna (1983) did not follow-up on any of the active cases of nasal ulcerations, which occurred only in workers in the ‘high exposure’ group, they did note that one worker, who exhibited nasal atrophy, had no visible nasal lesions 4 months after termination of exposure.

U.S. EPA (1998) based its RfC of 0.008 $\mu\text{g}/\text{m}^3$ for exposure to chromic acid mists and dissolved Cr(VI) aerosols on the same study but established the LOAEL at 2 $\mu\text{g}/\text{m}^3$ and applied a total UF of 90 (3 each for the LOAEL to NOAEL and subchronic to chronic extrapolation, and 10 for intraspecies extrapolation). It was unclear why U.S. EPA (1998) chose UFs of 3 for LOAEL and subchronic extrapolations. It was also unclear why the total uncertainty factor was 90, rather than 100, which would be obtained by following the usual convention (that the value for uncertainty factors of “3” is actually 3.16, the square root of 10, although it is usually only quoted to 1 significant figure).

For comparison, a REL can be estimated from the Adachi *et al.* (1987) study in which mice were exposed to 1.81 mg/m^3 chromic acid mist 2 hr/day, twice a week for 12 months. Lesions were observed in treated mice throughout the respiratory tract; a NOAEL was not determined. Application of the exposure continuity adjustment (2/24 hr/day x 2/7 days/week), an RDDR of 2.26 (MMAD and sigma g roughly estimated at 5 and 3 μm , respectively), and a total UF of 300 (10 for LOAEL to NOAEL, 3 for interspecies, and 10 for intraspecies) yields a REL of 0.3 $\mu\text{g}/\text{m}^3$.

In addition to being inhaled, airborne hexavalent chromium can settle onto crops and soil and enter the body by ingestion. Thus, an oral chronic reference exposure level for soluble salts of metallic chromium(VI) is also required for assessing risks from stationary sources in the Air Toxics Hot Spots program.

C. Derivation of Chronic Oral Reference Exposure Level for Chromium VI (Based on U.S. EPA RfD)

<i>Study</i>	Mackenzie <i>et al.</i> , 1958
<i>Study population</i>	8 male and 8 female Sprague-Dawley rats
<i>Exposure method</i>	Drinking water
<i>Critical effects</i>	No adverse effects seen
<i>LOAEL</i>	None
<i>NOAEL</i>	2.4 mg/kg-day (converted from 25 mg/L of chromium as K ₂ CrO ₄)
<i>Exposure continuity</i>	Continuous
<i>Exposure duration</i>	1 year
<i>Average experimental exposure</i>	2.4 mg/kg-day (0.11 ppm Cr(VI))
<i>LOAEL uncertainty factor</i>	1
<i>Subchronic uncertainty factor</i>	1
<i>Interspecies uncertainty factor</i>	10
<i>Intraspecies uncertainty factor</i>	10
<i>Cumulative uncertainty factor</i>	100
<i>Oral reference exposure level</i>	0.02 mg/kg bw-day

The oral REL (0.02 mg/kg bw-day) and U.S. EPA's oral Reference Dose (RfD) of 0.003 mg/kg-day (U.S. EPA, 1998) are based on the same study by MacKenzie *et al.* (1958). No adverse effects were reported at any dose in the study. The highest dose group (25 mg/L) was selected for derivation of the oral REL and RfD based on the reported body weight of the rat (0.35 kg) and the reported average daily drinking water consumption for the rat (0.035 L/day). Because a LOAEL was not observed in the primary study, the subchronic NTP studies provide supporting evidence to justify a REL based on MacKenzie *et al.* (1958). Cr(VI) was administered in the diet of rats for 9 weeks and a NOAEL of 6 mg/kg-day was observed for slightly depressed MCV and MCH values (NTP, 1996a). The LOAEL was 24 mg/kg-day. The NTP (1996b, 1997) also observed slightly depressed MCV and MCH values in mice, but at higher Cr(VI) concentrations. While the changes are small and may be a mild adverse effect at best, the NTP (1997) noted that decreased MCV and MCH are indicators of iron deficiency and suggested that an interaction between chromium and iron is altering erythrocyte formation. The liver effects noted in female mice in the 9 week study (NTP, 1996b) were not observed in the mouse reproductive study (NTP, 1997). Therefore, the toxicological significance of this finding is uncertain.

U.S. EPA (1998) applied UFs of 3 for subchronic, 10 for intraspecies, 10 for interspecies, and a modifying factor of 3 (to account for concerns raised by the study of Zhang and XiLin (1987)) to the NOAEL for an RfD of 0.003 mg/kg-day. The criteria for use of modifying factors are not

well specified by U.S. EPA. Such modifying factors were not used by OEHHA. Because the exposure duration in the primary study was greater than 12% of the estimated lifespan of rats, OEHHA applied UF of 1 for extrapolation to chronic exposure.

U.S. EPA stated its confidence in the RfD as: Study - Low; Data Base - Low; and RfD - Low. Confidence in the chosen study is low because of the small number of animals tested, the small number of parameters measured, and the lack of toxic effect at the highest dose tested. Confidence in the database is low because the supporting studies are of equally low quality, and teratogenic and reproductive endpoints are not well studied. Low confidence in the RfD follows.

OEHHA notes that more reproduction/developmental studies have been published that support the RfD and oral REL since U.S. EPA published its findings (U.S. EPA, 1998). In general, these studies indicate that reproductive and developmental effects occur at doses greater than an order of magnitude above the NOAEL established by MacKenzie *et al.* (1958) and the NTP (1996a,b, 1997). However, the dose levels used were relatively high such that a NOAEL was typically lacking.

VII. Data Strengths and Limitations for Development of the REL

The major strength of the inhalation REL for chromic acid mist is the use of human data. The major uncertainties for this inhalation REL is the lack of controlled and quantified exposure data and the lack of a NOAEL in the key chromic acid study.

The suitably thorough analysis of lower airway effects and the development of a BMC from continuous data are strengths for the Cr(VI) dust inhalation REL. Limitations include the lack of comprehensive data on multi-organ effects, the lack of chronic studies, the lack of upper airway analysis in the key study, and the lack of quantified exposure data in humans. The animal studies by Glaser *et al.* (1990, 1986) suggest that the lower respiratory airway is a primary target for Cr(VI) dusts. However, occupational studies (Mancuso, 1951; Federal Security Agency, 1953; Machle and Gregorius, 1948; Wang *et al.*, 1994; Walsh, 1953) indicate that nasal lesions result from exposure to Cr(VI) dusts and may, in fact, be the most sensitive indicator of human toxicity resulting from exposure to soluble Cr(VI) dusts. However, this finding is attenuated by the fact that dermal exposure to chromic acid and Cr(VI) dusts due to poor hygienic practices of workers may overestimate the airborne concentrations necessary to result in nasal lesions.

The major strength for the oral REL is the consistency of the doses resulting in NOAELs and/or LOAELs among the major and supporting studies. The major limitations for the oral REL, other than the ones noted above by U.S. EPA, are the lack of lifetime exposure studies in experimental animals and the lack of adequate oral human exposure data.

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CHRONIC TOXICITY SUMMARY

CRESOL MIXTURES

<i>Compounds</i>	<i>Synonyms</i>	<i>CAS Reg. No.</i>
cresols	cresylic acid; tricresol; hydroxytoluene; methylphenol	1319-77-3
o-cresol	1-hydroxy-2-methylbenzene; 2-hydroxytoluene; 2-methylphenol	95-48-7
m-cresol	1-hydroxy-3-methylbenzene; 3-hydroxytoluene; 3-methylphenol	108-39-4
p-cresol	1-hydroxy-4-methylbenzene; 4-hydroxytoluene; 4-methylphenol	106-44-5

I. Chronic Toxicity Summary

<i>Inhalation reference exposure level</i>	600 µg/m³ (100 ppb)
<i>Critical effect(s)</i>	Neurotoxicity
<i>Hazard index target(s)</i>	Nervous system

II. Chemical Property Summary (HSDB, 1995; CRC, 1994, unless otherwise noted)

<i>Description</i>	Colorless in pure form; yellowish, brownish-yellow, or pinkish liquid
<i>Molecular formula</i>	C ₇ H ₈ O
<i>Molecular weight</i>	108.14 g/mol
<i>Boiling point</i>	191.0°C (o-cresol) 202°C (m-cresol) 201.9°C (p-cresol)
<i>Melting point</i>	29.8°C (o-cresol) 11.8°C (m-cresol) 35.5°C (p-cresol)
<i>Solubility</i>	Soluble in 50 parts water; miscible with alcohol, benzene, ether, glycerol, petroleum ether; soluble in vegetable oils, glycol
<i>Conversion factor</i>	4.42 µg/m ³ per ppb at 25°C

III. Major Uses and Sources

Cresol compounds (mixtures of the ortho-, meta- and para-isomers) can be obtained from coal tar and petroleum or synthesized by sulfonation or oxidation of toluene (HSDB, 1995). Crude cresol (commercial grade) contains approximately 20% o-cresol, 40% m-cresol, and 30% p-

cresol. Phenol and xylenols are present in small amounts as contaminants. Cresylic acid compounds are called cresol when the boiling point is below 204°C.

Cresols have a wide variety of uses including the manufacture of synthetic resins, tricresyl phosphate, salicylaldehyde, coumarin, and herbicides. Cresols also serve as components of degreasing compounds in textile scouring and paintbrush cleaners as well as fumigants in photographic developers and explosives. Cresols also function as antiseptics, disinfectants, and parasiticides in veterinary medicine. An approximate breakdown of cresol and cresylic acid use is 20% phenolic resins, 20% wire enamel solvents, 10% agricultural chemicals, 5% phosphate esters, 5% disinfectants and cleaning compounds, 5% ore flotation, and 25% miscellaneous and exports.

Any combustion process, which results in the generation of phenolic compounds (such as automobile exhaust or coal, wood, or trash smoke), may be a potential source of exposure to cresols. Cresols are also formed from the atmospheric photooxidation of toluene. However, under normal conditions low vapor pressure limits the inhalation hazard presented by cresols (HSDB, 1995). The annual statewide industrial emissions from facilities reporting under the Air Toxics Hot Spots Act in California based on the most recent inventory were estimated to be 8407 pounds of mixtures of cresols (cresylic acid), 3 pounds of m-cresol, and 3 pounds of o-cresol (CARB, 2000).

IV. Effects of Exposures to Humans

Brief exposure to 6 mg cresol/m³ resulted in irritation of the throat and nose, nasal constriction, and dryness in 8 of 10 subjects (Uzhdavini *et al.*, 1972).

Chemical burns may result from exposure to cresols (Pegg and Campbell, 1985). The lungs of humans exposed to cresols have shown signs of emphysema, edema, bronchopneumonia, and small hemorrhages (Clayton and Clayton, 1982). Skin contact has resulted in the development of white patches and blistering, eventually turning brown or black (Lefaux, 1968). Other reported effects include turbidity, inflammation, and fatty degeneration of the liver, nephritis, and hemorrhage of the epicardium and endocardium. An infant fatally exposed to ~20 ml of a 90% cresol solution dermally showed widespread edema of the internal organs, especially the brain and kidney (Green, 1975). The liver showed signs of centrilobular and midzonal necrosis.

Chronic systemic poisoning by any route of exposure may produce symptoms of vomiting, dysphagia, salivation, diarrhea, loss of appetite, headache, fainting, dizziness, and mental disturbances (Sittig, 1981). Skin rash and discoloration may also result from prolonged or repeated exposure of the skin. Death may result from severe damage to the liver and kidneys. Oral poisoning has resulted in kidney problems (likely from the direct action of cresol) and pancreatitis (from constriction of the pancreatic ducts) (Klimkiewicz *et al.*, 1974, as reported in HSDB, 1995).

V. Effects of Exposures to Animals

The effects of inhaled o-cresol were examined in several species (Uzhdavini *et al.*, 1972, as reported in ATSDR, 1992 and U.S. EPA, 1982). Cats exposed for 30 minutes to 5-9 mg o-cresol/m³ showed signs of respiratory irritation as indicated by increased parotid gland secretions. Exposure of mice for 2 hrs/day for 1 month to 50 mg o-cresol/m³ did not have an effect on mortality, however, heart muscle degeneration and degeneration of nerve cells and glial elements were observed.

Uzhdavini *et al.* (1972) exposed rats (both sexes, numbers not stated) by inhalation to 9.0 ± 0.9 mg o-cresol/m³, first for 2 months (6 hours/day, 5 days/week), then for 2 more months (4 hours/day, 5 days/week). Endpoints examined in rats included elementary conditioned defensive reflex, white blood cell levels, bone marrow elements, and liver function (as indicated by increased susceptibility to hexobarbital narcosis). Both cresol-exposed and control animals showed some loss of the defensive reflex; the effect occurred in all exposed animals before the end of the second month and in control animals at later times. White blood cell counts were elevated in male animals, peaked at the end of the exposure period, and returned to normal one month after cessation of exposure. Exposed animals also showed a statistically significant change in the leukoid-to-erythroid ratio in the bone marrow. Liver toxicity was suggested by an extension in the duration of hexobarbital narcosis in treated animals. Although guinea pigs were similarly evaluated for changes in blood cell counts and ECG, scant reporting of experimental detail limits the usefulness of this portion of the study.

NR rats were exposed by inhalation to 0.0052 or 0.05 mg tricresol/m³ for 3 months (Kurliandskii *et al.*, 1975; as described by U.S. EPA, 1982). The proportional composition of the compound was not specified. Effects observed in the high-dose group included decreased weight gain, increased central nervous system excitability, increased oxygen consumption, and histological changes in the lung and liver. Serum gamma-globulin levels were also reduced. No effects were observed in the low-dose group. Rats (6/group, sex unspecified) were also exposed for 24 hours to 0.01, 0.1, and 2.4 mg tricresol/m³ with a control group of 6 rats for each exposure group. The absorption of neutral red dye by lung tissue was used as an indicator of protein denaturation in the tissue. Significantly increased dye absorption over control animals was observed at both 2.4 and 0.1 mg tricresol/m³. The degree of dye absorption in the low-dose group was not significantly increased over controls.

In a 90-day subchronic toxicity study (U.S. EPA, 1986), 30 Sprague-Dawley rats/sex/dose were gavaged daily with 0, 50, 175, or 600 mg/kg/day p-cresol. Body and organ weights, food consumption, mortality, clinical signs of toxicity, and clinical pathology were evaluated. At 600 mg/kg/day, o-cresol showed 47% combined mortality (9/30 males, 19/30 females), and a 30% reduction in body weight at week 1 and 10% at necropsy. Kidney-to-body weight ratio was 13% higher than that of the control value at the end of the study. CNS effects such as lethargy, ataxia, coma, dyspnea, tremor, and convulsions were seen within 15 to 30 minutes after dosing; but recovery occurred within 1 hour post-gavage. At 450 mg/kg/day, combined mortality was 10% (1/10 male, 1/10 female). In the 175 mg/kg/day group, two animals exhibited tremors on day 1 of the study during the hour following gavage administration, and one of the two became comatose. At 50 mg/kg/day, no significant adverse effects were observed (USEPA, 1999a,b).

In a 90-day neurotoxicity study (U.S. EPA, 1987), 10 Sprague-Dawley rats/sex/dose were gavaged daily with o-cresol at 0, 50, 175, 450, or 600 mg/kg/day. In addition to the parameters evaluated above, various signs of neurotoxicity were monitored. The lowest dose of o-cresol caused clinical signs of CNS-stimulation post-dosing, such as salivation, rapid respiration, and hypoactivity; however, these symptoms were low in incidence and sporadic in nature. Higher doses of o-cresol (greater than 450 mg/kg/day) produced significant neurological events, such as increased salivation, urination, tremors, lacrimation, palpebral closure, and rapid respiration. Animals given high doses also showed abnormal patterns in the neurobehavioral tests. The NOAEL based on systemic toxicity was 50 mg/kg/day (USEPA, 1999a,b).

Dermal exposure of rats to 1.0-1.7 ml cresol/kg body weight for 1-2 hours resulted in skin discoloration and death of the animals (Campbell, 1941).

Exposure to high concentrations of toluene vapors, or to intravenous o-cresol, a toluene metabolite, at about 0.9 mg/min, caused excitation of the somatosensory evoked potential (SEP) and electroencephalograph (EEG) of Fischer 344 rats (Mattsson *et al.*, 1989). Both substances induced an increase in EEG beta activity and caused a large increase in activity at 5 Hz. Toluene exposed rats were lightly anesthetized, while o-cresol rats were conscious but hyperreactive. When exposure was continued, both sets of rats had involuntary muscle movements and tremors. Neither benzoic acid and hippuric acid, also metabolites of toluene, caused neuroexcitation. The authors concluded that metabolically derived cresols are plausible candidates for the neuroexcitatory properties of toluene.

In rat liver slices at equimolar concentrations, p-cresol was 5- to 10-times as toxic as the o- or m-isomers for cell killing (Thompson *et al.*, 1994). p-Cresol rapidly depleted intracellular glutathione levels, while the o- and m-isomers depleted it to a lesser extent. p-Cresol was metabolized to a reactive intermediate which bound covalently to protein. The reaction was inhibited by N-acetylcysteine.

The National Toxicology Program (NTP) sponsored reproductive toxicity tests of cresol isomers in Swiss CD-1 mice using the risk assessment by continuous breeding (RACB) protocol (Heindel *et al.*, 1997a, 1997b). For o-cresol the exposure concentrations in the continuous cohabitation task were 0.05%, 0.2%, and 0.5% in feed (approximately 60, 220, and 550 mg/kg/day (Heindel *et al.*, 1997a). At these doses o-cresol was not a reproductive toxicant. When a m-/p-cresol mixture was used at concentrations of 0.25, 1.0 and 1.5% in feed (approximately 370, 1500, and 2100 mg/kg/day), the m/p mixture was a reproductive toxicant, since (1) fewer F₁ pups per litter were produced, (2) both generations showed reduced pup weights, and (3) reproductive organs showed weight reductions. Unfortunately the responses were not dose-dependent and the mixture was judged not to be a selective reproductive toxicant. Oral gavage administration of o-, m-, or p-cresol, separately, in rats did not produce selective reproductive toxicity; i.e., for each of the cresol isomers, in the absence of parental toxicity, there was no reproductive toxicity. The NOEL for reproductive toxicity for each isomer was 175 mg/kg/day (Tyl 1989a, 1989b, 1989c).

VI. Derivation of Inhalation Chronic Reference Exposure Level

<i>Study</i>	U.S. EPA, 1987
<i>Study population</i>	Sprague-Dawley rats
<i>Exposure method</i>	Gavage at 0, 50, 175, 450, or 600 mg/kg-day
<i>Critical effects</i>	Decreased body weights and neurotoxicity (tremors, salivation, lacrimation, etc.)
<i>LOAEL</i>	175 mg/kg-day
<i>NOAEL</i>	50 mg/kg-day
<i>Exposure continuity</i>	Daily gavage
<i>Exposure duration</i>	90 days
<i>LOAEL uncertainty factor</i>	1
<i>Subchronic uncertainty factor</i>	3 (90 day study)
<i>Interspecies uncertainty factor</i>	10
<i>Intraspecies uncertainty factor</i>	10
<i>Cumulative uncertainty factor</i>	300
<i>U.S. EPA Reference Dose (RfD)</i>	0.17 mg/kg/day
<i>Route-to-route extrapolation factor</i>	3500 $\mu\text{g}/\text{m}^3$ per mg/kg/day
<i>Inhalation chronic REL</i>	600 $\mu\text{g}/\text{m}^3$ (100 ppb)

An RfD of 0.05 mg/kg/day was derived by the USEPA for both o-cresol and m-cresol (USEPA 1998a, 1998b; listed as 2-methylphenol and 3-methylphenol). The RfD for p-cresol was withdrawn by the USEPA. U.S. EPA used a subchronic uncertainty factor of 10 for a 90 day study in rats. In accordance with its approved methodology (OEHHA, 2000), OEHHA used a factor of 3.

The available literature on the observed toxicity of cresol compounds and cresol mixtures to humans by inhalation indicates that at high concentrations these compounds are initially toxic due to their ability to cause chemical burns and are therefore of concern at the site of contact. In humans occupationally exposed, inhalation exposure is reported to cause respiratory effects including the development of pneumonia, pulmonary edema, and hemorrhage (Clayton and Clayton, 1982). Other case reports of cresol toxicity to humans are confounded by the presence of other compounds, such as phenol, formaldehyde, and ammonia (Corcos, 1939; NIOSH, 1974). The only quantitative information from inhalation exposures to humans, however, comes from acute exposure studies showing irritation at 6 mg cresol/ m^3 (Uzhdavini *et al.*, 1972, as reported in ATSDR, 1992). Toxic effects reported in animals include bone marrow and liver toxicity in rats from 4 month exposure to 9 mg cresol/ m^3 (Uzhdavini *et al.*, 1972, as reported in U.S. EPA, 1982). Other animal studies have shown more systemic effects from inhalation exposure to cresols. Uzhdavini *et al.*, 1972 reported cardiac and nerve cell degeneration in mice exposed for 2 hour/day for 1 month to 50 mg o-cresol/ m^3 . Kurliandskii *et al.* (1975) (as reported in HSDB, 1995) observed decreased weight gain with histological changes in the liver and lungs of rats exposed for 3 months to 0.05 mg tricresol/ m^3 . Although this study reports adverse effects at levels below those observed in the Uzhdavini *et al.* (1972) study, limited experimental detail precludes the use of these data in the development of the chronic REL.

The only useful inhalation data for the development of a chronic REL are those showing hematological toxicity to the bone marrow of rats exposed for 4 months to o-cresol (Uzhdavini *et al.* (1972) as reported in U.S. EPA, 1982). These authors report a LOAEL of 9 mg tricresol/m³. OEHHA staff decided not to use this study. (1) A complete translation from the original Russian was not available so that only the interpretations of others were available. (2) Some endpoints tested are not commonly used in toxicology. And (3) some of the results reported were unusual (e.g., elevation of white blood cells in male but not female rats).

As noted above, the inhalation study conducted by Kurliandskii *et al.* (1975) suggests that adverse health effects occur in experimental animals at exposure levels considerably below those reported by Uzhdavini *et al.* (1972) (9 mg/m³ vs. 0.05 mg/m³). The report from which the lower level is drawn has limitations. Human subjects exposed briefly to levels below the LOAEL have reported respiratory irritation.

VII. Data Strengths and Limitations for Development of the REL

The strengths of the REL for cresols include the use of measured exposure data of animals exposed over a significant fraction of their lifetime. Major areas of uncertainty are route-to-route extrapolation, the lack of chronic human data, and the paucity of reproductive and developmental toxicity studies. Additional inhalation studies of cresols will be useful.

VIII. References

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CHRONIC TOXICITY SUMMARY

1,4-DICHLOROBENZENE

(*p*-dichlorobenzene; di-chloride; *p*-dichlorobenzol; Paradow; Paramoth; Parazene; *p*-chlorophenyl chloride)

CAS Registry Number: 106-46-7

I. Chronic Toxicity Summary

<i>Inhalation reference exposure level</i>	800 µg/m³ (100 ppb)
<i>Critical effect(s)</i>	General effects (reduced body weights and food consumption) in rats CNS effects (tremors) in rats Respiratory/dermal effects (nasal and ocular discharge) in rats Liver effects (increased liver weight) in rats, and Kidney effects (increased kidney weight) in rats.
<i>Hazard index target(s)</i>	Nervous system; respiratory system; alimentary system; kidney

II. Chemical Property Summary (HSDB, 1997; CRC, 1994)

<i>Description</i>	White crystals, monoclinic prisms
<i>Molecular formula</i>	C ₆ H ₄ Cl ₂
<i>Molecular weight</i>	147.01 g/mol
<i>Boiling point</i>	174°C
<i>Melting point</i>	52.7°C
<i>Vapor pressure</i>	10 torr @ 54.8°C
<i>Solubility</i>	Soluble in chloroform, carbon disulfide, alcohol, ether, acetone, benzene
<i>Conversion factor</i>	1 ppm = 6.0 mg/m ³ at 25°C

III. Major Uses and Sources

Commercial grade 1,4-dichlorobenzene (1,4-DCB) is available in the USA as a technical grade liquid, typically containing a small percentage (>0.1% by weight) of meta (1,3-DCB) and ortho (1,2-DCB) isomers; as a solution in solvent or oil suspension; or as crystalline material pressed into various forms (HSDB, 1997). Besides its role as an intermediate in the synthesis of various organics, dyes and pharmaceuticals, 1,4-dichlorobenzene is used as a space or garbage deodorizer for odor control. The insecticidal and germicidal properties of 1,4-dichlorobenzene are used to control fruit borers and ants, moths, blue mold in tobacco seed beds, and mildew and mold on leather or fabrics. In 1996, the latest year tabulated, the statewide mean outdoor

monitored concentration of 1,4-DCB was approximately 0.15 ppb (CARB, 1999). The annual statewide industrial emissions from facilities reporting under the Air Toxics Hot Spots Act in California based on the most recent inventory were estimated to be 30,577 pounds of dichlorobenzene (CARB, 2000).

IV. Effects of Human Exposure

Case reports of human exposure to 1,4-DCB include malaise, nausea, hepatic manifestations (yellow atrophy and cirrhosis), proteinuria, bilirubinuria, hematuria, and anemia. A woman exposed to 1,4-DCB for 6 years developed central nervous system effects, including severe cerebellar ataxia, dysarthria, weakness in all limbs, and hyporeflexia (U.S. EPA, 1985).

No epidemiologic studies of 1,4-DCB exposures were located.

V. Effects of Animal Exposure

Rats, rabbits and guinea pigs were exposed to 0, 96, 158, 341 or 798 ppm (0, 577, 950, 2050 or 4800 mg/m³) 1,4-DCB by inhalation 7 hours/day, 5 days/week for 6-7 months (Hollingsworth *et al.*, 1956). High dose animals showed marked tremors, weakness, loss of weight, eye irritation and unconsciousness. Liver and kidney changes included cloudy swelling and centrilobular cellular degeneration (liver). In another inhalation study in rats animals were exposed to 0, 75 or 500 ppm (0, 451 or 3006 mg/m³) for 5 hours/day, 5 days/week for 76 weeks (Riley *et al.*, 1980). The authors found increased kidney and liver weights in the high dose group. Thus 75 ppm was a NOAEL. Studies with oral exposure to 1,4-DCB, including the NTP (1987) chronic bioassay study (maximum dose of 300 mg/kg-day), have also found an increased incidence of renal and hepatic lesions (cellular degeneration and focal necrosis).

Three inhalation reproductive studies, one in rabbits (Hayes *et al.*, 1985), one in mice (Anderson and Hodge, 1976), and one in rats (Chlorobenzene Producers Assn., 1986), found minimal reproductive effects. In rabbits exposed on days 6-18 of gestation to 100, 300, and 800 ppm 1,4-DCB, only the differences in percentage of implantations resorbed and in percentage of litters with resorptions were significantly increased and only in the 300 ppm group (Hayes *et al.*, 1985). No reduction in reproductive performance was observed in mice exposed to 0, 75, 225, or 450 ppm 1,4-DCB for 6 hours/day for 5 days (Anderson and Hodge, 1976).

In a two-generation reproductive study (Chlorobenzene Producers Association, 1986), Sprague-Dawley rats P1 (28/sex/group) were exposed to 0, 50, 150 or 450 ppm (0, 301, 902, or 2705 mg/m³) of 1,4-DCB vapor, 6 hours/day, 7 days/week for 10 weeks, and then mated for 3 weeks. The second generation F1 weanlings were exposed to 1,4-DCB for 11 weeks and then mated. No developmental abnormalities were observed in pups examined. At 450 ppm significant decreases in live births, pup weights, and pup survival were seen in both the F1 and F2 generations. Non-reproductive effects observed in the parental males in the 150 and 450 ppm groups included significantly increased liver and kidney weights. All dose levels caused hyaline droplet nephrosis in post-pubescent males; but this change was associated with the formation of

alpha-2u-globulin, an abnormality considered specific for male rats with no relative human significance (U.S. EPA, 1991). The Chlorobenzene Producers Association reproductive study was chosen by the U.S. EPA to derive the RfC.

VI. Derivation of Chronic Reference Exposure Level

<i>Study</i>	Chlorobenzene Producers Association, 1986
<i>Study population</i>	Sprague-Dawley rats (28 rats/sex/group)
<i>Exposure method</i>	Discontinuous whole-body inhalation exposures (0, 50, 150 or 450 ppm)
<i>Critical effects</i>	Reduced body weights and food consumption; tremors; nasal and ocular discharge; increased liver and kidney weights
<i>LOAEL</i>	150 ppm
<i>NOAEL</i>	50 ppm
<i>Exposure continuity</i>	6 hr/day for 7 days/week
<i>Average experimental exposure</i>	13 ppm for NOAEL group (50 x 6/24)
<i>Human equivalent concentration</i>	13 ppm for NOAEL group (gas with systemic effects, based on RGDR = 1.0 using default assumption that $\lambda(a) = \lambda(h)$)
<i>Exposure duration</i>	10 weeks
<i>LOAEL uncertainty factor</i>	1
<i>Subchronic uncertainty factor</i>	3
<i>Interspecies uncertainty factor</i>	3
<i>Intraspecies uncertainty factor</i>	10
<i>Cumulative uncertainty factor</i>	100
<i>Inhalation reference exposure level</i>	0.1 ppm (100 ppb, 0.8 mg/m ³ , 800 µg/m ³)

The chronic REL for 1,4-dichlorochlorobenzene is also the U.S. EPA RfC. OEHHA agrees with the U.S. EPA analysis. A 3-fold subchronic uncertainty factor (instead of 10) was used by U.S. EPA because of data suggesting limited progression of hepatic lesions (Riley *et al.*, 1980). Ten weeks are also greater than 8% of a rat's two-year lifetime and thus in accord with OEHHA's use of a subchronic UF of 3 (OEHHA, 2000).

For comparison, Riley *et al.* (1980) found a chronic NOAEL of 75 ppm for kidney and liver effects in rats, which is equivalent to 11.2 ppm continuous exposure. Use of an RGDR of 1 and a total UF of 30 (3 for interspecies and 10 for intraspecies) results in a REL estimate of 0.4 ppm.

VII. Data Strengths and Limitations for Development of the REL

The major strengths of the REL for 1,4-dichlorochlorobenzene are the observation of a NOAEL and the demonstration of a dose-response relationship. The major uncertainties are the lack of human data and the lack of chronic, multiple-species health effects data.

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CHRONIC TOXICITY SUMMARY

1,1-DICHLOROETHYLENE*(DCE; 1,1-dichloroethene; VDC; vinylidene chloride)***CAS Registry Number: 73-35-4****I. Chronic Toxicity Summary**

<i>Inhalation reference exposure level</i>	70 $\mu\text{g}/\text{m}^3$ (20 ppb)
<i>Critical effect(s)</i>	Increased mortality; hepatic effects (mottled livers and increases in liver enzymes) in guinea pigs
<i>Hazard index target(s)</i>	Alimentary system

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

<i>Description</i>	Colorless liquid
<i>Molecular formula</i>	$\text{C}_2\text{H}_2\text{Cl}_2$
<i>Molecular weight</i>	96.95 g/mol
<i>Boiling point</i>	31.7°C
<i>Melting point</i>	-122.5°C
<i>Vapor pressure</i>	500 torr @ 20°C
<i>Solubility</i>	Soluble in water (2.5 g/L); miscible in organic solvents
<i>Conversion factor</i>	3.97 $\mu\text{g}/\text{m}^3$ per ppb at 25 °C

III. Major Uses and Sources

1,1-Dichloroethylene (1,1-DCE) is used in the production of polyvinylidene chloride copolymers (HSDB, 1994). 1,1-DCE containing copolymers include other compounds such as acrylonitrile, vinyl chloride, methacrylonitrile, and methacrylate. These copolymers are used in flexible packaging materials; as flame retardant coatings for fiber, carpet backing, and piping; as coating for steel pipes; and in adhesive applications. Flexible films for food packaging, such as SARAN and VELON wraps, use such polyvinylidene chloride copolymers. The annual statewide industrial emissions from facilities reporting under the Air Toxics Hot Spots Act in California based on the most recent inventory were estimated to be 2458 pounds of vinylidene chloride (CARB, 2000).

IV. Effects of Human Exposure

Limited information exists regarding the human health effects following exposure to 1,1-DCE. A few case reports and mortality studies have reported hepatotoxicity and nephrotoxicity after repeated, low-level exposures (USEPA, 1976; Ott *et al.*, 1976). However, these investigations were conducted in industrial settings with the possibility of mixed chemical exposures. In preliminary clinical findings reported by the EPA (1976), workers exposed to 1,1-DCE for 6 years or less had a high incidence of hepatotoxicity, with liver scans and measurements of liver enzymes revealing 50% or greater loss in liver function in 27 of 46 exposed workers. Unfortunately, no follow-up study was reported.

V. Effects of Animal Exposure

Several studies have reported on the subchronic or chronic toxicity of 1,1-DCE in laboratory animals exposed either via oral or inhalation routes. The liver is the primary target organ of 1,1-DCE toxicity following acute or chronic inhalation exposure. Such exposure is marked by both biochemical changes (alterations in serum enzyme levels) and histological changes (e.g., midzonal and centrilobular swelling, degeneration, and necrosis) (Gage, 1970; Lee *et al.*, 1977; Plummer *et al.*, 1990; Quast, 1976; Quast *et al.*, 1986). Unfortunately, these longer-term studies used only one or two doses or a limited number of animals.

Male and female rats exposed intermittently (6 hours/day, 5 days/week) to 125 or 200 ppm 1,1-DCE over 30 days exhibited centrilobular fatty degeneration or hepatocellular necrosis (Quast 1976, as cited by USDHHS, 1994). Two other studies identified hepatic changes in rats at lower concentrations of 1,1-DCE (6 hours/day, 5 days/week): cytoplasmic vacuolation after 30- or 90-day exposure to 25 or 75 ppm 1,1-DCE (Balmer *et al.*, 1976, as cited by USDHHS, 1994), and fatty changes after 6 months at 25 ppm 1,1-DCE (Quast *et al.*, 1986).

Laboratory animals appear less tolerant of continuous exposure (23-24 hours per day) than intermittent exposure. Beagle dogs exposed to 100 ppm 1,1-DCE for 8 hours/day, 5 days/week for 42 days had no evidence of hepatotoxicity, but continuous exposure to 48 ppm for 90 days caused liver changes (Prendergast *et al.*, 1967). Similarly, monkeys continuously exposed to 48 ppm for 90 days exhibited focal necrosis and hemosiderin deposition, while no liver toxicity was apparent following 42 days of intermittent exposure to 100 ppm 1,1-DCE (Prendergast *et al.*, 1967). Guinea pigs exposed to 1,1-DCE for 24 hours per day for 90 days (0, 5, 15, 25, or 48 ppm) displayed mottled livers at 15 ppm, and increased liver enzyme levels (serum glutamic-pyruvic transaminase (SGPT) and alkaline phosphatase (AP)) at 48 ppm. A NOAEL of 5 ppm based on liver changes (Prendergast *et al.*, 1967) is indicated by the results.

Data on continuously exposed guinea pigs from Prendergast *et al.* (1967)

<i>ppm 1,1-DCE (mg/m³)</i>	<i>Survival</i>	<i>Body weight change</i>	<i>Liver AP</i>	<i>SGPT</i>
0	312/314	+69.0%	0.08±0.03	10±5
5 (20)	43/45	+58.6%	0.08±0.03	11±3
15 (61)	12/15	+55.3%	Not reported	Not reported
25 (101)	12/15	+74.0%	Not reported	Not reported
48 (191)	8/15	+50.3%	0.19±0.04	>70

Additional adverse effects observed to a lesser extent in laboratory animals include respiratory and renal toxicity. Nephrotoxicity observed following chronic 1,1-DCE exposure included gross organ (increases in kidney weight) (Klimisch *et al.*, 1979; Quast *et al.*, 1986) and histological changes (tubular swelling, degeneration, and necrosis) (Klimisch *et al.*, 1979; Lee *et al.*, 1977; Prendergast *et al.*, 1967). Continuous exposure of rats to 48 ppm 1,1-DCE for 90 days caused nuclear hypertrophy of the renal tubular epithelium (Prendergast *et al.*, 1976). Mice exposed to 25 ppm 1,1-DCE 4 hours/day, 4 or 5 days/week, for 52 weeks displayed severe tubular nephrotoxicity (Maltoni *et al.*, 1985 as cited by USDHHS, 1994). Nasal irritation was observed in rats exposed to 200 ppm for 4 weeks (Gage 1970). But no respiratory effects were attributed to 1,1-DCE exposure in rats, monkeys, dogs, rabbits, or guinea pigs exposed to 100 ppm intermittently for 6 weeks (Prendergast *et al.*, 1967) or in rats exposed to 75 ppm for 18 months (Quast *et al.*, 1986).

Toxicokinetic studies in laboratory animals have demonstrated that 1,1-DCE is readily absorbed and rapidly distributed following inhalation exposure (Dallas *et al.*, 1983; McKenna *et al.*, 1978b). Following inhalation exposure to radioactively labeled 1,1-DCE, rats preferentially accumulate radioactivity in the kidney and liver (McKenna *et al.*, 1978b; Jaeger *et al.*, 1977). Glutathione (GSH) conjugation appears to be the major detoxification route for 1,1-DCE intermediates, and GSH-depleting experimental states, such as drugs and fasting, may tend to increase 1,1-DCE toxicity (Jaeger *et al.*, 1977; McKenna *et al.*, 1978; Reichert *et al.*, 1978). One study greatly increased 1,1-DCE induced lethality and hepatotoxicity in rats by pretreatment with acetaminophen (Wright and Moore, 1991).

VI. Derivation of Chronic Reference Exposure Level (REL)

<i>Study</i>	Prendergast <i>et al.</i> (1967)
<i>Study population</i>	Guinea pigs (15 per group, except 45 animals in 20 mg/m ³ group)
<i>Exposure method</i>	Continuous whole body inhalation (0, 20, 61, 101, or 189 mg/m ³)
<i>Critical effects</i>	Increased mortality at 61, 101, and 189 mg/m ³ ; hepatic effects (mottled livers and increases in SGPT and AP enzymes) noted at 189 mg/m ³
<i>LOAEL</i>	61 mg/m ³ (15 ppm)
<i>NOAEL</i>	20 mg/m ³ (5 ppm)
<i>Exposure continuity</i>	Continuous
<i>Exposure duration</i>	90 days
<i>Average experimental exposure</i>	20 mg/m ³ for NOAEL group
<i>Human equivalent concentration</i>	20 mg/m ³ for NOAEL group (gas with systemic effects, based on default assumption that RGDR = 1 using default assumption that lambda (a) = lambda (h))
<i>LOAEL uncertainty factor</i>	1
<i>Subchronic uncertainty factor</i>	10 (since guinea pig life-span is approx. 6 years)
<i>Interspecies uncertainty factor</i>	3
<i>Intraspecies uncertainty factor</i>	10
<i>Cumulative uncertainty factor</i>	300
<i>Inhalation reference exposure level</i>	0.07 mg/m ³ (70 µg/m ³ ; 0.02 ppm; 20 ppb)

The principal study (Prendergast *et al.*, 1967) identified adverse hepatic and/or renal effects in rats (15 or 45/group), guinea pigs (15 or 45/group), dogs (2 or 6/group), and monkeys (3, 9, or 21/group) exposed to inhaled 1,1-DCE. Continuous exposure to 1,1-DCE, 24 hours/day over 90 days, demonstrated more severe effects than intermittent exposure, 6 hours/day, 5 days/week for 6 weeks, in the species tested. Unlike the other available subchronic and chronic studies, this principal study included multiple exposure levels of 0, 5, 15, 25 and 48 ppm (0, 20, 61, 101, and 189 mg/m³). Mortality, hematologic and body weight data were well tabulated and presented in this study. Histopathologic evaluation was conducted on the heart, lung, liver, spleen and kidneys. Following continuous exposure, adverse hepatic effects included focal necrosis in monkeys (LOAEL = 189 mg/m³, NOAEL = 101 mg/m³), in dogs (LOAEL = 189 mg/m³, NOAEL = 101 mg/m³), and in rats (LOAEL = 189 mg/m³, NOAEL = 101 mg/m³); and altered lipid content and increases in SGPT and alkaline phosphatase in guinea pigs (LOAEL = 189 mg/m³, NOAEL = 20 mg/m³). Additionally, renal alterations were observed in rats as nuclear hypertrophy in the tubular epithelium (LOAEL = 189 mg/m³, NOAEL = 61 mg/m³). Monkeys exposed to 1,1-DCE also displayed a greater than 25% decrease in body weight (LOAEL 189 mg/m³, NOAEL 20 mg/m³). The subchronic study by Prendergast *et al.* (1967) was chosen over the chronic studies because of its better design, its use of continuous exposure, and its exhibition of toxic effects below the LOAELs reported in the other studies.

Although limited in number, the other chronic and subchronic studies available consistently demonstrate adverse hepatic effects following 1,1-DCE exposure (Lee *et al.*, 1977; Maltoni *et*

al., 1985; Plummer *et al.*, 1990; Quast *et al.*, 1986). Hepatocellular fatty change was observed in rats exposed to 25 ppm or 75 ppm 1,1-DCE intermittently (6 hrs/d, 5 d/wk) for 18 months. This mid-zonal fatty change was also observed at the 12-month interim sacrifice, but did not appear to progress in severity or incidence over time (Quast *et al.*, 1986). A more severe hepatocellular necrosis and renal tubular necrosis were observed in mice exposed to 55 ppm 1,1-DCE 6 hr/d, 5 d/week for 1 year (Lee *et al.*, 1977).

For comparison, Quast *et al.* (1986) determined a LOAEL of 25 ppm for liver effects of minimal severity in rats after 18 months exposure. Use of continuous time adjustment to 4.5 ppm, multiplication by an RGDR of 1, and division by a total UF of 100 (3 for LOAEL to NOAEL, 3 for interspecies, and 10 for intraspecies) results in an estimate of 45 ppb (200 $\mu\text{g}/\text{m}^3$).

VII. Data Strengths and Limitations for Development of the REL

Uncertainty factors are appropriate due to the limited number of subchronic and chronic inhalation studies (greater than 1 year duration) in laboratory animals. In addition, few industrial surveys and epidemiological studies are available on the adverse effects of 1,1-DCE in humans; these are limited by small sample size, short follow-up, and/or brief exposure periods. But this limited evidence does suggest an association between repeated exposure to 1,1-DCE and liver damage in humans (EPA, 1976), and the key study is an animal study which found adverse hepatic effects. No toxicokinetic data regarding the absorption, distribution, metabolism or excretion of 1,1-DCE in humans are available.

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CHRONIC TOXICITY SUMMARY

DIETHANOLAMINE

(DEA; 2,2'-iminodiethanol; 2,2'-iminobisethanol; diethylamine; 2,2'-aminodiethanol; 2,2'-dihydroxydiethylamine)

CAS Registry Number: 111-42-2

I. Chronic Toxicity Summary

<i>Inhalation reference exposure level</i>	3 $\mu\text{g}/\text{m}^3$ (0.6 ppb)
<i>Critical effect(s)</i>	Laryngeal lesions in rats
<i>Hazard index target(s)</i>	Respiratory system; cardiovascular system

II. Physical and Chemical Properties (Melnick and Thomaszewski, 1990; Dow, 1980; CRC, 1994)

<i>Description</i>	Colorless crystals
<i>Molecular formula</i>	$\text{C}_4\text{H}_{11}\text{NO}_2$
<i>Molecular weight</i>	105.14 g/mol
<i>Density</i>	1.097 g/cm ³ @ 20°C
<i>Boiling point</i>	268.8°C
<i>Melting point</i>	28°C
<i>Vapor pressure</i>	0.00014 torr @ 25°C
<i>Solubility</i>	Soluble in alcohol, water, acetone
<i>Conversion factor</i>	1 ppm = 4.3 mg/m ³ @ 25°C

III. Major Uses and Sources

Diethanolamine is used in the formation of soaps, emulsifiers, thickeners, wetting agents, and detergents in cosmetic formulations (Melnick and Thomaszewski, 1990; Knaak *et al.*, 1997). It is used as a dispersing agent in some agricultural chemicals, as an absorbent for acidic gases, as a humectant, as an intermediate in the synthesis of morpholine, as a corrosion inhibitor, and as a component in textile specialty agents (Beyer *et al.*, 1983). Diethanolamine is permitted in articles intended for use in production, processing, or packaging of food (CFR, 1981; cited in Melnick and Thomaszewski, 1990). It is also found in adhesives, sealants, and cutting fluids (Melnick and Thomaszewski, 1990). The annual statewide industrial emissions from facilities reporting under the Air Toxics Hot Spots Act in California based on the most recent inventory were estimated to be 1520 pounds of diethanolamine (CARB, 2000).

IV. Effects of Chronic Exposures to Humans

There have been no controlled or epidemiological studies of chronic diethanolamine exposure in humans. There is a single case report of occupational asthma determined to be due to the patient's handling of a cutting fluid containing diethanolamine (Piipari *et al.*, 1998). Specific bronchial provocation tests were done with the cutting fluid containing DEA and with DEA aerosol at two concentrations (0.75 mg/m³ and 1.0 mg/m³) below the occupational limit of 2.0 mg/m³. DEA caused asthmatic airway obstruction at both concentrations, but IgE-antibodies specific for DEA were not found.

V. Effects of Exposures in Animals

Diethanolamine replaces choline in phospholipids (Blum *et al.*, 1972). DEA also reversibly inhibits phosphatidylcholine synthesis by blocking choline uptake and competing for utilization in the CDP-choline pathway (Lehman-McKeeman and Gamsky, 1999). Systemic toxicity occurs in many tissue types including the nervous system, liver, kidney, and blood system.

Gamer *et al.* (1996) exposed groups of 26 Wistar rats (13 male and 13 female) head-nose to a liquid aerosol of DEA for six hours per working day for 90 days at target concentrations of 15, 150, and 400 mg/m³. Three of each sex were used for whole animal perfusion studies and the remaining 20 animals were examined for pathology. The study found no functional or morphological evidence of neurotoxicity. Retardation of body weight increase was observed in animals exposed to high concentrations. No systemic effects occurred at the low dose, but systemic effects in the liver, kidney, male reproductive system, and red blood cell occurred in the high concentration dose group. In the mid-dose group, mild liver and kidney effects were present. Local irritation of the larynx and trachea was found in the high and mid dose groups; irritating laryngeal effects were also detected in the low dose group. Based on this study 15 mg/m³ is a NOAEL for liver and kidney effects and a LOAEL for irritation of the larynx. The equivalent continuous exposure at the LOAEL is 2.7 mg/m³ (15 x 6/24 x 5/7).

Incidence of laryngeal lesions (Gamer *et al.*, 1996)

Aerosolized diethanolamine	Chronic inflammation of the larynx	Squamous hyperplasia	Focal squamous metaplasia of laryngeal epithelium at base of the epiglottis
0	None*	None	None
15 mg/m ³	4/20	0/20	20/20
150 mg/m ³	20/20	13/20	20/20
400 mg/m ³	20/20	17/20	20/20

* The report does not give control incidences. Assumed 0/20.

In an abstract Hartung *et al.* (1970) reported that inhalation by male rats of 6 ppm (25.8 mg/m³) DEA vapor 8 hours/day, 5 days/week for 13 weeks resulted in depressed growth rates, increased lung and kidney weights, and even some mortality. Rats exposed continuously for 216 hours (nine days) to 25 ppm (108 mg/m³) DEA showed increased liver and kidney weights, elevated

blood urea nitrogen (BUN), and increased serum glutamate oxaloacetate transferase (SGOT), an indicator of liver damage (Hartung et al., 1970). In studies at lower DEA levels, Eastman Kodak (1967) exposed dogs, weanling and adult rats, and guinea pigs to 0.26 ppm (1.1 mg/m³) DEA for 90 days and found no pathology attributable to DEA. In a 45-day study with 0.5 ppm (2.2 mg/m³) DEA they also found no pathology attributable to DEA except for a possible slight retardation in rat growth rate.

Gamer *et al.* (1993) exposed groups of 25 pregnant Wistar rats on gestation days 6-15 to a (nose-only) liquid aerosol of DEA at 10, 50 and 200 mg/m³. Maternal toxicity, indicated by vaginal hemorrhage in 8 of the dams on gestation day 14, and fetotoxicity, evidenced by a statistically significant ($p < 0.05$) increased incidence of total fetal skeletal variations, were observed at 200 mg/m³. No teratogenic effects were seen at any level. Thus 50 mg/m³ was a NOAEL for maternal toxicity and for embryo-fetal effects.

A 13-week drinking water study in rats (10 per sex per group) showed significant dose-dependent hematological changes following exposure to DEA at all concentrations tested: 320, 630, 1250, 2500, and 5000 ppm in males, and 160, 320, 630, 1250, and 2500 ppm in females. Hematological effects included decreased hemoglobin and mean corpuscular volume (Melnick *et al.*, 1994a). Similar hematological changes were observed following daily topical treatment. In addition to the hematological effects, female rats also showed dose-dependent spinal cord and medullary demyelination beginning at a drinking water concentration of 1250 ppm DEA. Male rats displayed demyelination beginning at 2500 ppm. Female rats gained significantly less weight than controls beginning at 63 mg/kg/day topical treatment. In a companion drinking water study (Melnick *et al.*, 1994b), mice (10 per sex per group) were exposed to concentrations of 0, 630, 1250, 2500, 5000, and 10,000 ppm DEA and displayed dose-dependent hepatotoxicity, nephrotoxicity, and cardiac toxicity. Daily topical treatment in a separate study resulted in skin lesions in mice. Significant hepatic toxicity was observed at all drinking water concentrations, and skin lesions were observed at all topical doses.

Data from female rats exposed to diethanolamine by Melnick *et al.* (1994)

Dose (ppm)	mg/kg/day DEA consumed	Survival	Mean bw change (g)	Hgb (g/dL)	Mean cell volume	Mean cell Hgb (pg)
0	0	10/10	120±6a	15.1±0.3	56±0.2	17.9±0.2
160	14	9/10	106±3	15.2±0.1	55±0.2**	17.8±0.1*
320	32	10/10	98±3**	13.8±0.1**	54±0.2**	17.7±0.1**
630	57	10/10	95±4**	13.0±0.1**	53±0.3**	17.2±0.1**
1250	124	10/10	85±4**	11.3±0.2**	51±0.3**	16.7±0.1**
2500	242	10/10	63±4**	10.50±.2**	49±0.2**	16.30±.1**

a Values are means±SEM; * $p < 0.05$ or ** $p < 0.01$ versus control group

Barbee and Hartung (1979a) found that repeated treatment of rats with 330 mg DEA/kg/day significantly inhibited formation of phosphatidyl choline and phosphatidyl ethanolamine in the liver as compared with control rats. In a subsequent study, Barbee and Hartung (1979b) noted changes in liver mitochondrial activity in rats (4 per group) following exposure to DEA in

drinking water for up to 5 weeks. Mitochondrial changes were observed at 42 mg/kg/day after 2 weeks.

Daily oral treatment of male rats with 0, 250, 500, or 750 mg/kg/day for 5 days, or 100 mg/kg/day for 14 days resulted in reduced activities of the liver enzymes microsomal hydroxylase and N-demethylase (Foster *et al.*, 1971).

In a developmental study Marty *et al.* (1999) administered DEA cutaneously to pregnant CD rats during gestation days 6-15 at doses of 0, 150, 500, and 1500 mg/kg/day. Dams exhibited reduced body weight at the highest dose, skin irritation and increased kidney weights at both 500 and 1500 mg/kg/day, and a slight microcytic anemia with abnormal red blood cell morphology at all 3 dose levels. The blood results are consistent with the results of topical application of DEA by Melnick *et al.* (1994b). Rat fetuses had increased incidences of six skeletal variations at 1500 mg/kg/day. Lower doses were without effect on the fetuses. Marty *et al.* (1999) also administered DEA cutaneously to pregnant New Zealand White rabbits on days 6-18 of gestation at 0, 35, 100, and 350 mg/kg/day. Dams administered the highest dose exhibited various skin lesions, reduced food consumption, and color changes in the kidneys, but no hematological changes. Body weight gain was reduced at ≥ 100 mg/kg/day. There was no evidence of maternal toxicity at 35 mg/kg/day and no evidence of developmental toxicity in rabbits at any dose. Developmental toxicity was observed only in the rat and only at doses causing significant maternal toxicity, including hematological effects. Due to a dose discrepancy, the authors adjusted the no observable effect level (NOEL) for DEA developmental toxicity to 380 mg/kg/day for rats. In rabbits, the embryonal/fetal NOEL was 350 mg/kg/day.

VI. Derivation of Chronic Reference Exposure Level (REL)

<i>Study</i>	Gamer <i>et al.</i> (1996)
<i>Study population</i>	Wistar rats (male and female)
<i>Exposure method</i>	Inhalation 6 h/day, 5 d/wk
<i>Critical effects</i>	Chronic inflammation and squamous hyperplasia and metaplasia of the larynx
<i>LOAEL</i>	15 mg/m ³
<i>NOAEL</i>	Not observed
<i>Exposure duration</i>	90 days
<i>Average experimental exposure</i>	2700 µg/m ³ for LOAEL group (15 mg/m ³ x 6h/24h x 5d/7d x 1000 µg/mg)
<i>LOAEL uncertainty factor</i>	3 (see below)
<i>Subchronic uncertainty factor</i>	3
<i>Interspecies uncertainty factor</i>	10
<i>Intraspecies uncertainty factor</i>	10
<i>Cumulative uncertainty factor</i>	1000
<i>Inhalation reference exposure level</i>	3 µg/m ³ (0.6 ppb)

No chronic inhalation studies with diethanolamine were located in the peer-reviewed literature. Thus the 90 day study by Gamer *et al.*, which found a LOAEL of 15 mg/m³ for irritation of the

rat larynx, was used to derive the REL. All 20 of the rats in the 15 mg/m³ exposure group showed focal squamous metaplasia of the laryngeal epithelium at the base of the epiglottis, and 4 of the 20 had inflammatory cells present in the larynx. The former lesion seemed to be very limited and did not justify use of the full LOAEL uncertainty factor of 10.

For comparison, the BASF (1993) developmental study by the inhalation route found a LOAEL of 200 mg/m³ DEA and a NOAEL of 50 mg/m³ for fetotoxic effects. The equivalent continuous exposure at the NOAEL is 12.5 mg/m³. Multiplying by an RGDR of 1 and dividing by an interspecies uncertainty factor (UF_A) of 3 and an intraspecies uncertainty factor (UF_H) of 10 results in a REL estimate of 40 µg/m³.

As another comparison, the study by Melnick *et al.* (1994a) shows dose-dependent adverse hematological and CNS effects in rats exposed to DEA in drinking water. Similar systemic effects were observed following dermal exposure. The Melnick *et al.* subchronic study was of the longest duration and was the most comprehensive report of the systemic effects of DEA in the literature. However, portal-of-entry effects of DEA have not been examined and should be addressed in future studies since this compound has irritant properties. The data from female rats were used since females were more sensitive than males to the hematologic effects of DEA. The LOAEL was 160 mg/L, or 14 mg/kg-day based on water consumption rates. Dividing by a LOAEL UF of 3, a subchronic UF of 3, an interspecies UF of 10, and an intraspecies UF of 10 (cumulative UF = 1000) results in a oral REL of 0.014 mg/kg-day. Using route-to-route extrapolation and assuming that a 70 kg person inhales 20 m³ of air per day leads to an inhalation REL estimate of 50 µg/m³ (10 ppb) DEA.

VII. Data Strengths and Limitations for Development of the REL

The diethanolamine database is relatively weak. Major areas of uncertainty are the lack of adequate human exposure data, the absence of a NOAEL in the major study, the lack of reproductive and developmental toxicity studies, and the lack of chronic inhalation, multiple-species, health effects data.

VIII. Potential for Differential Impacts on Children's Health

Since the proposed chronic REL of 3 µg/m³ based on laryngeal effects is much lower than the comparison REL of 40 µg/m³ based on fetotoxic effects, the REL should adequately protect infants and children. Diethanolamine is a respiratory irritant and thus might exacerbate asthma, which has a more severe impact on children than on adults. The large uncertainty factor of 1000 should protect against that potential hazard. However, there is no direct evidence in the literature to demonstrate that DEA exacerbates asthma or to quantify a differential effect of diethanolamine on the larynx or on other organs in infants and children.

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CHRONIC TOXICITY SUMMARY

N,N-DIMETHYLFORMAMIDE*(N-formyldimethylamine)***CAS Registry Number: 68-12-2****I. Chronic Toxicity Summary**

<i>Inhalation reference exposure level</i>	80 µg/m³ (30 ppb)
<i>Critical effect(s)</i>	Liver dysfunction and respiratory irritation in humans
<i>Hazard index target(s)</i>	Alimentary system, respiratory system

II. Chemical Property Summary (HSDB, 1994)

<i>Description</i>	Colorless to very slightly yellow liquid
<i>Molecular formula</i>	C ₃ H ₇ NO
<i>Molecular weight</i>	73.09 g/mol
<i>Boiling point</i>	153°C
<i>Melting point</i>	-61°C
<i>Vapor pressure</i>	3.7 torr @ 25°C
<i>Solubility</i>	Soluble in alcohol, ether, acetone, benzene, and chloroform; miscible with water
<i>Conversion factor</i>	2.99 µg/m ³ per ppb at 25°C

III. Major Uses and Sources

Dimethylformamide (DMF) is primarily used as a solvent in the production of polyurethane products and acrylic fibers. It is also used in the pharmaceutical industry, in the formulation of pesticides, and in the manufacture of synthetic leathers, fibers, films, and surface coatings (Howard, 1993; Gescher, 1993; Redlich *et al.*, 1988). DMF may be emitted to the environment as a result of its use in a variety of petrochemical industries (Howard, 1993). The annual statewide industrial emissions from facilities reporting under the Air Toxics Hot Spots Act in California based on the most recent inventory were estimated to be 18,249 pounds of DMF (CARB, 2000).

IV. Effects of Human Exposure

Among 100 workers occupationally exposed to DMF for at least one year (mean exposure of 5 years; range = 1-15 years), a statistically significant incidence of hepatic impairment, as indicated by elevated gamma-glutamyl transpeptidase levels and digestive disturbances, was

noted (Cirila *et al.*, 1984). Other changes, that were not statistically significant, included increased SGOT and SGPT and enlarged livers. The mean time-weighted average concentration of DMF was 22 mg/m³ (range = 8-58 mg/m³). Symptoms of irritation occurring only during work at statistically significantly higher incidences included watery eyes, dry throat, and coughing. Also, the exposed workers reported a reduced sense of smell and dry coughs at home with a statistically significant difference as compared to controls. Several of the DMF exposed workers also reported alcohol intolerance characterized by a disulfiram-type reaction (facial flushing and palpitations following alcohol ingestion). Alcohol consumption, a potential confounder, was controlled for in the study design.

A similar study was conducted on workers who had been employed in an acrylic acid fiber plant for more than 5 years (Cantenacci *et al.*, 1984). Concentrations to which the workers were exposed were characterized as either an 8-hour TWA of 18 mg/m³ or an 8-hour TWA of 3 mg/m³. Measures of liver function including SGOT, SGPT, gamma-glutamyl transferase, and alkaline phosphatase levels were not significantly different between exposed and unexposed workers. However, the U.S. EPA cautions that because only 54 matched pairs of workers were examined, the power of this study was not high enough to reliably detect a difference in enzyme levels.

Redlich *et al.* (1988) characterized a plant-wide outbreak of liver disease among workers in a factory coating fabric with polyurethane. Fifty-eight of 66 (88%) workers participated and each had standard liver screening function tests done at least once. At the work site DMF was being used in poorly ventilated areas without appropriate skin protection. No other major known hepatotoxic exposure was identified. Overall, 36 of 58 (62%) workers tested had elevations of either aspartate aminotransferase (AST) or alanine aminotransferase (ALT) levels. Enzyme abnormalities occurred almost exclusively in production workers (35 out of 46 abnormal). Only 1 of 12 non-production workers showed elevations in enzyme levels ($p < 0.0001$). Serologic tests excluded known infectious causes of hepatitis in all but 2 workers. Changes, characteristic of liver injury, were confirmed by histologic examination of biopsy specimens from 4 workers. Improvement in liver enzyme abnormalities and symptoms in most patients were seen, after modification of work practices and removal of workers most severely affected from exposure. However, some patients showed persistent elevations of enzyme levels. No measurements or estimates of DMF exposure levels were reported.

Wang *et al.* (1991) investigated the prevalence of liver injury associated with DMF exposure in 183 of 204 (76%) employees of a synthetic leather factory by performing medical examinations, liver function tests, and creatine phosphokinase (CPK) determinations. Air concentrations were measured with personal samplers and gas chromatography. The concentration of DMF in air to which each worker was exposed was categorized as high (DMF exposure index 2: 25-60 ppm; 75-180 mg/m³), medium (index 1: 10-40 ppm), and low (index 0: <10 ppm). High exposure concentrations were significantly associated with elevated alanine aminotransferase (ALT) levels (i.e., greater than or equal to 35 International Units/liter), a result that did not change after stratification by hepatitis B carrier status. Logistic regression analysis indicated that exposure to high DMF levels was associated with elevated ALT ($p = 0.01$), whereas hepatitis B surface antigen (HBsAg) was slightly but independently associated with elevated ALT ($p = .07$). Workers with normal ALT values had significantly higher mean ALT and aspartate

aminotransferase (AST) activities, especially among those who were not HBsAg carriers. A significant association existed between elevated CPK levels and exposure to DMF. However, an analysis of the CPK isoenzyme among 143 workers did not reveal any specific damage to muscles. Thus the authors ascribed the liver injury to DMF.

U.S. EPA (1994) states that subjective evidence of liver toxicity, such as digestive impairment and alcohol intolerance, is often observed at exposures below those that cause clinical changes in liver enzymes. Thus, the symptoms may be more sensitive indicators of hepatic impairment.

Three unexplained cases of small-for-date third trimester intrauterine deaths were observed in a group of women working as quality control analysts in the pharmaceutical industry (Farquhason *et al.*, 1983). This represented a 30% stillbirth rate as compared with the average for the general population of about 0.26%. While the authors concluded that the occurrence of stillbirth in these women was not likely due to chance, the effects cannot be solely attributed to DMF because the women were exposed to other agents in addition to DMF.

V. Effects of Animal Exposure

Malley *et al.* (1994) exposed male and female Crl:CD rats and mice to 0, 25, 100, or 400 ppm DMF for 6 hr/day, 5 days/week for 18 months (mice) or 2 years (rats). No compound-related effects on clinical observations or survival were observed. Body weights of rats exposed to 100 (males only) and 400 ppm were reduced, while body weights were increased in 400 ppm mice. No hematologic changes were observed in either species. Serum sorbitol dehydrogenase activity was increased in rats exposed to 100 or 400 ppm. DMF-related morphological changes were observed only in liver. Exposure of rats to 100 and 400 ppm produced increased relative liver weights, centrilobular hepatocellular hypertrophy, lipofuscin/hemosiderin accumulation in Kupffer cells, and centrilobular single cell necrosis (400 ppm only). In mice, increased liver weights (100 ppm males, 400 ppm both sexes), centrilobular hepatocellular hypertrophy, accumulation of lipofuscin/hemosiderin in Kupffer cells, and centrilobular single cell necrosis were observed in all exposure groups. These observations occurred in a dose-response fashion and were minimal at 25 ppm. No increase in hepatic cell proliferation was seen in mice or female rats. Slightly higher proliferation was seen in male rats exposed to 400 ppm at 2 weeks and 3 months but not at 12 months. Thus 25 ppm was a chronic NOAEL for both rats and mice.

A developmental toxicity study using three species (mice, rabbits, and rats) and four routes of administration (oral, inhalation, dermal, and intraperitoneal) identified the rabbit as the most sensitive of the three species. Groups of 15 pregnant rabbits were exposed for 6 hours per day on days 8-20 of gestation to 50, 150, or 450 ppm (150, 449, or 1350 mg/m³) DMF (Hellwig *et al.*, 1991). Slight maternal toxicity, as indicated by non-statistically significant decreases in maternal body weight gain, was observed in the 450 ppm exposure group. An increased number of total malformations per litter was observed in the 450 ppm exposure group. Malformations observed at statistically higher incidences compared to controls included hernia umbilicalis, external variations, pseudoankylosis of the forelimbs, and skeletal variation and retardation. The authors conclude that there was a clear teratogenic effect in rabbits following maternal exposure to 450 ppm DMF and a marginal effect following exposure to 150 ppm DMF. A NOAEL of

50 ppm for fetal and maternal effects was reported. Inhalation exposure to 150 ppm was calculated by the authors to approximate a daily dose of 45 mg/kg/day, which coincides with previous work on this compound in this species.

VI. Derivation of Chronic Reference Exposure Level

<i>Study</i>	Cirla <i>et al.</i> , 1984; Catenacci <i>et al.</i> , 1984
<i>Study population</i>	Occupationally exposed workers
<i>Exposure method</i>	Discontinuous inhalation exposures
<i>Critical effects</i>	Digestive disturbances and slight hepatic changes
<i>LOAEL</i>	22 mg/m ³
<i>NOAEL</i>	Not observed
<i>Exposure continuity</i>	8 hr/day (10 m ³ /day), 5 days/week (assumed)
<i>Average occupational exposure</i>	7.9 mg/m ³ for LOAEL group (22 x 10/20 x 5/7)
<i>Human equivalent concentration</i>	7.9 mg/m ³
<i>Exposure duration</i>	5 years (mean exposure duration)
<i>LOAEL uncertainty factor</i>	3
<i>Subchronic uncertainty factor</i>	3
<i>Interspecies uncertainty factor</i>	1
<i>Intraspecies uncertainty factor</i>	10
<i>Cumulative uncertainty factor</i>	100
<i>Inhalation reference exposure level</i>	0.08 mg/m ³ (80 µg/m ³ , 0.03 ppm, 30 ppb)

The U.S. EPA (1994) based its RfC of 30 µg/m³ on the same study but included a Modifying Factor (MF) of 3 due to lack of reproductive toxicity data in the DMF database. The criteria for use of modifying factors are not well specified by U.S. EPA. Such modifying factors were not used by OEHHA. Intermediate uncertainty factors were used for LOAEL to NOAEL and subchronic to chronic extrapolation because of the mild nature of the effects observed and the less than chronic exposure duration.

For comparison Hellwig *et al.* (1991) found a developmental NOAEL of 50 ppm in rabbits exposed 6 hours per day on gestation days 8-20, equivalent to continuous exposure of 12.5 ppm. Multiplication by an RGDR of 1 and division by a UF of 30 (3 for interspecies and 10 for intraspecies) results in a REL estimate of 400 ppb. The NOAEL of 25 ppm for rats and mice in the chronic study of Malley *et al.* (1994) leads to a REL estimate of 150 ppb.

VII. Data Strengths and Limitations for Development of the REL

The major strength of the REL for N,N-dimethylformamide is the availability of human health effects data over several years of exposure. The major uncertainties are the difficulty in estimating exposure patterns and magnitude, the lack of a NOAEL observation, and the lack of complete reproductive and developmental toxicity data.

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CHRONIC TOXICITY SUMMARY

1,4-DIOXANE

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

CAS Registry Number: 123-91-1

I. Chronic Toxicity Summary

<i>Inhalation reference exposure level</i>	3,000 µg/m³ (800 ppb)
<i>Critical effects</i>	Liver, kidney, hematologic changes in rats
<i>Hazard index target(s)</i>	Alimentary system; kidney; circulatory system

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

<i>Description</i>	Colorless liquid with a faint, pleasant odor
<i>Molecular formula</i>	C ₄ H ₈ O ₂
<i>Molecular weight</i>	88.10 g/mol
<i>Boiling point</i>	101.5 °C
<i>Melting point</i>	11.8°C
<i>Vapor pressure</i>	37 torr @ 25°C
<i>Solubility</i>	Miscible with water, aromatic solvents, and oils
<i>Kow</i>	0.537
<i>Conversion factor</i>	3.60 µg/m ³ per ppb at 25°C

III. Major Uses and Sources

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

IV. Effects of Human Exposure

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

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

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

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

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

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

V. Effects of Animal Exposure

In rats, the major metabolite of dioxane is HEAA, which is excreted through the kidneys (Braun and Young, 1977). Exposure to dioxane by ingestion results in saturation of metabolism above

100 mg/kg given in single dose. Saturation of metabolism was also observed as low as 10 mg/kg if dioxane was administered in multiple doses. Dioxane itself is not cleared through the kidney. A decrease in metabolic clearance with increasing dose (iv) has been interpreted as the saturation of metabolism at the higher doses (Young *et al.*, 1978).

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

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

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

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

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

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

VI. Derivation of Chronic Reference Exposure Level (REL)

<i>Study</i>	Torkelson <i>et al.</i> (1974)
<i>Study populations</i>	Rats
<i>Exposure method</i>	Discontinuous inhalation
<i>Critical effects</i>	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 <i>et al.</i> 1974) and humans (Thiess, <i>et al.</i> , 1976, described by NIOSH, 1977).
<i>LOAEL</i>	Not observed in inhalation studies
<i>NOAEL</i>	111 ppm
<i>Exposure continuity</i>	7 h/d x 5 days/wk
<i>Average experimental exposure</i>	23 ppm (111 x 7/24 x 5/7)
<i>Human equivalent concentration</i>	23 ppm (gas with systemic effects, based on RGDR = 1.0 using default assumption that $\lambda(a) = \lambda(h)$)
<i>Exposure duration</i>	2 years
<i>LOAEL uncertainty factor</i>	1
<i>Subchronic exposure</i>	1
<i>Interspecies uncertainty factor</i>	3
<i>Intraspecies uncertainty factor</i>	10
<i>Cumulative uncertainty factor</i>	30
<i>Inhalation reference exposure level</i>	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**CHRONIC TOXICITY SUMMARY***EPICHLOROHYDRIN***(1-chloro-2,3-epoxy-propane)***CAS Registry Number: 106-89-8****I. Chronic Toxicity Summary**

<i>Inhalation reference exposure level</i>	3 $\mu\text{g}/\text{m}^3$ (0.8 ppb)
<i>Critical effects</i>	Histological changes in nasal turbinates in rats
<i>Hazard index target(s)</i>	Respiratory system; eyes

II. Physical and Chemical Properties (HSDB, 1997; CRC, 1994)

<i>Description</i>	Colorless liquid
<i>Molecular formula</i>	$\text{C}_3\text{H}_5\text{ClO}$
<i>Molecular weight</i>	92.52 g/mol
<i>Density</i>	1.181 g/cm ³ @ 20° C
<i>Boiling point</i>	117° C
<i>Melting point</i>	-26° C
<i>Vapor pressure</i>	13 torr @ 20° C
<i>Solubility</i>	Slightly soluble in water, soluble in most organic solvents
<i>Conversion factor</i>	1 ppm = 3.78 mg/m ³ @ 25° C

III. Major Uses and Sources

Epichlorohydrin is a major raw material used in the manufacture of epoxy and phenoxy resins. It is also used as a solvent and in the synthesis of glycerol. Other uses include that of insect fumigation and as a chemical intermediate for the formation of glycidyl acrylate derivatives such as those used in the formation of eyeglass lenses (HSDB, 1994). The annual statewide industrial emissions from facilities reporting under the Air Toxics Hot Spots Act in California based on the most recent inventory were estimated to be 4841 pounds of epichlorohydrin (CARB, 2000).

IV. Effects of Exposures to Humans

Studies of male reproductive function have shown no evidence of decreased sperm counts in populations occupationally exposed to epichlorohydrin (Milby *et al.*, 1981).

V. Effects of Exposures in Animals

Rats were exposed for 136 weeks (6 hours/day, 5 days/week) to 0, 10, 30, or 100 ppm (0, 38, 113, or 380 mg/m³) epichlorohydrin (Laskin *et al.*, 1980). Kidney damage in the form of renal tubular degeneration and dilatation was observed in rats exposed to 30 ppm or greater. The observation of severe inflammation in the nasal passages of 90% of the control animals, as well as in the treated animals, prevented comparison of this effect between the two groups.

A subchronic exposure of rats to 9, 17, 27, 56, or 120 ppm (34, 64, 102, 212, or 454 mg/m³) for 6 hours/day, 5 days/week for 11-19 exposures showed evidence of extrarrespiratory effects. These included liver congestion and necrosis and tubular atrophy in the kidneys at the highest concentration (Gage, 1959). Lethargy and weight loss were observed at 56 ppm.

A study on the effects of epichlorohydrin exposure for 10 weeks (6 hours/day, 5 days/week) on male and female fertility in rats and rabbits showed that male rats, exposed to 50 ppm (189 mg/m³), were significantly less fertile than controls, as measured by successful matings to unexposed females (John *et al.*, 1979; 1983a). No histological changes were observed in the testes of the male rats at the end of exposure. No significant effects on fertility occurred in the exposed female rats. Degenerative changes in the nasal epithelium were observed in the female rats exposed to 25 ppm (94.5 mg/m³), and in both sexes at 50 ppm.

A teratology study was carried out in rats and rabbits exposed to 0, 2.5, or 25 ppm (0, 9.5, or 95 mg/m³) epichlorohydrin 7 hours/day during the critical days of gestation. There were no significant differences between controls and treated animals in the incidence of developmental defects, in maternal toxicity, or in histopathology of the lungs, nasal turbinates, or trachea (John *et al.*, 1983b).

Mice and rats (10/sex/concentration/strain) were exposed to 0, 5, 25, or 50 ppm (0, 19, 95, or 190 mg/m³) epichlorohydrin for 6 hours/day, 5 days/week for 90 days (Quast *et al.*, 1979). Animals were observed for clinical signs of toxicity and were measured biweekly for body weight changes. Body weight measurements, clinical chemistry, hematology, and urinalysis were conducted. Gross and histopathological examinations were performed at the end of the experiment. Exposures of rats to 25 and 50 ppm epichlorohydrin resulted in inflammation, focal erosions, hyperplasia, and metaplasia in the nasal turbinates. No adverse effects were observed in rats exposed to 5 ppm (19 mg/m³). Mice similarly showed focal erosion, hyperplasia and metaplasia in the epithelium of the nasal turbinates when exposed to 25 ppm epichlorohydrin or greater.

VI. Derivation of Chronic Reference Exposure Level

<i>Study</i>	Quast <i>et al.</i> (1979)
<i>Study population</i>	Rats and mice (10 per sex per concentration)
<i>Exposure method</i>	Discontinuous whole-body inhalation
<i>Critical effects</i>	Inflammation, focal erosions, hyperplasia, and metaplasia in the nasal turbinates
<i>LOAEL</i>	25 ppm (94.5 mg/m ³)
<i>NOAEL</i>	5 ppm (19 mg/m ³)
<i>Exposure continuity</i>	6 hours/day, 5 days/week
<i>Exposure duration</i>	90 days
<i>Average experimental exposure</i>	0.89 ppm (5 x 6/24 x 5/7)
<i>Human equivalent concentration</i>	0.083 ppm (gas with extrathoracic respiratory effects, RGDR = 0.093, based on MVA = 0.14 m ³ /day, MVh = 20 m ³ /day, SAa(ET) = 15 cm ² , SAh(ET) = 200 cm ²)
<i>LOAEL uncertainty factor</i>	1
<i>Subchronic uncertainty factor</i>	3
<i>Interspecies uncertainty factor</i>	3
<i>Intraspecies uncertainty factor</i>	10
<i>Cumulative uncertainty factor</i>	100
<i>Inhalation reference exposure level</i>	0.0008 ppm (0.8 ppb; 0.003 mg/m ³ ; 3 µg/m ³)

The U.S. EPA (1994) based its RfC of 1 µg/m³ on the same study but used a subchronic UF of 10 for a 90 day study instead of 3 (OEHHA, 2000).

VII. Data Strengths and Limitations for Development of the REL

The strengths of the inhalation REL for epichlorohydrin include the availability of controlled exposure inhalation studies in multiple species at multiple exposure concentrations and with adequate histopathological analysis and the observation of a NOAEL. Major areas of uncertainty are the lack of adequate human exposure data, the lack of chronic inhalation exposure studies, the limited reproductive toxicity data, and the small groups tested in the study.

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CHRONIC TOXICITY SUMMARY

1,2-EPOXYBUTANE

(1-butene oxide; 1,2-butene oxide; 1,2-butylene oxide; 1,2-epoxybutane; 2-ethyloxirane; ethylethylene oxide; NCI-C55527)

CAS Registry Number: 106-88-7

I. Chronic Toxicity Summary

<i>Inhalation reference exposure level</i>	20 µg/m³ (6 ppb)
<i>Critical effect(s)</i>	Degenerative lesions of the nasal cavity in mice
<i>Hazard index target(s)</i>	Respiratory system; cardiovascular system

II. Physical and Chemical Properties (HSDB, 1997)

<i>Description</i>	Colorless liquid with disagreeable odor
<i>Molecular formula</i>	C ₄ H ₈ O
<i>Molecular weight</i>	72.12 g/mol
<i>Density</i>	0.837 g/cm ³ @ 17°C
<i>Boiling point</i>	63.3°C
<i>Melting point</i>	Not available (CRC, 1994)
<i>Vapor pressure</i>	176 torr @ 25°C
<i>Solubility</i>	Soluble in ethanol, ether, acetone, water
<i>Odor threshold</i>	Unknown
<i>Conversion factor</i>	1 ppm = 2.95 mg/m ³

III. Major Uses or Sources

1,2-Epoxybutane is used as a chemical intermediate, acid scavenger, and stabilizer for chlorinated solvents (Reprotex, 1994). It is highly reactive, flammable, and undergoes exothermic polymerization reactions in the presence of acids, bases, and some salts. It is less volatile than ethylene oxide or propylene oxide (Reprotex, 1994). The annual statewide industrial emissions from facilities reporting under the Air Toxics Hot Spots Act in California based on the most recent inventory were estimated to be 6105 pounds of 1,2-epoxybutane (CARB, 2000).

IV. Effects of Human Exposure

No human toxicological data were found for 1,2-epoxybutane.

V. Effects of Animal Exposure

F344/N rats (50/sex) were exposed to 0, 200, or 400 ppm EBU for 6 hours/day, 5 days/week for 2 years (NTP, 1988). Survival was impaired and concentration-related increases of inflammation, respiratory epithelial hyperplasia, olfactory sensory epithelial atrophy, and hyperostosis of the nasal turbinate bone cavity were observed in male and female rats exposed to either concentration.

B6C3F1 mice (50/sex) were exposed to 0, 50, or 100 ppm EBU for 6 hours/day, 5 days/week for 2 years (NTP, 1988). Survival and body weight gain were reduced significantly at 100 ppm in both sexes. Significant concentration-related increases in incidence of chronic inflammation, epithelial hyperplasia, and erosion of the nasal cavity were noted in both sexes at either concentration. Increases in granulocytic hyperplasia and splenic hematopoiesis were noted at both concentrations in female mice.

Number of mice with lesions in the nasal cavity and olfactory sensory epithelium (NTP, 1988)

Sex	Males			Females		
EBU concentration	0 ppm	50 ppm	100 ppm	0 ppm	50 ppm	100 ppm
Number of mice studied	49	49	50	50	50	48
Nasal cavity						
Chronic inflammation	0	33	40	0	39	44
Erosion	0	7	17	0	16	24
Regeneration	0	15	17	0	14	15
Epithelial hyperplasia	0	32	45	1	34	35
Squamous metaplasia	1	24	41	0	34	41
Squam. cell papilloma	0	0	1	0	0	0
Olfactory sensory epithelium – atrophy	0	13	32	0	25	35

Male and female mice exposed to 800 ppm (2360 mg/m³) EBU for 6 hours/day, 5 days/week, for 13 weeks were listless after the first exposure (NTP, 1988). Animals from this group all died by the end of the 13-week exposure. Renal tubular necrosis, and thymic and splenic atrophy were seen in mice exposed to 800 ppm; decreased liver weights were observed following exposure of mice to 400 ppm (1180 mg/m³) or more. Inflammation of the nasal turbinates was seen in female mice exposed to 100 ppm (295 mg/m³) or more. No inflammation was observed in controls.

Miller *et al.* (1981) exposed rats and mice of either sex to 0, 75, 150, or 600 ppm (0, 221, 442, or 1770 mg/m³) EBU 6 hours/day, 5 days/week, for 13 weeks. In this study, no treatment-related effects were noted except for histological lesions in the nasal mucosal epithelium and reduced specific gravity in the urine of rats treated with 600 ppm.

Wolf (1961) observed increased lung weights in rats exposed to 800 ppm of a mixture of epoxybutane isomers. No increase in lung weight was seen at 400 ppm.

Sikov *et al.* (1981) conducted experiments to determine the reproductive toxicity of EBU in rats and rabbits. Rats were exposed to 0, 250, or 1000 ppm (0, 738, or 2950 mg/m³) 1,2-epoxybutane for 7 hours/day, 5 days/week for 3 weeks prior to gestation, or for 7 hours/day on days 1-19 of gestation. Maternal toxicity in the form of 10% weight loss was observed in rats exposed to 1000 ppm. One death out of 42 occurred in the dams exposed to 1000 ppm. No adverse histological, reproductive, or developmental effects were seen at any concentration. Exposure of rabbits on days 1-24 of gestation to the same concentrations as in the rat experiment showed more severe effects at lower concentrations than those observed in rats. In the rabbits, 6 out of 48 dams died during exposure to 250 ppm, and 14 out of 24 died at 1000 ppm. Extensive maternal mortality in this study prevented evaluation of the reproductive and developmental effects.

VI. Derivation of Chronic Reference Exposure Level

<i>Study</i>	National Toxicology Program (NTP, 1988)
<i>Study population</i>	Rats and mice
<i>Exposure method</i>	Discontinuous inhalation to 0, 50, or 100 ppm EBU
<i>Critical effects</i>	Damage to the upper respiratory epithelium was observed in both species at all concentrations. Mice also showed an increased incidence of granulocytic hyperplasia and splenic hematopoiesis at both concentrations, possibly due to inflammation in the upper respiratory tract.
<i>LOAEL</i>	50 ppm (mice)
<i>NOAEL</i>	Not observed
<i>Exposure continuity</i>	6 hours/day, 5 days/week
<i>Exposure duration</i>	2 years
<i>Average experimental exposure</i>	8.9 ppm for LOAEL group (50 x 6/24 x 5/7)
<i>Human equivalent concentration</i>	1.8 ppm for LOAEL group (gas with extrathoracic respiratory effects, RGDR = 0.20, based on MVa = 0.06 m ³ /day, MVh = 20 m ³ /day, SAa(ET) = 3.0 cm ² , SAh(ET) = 200 cm ²)
<i>LOAEL uncertainty factor</i>	10 (high incidence of adverse effects)
<i>Subchronic uncertainty factor</i>	1
<i>Interspecies uncertainty factor</i>	3
<i>Intraspecies uncertainty factor</i>	10
<i>Cumulative uncertainty factor</i>	300
<i>Inhalation reference exposure level</i>	0.006 ppm (6 ppb; 0.02 mg/m ³ ; 20 µg/m ³)

The chronic REL is also the U.S. EPA RfC (U.S. EPA, 1994). OEHHA staff reviewed and agreed with U.S. EPA's analysis of the data.

VII. Data Strengths and Limitations for Development of the REL

The strengths of the inhalation REL for 1,2-epoxybutane include the availability of controlled exposure inhalation studies in multiple species at multiple exposure concentrations and with adequate histopathological analysis. Major areas of uncertainty are the lack of adequate human exposure data and the lack of observation of a NOAEL in the key study.

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CHRONIC TOXICITY SUMMARY

ETHYL CHLORIDE*(Chloroethane; monochloroethane; ether hydrochloric)***CAS Registry Number: 75-00-3****I. Chronic Toxicity Summary**

<i>Inhalation reference exposure level</i>	30,000 µg/m³ (10,000 ppb)
<i>Critical effect(s)</i>	Delayed fetal ossification in mice
<i>Hazard index target(s)</i>	Teratogenicity; alimentary system

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

<i>Description</i>	Colorless gas
<i>Molecular formula</i>	C ₂ H ₅ Cl
<i>Molecular weight</i>	64.52
<i>Density</i>	0.9214 g/cm ³ @ 0°C
<i>Boiling point</i>	12.3 °C
<i>Melting point</i>	-138.7 °C
<i>Vapor pressure</i>	1000 torr @ 20 °C
<i>Conversion factor</i>	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/m³ 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/m³ (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/m³ (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/m³).

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/m³) 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

<i>Study</i>	Scortichini <i>et al.</i> , 1986
<i>Study population</i>	Mice
<i>Exposure method</i>	Discontinuous whole-body inhalation (on days 6-15 of gestation)
<i>Critical effects</i>	Delayed ossification of skull foramina
<i>LOAEL</i>	13,000 mg/m ³
<i>NOAEL</i>	4,000 mg/m ³
<i>Exposure continuity</i>	6 hours per day
<i>Exposure duration</i>	Days 6-15 of gestation
<i>Average experimental exposure</i>	1,000 mg/m ³ for NOAEL group (4000 x 6/24)
<i>Human equivalent concentration</i>	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))
<i>LOAEL uncertainty factor</i>	1
<i>Subchronic uncertainty factor</i>	1
<i>Interspecies uncertainty factor</i>	3
<i>Intraspecies uncertainty factor</i>	10
<i>Cumulative uncertainty factor</i>	30
<i>Inhalation reference exposure level</i>	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/m³. 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 histopathological 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 TOXICITY SUMMARY***ETHYLBENZENE***(Phenylethane; NCI-C56393)***CAS Registry Number: 100-41-4****I. Chronic Toxicity Summary**

<i>Inhalation reference exposure level</i>	2000 µg/m³ (400 ppb)
<i>Critical effect(s)</i>	Liver, kidney, pituitary gland in mice and rats
<i>Hazard index target(s)</i>	Alimentary system (liver); kidney; endocrine system

II. Physical and Chemical Properties (HSDB, 1994)

<i>Description</i>	colorless liquid
<i>Molecular formula</i>	C ₈ H ₁₀
<i>Molecular weight</i>	106.16 g/mol
<i>Boiling point</i>	136.2°C
<i>Melting point</i>	-95°C
<i>Vapor pressure</i>	10 torr @ 25.9°C
<i>Density</i>	0.867 g/cm ³ @ 20°C
<i>Solubility</i>	Soluble in ethanol and ether, low solubility in water (0.014 g/100 ml at 15°C)
<i>Conversion factor</i>	1 ppm = 4.35 mg/m ³

III. Major Uses or Sources

Ethylbenzene is used as a precursor in the manufacture of styrene (HSDB, 1994). It is also used in the production of synthetic rubber, and is present in automobile and aviation fuels. It is found in commercial xylene (Reprotext, 1994). In 1996, the latest year tabulated, the statewide mean outdoor monitored concentration of ethylbenzene was approximately 0.4 ppb (CARB, 1999a). The latest annual statewide emissions from facilities reporting under the Air Toxics Hot Spots Act in California, based on the most recent inventory, were estimated to be 161,846 pounds of ethylbenzene (CARB, 1999b).

IV. Effects of Human Exposure

Studies on the effects of workplace exposures to ethylbenzene have been complicated by concurrent exposures to other chemicals, such as xylenes (Angerer and Wulf, 1985). Bardodej

and Cirek (1988) reported no significant hematological or liver function changes in 200 ethylbenzene production workers over a 20-year period.

V. Effects of Animal Exposure

Rats and mice (10/sex/group) were exposed to 0, 100, 250, 500, 750, and 1000 ppm (0, 434, 1086, 2171, 3257, and 4343 mg/m³) ethylbenzene 6 hours/day, 5 days/week for 90 days (NTP, 1988; 1989; 1990). Rats displayed significantly lower serum alkaline phosphatase in groups exposed to 500 ppm or higher. Dose-dependent increases in liver weights were observed in male rats beginning at 250 ppm, while this effect was not seen until 500 ppm in the females. An increase in relative kidney weights was seen in the 3 highest concentrations in both sexes. Minimal lung inflammation was observed in several of the treatment groups, but this phenomenon was attributed to the presence of an infectious agent rather than to ethylbenzene exposure. The mice in this study did not show any treatment-related effects except for elevated liver and kidney weights at 750 and 1000 ppm, respectively.

Rats and mice were exposed to ethylbenzene (greater than 99% pure) by inhalation for 2 years (NTP, 1999; Chan *et al.*, 1998). Groups of 50 male and 50 female F344/N rats were exposed to 0, 75, 250, or 750 ppm, 6 hours per day, 5 days per week, for 104 weeks. Survival of male rats in the 750 ppm group was significantly less than that of the chamber controls. Mean body weights of 250 and 750 ppm males were generally less than those of the chamber controls beginning at week 20. Mean body weights of exposed groups of females were generally less than those of chamber controls during the second year of the study. In addition to renal tumors, the incidence of renal tubule hyperplasia in 750 ppm males was significantly greater than that in the chamber controls. The severity of nephropathy in 750 ppm male rats was significantly increased relative to the chamber controls. Some increases in incidence and severity of nephropathy were noted in all exposed female rats, but these were statistically significant only at 750 ppm.

Groups of 50 male and 50 female B6C3F1 mice were exposed to 0, 75, 250, or 750 ppm ethylbenzene by inhalation, 6 hours per day, 5 days per week, for 103 weeks. Survival of exposed mice was similar to controls. Mean body weights of females exposed to 75 ppm were greater than those of the chamber controls from week 72 until the end of the study. In addition to lung and liver tumors, the incidence of eosinophilic liver foci in 750 ppm females was significantly increased compared to that in the chamber controls. There was a spectrum of nonneoplastic liver changes related to ethylbenzene exposure in male mice, including syncytial alteration of hepatocytes, hepatocellular hypertrophy, and hepatocyte necrosis. The incidences of hyperplasia of the pituitary gland pars distalis in 250 and 750 ppm females and the incidences of thyroid gland follicular cell hyperplasia in 750 ppm males and females were significantly increased compared to those in the chamber control groups. Based on an evaluation of all the non-cancer data in mice and rats OEHHA staff selected 75 ppm as the NOAEL for the NTP (1999) study.

Rats (17-20 per group) were exposed to 0, 600, 1200, or 2400 mg/m³ for 24 hours/day on days 7 to 15 of gestation (Ungvary and Tatrai, 1985). Developmental malformations in the form of “anomalies of the uropoietic apparatus” were observed at the 2400 mg/m³ concentration.

Skeletal retardation was observed in all exposed groups compared with controls. The incidence of skeletal abnormalities increased with higher concentrations of ethylbenzene.

Rabbits exposed by these investigators to the same concentrations as the rats on days 7 to 15 of gestation, exhibited maternal weight loss with exposure to 1000 mg/m³ ethylbenzene. There were no live fetuses in this group for which abnormalities could be evaluated. No developmental defects were observed in the lower exposure groups.

Rats (78-107 per group) and rabbits (29-30 per group) were exposed for 6 or 7 hours/day, 7 days/week, during days 1-19 and 1-24 of gestation, respectively, to 0, 100, or 1000 ppm (0, 434, or 4342 mg/m³) ethylbenzene (Andrew *et al.*, 1981; Hardin *et al.*, 1981). No effects were observed in the rabbits for maternal toxicity during exposure or at time of necropsy. Similarly, no effects were seen in the fetuses of the rabbits. The only significant effect of ethylbenzene exposure in the rabbits was a reduced number of live kits in the 1000 ppm group. A greater number and severity of effects were seen in rats exposed to 1000 ppm ethylbenzene. Maternal rats exposed to 1000 ppm exhibited significantly increased liver, kidney, and spleen weights compared with controls. Fetal rats showed an increase in skeletal variations at the 1000 ppm concentration, but the results of the 100 ppm exposure were not conclusive.

Clark (1983) found no significant effects on body weight, food intake, hematology, urinalysis, organ weights or histopathology in rats (18 per group) exposed to 100 ppm (434 mg/m³) ethylbenzene for 6 hours/day, 5 days/week, for 12 weeks.

Degeneration of the testicular epithelium was noted in guinea pigs and a rhesus monkey exposed to 600 ppm (2604 mg/m³) for 6 months (Wolf *et al.*, 1956). No effects were reported for female monkeys exposed to the same conditions.

Cragg *et al.* (1989) exposed mice and rats (5/sex/group) to 0, 99, 382, and 782 ppm (0, 430, 1659, and 3396 mg/m³) 6 hours/day, 5 days/week for 4 weeks. Some evidence of increased salivation and lacrimation was seen in the rats exposed to 382 ppm. No other gross signs of toxicity were observed. Both male and female rats had significantly enlarged livers following exposure to 782 ppm. Female mice also showed a significant increase in liver weight at this concentration. No histopathological lesions were seen in the livers of these mice.

Dose-dependent induction of liver cytochrome P450 enzymes in rats by ethylbenzene was observed by Elovaara *et al.* (1985). Rats (5 per group) were exposed to 0, 50, 300, or 600 ppm (0, 217, 1302, or 2604 mg/m³) ethylbenzene for 6 hours/day, 5 days/week for 2, 5, 9, or 16 weeks. Cytochrome P450 enzyme induction, and microscopic changes in endoplasmic reticulum and cellular ultrastructure were evident at all ethylbenzene concentrations by week 2, and persisted throughout the exposure. Liver weights were not elevated in these studies.

VI. Derivation of the Chronic Reference Exposure Level

<i>Study</i>	NTP, 1999; Chan <i>et al.</i> , 1998
<i>Study population</i>	Male and female rats and mice (50 per group)
<i>Exposure method</i>	Discontinuous inhalation
<i>Critical effects</i>	Nephrotoxicity, body weight reduction (rats) hyperplasia of the pituitary gland; liver cellular alterations and necrosis (mice)
<i>LOAEL</i>	250 ppm
<i>NOAEL</i>	75 ppm
<i>Exposure continuity</i>	6 hours/day, 5 days/week
<i>Exposure duration</i>	103 weeks.
<i>Average experimental exposure</i>	13 ppm for NOAEL group
<i>Human equivalent concentration</i>	13 ppm for NOAEL group (gas with systemic effects, based on RGDR = 1.0 using default assumption that lambda (a) = lambda (h))
<i>LOAEL uncertainty factor</i>	1
<i>Subchronic uncertainty factor</i>	1
<i>Interspecies uncertainty factor</i>	3
<i>Intraspecies uncertainty factor</i>	10
<i>Cumulative uncertainty factor</i>	30
<i>Inhalation reference exposure level</i>	0.4 ppm (400 ppb; 2 mg/m ³ ; 2,000 µg/m ³)

The REL is based on a lifetime toxicity/carcinogenesis study. The NOAEL for non-neoplastic effects in the study was 75 ppm, and the LOAEL was 250 ppm. Some shorter duration studies discussed above (e.g. NTP, 1988, 1989, 1990) identify higher concentrations as NOAELs, but the study used (NTP 1999) is the most recent available and is considered the most reliable for assessing chronic effects.

U.S. EPA based its RfC on developmental toxicity studies in rats and rabbits (Andrew *et al.*, 1981; Hardin *et al.*, 1981; U.S. EPA, 1994). The NOAEL in the studies was 100 ppm, and the LOAEL was 1000 ppm. In accordance with its methodology, U.S. EPA did not use a time-weighted average concentration for the discontinuous exposure experiment since the key effect was developmental toxicity. If OEHHA methodology is followed (which includes the time-weighted averaging of the exposure concentrations, and uncertainty factors of 3 (interspecies, with RGDR = 1) and 10 (intraspecies), this study would indicate a REL of 0.6 ppm (3 mg/m³). The study by Ungvary and Tatrai (1985) reported a NOAEL of 600 mg/m³ for developmental and maternal effects in several species. However, the reporting and general quality of this paper create less confidence in its results.

For comparison to the proposed REL of 0.4 ppm, Clark (1983) found no significant effects in rats exposed to 100 ppm ethylbenzene 6 h/day, 5 d/week, for 12 weeks. This NOAEL can be time-adjusted to 18 ppm, then divided by a subchronic UF of 3, an interspecies UF of 3, and an intraspecies UF of 10 which results in a REL of 0.2 ppm. (The default value of 1 for RGDR was used). It appears that the proposed REL provides a sufficient margin of safety to provide

protection against the reported developmental effects (Andrew *et al.*, 1981; Hardin *et al.*, 1981; Ungvary and Tatrai, 1985)

VII. Data Strengths and Limitations for Development of the REL

The strengths of the inhalation REL for ethylbenzene include the availability of controlled exposure inhalation studies in multiple species at multiple exposure concentrations and with adequate histopathological analysis, and the observation of a NOAEL in lifetime chronic inhalation exposure studies. The major area of uncertainty is the lack of adequate human exposure data.

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CHRONIC TOXICITY SUMMARY

ETHYLENE DIBROMIDE

(1,2-dibromoethane; dibromoethane; alpha, beta-dibromoethane; EDB; ethylene bromide; glycol bromide)

CAS Registry Number: 106-93-4

I. Chronic Toxicity Summary

<i>Inhalation reference exposure level</i>	0.8 µg/m³ (0.1 ppb)
<i>Critical effect(s)</i>	Decreased sperm count/ejaculate, decreased percentage of viable and motile sperm, increased semen pH, and increased proportion of sperm with specific morphological abnormalities in human males
<i>Hazard index target(s)</i>	Reproductive system

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

<i>Description</i>	Colorless, heavy, nonflammable liquid with a mildly sweet, chloroform-like odor.
<i>Molecular formula</i>	C ₂ H ₄ Br ₂
<i>Molecular weight</i>	187.88 g/mol
<i>Boiling point</i>	131-132°C
<i>Melting point</i>	9.9°C
<i>Vapor pressure</i>	0.11 torr at 20°C
<i>Solubility</i>	Slightly soluble in water (3400 mg/L water at 20°C). Miscible with most organic solvents.
<i>Conversion factor</i>	7.68 µg/m ³ per ppb at 25°C

III. Major Uses and Sources

Ethylene dibromide (EDB) is used as a solvent for resins, gums, and waxes, and as a chemical intermediate in the synthesis of dyes and pharmaceuticals (HSDB, 1995). EDB was once widely used as a fumigant for the control of pests in the U.S. Because of concerns regarding its carcinogenicity, the agricultural uses of EDB were banned in 1983 (RECT, 1988). EDB was also commonly used as a gasoline additive to scavenge inorganic lead compounds. The transition to the use of lead-free gasoline has drastically curtailed the use of EDB in this country (REPROTOX, 1995). EDB is now used mainly in industry. EDB may be formed naturally in

the ocean as a result of macro algae growth. Exposure to the general population, via inhalation, may occur in the vicinity of industries and in industrial settings where this compound is manufactured and used. The annual statewide industrial emissions from facilities reporting under the Air Toxics Hot Spots Act in California based on the most recent inventory were estimated to be 1179 pounds of EDB (CARB, 2000).

IV. Effects of Human Exposures

Pharmacokinetic studies of EDB in humans could not be found in the literature. However, *in vitro* studies of EDB metabolism in human liver samples have been performed (Wiersma *et al.*, 1986). These experiments have shown that the enzyme systems known to metabolize EDB in rodent liver also metabolize EDB in the human liver. EDB was metabolized by human liver cytosolic glutathione S-transferases (GST), microsomal GST, and microsomal mixed function oxidases (MFO). MFO activity resulted in adducts irreversibly bound to protein, while GST activity was mostly responsible for adducts irreversibly bound to DNA. Rodent liver enzymes similarly activate EDB to metabolites that bind to cellular macromolecules. In human fetal liver (16-18 weeks gestation) cytosolic GST was also found to metabolize EDB with high efficiency (Kulkarni *et al.*, 1992). Since detoxification via MFO activity may be limited at this stage of development, the results suggest that the human fetus and neonate may be at greater risk from EDB toxicity than adults.

A study of mortality from cancer and respiratory diseases was conducted among 161 employees exposed to EDB in 2 production units operated from 1942 to 1969 and from the mid-1920s to 1976, respectively (Ott *et al.*, 1980). No apparent connection was found between mortality due to respiratory diseases and exposure to EDB, when compared to U.S. white male mortality figures.

Due to the structural similarity of EDB to dibromochloropropane (DBCP), a known toxic agent in human male reproductive organs, a number of epidemiological studies concerning male reproduction and spermatogenesis were conducted.

In a study of 59 employees exposed to EDB at the Ethyl Corporation plant in Magnolia, Arkansas, the sperm counts of the exposed men were divided into 2 groups depending on estimated exposure (Ter Haar, 1980). Twenty percent of the low exposure group (<0.5 ppm) had sperm counts below 40 million, whereas 42% of the high exposure group (0.5 to 5 ppm) had sperm counts below this figure. The sperm counts were intermediate between counts reported for 2 types of U.S. samples (for normal men). The observed births among the two exposure groups were found to be similar to the number of expected births. The author determined that EDB had no effect on sterility or reproduction in the workers. Weaknesses of this study include the small population of exposed workers and the lack of a concurrent unexposed control group. Taking these defects of the study into account, Dobbins (1987) concluded that the results provide evidence that EDB exposure between 0.5 and 5.0 ppm is associated with lower sperm counts.

A comparison of observed marital fertility with expected fertility (based on U.S. fertility rates) was conducted among 297 men working at 4 U.S. plants that manufacture EDB (Wong *et al.*,

1979). Fertility was 20% below expected for the four plants combined. This was largely due to one plant (plant D), which was 49% below the expected level. After omitting the incidence of vasectomies and hysterectomies among married couples, observed fertility was still 39% below the expected figure for plant D but was now no longer statistically significant. Exposure levels of EDB at plant D were not known but were estimated to be no more than 5 ppm. Later review determined that expected (control) levels of fertility and the power of the study were too low, resulting in the inability to identify a possible adverse effect (Dobbins, 1987). The lower fertility at plant D indicates that EDB has the potential to reduce fertility, but the extent of the reduction cannot be estimated from this study. Further treatment of the data by a method that uses the proper statistical adjustments of reproductive experience in the U.S. population (used as the control) suggests borderline significance for reduced fertility among the combined workers at the four plants (Wong *et al.*, 1985). The fertility evaluation indicates that more in-depth epidemiologic or physiologic studies are needed.

Semen analysis of 83 pineapple workers at two plantations was performed by Rogers and associates (1981). EDB-exposed workers were removed from each group and placed in a separate group. The remaining two groups of workers acted as control groups. Sperm counts, motility, and morphology were similar among the three groups. However, 43.8% of exposed workers had abnormally low counts (<40 million/ml), while abnormally low sperm counts of controls were 34.2% and 17.8%. Of the four exposed workers that had fertility tests done, all tested in the infertile range. Forty percent or less tested in the infertile range among the control groups. The results suggest that workers exposed to EDB had reduced sperm counts, but exposure levels were not known.

Semen analysis among 46 men employed in the papaya fumigation industry was conducted to determine if EDB affected semen quality (Ratcliff *et al.*, 1987; Schrader *et al.*, 1987). Average duration of exposure was 5 years and the geometric mean breathing zone exposure to airborne EDB was 88 ppb (8 hr time weighted average) with peak exposures of up to 262 ppb. The comparison group consisted of 43 unexposed men from a nearby sugar refinery. Following consideration of confounding factors, statistically significant decreases in sperm count/ejaculate, the percentage of viable and motile sperm, and increases in the proportion of sperm with specific morphological abnormalities (tapered heads, absent heads, and abnormal tails) were observed among exposed men. Semen pH was significantly more alkaline than that of unexposed workers. Other measured sperm quality parameters were unchanged. This study suggests that EDB can result in reproductive impairment. However, no measurement of male fertility was conducted.

In a study that examined similar indices of semen quality, 6 week exposure of 10 forestry workers to EDB (60 ppb time weighted average, with peak exposures of up to 2165 ppb) resulted in decreased semen volume and slower sperm velocity (Schrader *et al.*, 1988). Six unexposed men were used as controls. The researchers suggest that short-term exposure to EDB results in decreased sperm velocity, while long-term exposure, as in the previous study of EDB-exposed papaya workers, results in sperm immotility and cell death.

V. Effects of Animal Exposures

EDB is readily and rapidly absorbed from the lung when breathed as a vapor, from the GI tract when taken orally, or through the skin when applied dermally (HSDB, 1995). In rats, the rate of absorption of EDB from the respiratory tract reached a plateau within 10 to 20 minutes following exposure to 75 ppm EDB for up to 2 hours (Stott and McKenna, 1984). About 58% of the EDB was absorbed. Intraperitoneal injection of [¹⁴C]EDB into guinea pigs resulted in the highest concentrations in liver, kidneys, and adrenals (Plotnick and Conner, 1976). Sixty-five percent of the dose was excreted as metabolites in urine, 3% in feces, and 12% excreted unchanged in expired air. In rats, the highest concentrations of [¹⁴C]EDB label were found in liver, kidney and spleen following an oral dose of 15 mg/kg body wt (Plotnick *et al.*, 1979). Studies with rats have provided evidence that 2 pathways of metabolic bioactivation exist for EDB (RECT, 1988). The oxidative pathway yields the metabolite 2-bromo-acetaldehyde, which is associated with cell macromolecule binding and liver damage. The conjugative pathway principally yields glutathione products, such as *S*-(2-bromoethyl)-glutathione, which are mainly responsible for DNA binding and mutagenesis. In rats, orally administered EDB is excreted primarily in the urine as mercapturic acid derivatives (Jones and Edwards, 1968). The biologic half-life for elimination of [¹⁴C]EDB in rats is 5.1-5.6 hours (Watanabe *et al.*, 1978) and less than 48 hours in mice and guinea pigs (HSDB, 1995). Besides the small amount irreversibly bound to cell macromolecules and DNA, EDB shows little, if any, bioaccumulation in mammalian systems.

In a subchronic toxicity study of experimental animals, rats and guinea pigs were given EDB by oral administration for about 4 months (Aman *et al.*, 1946). Body weights and mortality of animals at or below an average daily dose of 40-50 mg/kg body wt-day were unaffected. However, only one control animal/species was used, the dosing regimen was not well described, and pathologic examination was apparently not performed.

Subchronic exposure of rats (20/sex/group) to 50 ppm EDB for as many as 63 seven-hour exposures in 91 days resulted in no significant change in body weights (Rowe *et al.*, 1952). Liver and kidney weights were increased in both sexes while testis weights were decreased in males. Also, lung weights in males were elevated and spleen weights in females were decreased. Histopathological examination revealed no changes. Guinea pigs (8/sex/group) subjected to as many as 57 seven-hour exposures of 50 ppm EDB in 80 days exhibited reduced body weights. Organ weights were unchanged, but microscopic examination of the livers showed slight central fatty degeneration. In kidneys, slight interstitial congestion and edema with slight parenchymatous degeneration of the tubular epithelium were observed. Four rabbits exposed to 59 seven-hour sessions at 50 ppm in 84 days showed no signs of adverse effects. Clinical signs of monkeys exposed to 50 ppm EDB (49 seven-hour exposures in 70 days) included an ill, unkempt appearance and nervousness. Slight central fatty degeneration in livers was observed, but pathology was not seen in other tissues. Exposure of the same four species to 25 ppm EDB for up to 220 days (145 to 156 seven-hour exposures) showed no signs of adverse effects.

In a 13-week inhalation study, 5 Fischer 344 albino rats/group/sex and 10 B6C3F1 mice/group/sex were exposed to 0, 3, 15, or 75 ppm EDB for 6 hr/day, 5 days/week (Reznik *et al.*, 1980). At 75 ppm, rats and mice exhibited severe necrosis and atrophy of the olfactory epithelium in the nasal cavity. Squamous metaplasia, hyperplasia and cytomegaly of the

epithelium were also seen in nasal turbinates, larynx, trachea, bronchi, and bronchioles. Minor alterations were seen in the nasal cavity of only a few male and female rats at 15 ppm. No compound-related lesions were observed in the olfactory and respiratory epithelium at 3 ppm. No lesions were seen in other tissues at any dose.

In another 13-week inhalation study, 40 male and 20 female CDF(F344) rats/group were exposed to 0, 3, 10, or 40 ppm EDB 6 hr/day, 5 days/week (Nitschke *et al.*, 1981). Male rats in the 40 ppm group exhibited decreased weight gain throughout most of the exposure period. However, reduced weight gain was never more than 6-8% below control levels. With the exception of decreased specific gravity of urine in females of the 40 ppm group, no treatment-related changes were observed in any rat group with respect to urinalysis, hematology, and clinical chemistry. At the end of 13 weeks, relative liver and kidney weights of males exposed to 40 ppm EDB were significantly elevated, while relative liver weights of females in the two highest exposure groups were significantly elevated. Absolute liver weight of females in the 40 ppm group was also significantly elevated. Histopathological examination revealed lesions primarily confined to the anterior sections of the nasal turbinates. Hyperplasia and nonkeratinizing squamous metaplasia of the respiratory epithelium were observed in nasal turbinates of rats exposed to 40 ppm EDB. Only slight epithelial hyperplasia of nasal turbinates was noted at 10 ppm. No treatment related effects were seen at 3 ppm. Livers of females in the 40 ppm group showed a slight increase in fat. After an 88 day recovery period, there was a reversion to normal of the nasal turbinates in all but one rat.

In what was originally scheduled to be a lifetime exposure study, 50 Osborne-Mendel rats/group/sex and 50 B6C3F1 mice/group/sex were administered EDB 5 days/week by gastric lavage over a substantial portion of their life-span (NCI, 1978). Twenty untreated controls/sex and 20 vehicle controls/sex of each species were included in the study. Rats received initial doses of 80 and 40 mg/kg body wt-day for the first 17 weeks. Due to high mortality, dosing of high dose rats was discontinued for 13 weeks and resumed on week 30 at 40 mg/kg body wt-day. In week 42, all intubations of low and high dose rats ceased for 1 week followed by 4 weeks of dose administration. All surviving, treated male rats were necropsied in week 49; all surviving, treated female rats were necropsied in week 61. The resulting time-weighted average dose over the test period was 38 and 41 mg/kg body wt-day for low and high dose males, respectively, and 37 and 39 mg/kg body wt-day for low and high dose females, respectively. Mice received initial doses of 120 and 60 mg/kg body wt-day. In weeks 11-13, high and low doses were increased to 200 and 100 mg/kg body wt-day, respectively. Original dose levels were resumed after week 13. At week 40, administration of EDB was decreased to 60 mg/kg body wt-day for high dose mice. EDB administration was discontinued at week 54 with necropsy occurring at week 78 for males and high dose females. Low dose female mice were observed for 37 weeks after intubation ceased. The resulting time-weighted average dose over the test period was 62 and 107 mg/kg body wt-day for low and high dose mice, respectively. In rats, clinical signs by week 5 included reddened ears and hunched back in all treatment groups. By week 10, all treated rats had reduced body weights ($\geq 10\%$). Both female and male rats exhibited dose-dependent mortality. Many of the deaths occurred during or shortly after intubation, suggesting an acute toxic reaction. Pathology revealed hyperkeratosis and acanthosis of the forestomach in high dose males and females and in one low dose female. A small number of rats in both treatment groups showed adrenal cortex degeneration and peliosis of the liver (hepatitis). Dosed males showed

early development of testicular atrophy. In mice, dose-related body weight reduction and mortality were observed. Clinical signs included alopecia, thin, hunched appearance, soft feces and body sores. Hyperkeratosis and acanthosis of the forestomach were seen in high dose male and female mice. One incidence each of hyperkeratosis (in a female) and acanthosis (in a male) was seen at the low dose. Splenic changes were present in high dose mice and testicular atrophy was present in high dose males.

In a long-term inhalation exposure study, F344 rats and B6C3F₁ mice were exposed to 0, 10, or 40 ppm EDB 6 hr/day, 5 days/week for up to 103 weeks (NTP, 1982). In male and female rats, the high dose groups had reduced body weights and increased mortality that began at about week 60. The treatment-related non-neoplastic pathology included hepatic necrosis (both sexes), epithelial hyperplasia and suppurative inflammation throughout the respiratory system (both sexes), and nephropathy (males only). Toxic nephropathy and mineralization were also seen in high dose female rats. Testicular degeneration and atrophy occurred with greater frequency in exposed rats and may be related to observed testicular tumors. Spermatic granulomas were also more frequently seen in high-dose males. Degeneration of the adrenal cortex appeared to be dose-related in females, but only one incidence each was seen in low and high dose males. Increased incidence of retinal atrophy was observed in exposed females. In mice, body weights were reduced at the high dose in both males and females. Many of the high dose animals exhibited a progressive weakness of the limbs or body during the second year. Increased mortality occurred in a dose-related manner in females and was significantly greater in low dose males. Non-neoplastic pathology included epithelial hyperplasia throughout the respiratory system and serous and suppurative inflammation of the nasal cavity in exposed mice. In all male mice, the principal cause of death was urinary bladder inflammation. However, bladder epithelial hyperplasia was only seen in exposed animals. An increased incidence of suppurative inflammation of the prostate was present but was also seen in controls. Dose-related spleen hematopoiesis was observed in females.

Another long-term inhalation study investigated the effects of 0 or 20 ppm EDB (7 hr/day, 5 days/week) on 48 Sprague-Dawley rats/sex/group for 18 months (Wong *et al.*, 1982). Significantly lower body weight gains (>10% difference from controls) occurred by the 15th month in males, and by the 18th month in females. Significantly reduced food consumption was not apparent. Increased mortality rates in both sexes occurred beginning in the 12th month of EDB exposure. All hematological findings were within normal ranges. The only recorded non-neoplastic gross or microscopic finding was atrophy of the spleen in males, which may be related to tumor formation (hemangiosarcoma). The nasal cavity was not examined.

In a study of the effect of EDB on sperm production in bulls (Isreal-Friesian breed), 4 calves were fed 2 mg/kg body wt-day for 12 months (Amir and Volcani, 1965). The bulls were then given EDB in gelatin capsules every other day for 2-4 months longer. EDB did not appear to affect the growth, health, and libido of the bulls. However, semen density and motility were significantly lower compared to untreated control bulls of the same age. Many abnormal spermatozoa were also present in treated bulls. A NOAEL for this effect was apparently not determined. Cessation of EDB administration resulted in normal sperm within 10 days to 3 months. Further studies confirmed that EDB adversely affected sperm production without any other apparent effects on bulls (Amir and Volcani, 1967; Amir and Ben-David, 1973). However,

feeding rams 2-5 mg/kg body wt-day for 120 days did not result in any effect on sperm or on the health of the animal (Amir, 1991).

Female B6C3F1 mice (10/group) were given 31.25, 62.5, or 125 mg/kg EDB by gastric lavage 5 days/week for 12 weeks (Ratajzak *et al.*, 1995). At the highest dose, EDB significantly prolonged intervals between estrus, decreased hemoglobin and hematocrit levels, and increased cholesterol, triglycerides, total protein, and albumin. The highest dose also caused an immunosuppressive effect by lowering the *in vitro* splenic lymphocyte response to T- and B-cell mitogens.

In a developmental toxicity study, 15-17 pregnant Charles River CD rats and 17-19 pregnant CD mice were exposed to 0, 20, 38, and 80 ppm EDB by inhalation 23 hr/day during days 6 to 16 of gestation (Short *et al.*, 1978). A significant increase in mortality occurred in adult rats exposed to 80 ppm EDB and in adult mice exposed to 38 and 80 ppm EDB. Mice exposed to the highest dose experienced 100% mortality. Reduced body weights and feed consumption occurred in both species at all doses tested. Fetal mortality was increased in rats at the highest dose and in mice at 38 ppm. Reduced fetal body weights occurred at 38 ppm in rats and at all exposure levels in mice. No anomalies were seen in rat fetuses. An increase in runts at 38 ppm and a dose-dependent increase in skeletal anomalies were observed among mouse fetuses. However, these anomalies were characteristic of delayed development and occurred at doses that adversely affected maternal welfare. Therefore, these effects are indicative of fetal toxicity rather than teratogenicity.

Male reproductive toxicity of EDB has been evaluated in some other experimental animals. New Zealand white rabbits, dosed subcutaneously with 0, 15, 30, or 45 mg/kg body wt-day, showed adverse effects at the highest dose (Williams *et al.*, 1991). Increased mortality, increased serum enzymes, and liver damage were observed at this dose level. With respect to sperm quality, sperm velocity, motility, and motion parameters were reduced at the highest dose. A dose related decrease in semen pH was also noted. However, male fertility and fetal structural development were unaffected.

A dominant lethal assay in mice was negative following a single intraperitoneal injection of 100 mg EDB/kg body wt (Barnett *et al.*, 1992). Germ cell tests did not indicate that EDB was a germ cell mutagen in male mice.

VI. Derivation of Chronic Reference Exposure Level (REL)

<i>Study</i>	Ratcliff <i>et al.</i> , 1987
<i>Study population</i>	46 exposed men, 43 unexposed men; 89 total
<i>Exposure method</i>	Variable workplace breathing zone airborne exposure (88 ppb geometric mean 8-hour time weighted average (TWA) exposure with peak exposures up to 262 ppb)
<i>Critical effects</i>	Reproductive toxicity; decreased sperm count/ejaculate, decreased percentage of viable and motile sperm, increased semen pH, and increased proportion of sperm with specific morphological abnormalities (tapered heads, absent heads, and abnormal tails) in human males
<i>LOAEL</i>	88 ppb
<i>NOAEL</i>	Not observed
<i>Exposure continuity</i>	8 hr/day (10 m ³ /day occupational inhalation exposure rate), 5 days/week
<i>Exposure duration</i>	Average, 4.9 years (with standard deviation of 3.6 years)
<i>Average experimental exposure</i>	31 ppb for LOAEL group (88 x 10/20 x 5/7)
<i>Human equivalent concentration</i>	31 ppb
<i>LOAEL uncertainty factor</i>	10
<i>Subchronic uncertainty factor</i>	3
<i>Interspecies factor</i>	1
<i>Intraspecies factor</i>	10
<i>Cumulative uncertainty factor</i>	300
<i>Inhalation reference exposure level</i>	0.1 ppb (0.0008 mg/m ³ , 0.8 µg/m ³)

The primary study by Ratcliff and associates (1987) found significant changes in sperm quality indices of papaya workers exposed to EDB vapors for an average of nearly 5 years. No other health effects were apparent. A level of EDB at which no toxicity was observed (NOAEL) was not determined.

In addition to the primary study of Ratcliff *et al.* (1987), several other epidemiological studies together strongly suggest a correlation between EDB exposure and male reproductive toxicity (Ter Haar, 1980; Wong *et al.*, 1979; Wong *et al.*, 1985; Rogers *et al.*, 1981; Schrader *et al.*, 1988). This lesion appears to occur in humans at concentrations at which other toxic effects are not seen. EDB also shares some structural similarity to dibromochloropropane (DBCP), a known reproductive toxicant in human males. The evidence for male reproductive toxicity of EDB is not as strong as that for DBCP, probably because EDB is not as potent as DBCP in producing this toxic effect. However, animal studies demonstrate testicular toxicity and the number of studies indicating a connection between male reproductive toxicity and EDB exposure cannot be ignored for the development of the REL.

Chronic oral exposure of bulls to EDB results in similar toxic effects at low concentrations (equivalent to 0.9 ppm) without affecting the general health of the animal (Amir and Volcani, 1965; Amir, 1991). However, the small sample size and the lack of a dose-response effect and an observed NOAEL limits the usefulness of this study. Long-term studies of EDB toxicity in other experimental animals also lack the determination of a NOAEL (NCI, 1978; NTP, 1982). Evidence of testicular atrophy was found in other long-term studies with experimental animals, but at concentrations that also produced toxic effects in other organ systems.

For comparison with the proposed REL based on a human study, the NTP (1982) chronic inhalation study established a LOAEL (10 ppm) for liver, kidney, eyes, and the respiratory, male reproductive, and endocrine system in rats. A LOAEL was established in mice for mortality, spleen changes in females, and respiratory system toxicity. A NOAEL was not established for either species. Use of a time adjustment (6/24 hr/day, 5/7 day/week), an RGDR of 1, and a total uncertainty factor of 300 (an interspecies UF of 3, a LOAEL to NOAEL UF of 10, and an intraspecies UF of 10) resulted in an estimated REL of 6 ppb (50 $\mu\text{g}/\text{m}^3$).

VII. Data Strengths and Limitations for Development of the REL

The strengths of the inhalation REL for ethylene dibromide include the use of human exposure data from workers exposed over a period of years, and the presence of the toxic endpoint (male reproductive system) in several experimental animal species. Major areas of uncertainty are the lack of observation of a NOAEL, the uncertainty in estimating occupational exposure, the potential variability in occupational exposure concentration, and the limited nature of the study (fertility was not actually tested). The database for chronic toxicity of EDB in experimental animals would be enhanced if the proper doses were chosen to determine a NOAEL.

VIII. Potential for Differential Impacts on Children's Health

Little fetal toxicity was observed when pregnant rats and mice were exposed to 20 ppm EDB during gestation (Short *et al.*, 1978). Thus the REL of 0.1 ppb should adequately protect infants and children. However, we do not know if adolescent boys would be more sensitive than men to this alkylating agent. Differences in metabolic capability between infants and older children and adults may result in either more or less toxicity of EDB. Both oxidative and conjugated metabolites are toxic. Infants may produce proportionately more conjugate than oxidized metabolite relative to adults.

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CHRONIC TOXICITY SUMMARY

ETHYLENE DICHLORIDE*(1,2-dichloroethane)***CAS Registry Number: 107-06-2****I. Chronic Toxicity Summary**

<i>Inhalation reference exposure level</i>	400 µg/m³ (100 ppb)
<i>Critical effect(s)</i>	Hepatotoxicity; elevated liver enzyme levels in serum of rats.
<i>Hazard index target(s)</i>	Liver

II. Physical and Chemical Properties (HSDB, 2000; CRC, 1994)

<i>Description</i>	Clear, colorless, oily liquid
<i>Molecular formula</i>	C ₂ H ₄ Cl ₂
<i>Molecular weight</i>	98.97 g/mol
<i>Density</i>	1.2351 g/cm ³ @ 20°C
<i>Boiling point</i>	57.4°C
<i>Melting point</i>	-96.9°C
<i>Vapor pressure</i>	64 torr @ 20°C
<i>Solubility</i>	Slightly soluble in water (0.869 g/100 ml at 20°C); miscible with alcohol; soluble in ordinary organic solvents
<i>Conversion factor</i>	1 ppm = 4.05 mg/m ³

III. Major Uses or Sources

Ethylene dichloride (EDC) is used primarily in the production of vinyl chloride monomer (HSDB, 2000). It is also an intermediate in the manufacture of trichloroethane and fluorocarbons and is used as a solvent. In California, EDC is also used as a reactant carrier in the production of solid fuel (CARB, 1997). EDC was commonly used as a gasoline additive to scavenge inorganic lead compounds. The transition to the use of lead-free gasoline has essentially eliminated the use of EDC as a fuel additive in this country. EDC was also used as a soil fumigant but is no longer registered for this use on agricultural products in the United States. The annual statewide industrial emissions from facilities reporting under the Air Toxics Hot Spots Act in California based on the most recent inventory were estimated to be 24,935 pounds of ethylene dichloride (CARB, 2000).

IV. Effects of Human Exposure

Toxicological data resulting solely from long-term exposure to EDC in humans are lacking. Nausea, vomiting, dizziness, and unspecified blood changes were reported in a study of workers exposed to levels of 10-37 ppm EDC (Brzozowski *et al.*, 1954). Kozik (1957) reported adverse central nervous system and liver effects in workers occupationally exposed to concentrations of 16 ppm EDC and below. Rosenbaum (1947) also reported nervous system effects in a study of 100 Russian workers exposed for less than 5 years to concentrations of EDC less than 25 ppm.

Immediately following a 30-minute exposure to an unknown concentration of EDC, a 51 year-old male was somnolent and experienced vomiting (Nouchi *et al.*, 1984). Delirious and trembling, the worker was admitted to the hospital 20 hours post-exposure. The liver was palpable, but serum liver enzymes were normal. The patient lapsed into a coma 3.5 hours following admission to the hospital. A marked elevation in serum liver enzymes was noted on the second day of hospitalization, 35 hours post-exposure. Multiple organ failure occurred on the fourth day of hospitalization and the patient died of arrhythmia. At autopsy, the lungs were congested and edematous. Diffuse degenerative changes were observed in the myocardium. Extensive centrilobular necrosis was observed in the liver, and acute centrilobular necrosis was observed in the kidney. Nerve cells in the brain, including Purkinje cells, appeared shrunken with pyknotic nuclei. The latency period for hepatotoxicity of approximately 20 hours suggests that metabolism of the compound yields the reactive agent (see below).

V. Effects of Animal Exposure

As with humans, the absorption and distribution of EDC in rats following ingestion or inhalation is rapid and complete (IARC, 1999). Metabolism in rats and mice is extensive with 85% of the metabolites appearing in urine. Metabolism occurs predominantly via two pathways, one catalyzed by cytochrome P450 and one by glutathione S-transferase. The direct conjugation with glutathione catalyzed by glutathione S-transferase may ultimately result in the putative alkylating agent (episulfonium ion) primarily responsible for toxicity and carcinogenicity. Evidence for DNA-damaging metabolites resulting via the P450 pathway exists (IARC, 1999). However, this pathway appears to be a minor route for toxic metabolite formation.

Acute exposure in mice resulted in toxic effects similar to those seen in the human case study presented above, including liver and kidney damage (Francovitch *et al.*, 1986). Acute EDC exposure exhibits a steep dose-response curve with respect to mortality. However, the long-term exposure studies were notable for the limited organ toxicity and mortality observed in comparison to acute studies (IARC, 1999).

Male and female rats (50 per sex) were exposed to 50 ppm EDC 7 hours per day, 5 days per week for 2 years (Cheever *et al.*, 1990). Absolute and relative liver weights were not significantly different from controls. Daily observations, gross pathology, and extensive histopathology revealed no differences from controls other than a slight increase in unspecified testicular lesions in the EDC group. Additional rats were exposed to 50 ppm EDC with 0.05% disulfiram (a non-carcinogen used extensively in the rubber industry and as a treatment

(Antabuse) for alcoholism) in the diet. Disulfiram treatment resulted in increased number of tumors, increased blood levels of EDC, and increased liver (primarily bile duct cysts) and kidney (chronic nephropathy) lesions. It was concluded that some pathways responsible for metabolism of EDC were inhibited by disulfiram, resulting in increased EDC blood levels and bioactivation to toxic metabolites via other metabolic pathways.

Rats (8-10 per sex per group) were exposed to 0, 5, 10, 50, and 150-250 ppm EDC 7 hours per day, 5 days per week for up to 18 months (Spreafico *et al.*, 1980). Serum chemistry measurements were taken after 3, 6, 12, and 18 months of exposure. Rats to be examined after 3, 6 and 18 months of exposure were 3 months of age at the beginning of the experiment, and rats to be examined after 12 months of exposure were 14 months of age at the beginning of the experiment. Complete histological exams were conducted but non-cancer effects were not discussed. No consistent treatment-related changes in serum chemistry parameters were observed at 3, 6, or 18 months of exposure. However, rats exposed to higher levels of EDC for 12 months exhibited changes in serum chemistry indicative of chronic liver damage, primarily increased alanine aminotransferase (ALT) levels at the two highest exposures. Lactate dehydrogenase (LDH) and aspartate aminotransferase (AST) levels were significantly decreased, but did not appear to be dose-related. γ -Glutamyl transpeptidase levels were elevated but at non-significant levels. Indicators of kidney toxicity included increased blood urea nitrogen levels in the 150 ppm group and increased uric acid levels at the two highest exposures. However, the control values for both of these parameters were significantly lower than that seen in rats tested at other times in this study. Thus, the toxicological significance is questionable. Cholesterol was reduced significantly at the higher exposure levels but the toxicological significance of this finding was unknown. The marked difference between serum chemistry parameters following 12 months of exposure, compared to those following 3, 6, and 18 months of exposure, may be due to the considerable difference in the age of the rats at the start of exposure. This study identifies a 12-month LOAEL of 50 ppm and a NOAEL of 10 ppm in rats.

A study examining the interaction between 1,2-dichloroethane and disulfiram (DSF) exposed rats to EDC concentrations of 150, 300, or 450 ppm 5 days per week for 30 days (Igwe *et al.*, 1986a; Igwe *et al.*, 1986b). Increased liver weights and increased 5-nucleotidase (5-NT) activity were observed in rats following exposure to 450 ppm EDC (the LOAEL for this study). This study also determined that the interaction between DSF and EDC greatly increased the toxicity of EDC (i.e., increased serum activities of SDH, APT, and 5-NT, bilateral testicular atrophy, periportal necrosis and cytoplasmic swelling of hepatocytes, and bile duct proliferation). Therefore, any person exposed to DSF either occupationally or therapeutically is likely to be more susceptible to the effects of EDC toxicity.

Rats, rabbits, guinea pigs, dogs, cats, and monkeys were used in exposures ranging from approximately 100 to 1000 ppm EDC (Heppel *et al.*, 1946). At the highest experimental concentration of 963 ppm, high mortality was observed in rats, rabbits, and guinea pigs following exposure 7 hours per day, 5 days per week for two weeks or less. At 963 ppm guinea pigs exhibited lacrimation and inactivity during exposure; pulmonary congestion was noted at autopsy. Rats exposed to this concentration exhibited degenerative proliferative changes in the renal tubular epithelium and splenitis. Pulmonary congestion and focal hemorrhage were also noted in 2 of 4 rats examined. While 4 of 6 cats exposed to this concentration survived until

sacrifice 11 weeks following termination of exposure, congestion and fatty infiltration of the liver were observed at necropsy. Due to high mortality in the rodents at the higher concentration, a subsequent experiment exposed rats and guinea pigs 7 hours per day, 5 days per week to 100 ppm EDC for four months. No increase in mortality or effects on growth was observed in rats exposed to this concentration. The rats were successfully bred and their pups were exposed with the dams. No significant findings were observed upon gross and histological examinations of 10/39 exposed and 10 control rats. This study is severely limited by the methods used to determine the exposure concentration and by the lack of quantitative measurements of toxicity other than death. This study does, however, indicate that fatty infiltration of the liver is one indication of toxicity following multiple exposures to EDC.

In developmental toxicity studies summarized by Zhao *et al.* (1997), rats were exposed to 0, 24.8, and 207.6 mg/m³ (equivalent to 0, 6, and 51 ppm) EDC for 6 hr/day from two weeks before mating and throughout gestation. Statistically significant increases in pre-implantation loss and decreased male pup weights were observed at the highest dose. Gross skeletal and visceral malformations were not found.

In a developmental study by Payan *et al.* (1995), Sprague-Dawley rats were exposed to 150, 200, 250, or 300 ppm EDC for 6 hrs/day from day 6 to 20 of gestation. Maternal toxicity (reduced body weight gain; death of two females) was observed at the highest exposure. Statistically significant evidence of altered growth and teratogenic effects were not observed at any concentration.

Rao *et al.* (1980) exposed rats and rabbits to 100 or 300 ppm EDC for 7 hr/day on days 6 through 15 (rats) or 6 through 18 (rabbits) of gestation. Maternal toxicity (mortality) was observed in rabbits at 100 ppm, and both species at 300 ppm. One rat exhibited resorption of all implantations at the maternally-toxic dose. Otherwise, no fetotoxic or teratogenic effects were observed in either species. In a reproduction study, rats were exposed to 25, 75, or 150 ppm EDC 6 hr/day, 5 days/week for 60 days before breeding. Exposure following this period was 6 hr/day, 7 days/week. Maternal animals were not exposed to EDC from gestational day 21 through day 4 postpartum. EDC had no effect on reproduction over one generation within two litters.

In a two-generation study conducted by Lane *et al.* (1982), ICR Swiss mice were administered 30, 90, or 290 mg/L EDC in drinking water (equivalent to about 5, 15, or 50 mg/kg bw/day) starting five weeks before mating of the F₀ generation. No treatment-related effects on fertility, gestation, viability, weight gain, or lactation indices were noted. EDC exposure did not result in teratogenic or dominant lethal effects.

No gross or histopathological indications of hepato- or nephrotoxicity were observed in Osborn-Mendel rats (47 or 95 mg/kg bw/day, 5 days/week for both sexes) or B6C3F1 mice (97 or 195 mg/kg bw/day, 5 days/week for males; 149 or 299 mg/kg bw/day, 5 days/week for females), which were given EDC via gavage for 78 weeks (NCI, 1978). However, rats of each sex and female mice had significantly reduced survival at the highest dose.

In a comparative study of the toxicity of EDC, Morgan *et al.* (1990) administered 0, 500, 1000, 2000, 4000, and 8000 ppm in drinking water to several species of rats for 13 weeks. A statistically significant increase in kidney weight was observed in male and female Fischer 344/N rats administered 1000 ppm or greater in drinking water. However, minimal histological damage was observed only in the kidney of female Fischer 344/N rats. A statistically significant decrease in body weight was observed in rats administered 8000 ppm. Significant decreases in absolute and relative kidney weight were observed in male and female rats administered concentrations of 1000 ppm EDC. A significant increase in relative liver weight was observed in male rats administered 2000 ppm EDC and greater and female rats administered 4000 ppm EDC and greater. Similar but less marked toxicity was observed in the Sprague-Dawley and Osborne-Mendel rats administered 1000 ppm. Additionally, rats were administered EDC in corn oil by gavage at doses of 0, 30, 60, 120, 240, and 480 mg/kg for 13 weeks (Morgan *et al.*, 1990). Rats administered EDC by gavage exhibited high mortality in the higher dose groups. Statistically significant increases in kidney weights were observed in surviving male rats administered EDC and in female rats administered 120 or 240 mg/kg. However, no histological damage to the liver or kidney was observed.

VI. Derivation of Chronic Reference Exposure Level (REL)

<i>Study</i>	Spreafico <i>et al.</i> , 1980.
<i>Study population</i>	Rats (8-10 per sex/group)
<i>Exposure method</i>	Discontinuous whole-body inhalation exposures (0, 5, 10, 50, or 150-250 ppm)
<i>Critical effects</i>	Significant elevation in liver enzymes
<i>Exposure duration</i>	12 months
<i>Exposure continuity</i>	7 hours/day, 5 days/week
<i>LOAEL</i>	50 ppm
<i>NOAEL</i>	10 ppm
<i>Average experimental exposure</i>	2.1 ppm for NOAEL group (10 x 7/24 x 5/7)
<i>Human equivalent concentration</i>	3.2 ppm for NOAEL group (gas with systemic effects, based on RGDR = 1.5 for lambda (a) : lambda (h)) (Gargas <i>et al.</i> , 1989)
<i>LOAEL uncertainty factor</i>	1
<i>Subchronic uncertainty factor</i>	1
<i>Interspecies uncertainty factor</i>	3
<i>Intraspecies uncertainty factor</i>	10
<i>Cumulative uncertainty factor</i>	30
<i>Inhalation reference exposure level</i>	0.1 ppm (100 ppb; 0.4 mg/m ³ ; 400 µg/m ³)

Cheever *et al.* (1990) and Spreafico *et al.* (1980) were the only chronic inhalation exposure studies found in the literature that presented non-cancer effects. No reproductive and developmental effects were observed in studies published in peer-reviewed journals. The study by Spreafico *et al.* (1980) was chosen for REL development based on the utilization of multiple exposure levels and the observation of a NOAEL and a LOAEL for liver effects.

The Agency for Toxic Substances and Disease Registry (ATSDR) calculated a chronic inhalation minimal risk level (MRL) for EDC of 0.2 ppm (ATSDR, 1994). The calculation was based on the study by Cheever *et al.* (1990), which determined a free-standing NOAEL of 50 ppm for lack of liver effects. A LOAEL was not determined. To derive the MRL, the ATSDR applied uncertainty factors (UFs) of 10 each for intraspecies and interspecies variability, and a modifying factor of 3 to account for database deficiencies, to the NOAEL of 50 ppm. The criteria for use of modifying factors are not well specified by ATSDR. Such modifying factors were not used by OEHHA. A continuity correction for discontinuous exposure was not applied. The resulting MRL was 0.2 ppm (0.7 mg/m³).

For comparison to the proposed REL, a REL developed by OEHHA based on the free-standing NOAEL of 50 ppm determined in rats by Cheever *et al.* (1990) would include a continuity correction (50 ppm x 7/24 x 5/7) resulting in an equivalent continuous level of 10.42 ppm. Application of an RGDR = 1.5 and UFs of 3 for interspecies and 10 for intraspecies differences result in a REL of 0.5 ppm (2 mg/m³).

VII. Data Strengths and Limitations for Development of the REL

The strengths of the inhalation REL for ethylene dichloride include the availability of chronic inhalation exposure data, the relatively large number of exposure levels at lower concentrations (allowing for better elucidation of the dose-response relationship for hepatotoxicity), and the observation of a NOAEL. Major areas of uncertainty are the lack of adequate human exposure data, the small groups tested in the key study, and the lack of health effects data from multiple species.

The small number of animals per group and the relatively modest clinical chemistry findings observed in the Spreafico *et al.* (1980) study may have resulted in false-positives, false-negatives, and lack of clear dose-response relationships. Repeating the study in one or more experimental animal species with full histopathological examination of organs and a greater number of animals/dose would significantly enhance the chronic toxicity database for EDC.

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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**

<i>Chronic reference exposure level</i>	400 µg/m³ (200 ppb)
<i>Critical effects</i>	Respiratory irritation in human volunteers
<i>Hazard index target(s)</i>	Respiratory system; kidney; teratogenicity

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

<i>Description</i>	Clear, colorless, odorless liquid
<i>Molecular formula</i>	C ₂ H ₆ O ₂
<i>Molecular weight</i>	62.07 g/mol
<i>Density</i>	1.1088-1.1135 g/cm ³ @ 20° C
<i>Boiling point</i>	197.6° C
<i>Melting point</i>	-13° C (CRC, 1994)
<i>Vapor pressure</i>	0.06 torr @ 20°C; 0.092 torr @ 25°C
<i>Solubility</i>	Soluble in water and ethanol; slightly soluble in ether. Insoluble in benzene and petroleum ether.
<i>Conversion factor</i>	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/m³) for 30 days was without effect (Wills *et al.*, 1974). The actual concentrations measured in the exposure chamber were:

Days	Concentration of ethylene glycol in air, mg/m ³		
	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/m³), and became quickly intolerable at 123 ppm (308 mg/m³). 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 hemoglobin 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/m³ or continuously to 12 mg/m³ for 90 days. At 10 mg/m³ 2 rabbits had conjunctivitis and liver changes were noted in a few animals of the other species. At 57 mg/m³ 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/m³ 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.

<i>Mortality in Coon et al. (1970)</i>			<i>Number died/number exposed</i>				
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/m³ 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/m³. 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/m³. The NOAEL for maternal toxicity in rats was 888 mg/m³, while the NOAEL for fetotoxicity was 119 mg/m³.

In mice, reduced body weight and gravid uterine weight during and after the exposure were observed at the 888 and 2090 mg/m³ concentrations. Increased nonviable implants per litter and reduced fetal body weights were also observed in groups exposed to 888 or 2090 mg/m³. External, visceral, skeletal, and total malformations were increased in the 888 and 2090 mg/m³ groups. The NOAEL for these effects in mice was 119 mg/m³.

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/m³. The NOAEL for maternal effects (increased kidney weight) was 500 mg/m³, and the NOAEL for fetal toxicity (skeletal variations and fused ribs) was 1000 mg/m³. 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

<i>Study</i>	Wills <i>et al.</i> (1974)
<i>Study population</i>	Human volunteer prisoners
<i>Exposure method</i>	Discontinuous whole-body inhalation
<i>Critical effects</i>	Respiratory tract irritation
<i>LOAEL</i>	75 ppm
<i>NOAEL</i>	20 ppm
<i>Exposure continuity</i>	20 hours/day
<i>Exposure duration</i>	30 days
<i>Average exposure</i>	16.7 ppm for NOAEL group (20 x 20/24)
<i>Human equivalent concentration</i>	16.7 ppm
<i>LOAEL uncertainty factor</i>	1
<i>Subchronic uncertainty factor</i>	10
<i>Interspecies factor</i>	1
<i>Intraspecies factor</i>	10
<i>Cumulative uncertainty factor</i>	100
<i>Inhalation reference exposure level</i>	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/m³ and intense and intolerable irritation occurring at 308

mg/m³. The volunteers were followed for 6 weeks without any apparent long-term effects from the exposures. Although the irritation experienced in the human subjects appears to be an acute phenomenon and not a cumulative lasting effect, the subchronic uncertainty factor of 10 was retained to protect against other systemic effects associated with ethylene glycol such as kidney damage which may occur over a long-term exposure.

The chronic feeding study in rats by DePass *et al.* (1986a) showed significant chronic effects including reduced body weight, increased water intake, increased blood urea nitrogen and creatinine, reduced erythrocyte counts, reduced hematocrit and hemoglobin, increased neutrophil counts, increased urine volume, and reduced urine specific gravity and pH in rats exposed to a concentration of 1000 mg/kg/day. However, no effects were reported in mice. In contrast, reproductive and developmental toxicity studies in mice, rats, and rabbits have shown the mouse to be the most sensitive species for both terata and maternal toxicity endpoints (Tyl *et al.*, 1995a; Tyl *et al.*, 1993; Nepper-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 m³/day) from the feed in the 3-generation study by DePass *et al.* (1986b) are 700 mg/m³ and 3500 mg/m³ for the NOAEL and LOAEL, respectively.

For comparison with the proposed REL of 400 µg/m³ 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/m³) 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

ETHYLENE GLYCOL MONOETHYL ETHER*(2-ethoxyethanol; EGEE)***CAS Registry Number: 110-80-5****I. Chronic Toxicity Summary**

<i>Inhalation reference exposure level</i>	70 µg/m³ (20 ppb)
<i>Critical effect(s)</i>	Testicular degeneration and decreased hemoglobin in rabbits
<i>Hazard index target(s)</i>	Reproductive system; hematopoietic system

II. Chemical Property Summary (from HSDB, 1996; 1999)

<i>Description</i>	Colorless liquid; sweet, pleasant, ether-like odor
<i>Molecular formula</i>	C ₄ H ₁₀ O ₂
<i>Molecular weight</i>	90.12
<i>Boiling point</i>	135°C
<i>Vapor pressure</i>	3.8 torr @ 20°C; 5.31 torr at 25°C
<i>Solubility</i>	Miscible with water and organic solvents
<i>Conversion factor</i>	3.69 µg/m ³ per ppb at 25°C

III. Major Uses and Sources

Ethylene glycol monoethyl ether (EGEE) is a widely used solvent for nitrocellulose, dyes, inks, resins, lacquers, paints, and varnishes (HSDB, 1996). It is also a component of many cleaning agents, epoxy coatings, paints, hydraulic fluid, and is an anti-icing fuel additive in aviation. EGEE is also a chemical intermediate in the production of another solvent, ethylene glycol monoethyl ether acetate. The specific annual statewide industrial emissions of EGEE from facilities reporting under the Air Toxics Hot Spots Act in California based on the most recent inventory were estimated to be 443,748 pounds (CARB, 1999). (Many industries did not report emissions of specific glycol ethers. Thus there were also emitted 2,922,744 pounds of the general category glycol ethers, which can include EGEE.)

IV. Effects of Human Exposure

Sperm quality was examined in 37 workers exposed to EGEE by skin contact and/or inhalation in two buildings (Clapp *et al.*, 1987; Ratcliffe *et al.*, 1989). Exposure levels ranged from undetectable to 24 ppm with an average exposure level of 6 ppm in one building and 11 ppm in the other. A statistically significant difference in mean sperm count was observed between the

37 exposed male workers and 39 unexposed male workers. Semen volume and pH, viability, motility, velocity, and morphology were not significantly different between the two groups. The primary metabolite of EGEE, ethoxyacetic acid, was identified in the urine of exposed but not control workers. Both exposed and control subjects had significantly lower sperm counts than historical controls. Furthermore, members of both groups may have been exposed to other compounds including metals, solvents, heat, and vibration.

Welch and Cullen (1988) evaluated shipyard painters exposed to ethylene glycol ethers (EGEE and EGME). Air concentrations at the workplace were estimated based on 102 samples over six shifts in Sparer *et al.* (1988). Time-weighted average (TWA) exposures to EGEE ranged from 0 to 80.5 mg/m³ with a mean of 9.9 mg/m³. TWA exposures to EGME ranged from 0 to 17.7 mg/m³ (mean = 2.6 mg/m³). The authors note that during the time period of measurement, painting activities were unusually low and previous NIOSH analyses indicated considerably higher exposures. Ninety-four painters and 55 controls answered a medical and environmental exposure questionnaire including work history and provided blood, urine, and in some cases semen samples. Mean hemoglobin levels, total cell counts and differential counts did not differ between exposed and control. However, the authors found that the lowest quartile of hemoglobin was mostly painters and the lowest polymorphonuclear leukocyte counts were in painters. Nine painters were considered anemic and five were considered granulocytopenic. The authors note that the absence of a significant difference in the group as a whole and the inability to detect a dose-response pattern in the exposed group make a strong conclusion unwarranted.

Welch *et al.* (1988) evaluated the semen samples from the workers in the cohort from Welch and Cullen (1988). Sperm concentration, velocity, motility, morphology, morphometry, and viability were measured. Although not statistically significant, the measures of sperm count tended to be lower in the painters with a $p = 0.10$ for density and $p = 0.11$ for count. When nonsmokers were analyzed separately from smokers, the number of oligospermic painters was larger than that in controls at $p = 0.05$. There was no difference between controls and exposed men who were smokers. The authors state that although mean values of sperm count did not differ significantly between controls and exposed groups, biologically important differences were seen when the proportion of men with oligospermia was examined. The proportion of painters with azoospermia was 5% with only 1% expected based on other population surveys. The authors note that to create a dose-response model for an effect of glycol ethers on semen parameters would require description of the exposure of each individual 3 to 6 months prior to sampling. The painters moved frequently from one exposure area to the next, making exposure assessment particularly difficult in this cohort.

Cullen *et al.* (1992) conducted a histopathologic analysis of the bone marrow and circulating blood cells in the workers previously examined in Welch *et al.* (1988). The objects of the study included: 1) to exclude other causes for granulocytopenia and depressed hemoglobin levels noted in some painters exposed to ethylene glycol ethers, 2) to determine if subclinical evidence of hematologic damage is present in healthy coworkers, and 3) to identify host or exogenous factors which may increase the risk of hematologic damage in glycol ether exposed painters. Workers were grouped as follows: Group I consisted of those painters that had anemia or granulocytopenia in the Welch and Cullen (1988) study; Group II consisted of exposed painters with normal hematology; Group III consisted of unexposed controls. A battery of hematologic

and biochemical parameters were measured and a questionnaire was completed to determine occupational exposure status, health status and drug and alcohol consumption. All hematologic parameters were normal in all groups. Tests of liver, renal, and thyroid function were normal in all groups. Bone marrow histology showed no differences between groups. One biochemical parameter, pyruvate kinase activity, was lower in Group I than Groups II and III ($p = 0.05$). Depression of red cell pyruvate kinase did not vary by race and was lower in every subject in Group I by more than one standard deviation. Low pyruvate kinase is the most consistent red cell enzyme defect noted in acquired hematologic disorders.

V. Effects of Animal Exposure

Sprague-Dawley rats (15/sex/group) and New Zealand white rabbits (10/sex/group) were exposed to 0, 25, 103, or 403 ppm EGEE by inhalation for 6 hours/days, 5 days/week, for 13 weeks (Barbee *et al.*, 1984). Animals were physically examined weekly and, at the end of the study, hematology, clinical chemistry, and histopathological examination were performed. No histopathological changes in the respiratory tract were found. Among rabbits, body weight was reduced in the high-dose group males and females. In the 25 ppm dose group, adrenal weight was reduced significantly among males, although this effect was not found to be dose-related. Among males in the high-dose group, testes weights were significantly reduced with a corresponding degenerative change to the seminiferous tubule epithelium. No effect on spermatogenic activity was found, however. Significant hematological effects observed at the high-dose included decreased hemoglobin, hematocrit, and erythrocyte count.

Teratologic effects in pregnant rats from the inhalation of EGEE were reported (Tinston *et al.*, 1983a). The results of this study were presented in summary form (Doe, 1984). Wistar rats (24/group) were exposed to target concentrations of 0, 10, 50, or 250 ppm EGEE for 6 hours/day during gestational days 6-15 and the animals were sacrificed on day 21. Maternal toxicity was observed in the high-dose group with decreased hemoglobin, hematocrit, and mean corpuscular volume. Significant increases in preimplantation loss occurred in the 10 and 50 ppm dose groups, however the absence of this effect at 250 ppm indicated a poor dose-response, and because implantation occurred on the first day of exposure, the relatedness of the effect to exposure is in question. Post-implantation loss was also increased in the mid-dose group, however, no corresponding decrease in intrauterine death was observed in this group. Minor skeletal defects, particularly delayed ossification, were widely observed in the fetuses of mothers exposed to 250 ppm EGEE. Delayed ossification of the cervical vertebrae and sternbrae and the presence of extra ribs was significantly increased in both the 50 and 250 ppm dose groups.

Teratologic effects on pregnant rabbits from inhalation exposure to EGEE were also reported (Tinston *et al.*, 1983b; also summarized by Doe, 1984). Dutch rabbits (24/group) were exposed to 0, 10, 50, or 175 ppm EGEE for 6 hours/day during gestational days 6-18, with sacrifice occurring on gestational day 29. There were no indications of maternal toxicity or litter effects. A statistically significant increase in minor defects and skeletal variants was found in fetuses in the 175 ppm dose group. Other slightly increased incidences of defects in the lower dose groups alone, including extra ribs and partial ossification of the vertebrae, were not considered treatment-related.

Behavioral teratogenic effects were examined in pregnant Sprague-Dawley rats (14 or 15/dose group) exposed to 0 or 100 ppm EGEE for 7 hours/day through gestational days 7-13 (early) or days 14-20 (late) (Nelson *et al.*, 1981). No maternal toxicity was observed and fetal weights were unchanged, although mean gestational length was increased in rats exposed on gestational days 14-20. Six tests (ascent, rotorod, open field, activity wheel, avoidance conditioning, and operant conditioning) were selected to measure motor, sensory, and cognitive function at several stages of development. The offspring of the rats exposed during days 7-13 exhibited impaired performance on the rotorod test (a test of neuromuscular ability) and increased latency in an open field test (a test of exploratory activity) as compared to controls. The offspring of rats exposed during days 14-20 of gestation exhibited decreased activity on an activity wheel (a test of circadian activity). Also, avoidance conditioning revealed that these pups received shocks of a greater number and duration than controls. Neurochemical differences between the prenatally exposed and control pups were measured in newborns and in pups 21 days of age. In newborns from both EGEE-exposed groups, total brain norepinephrine was decreased. In 21-day old pups of both groups, norepinephrine and dopamine levels in the cerebrum were increased. Serotonin level was increased in the cerebrum of the late exposure group only. The authors concluded that there were behavioral and neurochemical alterations in offspring of rats following prenatal exposure to 100 ppm EGEE, however the study design was inadequate to detect gross teratologic anomalies. In a dose range-finding study, two sets of pregnant rats (3-4/group) were exposed during the gestational days 7-13 or 14-20 to 0, 200 (late group only), 300, 600, 900, or 1200 ppm EGEE for 7 hours/day. Increased fetal and pup mortality was observed in all groups exposed to EGEE.

Behavioral and neurochemical effects on the offspring of pregnant S-D rats exposed to 0 or 200 ppm EGEE on gestational days 7-13 were reported (Nelson *et al.*, 1982a; Nelson *et al.*, 1982b). Pregnancy duration was significantly increased in exposed dams. Significantly increased levels of norepinephrine and dopamine were observed in the 21-day old offspring of EGEE-exposed animals. Behavioral changes in pups of treated dams included decreased neuromotor ability and decreased activity.

An investigation into teratologic effects of EGEE was conducted by exposing pregnant rats and rabbits to EGEE by inhalation on gestational days 0-19 (Andrew *et al.*, 1981). Rats (37/group) were exposed to 0, 202, or 767 ppm EGEE for 7 hours/day. All fetuses were resorbed and maternal weight gain was reduced in the high-dose group. In the mid-dose group, a decrease in fetal weight and size (crown-rump length) was observed. Minor skeletal defects and variants and cardiovascular defects were increased in the mid-dose group. Rabbits (29/group) were exposed to 0, 16, or 617 ppm EGEE for 4 hours/day. Maternal weight gain and food intake were decreased in exposed animals. The incidence of fetal resorptions was increased in both the mid- and high-dose group animals. Major cardiovascular defects and minor skeletal defects (extra ribs, delayed ossification) were significantly increased in the mid-dose group. Andrew *et al.* (1981) also examined reproductive effects by exposing female Wistar rats (37/group) to 1, 150, or 649 ppm EGEE 7 hours/day, 5 days/week for 3 weeks before mating with untreated males. No significant effects were observed.

VI. Derivation of Reference Exposure Level

<i>Study</i>	Barbee <i>et al.</i> , 1984
<i>Study population</i>	Rabbits
<i>Exposure method</i>	Discontinuous inhalation
<i>Critical effects</i>	Testicular degeneration and decreased hemoglobin levels
<i>LOAEL</i>	403 ppm
<i>NOAEL</i>	103 ppm
<i>Exposure continuity</i>	6 hr/day, 5 days/week
<i>Exposure duration</i>	13 weeks
<i>Average experimental exposure</i>	18.4 ppm (68 mg/m ³) for the NOAEL group
<i>Human equivalent concentration</i>	18.4 ppm (68 mg/m ³) for the NOAEL group (gas with systemic effects, based on RGDR = 1.0 using default assumption that lambda (a) = lambda (h))
<i>Subchronic uncertainty factor</i>	10
<i>LOAEL uncertainty factor</i>	1
<i>Interspecies factor</i>	10 (see explanation below)
<i>Intraspecies factor</i>	10
<i>Cumulative uncertainty factor</i>	1000
<i>Inhalation reference exposure level</i>	0.02 ppm (20 ppb, 0.07 mg/m ³ , 70 µg/m ³)

The reproductive effects observed in the subchronic inhalation study of Barbee *et al.* (1984) were determined by the US EPA (U.S. EPA, 1990) to be the most sensitive endpoints due to EGEE exposure and resulted in a reference concentration (RfC) of 0.2 mg/m³ (0.06 ppm). OEHHA staff concurred regarding the basis of the U.S. EPA RfC but differed in the application of the interspecies uncertainty factor. Reduced testes weight and testicular degeneration were found in rabbits exposed to EGEE at 403 ppm for 13 weeks. Changes in hematological parameters including decreased hemoglobin, hematocrit, and erythrocyte count were also observed at this dose. A gas:extrarrespiratory effect ratio of 1.0 was used to calculate a human equivalency concentration (HEC) in the absence of information relating the effect in rabbits relative to humans.

For a comparison with the proposed REL of 60 ppb (200 µg/m³) based on testicular degeneration, a REL can be calculated from the LOAEL of 202 ppm observed in the teratology study of Andrew *et al.* (1981). The 7 h exposure to 202 ppm is time-adjusted to a continuous exposure of 59 ppm. Using a RGDR of 1 for a systemic effect, a UF_L of 10, a UF_A of 3 and a UF_H of 10 results in an estimated REL of 200 ppb (700 µg/m³). Nelson *et al.* (1981) found a LOAEL of 100 ppm for neurobehavioral developmental toxicity in rats exposed 7 hours per day on days 7 to 13 of gestation. The equivalent continuous exposure is 29 ppm. Using an RGDR of 1, a LOAEL UF of 10, an interspecies UF of 3, and an intraspecies UF of 10 results in a REL of 100 ppb (400 µg/m³).

Although reproductive toxicity has been reported in male workers occupationally exposed to EGEE (Clapp *et al.*, 1987; Ratcliffe *et al.*, 1989), potential confounding factors, particularly

exposure to other compounds, make the study inadequate for the development of the reference exposure level. However, for another comparison with the proposed REL of 60 ppb, if only EGEE caused the adverse reproductive effect, use of a mean concentration between the 2 buildings of 8 ppm for workplace exposure, extrapolation to an equivalent continuous exposure of 3 ppm, and division by 10 for a LOAEL (serious effect) and 10 for intraspecies variability result in a REL of 30 ppb ($100 \mu\text{g}/\text{m}^3$).

Another comparison with the proposed REL of 60 ppb can be made using the study of Welch *et al.* (1988), who studied shipyard painters exposed to both EGEE and EGME. The authors examined the semen of 73 painters and 40 non-exposed shipyard employees. The men supplied demographic characteristics, medical conditions, personal habits, and reproductive history; underwent a physical examination; and provided a semen sample. An industrial hygiene survey showed that the painters were exposed to EGEE at a time-weighted average (TWA) concentration varying from 0 to $80.5 \text{ mg}/\text{m}^3$ (mean = $9.9 \text{ mg}/\text{m}^3$), and to EGME at a TWA concentration varying from 0 to $17.7 \text{ mg}/\text{m}^3$ (mean = $2.6 \text{ mg}/\text{m}^3$). The painters had an increased prevalence of oligospermia and azospermia and an increased odds ratio for a lower sperm count per ejaculate. (The results were controlled for smoking.) Adding the mean levels together results in a total glycol ether concentration of $12.5 \text{ mg}/\text{m}^3$, which is equivalent to a continuous exposure of $4.5 \text{ mg}/\text{m}^3$. Division by a UF of 10 for a LOAEL and by another of 10 for intraspecies variability results in a REL of $40 \mu\text{g}/\text{m}^3$ (10 ppb). A similar REL would be calculated using the report by Cullen *et al.* (1992) of depression in red cell pyruvate kinase among anemic and granulocytopenic painters. Since exposure in these studies was to both EGEE and EGME, and exposure assessment was made difficult by frequent job movement and other factors, these studies were not deemed suitable for developing a REL. However, the possibility that humans are more susceptible to EGEE toxicity is raised by the series of studies by Welch *et al.* (1988) and Welch and Cullen (1988) such that we have deviated from the RfC and opted to use an interspecies uncertainty factor of 10 rather than 3 as would usually be the case with an HEC adjustment.

VII. Data Strengths and Limitations for Development of the REL

The strengths of the inhalation REL for EGEE include the availability of subchronic inhalation exposure data from a well-conducted study with histopathological analysis, and the observation of a NOAEL. The observation in several studies noted above of both hematological abnormalities and sperm abnormalities in exposed workers, although difficult to use in a quantitative risk assessment, provide support for the REL developed from animals. In addition, several comparative calculations indicate that RELs based on other studies are generally in agreement with that based on Barbee *et al.* (1984). Major areas of uncertainty are the lack of adequate human exposure data and the lack of chronic inhalation exposure studies.

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CHRONIC TOXICITY SUMMARY

**ETHYLENE GLYCOL MONOETHYL ETHER
ACETATE**

(EGEEA; 1-acetoxy-2-ethoxyethane; 2-ethoxyethanol acetate; 2-ethoxyethyl acetate; acetic acid, 2-ethoxyethyl ester; beta-ethoxyethyl acetate; Cellosolve[®] acetate; ethoxy acetate; ethyl Cellosolve[®] acetate; Poly-solv[®] EE acetate; ethyl glycol acetate; oxitol acetate)

CAS Registry Number: 111-15-9

I. Chronic Toxicity Summary

<i>Inhalation reference exposure level</i>	300 µg/m³ (60 ppb)
<i>Critical effect(s)</i>	Teratogenicity and fetotoxicity in rabbits
<i>Hazard index target(s)</i>	Development

II. Chemical Property Summary (HSDB, 1996)

<i>Description</i>	Colorless liquid
<i>Molecular formula</i>	C ₆ H ₁₂ O ₃
<i>Molecular weight</i>	132.16 g/mol
<i>Boiling point</i>	156°C
<i>Vapor pressure</i>	2 torr @ 20°C
<i>Solubility</i>	Soluble in water (229 g/l at 20°C); sol. in alcohol, ether, acetone; miscible with olive oil, aromatic hydrocarbons
<i>Conversion factor</i>	5.41 µg/m ³ per ppb at 25°C

III. Major Uses and Sources

Ethylene glycol monoethyl ether acetate (EGEEA) is used in automobile lacquers where it retards "blushing" and evaporation and imparts a high gloss (HSDB, 1996). It is also used as a solvent for nitrocellulose, oils, and resins and as a component of varnish removers and wood stains. EGEEA is also used in the treatment of textiles and leather. The annual specific statewide industrial emissions of EGEEA from facilities reporting under the Air Toxics Hot Spots Act in California based on the most recent inventory were estimated to be 66,851 pounds (CARB, 1999).

IV. Effects of Human Exposure

No studies relating exposure to EGEEA to adverse health effects in humans were located in the literature.

Ten male volunteers were exposed to EGEEA by inhalation. Five were exposed to 14, 28, and 50 mg EGEEA/m³ and five to 28 mg/m³ for 4 hours (Groeseneken *et al.*, 1987a). Twenty-two percent of the absorbed dose was eliminated in the urine as ethoxyacetic acid within 42 hours. In another study, male volunteers exposed to EGEEA by inhalation under various conditions were found to eliminate some in the form of ethylene glycol monoethyl ether (EGEE) (Groeseneken *et al.*, 1987b).

V. Effects of Animal Exposure

Pregnant rabbits (24 or 25/group) were exposed to 0, 25, 100, or 400 ppm EGEEA by inhalation for 6 hours/day on gestational days 6-18 (Tinston *et al.*, 1983; reviewed in Doe, 1984). The animals were killed on gestational day 29. Maternal effects (decreased weight gain, decreased food consumption, decreased hemoglobin) were observed in the high-dose group. The number of rabbits with total fetal resorptions was increased in the 400 ppm dose group, accompanied by a decrease in weight in surviving fetuses. A reduction in average fetal weight was also observed at 100 ppm EGEEA, but this effect may relate to the increased litter size among dams in this dose group. Evidence of teratogenicity was observed in the 400 ppm dose group, with increased major malformations of the vertebral column. Both 400 and 100 ppm EGEEA were found to be fetotoxic as indicated by retarded ossification. No statistically significant effects were observed in the 25 ppm dose group, although a single case of a major defect (kidney agenesis) was observed in both the 25 and 400 ppm EGEEA dose groups.

Rats (10/sex/dose) and rabbits (2/sex/dose) were exposed for 4 hours/day, 5 days/week for 10 months to 0 or 200 ppm EGEEA (Truhaut *et al.*, 1979). Observation of body weight gain, hematology, clinical chemistry, and gross pathology revealed no toxic effects among treated animals. Among male rats and rabbits, "discrete lesions of tubular nephritis with clear degeneration of the epithelium with hyaline and granular tubular casts" were observed. Four hour exposure to 2000 ppm EGEEA resulted in transient hemoglobinuria and hematuria in rabbits (2/sex/dose), but not rats (10/sex/dose). No pathological lesions were observed following a 2 week observation period.

Dogs were exposed to 600 ppm EGEEA for 7 hours/day for 120 days (Carpenter *et al.*, 1956; Gingell *et al.*, 1982). Hematological, clinical chemistry, and histopathological examination revealed no adverse effects.

Pregnant rats and rabbits (24/group) were exposed to nominal concentrations of 0, 50, 100, 200 or 300 ppm EGEEA by inhalation during gestational days 6-15 and sacrificed on gestational day 21 (Union Carbide Corporation, 1984). Maternal effects in rats included increased absolute liver weights (all treated groups); increased relative liver weights, and decreased RBC count, hemoglobin, hematocrit, and RBC size (all but low-dose group); decreased food consumption, increased white blood cell count, and decreased platelet count (200 and 300 ppm groups). An increase in the number of non-viable implantations per litter was observed at 300 ppm and

decreased average fetal body weight per litter was observed at 200 and 300 ppm EGEEA. Visceral and skeletal malformations were widely observed at both 200 and 300 ppm EGEEA. Among rabbits, maternal effects included decreased platelets (100, 200, and 300 ppm); decreased weight gain, decreased gravid uterine weight, increased number of dams with non-viable implants, and increased number of non-viable implants per litter (200 and 300 ppm); increased occult blood, increased mean corpuscular volume, decreased corpora lutea/litter and increased early resorptions/litter (300 ppm). Visceral and skeletal malformations were observed in the 100, 200, and 300 ppm EGEEA dose groups.

Pregnant rats were exposed to 0, 130, 390, or 600 ppm EGEEA for 7 hours/day on gestational days 7-15 (Nelson *et al.*, 1984). Dams were sacrificed on day 20. Complete resorption of litters was observed at 600 ppm. Skeletal and cardiovascular defects and decreased fetal weight and fetal resorptions were observed at 390 ppm EGEEA. Reduced fetal weights were also observed at 130 ppm EGEEA.

Ethylene glycol monoethyl ether acetate (0.35 ml = 2.6 mmole/treatment) or water was applied to the shaved skin of pregnant rats four times daily on days 7 to 16 gestation (Hardin *et al.*, 1984). EGEEA treated rats showed reduced body weight (from litter resorption) and significantly fewer live fetuses per litter. Litters from treated dams also showed significantly increased visceral malformations and skeletal variations.

VI. Derivation of Chronic Reference Exposure Level (REL)

<i>Study</i>	Tinston <i>et al.</i> , 1983
<i>Study population</i>	Rabbits
<i>Exposure method</i>	Discontinuous inhalation exposure
<i>Critical effects</i>	Fetotoxicity
<i>LOAEL</i>	100 ppm
<i>NOAEL</i>	25 ppm
<i>Exposure continuity</i>	6 hours/day, 7 days/week
<i>Exposure duration</i>	13 days
<i>Average experimental exposure</i>	6.2 ppm for NOAEL group (25 x 6/24)
<i>LOAEL uncertainty factor</i>	1
<i>Subchronic uncertainty factor</i>	1
<i>Interspecies factor</i>	10
<i>Intraspecies factor</i>	10
<i>Cumulative uncertainty factor</i>	100
<i>Inhalation reference exposure level</i>	0.06 ppm (60 ppb, 0.03 mg/m ³ , 300 µg/m ³)

A review of the literature on the toxicity of EGEEA indicates that the most sensitive endpoint of toxicity is that seen in experimental animals showing developmental effects from inhalation exposure during pregnancy. There are no adequate data associating exposures in humans with toxic effects for the development of a chronic reference exposure level. Separate studies in animals have demonstrated developmental toxicity. Reduced fetal weights were observed in rats exposed to 130 ppm EGEEA on gestational days 7-15 (Nelson *et al.*, 1984). Skeletal and

cardiovascular defects were observed at the next higher dose of 390 ppm EGEEA, and all litters were resorbed in the high-dose group. Visceral and skeletal defects were observed in all but the low-dose group (50 ppm EGEEA) in the litters of rabbit dams exposed to EGEEA on gestational days 6-15 (Union Carbide Corporation, 1984). Fetotoxicity, as indicated by retarded bone development, was observed in all but the low-dose group (25 ppm EGEEA) in the litters of rabbit dams exposed on gestational days 6-18 (Tinston *et al.*, 1983). The lowest dose levels showing developmental toxicity are those reported by Union Carbide Corporation (1984) and Tinston *et al.* (1983), with 100 ppm EGEEA showing developmental defects in the offspring of exposed dams. Since only the Tinston *et al.* (1983) study also showed an exposure level without effect (a NOAEL), this study has been selected for the development of the chronic REL.

VII. Data Strengths and Limitations for Development of the REL

Strengths of the database for EGEEA include the large number of animal studies available. Limitations include the lack of any human data for exposures longer than 4 hours and the lack of sperm count studies, a critical effect for the related compounds, EGEE and EGME. However, the REL calculated is similar to that for EGEE which is based on testicular degeneration.

VIII. References

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CHRONIC TOXICITY SUMMARY

ETHYLENE GLYCOL MONOMETHYL ETHER*(EGME; 2-methoxyethanol; 1-hydroxy-2-methoxyethane; methyl cellosolve)***CAS Registry Number: 109-86-4****I. Chronic Toxicity Summary**

<i>Inhalation reference exposure level</i>	60 µg/m³ (20 ppb)
<i>Critical effect(s)</i>	Testicular toxicity in rabbits
<i>Hazard index target(s)</i>	Reproductive system

II. Physical and Chemical Properties (HSDB, 1995)

<i>Description</i>	Colorless liquid
<i>Molecular formula</i>	C ₃ H ₈ O ₂
<i>Molecular weight</i>	76.09
<i>Density</i>	0.965 g/cm ³ @ 20° C
<i>Boiling point</i>	125°C
<i>Melting point</i>	-85.1°C
<i>Vapor pressure</i>	6.2 torr @ 20°C
<i>Solubility</i>	Miscible with water, alcohol, benzene, ether, acetone
<i>Conversion factor</i>	1 ppm = 3.1 mg/m ³ @ 25°C

III. Major Uses and Sources

Ethylene glycol monomethyl ether (EGME) is used as a solvent for cellulose acetate and resins (HSDB, 1995) as well as a solvent in the semiconductor industry. It is also used in dyeing leather and in the manufacture of photographic film. EGME is used as an anti-freeze in jet fuels. Quick drying varnishes, enamels, nail polishes, and wood stains may also contain EGME. The specific annual statewide industrial emissions of EGME from facilities reporting under the Air Toxics Hot Spots Act in California based on the most recent inventory were estimated to be 7398 pounds (CARB, 1999). (Many industries did not report emissions of specific glycol ethers. Thus there were also emitted 2,922,744 pounds of the general category glycol ethers, which can include EGME.)

IV. Effects of Human Exposure

Human exposures to ethylene glycol monomethyl ether have been associated with hematological and neurological abnormalities. To determine whether employees potentially exposed to ethylene glycol monomethyl ether during manufacturing and packaging had a higher prevalence of anemia, leukopenia, or sterility than an in-plant comparison group, a cross-sectional study was

conducted. Blood samples on 65 of 97 potentially exposed and control white males, and semen samples from a subset of 15 were analyzed. No gross abnormalities or clinically meaningful differences in hematological or fertility indices were noted. Decreased testicular size was reported in workers (who were exposed to an 8-hour TWA concentration of 0.42 ppm EGME or less) but it was not statistically significant (Cook *et al.*, 1982).

Cullen *et al.* (1983) studied possible bone marrow toxicity of workplace substances including dipropylene glycol monomethyl ether, EGME, and various aliphatic, aromatic and halogenated hydrocarbons used for offset and ultraviolet cured multicolor printing. Evaluation of seven co-workers of a printer with aplastic anemia indicated normal peripheral blood, but bone marrow specimens demonstrated clear patterns of injury in three while the others had nonspecific signs of marrow effect. The authors could not assign the changes to known risk factors and concluded that further evaluation of possible bone marrow toxicity resulting from exposure to glycol ethers and ultraviolet curing printing processes was warranted. This was done to some extent in their studies on shipyard painters below.

Welch and Cullen (1988) evaluated shipyard painters exposed to ethylene glycol ethers (EGEE and EGME). Air concentrations at the workplace were estimated based on 102 samples over six shifts in Sparer *et al.* (1988). Time-weighted average (TWA) exposures to EGEE ranged from 0 to 80.5 mg/m³ with a mean of 9.9 mg/m³. TWA exposures to EGME ranged from 0 to 17.7 mg/m³ (mean = 2.6 mg/m³). The authors note that during the time period of measurement, painting activities were unusually low and previous NIOSH analyses indicated considerably higher exposures. Ninety-four painters and 55 controls answered a medical and environmental exposure questionnaire including work history and provided blood, urine, and in some cases semen samples. Mean hemoglobin levels, total cell counts and differential counts did not differ between exposed and control. However, the authors found that the lowest quartile of hemoglobin was mostly painters and the lowest polymorphonuclear leukocyte counts were in painters. Nine painters were considered anemic and five were considered granulocytopenic. The authors note that the absence of a significant difference in the group as a whole and the inability to detect a dose-response pattern in the exposed group makes a strong conclusion unwarranted.

Welch *et al.* (1988) evaluated the semen samples from the workers in the cohort from Welch and Cullen (1988). Sperm concentration, velocity, motility, morphology, morphometry, and viability were measured. Although not statistically significant, the measures of sperm count tended to be lower in the painters with a $p = 0.10$ for density and $p = 0.11$ for count. When nonsmokers were analyzed separately from smokers, the number of oligospermic painters was larger than that in controls at $p = 0.05$. There was no difference between controls and exposed men who were smokers. The authors state that although mean values of sperm count did not differ significantly between controls and exposed groups, biologically important differences were seen when the proportion of men with oligospermia was examined. The proportion of painters with azoospermia was 5% with only 1% expected based on other population surveys. The authors note that to create a dose-response model for an effect of glycol ethers on semen parameters would require description of the exposure of each individual 3 to 6 months prior to sampling. The painters moved frequently from one exposure area to the next, making exposure assessment particularly difficult in this cohort.

Cullen *et al.* (1992) conducted a histopathologic analysis of the bone marrow and circulating blood cells in the workers previously examined in Welch *et al.* (1988). The objects of the study included: 1) to exclude other causes for granulocytopenia and depressed hemoglobin levels noted in some painters exposed to ethylene glycol ethers, 2) to determine if subclinical evidence of hematologic damage is present in healthy coworkers, and 3) to identify host or exogenous factors which may increase the risk of hematologic damage in glycol ether exposed painters. Workers were grouped as follows: Group I consisted of those painters that had anemia or granulocytopenia in the Welch and Cullen (1988) study; Group II consisted of exposed painters with normal hematology; Group III consisted of unexposed controls. A battery of hematologic and biochemical parameters were measured and a questionnaire was completed to determine occupational exposure status, health status and drug and alcohol consumption. All hematologic parameters were normal in all groups. Tests of liver, renal, and thyroid function were normal in all groups. Bone marrow histology showed no differences between groups. One biochemical parameter, pyruvate kinase activity, was lower in Group I than Groups II and III ($p = 0.05$). Depression of red cell pyruvate kinase did not vary by race and was lower in every subject in Group I by more than one standard deviation. Low pyruvate kinase is the most consistent red cell enzyme defect noted in acquired hematologic disorders.

Reversible neurological symptoms (apathy, fatigue, decreased appetite) and macrocytic anemia were observed in a worker following occupational dermal and inhalation exposure to an average concentration of 35 ppm EGME for 1-1.5 years (Cohen, 1984). The worker was also exposed to methyl ethyl ketone and propylene glycol monomethyl ether at concentrations of 1-5 ppm and 4.2-12.8 ppm, respectively.

Hematologic effects were also reported in three women employed in a factory working with glue consisting of 70% acetone and 30% EGME (Larese *et al.*, 1992). The women exhibited abnormally low white blood cell counts, relative lymphocytosis and macrocytosis. These hematological parameters returned to normal following cessation of exposure.

Older case reports support findings of neurological and hematological toxicity following occupational exposure to EGME (Greenburg *et al.*, 1938; Zavon, 1963; Parsons and Parsons, 1938).

V. Effects of Animal Exposure

A concentration dependent decrease in testes weight was observed in male rabbits exposed to 30, 100, or 300 ppm EGME 6 hours per day, 5 days per week for 13 weeks (Miller *et al.*, 1983). Degenerative changes in the germinal epithelium were observed in male rabbits of all exposed groups, but were not statistically significant at 30 ppm. Two of five male rabbits exposed to 300 ppm EGME died during the course of the study. Female rabbits were also exposed; two of five female rabbits exposed to 100 or 300 ppm EGME died during the course of the study. The animals died at different times of different causes and thus the authors were uncertain if the deaths were treatment related. Reduced body weight gain, pancytopenia (abnormal depression of all the cellular elements of the blood), and thymic atrophy were observed in rabbits of both sexes

exposed to 300 ppm EGME. No effects on the reproductive organs of the female rabbits were observed.

In the same study (Miller *et al.*, 1983) male and female rats were exposed to 30, 100, or 300 ppm EGME 6 hours per day, 5 days per week for 13 weeks. Moderate to severe degeneration of the germinal epithelium and seminiferous tubules was observed in male rats exposed to 300 ppm EGME. A significant decrease in body weight was observed in male rats exposed to 300 ppm and in female rats exposed to concentrations of EGME of 100 ppm or greater. Pancytopenia, lymphoid tissue atrophy, and decreased liver weights were observed in animals of both sexes exposed to the highest concentration. Also in the highest exposure group, mean values for total serum protein, albumin and globulins were lower than control values.

Doe *et al.* (1983) designed a two-part study to provide a rapid assessment of the effect of glycol ethers on some aspects of reproduction in the rat. Exposure to EGME was by inhalation at 100 and 300 ppm for 6 hr/day. First, pregnant females were exposed on Days 6 to 17 of gestation. Body weight gain was reduced in both groups. No litters were delivered in the 300-ppm group and only 9/20 rats in the 100-ppm group produced litters; the number, weight, and viability of the pups were reduced, but the pups appeared normal externally. Second, male rats were exposed for 10 days. There was a reduction in testicular weight accompanied by seminiferous tubular atrophy in the 300-ppm group. There were no effects at 100 ppm. Exposure at 300 ppm EGME caused significant reductions in white blood cell count, red blood cell count, hemoglobin concentration, hematocrit, and mean cell hemoglobin.

More recent data point to the immune system as a key endpoint of EGME toxicity. A statistically significant dose-related decrease in thymus weight was observed both in male rats administered drinking water containing 2000 and 6000 ppm EGME (161 or 486 mg/kg/day) and in female rats administered drinking water containing 1600 and 4800 ppm EGME (200 or 531 mg/kg/day) for 21 days (Exon *et al.*, 1991). Histopathological examination revealed thymic atrophy and loss of demarcation between the cortex and medulla. Decreased spleen cell numbers were observed in female rats at both dose levels and male rats at the high dose level. Male rats in the high dose group exhibited a statistically significant decrease in body weight gain. Testicular effects were also observed in exposed male rats.

Pregnant mice were exposed to 100, 150, or 200 mg/kg/day EGME on days 10-17 of gestation (Holladay *et al.*, 1994). Thymic atrophy and inhibition of fetal thymocyte maturation were observed in EGME-treated offspring examined on day 18 of gestation. Also, the ability of the EGME-treated fetal mouse liver cells to repopulate the spleen of irradiated mice was significantly impaired as compared to that of control fetal mouse liver cells.

VI. Derivation of Reference Exposure Level

<i>Study</i>	Miller <i>et al.</i> , 1983; U.S. EPA, 1995
<i>Study population</i>	Rats and rabbits
<i>Exposure method</i>	Inhalation (0, 30, 100, or 300 ppm)
<i>Critical effects</i>	Decreased testes weight and degenerative changes in the testicular germinal epithelium.
<i>LOAEL</i>	100 ppm
<i>NOAEL</i>	30 ppm
<i>Exposure continuity</i>	6 hours/day, 5 days/week
<i>Average experimental exposure</i>	5.4 ppm for NOAEL group
<i>Human equivalent concentration</i>	5.4 ppm for NOAEL group (gas with systemic effects, based on RGDR = 1.0 using default assumption that lambda (a) = lambda (h))
<i>Exposure duration</i>	13 weeks
<i>LOAEL uncertainty factor</i>	1
<i>Subchronic uncertainty factor</i>	10
<i>Interspecies uncertainty factor</i>	3
<i>Intraspecies uncertainty factor</i>	10
<i>Cumulative uncertainty factors</i>	300
<i>Inhalation reference exposure level</i>	0.02 ppm (20 ppb; 0.06 mg/m ³ ; 60µg/m ³)

The REL is based on the same study on which U.S. EPA based its RfC. However, OEHHA declined to use a modifying factor because the criteria for use of such factors are not well described by U.S. EPA. However, since rabbits were the more sensitive species and live 6 years (312 weeks), a 13 week study in rabbits merits a subchronic UF of 10.

A comparison with the proposed REL for EGME of 20 ppb (60 µg/m³) can be made using the occupational study of Welch *et al.* (1988) of the semen of shipyard painters exposed to both EGEE and EGME. The men supplied demographic characteristics, medical conditions, personal habits, and reproductive history; underwent a physical examination; and provided a semen sample. The painters were exposed to EGEE at a TWA concentration of 0 to 80.5 mg/m³ (mean = 9.9 mg/m³, and to EGME at a TWA concentration of 0 to 17.7 mg/m³ (mean = 2.6 mg/m³). The painters had an increased prevalence of oligospermia and azoospermia and an increased odds ratio for a lower sperm count per ejaculate compared to shipyard employees who were not painters. (The results were controlled for smoking.) Adding the mean exposure levels together results in a total glycol ether concentration (EGME + EGEE) of 12.5 mg/m³, equivalent to a continuous exposure of 4.5 mg/m³. Division by a UF of 10 for a LOAEL and by another of 10 for human intraspecies variability results in a REL of 40 µg/m³ (10 ppb), similar to the REL based on rabbits. Since exposure was primarily to EGEE with co-exposure to EGME, and exposure assessment was difficult to quantify, this study was not deemed suitable for developing a REL. Nonetheless, the REL developed using this study is close in value to the proposed REL of 20 ppb.

VII. Data Strengths and Limitations for Development of the REL

The strengths of the inhalation REL for EGME include the availability of subchronic inhalation exposure data from a well-conducted study with histopathological analysis and the observation of a NOAEL. In addition, there are a number of human studies showing similar toxicological endpoints to those demonstrated in animal studies. Major areas of uncertainty are the lack of adequate human exposure data, and the lack of chronic inhalation exposure studies.

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CHRONIC TOXICITY SUMMARY

ETHYLENE GLYCOL MONOMETHYL ETHER ACETATE

(EGMEA; 2-methoxyethanol acetate; 2-methoxyethylester acetic acid; methyl glycol acetate; methyl Cellosolve[®] acetate)

CAS Registry Number: 110-49-6

I. Chronic Toxicity Summary

<i>Inhalation reference exposure level</i>	90 µg/m³ (20 ppb)
<i>Critical effect(s)</i>	Reproductive (testicular) toxicity in rabbits (EGME)
<i>Hazard index target(s)</i>	Reproductive system

II. Chemical Property Summary (HSDB, 1995)

<i>Description</i>	Colorless liquid
<i>Molecular formula</i>	C ₅ H ₁₀ O ₃
<i>Molecular weight</i>	118.3 g/mol
<i>Boiling point</i>	144-145°C
<i>Vapor pressure</i>	2 torr @ 20°C
<i>Solubility</i>	Miscible with water, organic solvents, oils
<i>Conversion factor</i>	4.83 µg/m ³ per ppb at 25°C

III. Major Uses and Sources

Ethylene glycol monomethyl ether acetate (EGMEA) is used as a solvent for nitrocellulose, cellulose acetate, and various other gums, resins, waxes, and oils (HSDB, 1995). It is also used in the semiconductor industry and in textile printing, photographic films, lacquers, and silk-screening inks. The annual specific statewide industrial emissions of EGMEA from facilities reporting under the Air Toxics Hot Spots Act in California based on the most recent inventory were estimated to be 3,060 pounds (CARB, 1999).

IV. Effects of Human Exposure

Developmental defects have been described in the offspring of a mother who was occupationally exposed to EGMEA during pregnancy (Bolt and Golka, 1990). The mother was exposed during pregnancy by skin absorption and inhalation for approximately 1-4 hours/day to 1-2 liters of EGMEA. Her first child was born with congenital hypospadias, chordee, micropenis, and

scrotum bifida and her second child (3 years later) was born with chordee, cryptorchidism, penile hypospadias and scrotum bifida. Both children had normal karyotypes. No estimates of exposure were made.

A single case report described allergic dermatitis which may have developed from contact with EGMEA (Jordan and Dahl, 1971). A 58-year-old woman developed dermatitis on the nose possibly from contact with EGMEA on her eyeglasses. Ethylene glycol monoethyl ether acetate (EGEEA) was also present.

V. Effects of Animal Exposure

Cats, rabbits, guinea pigs, and mice were repeatedly exposed by inhalation for 8 hours daily to 500 and 1000 ppm EGMEA (Gross, 1943; as described by Gingell *et al.*, 1982). This exposure regimen was fatal to cats at 500 ppm EGMEA. Death occurred after the animals showed slight narcosis. Similarly, exposure to 1000 ppm EGMEA produced deaths among rabbits, guinea pigs, and mice within a few days. Kidney toxicity was observed in animals in both dose groups. Repeated 4- and 6-hour exposure of cats to 200 ppm EGMEA resulted in decreased "blood pigments" and red blood cell counts.

The toxic effects of EGMEA were examined in male mice treated by gastric intubation 5 days/week for 5 weeks with 0, 62.5, 125, 250, 500, 1000, or 2000 mg EGMEA/kg/day (Nagano *et al.*, 1984). Dose-related testicular atrophy was observed at doses above 250 mg EGMEA/kg/day. Decreased white blood cell counts were observed in all EGMEA-exposed groups.

EGMEA was readily converted *in vitro* to ethylene glycol monomethyl ether (EGME) by the nasal mucosal carboxylesterases of mice and rabbits (Stott and McKenna, 1985). The enzyme activity in the nasal mucosa was equal to that of the liver and greater than that of the kidney and lung.

A concentration dependent decrease in testes weight was observed in male rabbits exposed to 30, 100, or 300 ppm ethylene glycol monomethyl ether (EGME) 6 hours/day, 5 days/week for 13 weeks (Miller *et al.*, 1983). Degenerative changes in the germinal epithelium were observed in male rabbits of all exposed groups, but the changes were not statistically significant at 30 ppm. Two of five male rabbits exposed to 300 ppm EGME died during the course of the study. Female rabbits were also exposed; two of five female rabbits exposed to 100 or 300 ppm EGME died during the course of the study. Reduced body weight gain, pancytopenia (abnormal depression of all the cellular elements of the blood), and thymic atrophy were observed in rabbits of both sexes exposed to 300 ppm EGME. No effects on the reproductive organs of the female rabbits were observed.

In the same study male and female rats were exposed to 30, 100, or 300 ppm EGME 6 hrs/day, 5 days/week for 13 weeks. Moderate to severe degeneration of the germinal epithelium and seminiferous tubules was observed in male rats exposed to 300 ppm EGME. A significant decrease in body weight was observed in male rats exposed to 300 ppm and in female rats

exposed to concentrations of EGME of 100 ppm or greater. Pancytopenia, lymphoid tissue atrophy, and decreased liver weights were observed in animals of both sexes exposed to the highest concentration. Also in the highest exposure group, mean values for total serum protein, albumin and globulins were lower than control values.

VI. Derivation of Chronic Reference Exposure Level (REL)

<i>Study</i>	Miller <i>et al.</i> , 1983 (see below)
<i>Study population</i>	Rabbits
<i>Exposure method</i>	Discontinuous inhalation exposure (0, 30, 100, or 300 ppm EGME)
<i>Critical effects</i>	Testicular effects
<i>LOAEL</i>	100 ppm EGME
<i>NOAEL</i>	30 ppm EGME
<i>Exposure continuity</i>	6 hr/day, 5 days/week
<i>Exposure duration</i>	13 weeks
<i>Average experimental exposure</i>	5.4 ppm EGME for NOAEL group (30 x 6/24 x 5/7)
<i>Human equivalent concentration</i>	5.4 ppm EGME for NOAEL group (gas with systemic effects, based on RGDR = 1.0 using default assumption that lambda (a) = lambda (h))
<i>LOAEL uncertainty factor</i>	1
<i>Subchronic uncertainty factor</i>	10
<i>Interspecies factor</i>	3
<i>Intraspecies factor</i>	10
<i>Cumulative uncertainty factor</i>	300
<i>Inhalation reference exposure level</i>	0.02 ppm (20 ppb, 0.06 mg/m ³ , 60 µg/m ³) EGME 90 µg/m ³ EGMEA (20 ppb) (60 x MW _{EGMEA} / MW _{EGME})

Data relating specific EGMEA exposure levels to toxicity in humans are not available for the development of a chronic REL. Data from experimental animals indicate that EGMEA is toxic to the hematopoietic and reproductive systems (Gross, 1943; Nagano *et al.*, 1984), however good, quantitative data relating chronic exposure to toxicity are lacking. Because of evidence that EGMEA is readily converted to EGME by several organ systems (Stott and McKenna, 1985) and since the scant data on EGMEA toxicity in animals indicate that the spectrum of toxicity of the two compounds is similar, the chronic REL was derived based upon the assumption of equimolar toxicity of EGMEA and EGME.

VII. Data Strengths and Limitations for Development of the REL

The strengths of the inhalation REL for EGMEA include the availability of subchronic inhalation exposure data from a well-conducted study of EGME as well as a number of supportive human studies on EGME showing the same toxicological endpoint, and the observation of a NOAEL.

Major areas of uncertainty are the assumption that EGMEA toxicity is comparable to that of EGME, the lack of adequate human exposure data, and the lack of chronic inhalation exposure studies.

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CHRONIC TOXICITY SUMMARY

ETHYLENE OXIDE*(oxirane, dimethylene oxide, epoxyethane)***CAS Registry Number: 75-21-8****I. Chronic Toxicity Summary**

<i>Inhalation reference exposure level</i>	30 µg/m³ (18 ppb)
<i>Critical effect(s)</i>	Neurotoxicity in rats
<i>Hazard index target(s)</i>	Nervous system

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

<i>Description</i>	Colorless gas
<i>Molecular formula</i>	C ₂ H ₄ O
<i>Molecular weight</i>	44.06 g/mol
<i>Density</i>	1.80 g/L @ 25°C
<i>Boiling point</i>	10.6°C
<i>Melting point</i>	-111.6°C
<i>Vapor pressure</i>	1095 torr @ 20°C
<i>Conversion factor</i>	1 ppm = 1.80 mg/m ³

III. Major Uses or Sources

The majority of all ethylene oxide (EtO) produced is used as a chemical intermediate in the production of various compounds including ethylene glycol, glycol ethers, and non-ionic surfactants (ATSDR, 1990). EtO is also used as a fumigant for food and cosmetics, and in hospital sterilization of surgical equipment and heat sensitive materials such as plastics. The annual statewide industrial emissions from facilities reporting under the Air Toxics Hot Spots Act in California based on the most recent inventory were estimated to be 43,972 pounds of ethylene oxide (CARB, 2000).

IV. Effects of Human Exposure

Ten hospital sterilizer workers were matched with controls and examined for physical and neuropsychological health (Estrin *et al.*, 1990). The workers had operated sterilizers using 12% EtO and 88% Freon for an average of 5 years (range 0.5-10 years). Regular monitoring of workroom air had not been done. Measurements at the time of the study indicated concentrations of 15 ppm EtO or less. However, a second measurement showed an air concentration of 250 ppm EtO. A significantly greater percent of exposed workers exhibited a

bilateral reflex reduction in the ankle compared to controls. Nerve conduction tests did not identify significant differences between control and exposed workers, but a highly significant reduction ($p = 0.009$) in finger tapping speed was observed in exposed workers. The exposed group also performed more poorly on tests of spatial and visual abilities, and on tests of visual motor function. The results extended previous work by the same group (Estrin *et al.*, 1987).

Cognitive impairment and personality dysfunction were observed more frequently in hospital workers chronically exposed to EtO, compared to a control group (Klees *et al.*, 1990). A group of 22 hospital workers, who had been exposed to an 8-hour TWA of 4.7 ppm EtO for a mean of 6.13 years (range 1-11 years), were matched with 24 control subjects. Neuropsychological function in the workers was classified as either normal or impaired on the basis of the questionnaires and of neuropsychological tests by 2 clinical psychologists (who were unaware of exposure status). (If the classification of the two clinicians did not agree, the subject was classified as “disagreement.” Disagreement occurred in 7/23 (30%) of the controls and 10/22 (45%) of the exposed.) Exposed subjects were significantly more frequently classified as impaired (5/12) compared to controls (1/16) ($\chi^2 = 6.0861$; $p < 0.05$). The Klees *et al.* (1990) study cites several earlier case reports of EtO neurotoxicity.

Recent studies have identified hemoglobin adducts, sister chromatid exchanges, and other hematological effects as indicators of ethylene oxide exposure (Ribeiro *et al.*, 1994; Sarto *et al.*, 1991). However, a recent study of 68 female workers from 9 hospitals in the U.S. and one in Mexico not only reports biological indicators of ethylene oxide exposure, but also provides a complete blood count with differential (Schulte *et al.*, 1995). The workers were classified as low- or high-exposure based on a mean 8-hour time weighted average of 0.08 or 0.17 ppm EtO. The mean length of employment for workers from U.S. hospitals was 5.5 and 10 years for low- and high-exposure workers, respectively. The mean length of employment in low- and high-exposure workers from the hospital in Mexico was 5.9 and 4.2 years, respectively. In workers from U.S. hospitals only, statistically significant decreases in hematocrit and hemoglobin were observed in high-exposure workers compared to low-exposure workers. Also, a statistically significant increase in lymphocytes and a significant decrease in neutrophils were observed in high-exposure workers compared to controls. In the workers from the hospital in Mexico, a significant relationship of EtO exposure and elevated neutrophil count was observed using regression.

At least 2 epidemiological reports indicate a possible association of EtO exposure and spontaneous abortion. Hemminki *et al.* (1982) analyzed spontaneous abortions in Finnish hospital sterilizing staff using data from a postal questionnaire and from a hospital discharge register. The study included all sterilizing staff employed in Finnish hospitals in 1980; the controls were nursing auxiliaries. When the women were involved in sterilizing procedures during their pregnancies, the frequency of spontaneous abortion was 16.7% versus 5.6% for the non-exposed pregnancies. The independent analysis of spontaneous abortions using the hospital discharge register confirmed the findings. Thus two analyses suggested that EtO exposure may carry a risk of spontaneous abortion among sterilizing staff.

More recently Rowland *et al.* (1996) sent questionnaires to 7,000 dental assistants (ages 18-39 years) registered in California in 1987. Of these, 4,856 responded (69%). They analyzed 1,320

women whose most recent pregnancy was conceived while working full-time. Thirty-two reported exposure to EtO; unexposed dental assistants comprised the comparison group. Among exposed women, the age-adjusted relative risk (RR) of spontaneous abortion was 2.5 [95% (CI) = 1.0-6.3]. The RR for pre-term birth was 2.7 (95% CI = 0.8-8.8) and the RR for post-term birth was 2.1 (95% CI = 0.7-5.9). The RR of any of these adverse outcomes among exposed women was estimated to be 2.5 (95% CI = 1.0-6.1). These results also indicate a possible relationship of EtO and spontaneous abortion.

V. Effects of Animal Exposure

A 2 year inhalation bioassay exposed groups of 80 male rats to 0, 50, or 100 ppm EtO 7 hours per day, 5 days per week for 104 weeks (Lynch *et al.*, 1984). Mean body weights were significantly lower and mortality was significantly higher in both exposure groups. Inflammatory lesions of the lung, nasal cavity, trachea, and inner ear were observed more frequently in EtO exposed rats. Skeletal muscle myopathy, consisting of atrophy and degeneration of skeletal muscle fibers, was observed more frequently in rats exposed to 100 ppm EtO compared to controls. Neoplastic changes were also observed in EtO exposed rats.

Mice (30 per sex) were exposed to 0, 10, 50, 100, or 250 ppm EtO for 6 hours per day, 5 days per week, for 10 weeks (males) or 11 weeks (females) (Snellings *et al.*, 1984). Neuromuscular screening was conducted, and samples of urine and blood were collected. A significantly greater percent of exposed mice exhibited abnormal posture during gait and reduced locomotor activity. A dose-response was observed for these effects, with significant changes at 50 ppm and greater. An abnormal righting reflex was observed in a significantly greater percent of mice exposed to 100 ppm and above. Reduced or absent toe and tail pinch reflexes were observed in a significantly greater percent of mice exposed to 250 ppm EtO. Hematological changes observed in mice exposed to 250 ppm include slight, yet significant, decreases in red blood cell count, packed cell volume, and hemoglobin concentration. Absolute and relative spleen weights were significantly decreased in female mice exposed to 100 and 250 ppm and in male mice exposed to 250 ppm EtO. A significant increase in relative liver weight was observed in female mice exposed to 250 ppm EtO. Male mice exhibited a significant decrease in body weight at 10, 50, and 250 ppm and a significant decrease in absolute testes weights at 50, 100, or 250 ppm EtO. This study indicates a subchronic NOAEL for neurological effects of 10 ppm EtO.

In a study of the testicular effects of EtO, male rats were exposed to 500 ppm EtO 6 hours per day, 3 days per week for 2, 4, 6, or 13 weeks (Kaido *et al.*, 1992). An awkward gait was observed in rats after 6-9 weeks of exposure. Although no significant changes in body weight were observed, a statistically significant dose-related decrease in testes weight was observed at 4, 6, and 13 weeks of exposure. Progressive degeneration and loss of germ cells were also observed during the 13 week exposure. While severe loss of germ cells and marked morphological changes in remaining germ cells were observed at 6 weeks of exposure, some intact spermatids were observed at 13 weeks of exposure. This suggests that recovery of spermatogenesis occurred.

Saillenfait *et al.* (1996) studied the developmental toxicity of EtO in pregnant Sprague-Dawley rats using inhalation exposure during gestation days 6 to 15. Two protocols were used: (1)

exposure for 0.5 hr once a day to 0, 400, 800, or 1200 ppm EtO; or (2) exposure for 0.5 hr three times a day to 0, 200, or 400 ppm EtO or to 0, 800, or 1200 ppm EtO. The second protocol caused fetal toxicity as indicated by reduced fetal weight at 800 ppm (the LOAEL for this endpoint) and at 1200 ppm, and overt maternal toxicity manifested as reduced body weight gain at 1200 ppm. No embryolethality or teratogenicity occurred in either exposure protocol.

VI. Derivation of Chronic Reference Exposure Level (REL)

<i>Study</i>	Snellings <i>et al.</i> , 1984
<i>Study population</i>	Male and female B6C3F1 mice
<i>Exposure method</i>	Inhalation chamber exposure to 0, 10, 50, 100, or 250 ppm ethylene oxide
<i>Critical effects</i>	Impaired neurological function
<i>LOAEL</i>	50 ppm
<i>NOAEL</i>	10 ppm
<i>Exposure continuity</i>	6-hours/day, 5 days/week
<i>Exposure duration</i>	10 weeks (males), or 11 weeks (females)
<i>Average experimental exposure</i>	1.79 ppm (10 x 8/24 x 5/7)
<i>Human equivalent concentration</i>	1.79 ppm ((gas with systemic effects, based on RGDR = 1.0 using default assumption that $\lambda(a) = \lambda(h)$)
<i>LOAEL uncertainty factor</i>	1
<i>Subchronic uncertainty factor</i>	3
<i>Interspecies uncertainty factor</i>	3
<i>Intraspecies uncertainty factor</i>	10
<i>Cumulative uncertainty factor</i>	100
<i>Inhalation reference exposure level</i>	18 ppb (30 $\mu\text{g}/\text{m}^3$)

Snellings *et al.* (1984) found a subchronic NOAEL of 10 ppm for neurological effects in mice. A neuromuscular screening test indicated that certain reflex responses and locomotor activities were altered in EtO-exposed animals. Human studies have also indicated neurological impairment in ethylene oxide exposed workers.

VII. Data Strengths and Limitations for Development of the REL

The strengths of the inhalation REL for ethylene oxide include the use of an animal study with both a LOAEL and a NOAEL and the use of an endpoint seen in both animals and humans.

Major areas of uncertainty are the short time-frame of the key study, the lack of an appropriate human study, and the limited number of developmental toxicity studies.

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CHRONIC TOXICITY SUMMARY

FLUORIDES *including* HYDROGEN FLUORIDE

(hydrofluoric acid (aqueous solution); hydrogen fluoride (as a gas);
fluoride salts (particulates or in solution))

CAS Registry Number: 7664-39-3

I. Chronic Toxicity Summary

<i>Inhalation reference exposure level</i>	14 $\mu\text{g HF/m}^3$ (17 ppb); 13 $\mu\text{g F/m}^3$
<i>Oral reference exposure level</i>	0.04 mg/kg-day
<i>Critical effect(s)</i>	Skeletal fluorosis
<i>Hazard index target(s)</i>	Bone and teeth; respiratory system

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

<i>Description</i>	Colorless gas (HF), or as particulates
<i>Molecular formula</i>	HF
<i>Molecular weight</i>	20.0 g/mol
<i>Density</i>	0.83 g/L @ 25°C
<i>Boiling point</i>	19.54°C
<i>Melting point</i>	-83.1°C
<i>Vapor pressure</i>	400 torr @ 2.5°C
<i>Solubility</i>	Soluble in water and alcohol
<i>Conversion factor</i>	1 ppm = 0.83 mg/m ³ @ 25°C

III. Major Uses or Sources

Hydrofluoric acid (HF) is a colorless, fuming liquid with a sharp, penetrating odor (Fairhall, 1949). This acid is used in the glass etching, electronic, microelectronic, and petroleum refining and chemical industries (Bertolini, 1992). These industries use HF in the manufacture of such things as computer chips (an important industry in California), phosphate fertilizer, metal cans, plastics, refrigerant chemicals (fluorocarbons), inorganic chemicals, soaps and detergents, high-octane gasoline, and aircraft parts (Wohlslagel *et al.*, 1976; Wing *et al.*, 1991). HF is also used in commercial rust removal products. Another high profile use of HF in California has been as a catalyst in petroleum alkylation to make high-octane gasoline. HF is also a product of combustion of any F containing materials; as such, it is produced during structural fires.

Sodium fluoride has been used as a topical and ingested anticaries agent due to its ability to harden tooth enamel during development. The optimal doses are not well established, but have been suggested to be approximately 0.080 mg/kg/day for 7 to 9 month old infants decreasing to 0.034 mg/kg/day at 13 years of age (Shulman *et al.*, 1995). A dose of 1.0 mg F ingested per day was reported to reduce dental caries 43%, and to be associated with a greatly increased rate of minor tooth mottling which caused no esthetic damage (Van Nieuwenhuysen and D'Hoore, 1992). Many communities in California routinely add fluoride to the drinking water. The California Department of Health Services has adopted regulations that establish standards for the addition of F (CDHS, 2002). Any public water system using fluoridation must maintain F levels within the range established for its climate. The ranges vary according to average air temperatures, since people in cooler climates typically drink less water per day than people in warmer climates. Thus, in cooler areas, more F is required to provide the same dental benefit. For 2001-2002, F levels in San Francisco municipal water ranged from 0.65 to 1.1 ppm, while in Los Angeles the range was 0.44 to 0.83 ppm (CDHS, 2002).

The annual statewide industrial emissions from facilities reporting under the Air Toxics Hot Spots Act in California based on the most recent inventory were estimated to be 48,221 pounds of fluorides and compounds, and 62,670 pounds of hydrogen fluoride (CARB, 2000).

IV. Effects of Human Exposure

The chronic exposure to fluorides, including HF, and the incidence of minimal osseous changes were studied in the workplace by Derryberry *et al.* (1963). In this study, the 8-hour time-weighted average fluoride exposure was calculated for the employment period of each of 74 male workers (30 Caucasian, 44 African-American). The overall average fluoride exposure in these workers was measured as a time-weighted average of 2.81 mg F/m³. In comparison, the 17 workers within this group who had evidence of minimally increased bone density had an average fluoride exposure of 3.38 mg F/m³. The other workers were exposed to an average measured concentration of 2.64 mg F/m³. In addition, urinary fluoride levels were greater in the 17 individuals with greatest exposure compared to the remaining 57 workers (average = 5.18 mg F/L vs. 4.53 mg F/L). No differences between exposed and unexposed individuals were observed for gastrointestinal, cardiovascular, or hematologic systems, or in a physical exam. A statistically significant ($p < 0.05$) increase in the incidence of acute respiratory disease as determined from past medical histories was observed in fluoride-exposed individuals (19/74 vs. 8/67 in controls); radiographic examination revealed a difference of lesser significance ($p < 0.10$) for pulmonary changes (11/74 vs. 4/67). No pulmonary function tests were reported.

An analysis of these data by OEHHA (see derivation section below) showed a statistically significant relationship between air fluoride and the minimal bone density increases. The raw data from the Derryberry *et al.* (1963) study are shown in Table 1. A Pearson correlation matrix of the variables measured in the Derryberry *et al.* study indicated that bone density was best correlated with mean air fluoride level, and to a lesser extent with the age of the individual. A log-logistic regression using the log air fluoride concentration as the independent variable showed a significant ($p < 0.033$) relationship between increasing air fluoride concentrations and probability of skeletal fluorosis. The parameters for the regression were $\beta_0 = -2.3468$ (std. error

= 0.6462), and $\beta_1 = 1.1736$ (std error = 0.5508); the odds ratio for the occurrence of skeletal fluorosis was 3.24. Years of exposure were not correlated with increased bone-density, according to a Pearson Correlation procedure ($p = 0.63$). Bone density has been shown to decrease with age after the age of 40 among normal, non-fluoride-exposed males (Runge *et al.*, 1979). As expected, age was very highly correlated with years exposed ($p < 0.00001$). Therefore including years exposed in the dose-metric likely introduces a confounding variable (see discussion in Section VI.). In addition, Runge *et al.* (1979) found no association between years exposed and mineral content or bone width among 245 aluminum smelter workers exposed to 2.75 or 3.2 mg F/m³. For these reasons, years exposed were not used as the dose-metric for bone-density in this analysis.

Although a threshold was not readily apparent from the logistic regression model, grouping the 74 individuals by air fluoride exposure level into quintiles of 15 each with one group of 14, allowed for a comparison of group mean responses (Table 2). The 14 employees exposed to a time-weighted average concentration of 1.07 mg F/m³ did not exhibit bone density changes. An analysis of the grouped responses using a binomial distribution showed a probability of $p = 0.008$ for obtaining 4/15 increased bone density observations in the 2.34 mg/m³ group, and a probability of $p = 0.047$ for obtaining 3/15 positive observations in the 1.89 mg F/m³ group. The 1.89 mg F/m³ group was therefore considered a LOAEL for chronic skeletal fluorosis, and the 1.07 mg/m³ group was considered a NOAEL. The above probabilities assume that a chance occurrence is, at most, 1 in 18 of skeletal fluorosis or other cause leading to an abnormally dense x-ray in the general population. Since osteosclerosis is a rare condition that is associated with several types of hematological malignancies such as myeloid leukemia, the actual incidence of conditions leading to osteosclerosis is far below 1 in 18. This lends strong support to the consideration of 1.89 mg/m³ as a LOAEL for skeletal fluorosis.

Table 1. Data on worker exposure to fluoride from Derryberry *et al.* (1963)

Observation #	ID	Bone density	Years exposed	Urine max F (mg F/L)	Urine min F (mg F/L)	Mean urinary F (mg F/L)	Age (years)	Air fluoride (mg/m ³)	OEHHA exposure grouping
1	119	normal	18.5	43.0	2.8	14.7	58	8.16	5
2	0	normal	8.4	24.7	5.3	9.6	42	3.19	4
3	41	normal	15.8	35.0	2.5	9.1	35	3.29	4
4	147	minimally increased	9.6	17.1	2.1	8.9	60	5.98	5
5	120	normal	16.7	20.5	3.4	8.6	55	3.29	4
6	54	minimally increased	17.0	44.0	4.0	8.6	56	7.73	5
7	148	normal	10.5	14.0	3.7	8.4	41	8.32	5
8	314	minimally increased	14.4	22.7	1.7	8.3	56	3.24	4
9	29	normal	17.0	18.2	2.5	7.7	50	2.60	3
10	14	normal	14.3	19.4	2.1	6.3	46	2.33	3
11	115	normal	15.2	18.5	1.4	6.3	38	2.11	3
12	10	minimally increased	10.3	22.0	2.3	6.1	38	2.72	4
13	4	minimally increased	7.1	7.7	2.0	5.7	54	3.22	4
14	51	normal	14.9	42.0	0.8	5.6	46	3.18	4
15	94	normal	16.2	15.4	3.3	5.5	56	5.12	5
16	217	normal	7.1	7.1	2.6	5.3	42	2.54	3
17	281	minimally increased	7.8	8.6	1.1	5.2	36	3.79	4
18	114	normal	10.4	13.2	2.8	5.2	38	7.66	5
19	7	normal	7.8	9.1	2.2	5.1	43	2.91	4
20	308	normal	11.9	6.7	3.5	5.1	44	1.89	2
21	301	minimally increased	15.2	9.5	2.5	5	36	2.56	3
22	72	normal	25.9	13.7	2.1	4.9	55	5.55	5
23	241	minimally increased	17.0	10.0	1.9	4.9	46	4.48	5
24	345	normal	10.5	7.1	2.0	4.9	47	1.49	1
25	26	normal	16.4	12.2	0.5	4.7	39	2.41	3
26	231	minimally increased	16.3	8.2	2.8	4.6	62	1.88	2
27	2	normal	24.7	8.9	2.1	4.6	46	3.53	4
28	295	normal	14.5	10.7	0.9	4.6	44	2.07	3
29	1	normal	8.9	5.9	2.4	4.5	30	1.92	2
30	203	minimally increased	18.2	6.8	1.6	4.4	43	2.66	3
31	63	normal	16.2	7.4	2.0	4.3	55	3.90	5
32	5	normal	4.5	11.5	1.9	4.3	43	1.12	1
33	460	normal	12.5	6.1	1.6	4.3	60	2.13	3

Observation #	ID	Bone density	Years exposed	Urine max F (mg F/L)	Urine min F (mg F/L)	Mean urinary F (mg F/L)	Age (years)	Air fluoride (mg F/m ³)	OEHHA exposure grouping
34	249	minimally increased	15.0	8.0	1.8	4.3	39	2.95	4
35	3	normal	7.6	14.5	2.1	4.3	31	3.90	5
36	322	normal	9.3	6.3	2.0	4.3	35	4.23	5
37	8	minimally increased	24.8	5.9	3.0	4.2	55	2.50	3
38	3	normal	15.2	12.2	2.1	4.2	42	1.14	1
39	309	normal	12.1	5.5	2.4	4.1	42	1.94	2
40	36	normal	9.1	13.2	0.8	4.1	33	1.94	2
41	45	normal	11.3	14.0	2.2	4.1	33	3.84	4
42	70	normal	17.9	8.0	1.0	3.9	44	4.00	5
43	250	minimally increased	9.8	6.7	1.5	3.9	35	1.78	2
44	38	normal	16.9	5.9	1.0	3.9	35	2.10	3
45	200	minimally increased	14.0	7.0	2.8	3.8	66	3.92	5
46	183	normal	9.8	4.9	2.2	3.7	48	1.67	2
47	32	normal	12.5	6.6	0.9	3.7	47	2.21	3
48	25	normal	13.6	5.5	1.5	3.7	44	1.86	2
49	21	normal	13.9	9.1	0.4	3.7	50	1.98	2
50	304	normal	13.4	5.0	2.1	3.7	36	2.62	3
51	132	normal	10.9	5.1	2.4	3.6	39	1.81	2
52	6	minimally increased	8.4	4.8	0.9	3.6	35	3.85	5
53	244	normal	16.6	7.1	1.4	3.6	62	2.87	4
54	30	normal	14.0	14.0	0.9	3.6	43	1.56	1
55	88	minimally increased	15.5	4.9	1.7	3.5	66	2.06	2
56	227	normal	16.6	5.7	1.0	3.5	41	1.18	1
57	271	normal	17.7	4.1	3.0	3.4	60	1.82	2
58	19	normal	13.9	10.0	1.8	3.4	41	1.32	1
59	190	normal	9.3	7.7	1.9	3.3	36	1.95	2
60	258	normal	17.8	5.6	1.6	3.2	58	0.87	1
61	278	normal	10.0	7.0	0.3	3.2	34	1.93	2
62	331	normal	12.8	5.6	1.5	3.1	34	1.23	1
63	91	normal	25.3	7.9	0.2	3.1	63	3.49	4
64	342	normal	18.5	6.0	1.3	3	40	2.73	4
65	261	normal	18.1	5.3	0.9	2.9	52	4.41	5
66	291	normal	13.5	4.5	1.5	2.8	34	2.14	3
67	149	normal	11.3	4.5	2.1	2.8	34	0.76	1
68	2	normal	24.7	4.5	1.5	2.7	51	1.15	1
69	4	normal	16.8	5.7	1.2	2.7	56	0.71	1
70	109	normal	8.3	5.1	0.8	2.7	36	1.89	2
71	242	normal	18.1	4.1	1.2	2.5	49	1.26	1

Observation #	ID	Bone density	Years exposed	Urine max F (mg F/L)	Urine min F (mg F/L)	Mean urinary F (mg F/L)	Age (years)	Air fluoride (mg F/m ³)	OEHHA exposure grouping
72	179	normal	18.9	3.9	1.0	2.4	46	0.50	1
73	325	minimally increased	11.8	5.0	0.5	2.2	40	2.10	3
74	159	normal	18.9	5.0	0.7	2.1	45	0.67	1

Table 2. Grouped mean exposure

Exposure group	Mean age ± SD	Mean air level mg F/m ³ ± SD	Number of responses	Probability of difference from group 1*
1	45.0 ± 7.0	1.07 ± 0.32	0/14**	Not Applicable
2	43.9 ± 11.2	1.89 ± 0.09	3/15***	0.047
3	43.0 ± 7.6	2.34 ± 0.23	4/15	0.008
4	45.9 ± 9.8	3.22 ± 0.35	5/15	0.001
5	48.5 ± 10.7	5.41 ± 1.72	5/15	0.001

* Probability of obtaining result assuming a chance occurrence of abnormally dense x-ray of, at most, 1 in 18 individuals, using a binomial distribution (Systat for Windows v.5.05, 1994).

** NOAEL

*** LOAEL ($p < 0.05$)

Largent *et al.* (1951) found a significant increase in bone density in the lower thoracic spine, with calcification extending into the lateral ligaments of 3 workers exposed for 17, 14, and 10 years to HF (concentrations not estimated).

A group of 74 men, who were occupationally exposed to unspecified concentrations of HF for an average of 2.7 years, reported occasions of upper respiratory irritation (Evans, 1940). Repeated chest X-rays over a 5-year period did not reveal any visible evidence of lung changes. The death rate of these workers from pneumonia and other pulmonary infections was the same as that of unexposed plant employees.

There are various reports of asthma and related respiratory effects in pot room workers in the primary aluminum smelting industry. Exposure to fluoride (among other materials such as sulfur trioxide and polycyclic aromatic hydrocarbons) was measured as a possible index of exposures related to this condition (Seixas *et al.*, 2000). However multiple exposures to respiratory irritants and other compounds which may affect immune response appear to be common in this work environment making it difficult to quantitatively relate the respiratory symptoms to inhaled HF or fluorides.

Workers in a warehouse containing HF retorts experienced transitory hyperemia of the skin on their face and hands (Dale and McCauley, 1948). Twenty four of the 40 workers had definite changes in the thickness and number of trabeculae in the upper and lower jaw.

Examinations of 107 pot room workers in two aluminum plants with airborne fluorides revealed 22 subjects with limited motion of the dorsolumbar spine, compared with none in a control group of 108 workers with no history of exposure to fluorides (Kaltreider *et al.*, 1972). In one plant, 76 of 79 workers had increased bone density as measured by roentgenogram, with diagnosis of slight to moderate fluorosis. Moderate and marked fluorosis was observed after 15 years employment. The 8-hour time-weighted average fluoride content in these workplaces was 2.4 to 6.0 mg/m³. Balazova (1971) measured significant fluoride uptake and distribution in children living near an aluminum smelter but reported no incidence of fluorosis.

No studies regarding the chronic irritant or respiratory effects of pure HF exposure in humans were available.

Fluoride ion produced by various fluorocarbons has been associated with toxicity to human kidney collecting duct cells leading to sodium and water disturbances (Cittanova *et al.*, 1996).

Oral supplementation of greater than 0.1 mg F/kg body weight daily has been associated with enamel fluorosis in young children (Forsman, 1977).

The Agency for Toxic Substances and Disease Registry (ATSDR, 2001) recently reviewed fluorides since they are found at hazardous waste sites which are candidates for remediation. The focus of this document was on oral exposure studies as that is the main concern for waste site remediation.

V. Effects of Chronic Exposures to Animals

Stokinger (1949) studied the subchronic effects of HF inhalation in several animal species. Animals (dogs, rabbits, rats, guinea pigs, and mice; 1 to 6 per group) were exposed to 0, 7.2 mg/m³, or 25.1 mg/m³ 6 hours/day, 6 days/week, for 30 days. Mortality, body weight, blood coagulation mechanisms, and gross pathology were measured. Exposure to 25.1 mg/m³ HF for 30 days resulted in degenerative testicular changes and ulceration of the scrotum in all 4 dogs and hemorrhage and edema in the lungs of 3 dogs. Pulmonary hemorrhage was also seen in 20 of 30 rats, and 4 of 10 rabbits. Renal cortical degeneration was observed in 27 of 30 rats. All of the rats and mice at the 25.1 mg/m³ concentration died. No mortality was observed in the other species tested. Blood fibrinogen levels were significantly increased in dogs, rats, and rabbits exposed to 25.1 mg/m³. Exposure to 7.2 mg/m³ HF resulted in pulmonary hemorrhage in 1 out of 5 dogs. No other significant effects were observed at the lower concentration.

Shusheela and Kumar (1991) administered male rabbits 10 mg NaF/kg-bw per day orally for 18 months (7 rabbits) or 29 months (3 rabbits), then studied the testis, epididymis, and vas deferens microscopically. After 29 months of F administration, the spermatogenic cells in the seminiferous tubules had degenerated and lacked spermatozoa. After both 18 and 29 months, cilia were lost from the epithelial cells lining the ductuli efferentes of the caput epididymidis. Stereocilia on the epithelial cells lining the vas deferens were also lost. In some regions of epithelia, the cell boundaries were not clear, and even appeared to be peeled off. Mucus droplets were abundant in the vas deferens of controls, but none were present in F treated rabbits.

Spermatogenesis ceased sometime between 18 and 29 months. The authors concluded that ingestion of a high concentration of F has adverse effects (including infertility) on the male rabbit reproductive system.

Ghosh *et al.* (2002) investigated the effects of NaF on steroidogenic and gametogenic activities in rat testes. Male Wistar rats were given 20 mg/kg/day NaF by gavage for 29 days. F treatment resulted in significantly lower relative wet weight of the testis, prostate, and seminal vesicle, decreased testicular delta(5),3beta-hydroxysteroid dehydrogenase (HSD) and 17beta-HSD activities, and significant lowering in plasma levels of testosterone. Epididymal sperm count was decreased significantly in F-treated rabbits and there were fewer mature luminal spermatozoa. Indicators of oxidative stress due to F included increased conjugated dienes in the testis, epididymis, and epididymal sperm pellet, and decreases of peroxidase and catalase in the sperm pellet. Thus F, at a dose encountered in drinking water in contaminated areas (at least of India), exerts an adverse effect on the male rat reproductive system. These effects on rats and rabbits (and dogs; see above) may be relevant to anecdotal reports of reproductive system malfunction in human chronic fluorosis.

Parameter	Control (n=6)	NaF (n=6)	p value
Body weight, final (g)	127.00±3.75	122.00±5.10	
Testis, relative weight (%)	1.522±0.034	1.923±0.081	< 0.05
Prostate, relative weight	0.297±0.043	0.148±0.014	< 0.05
Seminal vesicles, rel. weight	0.448±0.025	0.174±0.027	< 0.05
Testicular delta(5),3beta HSD	~28 ^a	~24 ^a	< 0.05 ^b
Testicular 17betaHSD	~29 ^a	~24 ^a	< 0.05 ^b
Plasma testosterone (ng/ml)	~2 ^a	~1 ^a	< 0.05 ^b
Epididymal sperm count (10 ⁶ /ml)	7.02±0.17	3.70±0.57	< 0.05

^a approximate values based on reading Figures 2 and 3 of paper; ^b p values of authors

Long *et al.* (2002) used ligand binding and Western blotting to study neuronal nicotinic acetylcholine receptors (nAChRs) in the brains of male and female Wistar rats ingesting 0.5 ppm (controls), 30 ppm, or 100 ppm F in their drinking water for 7 months. (All received 4 ppm F in their diet.) The brains of rats exposed to 100 ppm had significantly less binding sites for [³H]epibatidine, an analgesic agonist, but no change occurred at 30 ppm. Binding sites for [¹²⁵I]alpha-bungarotoxin, a competitive antagonist, were significantly decreased in the brains of rats exposed to both levels. The brain levels of the nAChR alpha4 subunit protein was significantly lowered by exposure to 100 ppm F. Alpha7 subunit protein was significantly decreased by both levels of F. No significant changes were seen in levels of the beta2 subunit protein. These nicotinic receptors have roles in learning and memory. Some of the effects were also seen in rat PC cells cultured for 48 h in up to 50 ppm F (Chen *et al.*, 2003). The results may help to explain anecdotal reports of nervous system symptoms in human chronic fluorosis (Waldbott, 1978).

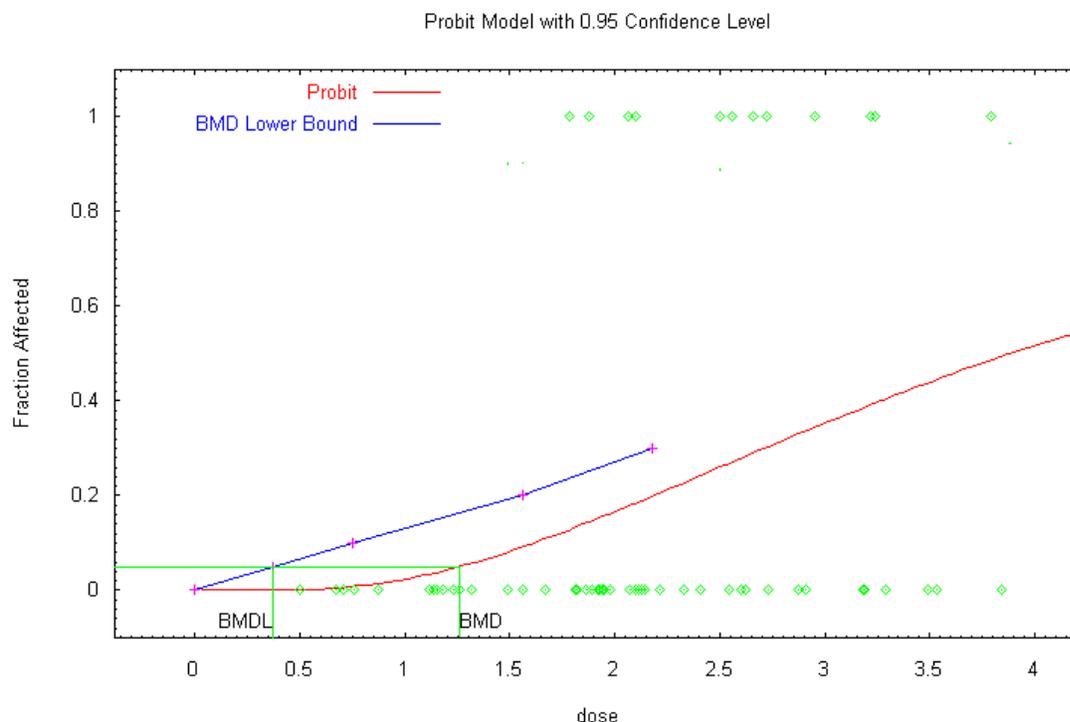
NTP (1990) exposed F344/N rats and B6C3F1 mice of both sexes for two years to 0, 25, 100, and 175 ppm sodium fluoride (NaF) in their drinking water. NaF caused a dose dependent whitish discoloration of the teeth in both rats and mice. Male rats had an increased incidence of

tooth deformities and attrition. NaF increased the dysplasia of dentine in both rats and mice. At the highest dose (175 ppm), osteosclerosis of long bones was increased in female rats. There was also equivocal evidence of carcinogenic activity of NaF in male rats based on four osteosarcomas in dosed animals (Bucher *et al.*, 1991). Other organ systems showed no dose-dependent effects.

VI. Derivation of Chronic Reference Exposure Level (REL)

<i>Study</i>	Derryberry <i>et al.</i> (1963)
<i>Study population</i>	74 fertilizer plant workers (67 unexposed control subjects)
<i>Exposure method</i>	Occupational
<i>Critical effects</i>	Increased bone density (skeletal fluorosis)
<i>LOAEL</i>	1.89 mg F/m ³ (1.98 mg HF/m ³)
<i>NOAEL</i>	1.07 mg F/m ³ (1.13 mg HF/m ³)
<i>BMC₀₅</i>	0.37 mg F/m ³ (0.39 mg HF/m ³)
<i>Exposure continuity</i>	8 hours/day, 5 days/week
<i>Exposure duration</i>	14.1 years (range = 4.5 to 25.9 years)
<i>Average exposure concentration</i>	0.14 mg HF/m ³ (0.39 x 10/20 x 5/7) or 0.13 mg F/m ³ (0.37 x 10/20 x 5/7)
<i>Human equivalent concentration</i>	0.14 mg HF/m ³ or 0.13 mg F/m ³
<i>LOAEL uncertainty factor</i>	1
<i>Subchronic uncertainty factor</i>	1
<i>Interspecies uncertainty factor</i>	1
<i>Intraspecies uncertainty factor</i>	10
<i>Cumulative uncertainty factor</i>	10
<i>Inhalation reference exposure level for F or HF</i>	0.013 mg F/m ³ (13 µg /m ³ ; 0.016 ppm; 16 ppb) or 0.014 mg HF/m ³ (14 µg /m ³ ; 0.017 ppm; 17 ppb)

OEHHA's analysis of the data in Derryberry *et al.* (1963) indicates a LOAEL of 1.89 mg/m³, and a NOAEL of 1.07 mg/m³. A benchmark concentration (BMC₀₅) of 0.37 mg/m³ was derived by fitting the probit model to the log dose in the U.S. EPA's BMDS (version 1.3) software, for the individual mean air exposure data and incidence data in Table 1 above. Individuals in the highest dose group (group 5 in Table 2) were not included in the model, since none of the models fit this range of exposures well. Several other models produced reasonable fits to the data, but the probit model with log-transformed dose was selected since it produced a good fit not only by statistical criteria ($p = 0.71$) but also, as determined by inspection, it fit the low dose curve shape better than other models. This model also has the advantage of biological plausibility, in that, since lower doses of fluoride have a beneficial or nutritional effect, a threshold type of response for adverse effects is clearly expected. A graphical representation of the fit is shown in Figure 1. Adjusting for exposure continuity and utilizing an intraspecies uncertainty factor of 10 (UF_H) results in a REL for F of 13 µg/m³.

Figure 1.

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Changes in bone density in association with fluoride exposure have been observed in several studies, and appear to be the most sensitive health effect for chronic exposure. The minimally increased bone density in the Derryberry study was significantly ($p < 0.04$, Fisher's Exact Test) associated with "other osseous changes," which reportedly included disc lesions, arthritis, and calcified ligaments. An increase in pulmonary changes in the workers with high bone density was marginally significant ($p < 0.06$) and included emphysema, fibrosis, and healed tuberculous lesions. Although dental fluorosis is a sensitive endpoint in many fluoride studies, the dental examinations of exposed workers in this study showed healthier teeth than in controls. The increased bone density observed was considered as indicating that adverse effects had occurred, based on the adverse effects associated with the increased density in the study, and on other research showing that increased bone density caused by fluoride exposure (75 mg sodium fluoride per day for four years) also leads to decreased bone strength and increased fragility (Riggs *et al.*, 1990). Symptoms of abdominal pain, backache, restricted joint movement, and respiratory symptoms have been associated with airborne fluoride exposures and bone density increases in industrial settings (Zhiliang *et al.*, 1987).

The absorption of particulate and gaseous fluorides is reported to be similar (Collings *et al.*, 1951). Therefore, it would be expected that the effects on bone density would be similar regardless of the form of fluoride.

As noted in the study description, Derryberry *et al.* (1963) did not find a good correlation between years of exposure to fluoride and bone density change. OEHHA reexamined the original individual data and confirmed that the presence of bone density changes showed a better correlation with mean air fluoride concentration than with years of exposure, or with the product of the individual values of mean air fluoride concentration and years of exposure. However, the product of exposure concentration and time did show a consistent pattern of cumulative incidence suggesting a dose-response relationship for this parameter. An attempt to derive a benchmark value by fitting the probit model to the log of (exposure duration*concentration) and response (presence or absence of bone density change) did not result in an acceptable fit, so a BMDL₀₅ could not be reported. However a maximum likelihood estimate of the benchmark (BMD₀₅) was found to be 6.04 (mg F*years/m³), with exclusion of the three highest values that appeared to be outliers to the main distribution. If this value is divided by the mean exposure duration for the data set of 14.1 years, a benchmark exposure concentration of 0.43 mg F/m³ is obtained. While this value is evidently less reliable than that obtained by fitting the mean exposure concentration, it is consistent with it, suggesting that, although other confounding factors related to age or duration prevent the demonstration of a relationship between the exposure/time integral and response in this data set, such a relationship probably does exist, as would be expected.

VII. Data Strengths and Limitations for Development of the REL

The major strengths of the key study for fluoride are the observation of health effects in a large group of workers exposed over many years, the availability of individual exposure estimates for each worker, and the identification of a NOAEL. The primary uncertainty in the study is the lack of a comprehensive health effects examination. Another source for concern is the potentially greater susceptibility of children to the effects of inhaled fluorides, considering the rapid bone growth in early years.

Derivation of Chronic Oral REL

In addition to being inhaled, airborne fluoride salts in particulate form can settle onto crops and soil and enter the body by ingestion. Thus an oral chronic reference exposure level (REL) for fluoride is also required in order to conduct a health risk assessment under the Air Toxics Hot Spots Act. California has developed a Public Health Goal (PHG) of 1 ppm (1,000 ppb) fluoride in drinking water (OEHHA, 1997). This level is intended to be an approximate year-round average. Thus it has properties similar to a chronic oral REL. (The PHG assumed that drinking water was the only source of fluoride since it was based on comparing communities with and without added fluoridation.)

<i>Study</i>	Dean, 1942; U.S. Public Health Service, 1991; National Research Council, 1993
<i>Study population</i>	Inhabitants of several U.S. cities
<i>Exposure method</i>	Drinking water
<i>Critical effects</i>	Dental fluorosis
<i>LOAEL</i>	2 ppm
<i>NOAEL</i>	1 ppm = 0.04 mg/kg-day*
<i>Exposure continuity</i>	Continuous
<i>Exposure duration</i>	Long-term
<i>Average experimental exposure</i>	1 ppm = 0.04 mg/kg-day
<i>LOAEL uncertainty factor</i>	1
<i>Subchronic uncertainty factor</i>	1
<i>Interspecies uncertainty factor</i>	1
<i>Intraspecies uncertainty factor</i>	1 (studies included children)
<i>Cumulative uncertainty factor</i>	1
<i>Oral reference exposure level</i>	0.04 mg/kg-day

* based on the assumption that an 18 kg child drinks 720 ml of water per day (OEHHA, 2000).

The PHG is based on a no-observed adverse-effect-level (NOAEL) of 1 mg/L for dental fluorosis in children (equivalent to 720 µg/day from drinking water for an 18 kg child drinking 40 ml/kg body weight/day of water). Moderate to severe dental fluorosis is rare when the drinking water fluoride level is near 1 mg/L, but begins to become significant at concentrations close to 2 mg/L. Since the study involved long term exposure to humans including children, a sensitive population, the cumulative uncertainty factor was 1. If one were to do a route-to-route extrapolation from this oral REL using the specific parameters for an 18 kg child breathing 4.2 m³/day, an equivalent inhalation REL would be about 170 µg/m³. Thus, the inhalation REL of 13 µg/m³ based on the adult occupational data is likely to be protective of children.

VIII. Potential for Differential Impacts on Children's Health

The critical effect for inhalation exposures is skeletal fluorosis. Since infants' and children's skeletons are developing, they may be more sensitive to this effect. This applies with particular importance to the teeth, and it is established that excessive exposure to fluoride during the period of tooth development in infancy and childhood causes dental fluorosis (Dean, 1942; U.S. Public Health Service, 1991; NRC, 1993). The oral REL and the California PHG for fluoride in drinking water are based on dental fluorosis. Although the inhalation chronic REL proposed is based on a study in adults, the inhalation chronic REL (see section VI) is lower than that implied by the oral REL and PHG. Since the oral REL and PHG are based on exposures throughout life, including the pre-natal period, infancy, and childhood, it is reasonable to conclude that the proposed inhalation REL is generally protective of infants and children, barring some unknown difference in toxicity between the two routes of exposure. The ratio of the intake at the PHG level in drinking water is closer to the effect level than the default intraspecies uncertainty factor of 10; this is to be expected since children are a sensitive subpopulation for the dental fluorosis effect.

Extensive interindividual variation in total fluoride intake ($930.7 \pm 391.5 \mu\text{g/day}$) was recently documented for a small group ($n = 11$) of healthy German children ages 3 to 6 years (Haftenberger *et al.*, 2001). Similar interindividual variation has also been reported for slightly younger children in Connorsville ($n = 14$) and Indianapolis, Indiana ($n = 29$) and in San Juan, Puerto Rico ($n = 11$) (Rojas-Sanchez *et al.*, 1999). Consideration should therefore be given to populations with exceptionally high fluoride intake due to locally elevated concentrations in drinking water, since some of these populations are already close to adverse effect levels of fluoride intake, and certain individuals in California experience dental fluorosis. For these individuals, even exposure to fluorides at the oral and/or inhalation RELs, which are acceptable in isolation, might be deleterious. The table below compares the data of Haftenberger *et al.* (2001) with recent estimates of F intake ranges in California (OEHHA, 1997).

Fluoride Intake (mg/day)

F in drinking water (mg/L)	F from drinking water	F from food	F from toothpaste	F from mouthwash	F from a supplement	Total F
Children (OEHHA)						
<0.3	0.1 - 0.3	0.1 - 0.5	0.2 - 1.2	0.1 - 0.5	0.5	1.0 - 3.0
0.7 - 1.2	0.7 - 1.2		0.2 - 1.2	0.1 - 0.5	0	1.1 - 4.6
Haftenberger						
0.25	(see food)	0.20±0.12	0.27±0.18	No data	0 - 1.0	0.93±0.39
Adults (OEHHA)						
<0.3	0.2 - 0.6	0.3 - 1.0	0.02 - 0.15	0.2 - 1.0	0	0.7 - 2.8
0.7 - 1.2	1.4 - 2.4	0.3 - 3.4	0.02 - 0.15	0.2 - 1.0	0	1.9 - 7.0

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CHRONIC TOXICITY SUMMARY

GLUTARALDEHYDE

(1,5-pentanedial; 1,5-pentanedione; glutaric dialdehyde; Aldesen; Cidex; Sonacide)

CAS Registry Number: 111-30-8

I. Chronic Toxicity Summary

<i>Inhalation reference exposure level</i>	0.08 µg/m³ (0.02 ppb)
<i>Critical effect(s)</i>	Squamous metaplasia of the respiratory epithelium in the nose of male and female mice
<i>Hazard index target(s)</i>	Respiratory system

II. Chemical Property Summary (HSDB, 1996; CRC, 1994; Chemfinder, 2000)

<i>Description</i>	Colorless liquid/oil
<i>Molecular formula</i>	C ₅ H ₈ O ₂
<i>Molecular weight</i>	100.12 g/mol
<i>Boiling point</i>	188°C (decomposes) (CRC, 1994)
<i>Melting point</i>	-6°C (Chemfinder, 2000)
<i>Solubility</i>	Soluble in water, alcohol, benzene
<i>Conversion factor</i>	4.1 µg/m ³ per ppb at 25°C

III. Major Uses and Sources

Glutaraldehyde is a chemical frequently used as a disinfectant and sterilizing agent against bacteria and viruses (2% solution), an embalming fluid and tissue fixative, a component of leather tanning solutions, and an intermediate in the production of certain sealants, resins, dyes, and electrical products (HSDB, 1996). For commercial purposes, solutions of 99%, 50%, and 20% are available. Glutaraldehyde is also an atmospheric reaction product of cyclohexene. The annual statewide industrial emissions from facilities reporting under the Air Toxics Hot Spots Act in California based on the most recent inventory were estimated to be 29,603 pounds of glutaraldehyde (CARB, 2000).

IV. Effects of Human Exposure

Evidence of the toxicity of glutaraldehyde to humans is limited to reports of occupational exposure from its use as a disinfectant and sterilizing agent. Frequently observed effects from exposure include skin sensitivity resulting in dermatitis, and irritation of the eyes and nose with accompanying rhinitis (Jordan *et al.*, 1972; Corrado *et al.*, 1986; Hansen, 1983; Wiggins *et al.*,

1989). Occupational asthma has also been reported among workers repeatedly exposed to glutaraldehyde, particularly respiratory technologists who use glutaraldehyde as a sterilizing agent for endoscopes (Chan-Yeung *et al.*, 1993; Stenton *et al.*, 1994; Gannon *et al.*, 1995). Quantitation of the exposure levels that led to glutaraldehyde sensitization was not available from the studies.

V. Effects of Animal Exposure

The histopathology of the respiratory tract in rats and mice exposed to glutaraldehyde by inhalation was examined (Gross *et al.*, 1994). F344 rats and B6C3F1 mice (20 animals of each sex and of each species at each exposure level for a total of 480 rodents) were continuously exposed to glutaraldehyde in recirculating exposure chambers at concentrations of 0, 62.5, 125, 250, 500, or 1000 ppb glutaraldehyde for one day, 4 days, 6 weeks, or 13 weeks. At termination, respiratory tract tissue as well as duodenum and any gross lesions were collected and formalin fixed. Animals were treated with tritiated thymidine two hours before termination to evaluate cell replication in certain respiratory tract tissues. Respiratory tract tissue sections were made as follows: transverse sections of the nose and trachea, frontal section of the carina, and longitudinal section of the lung. Ten male and 10 female mice exposed to 1000 ppb and one female mouse exposed to 500 ppb group died during the course of the study. Two male and 3 female rats exposed to 1000 ppb died during the course of the study. Histopathological examination of animals surviving to the end of the study entailed scoring the severity of the finding from “no response” to “very severe” response on a 0 to 5 scale. Unit length labeling index, the indicator of cell proliferation, was evaluated by autoradiography at two sites: the nasal vestibule and the dorsal atrioturbinates.

Lesions in animals treated with glutaraldehyde appeared primarily in the anterior third of the nose. Lesions were apparently more increased in mice compared to rats due to some level of “background” non-suppurative lesions in the rats. Mice were considered devoid of background lesions. In the 13-week study, female mice were the most sensitive, with lesions averaging a score of 2 (mild and clear, but of limited extent and/or severity). The lesions were characterized as neutrophilic infiltration primarily in the squamous epithelium of the vestibule, with thickening of the epithelium leading to loss of the characteristic surface grooves. Both cell size and number were reported to be increased. Lesions were generally found to increase in nature and severity with increased time and level of exposure. Obstruction of the nasal vestibule was thought to account for the mortality of animals in the higher dose groups. In female mice at 13 weeks, all glutaraldehyde dose groups showed the accumulation of eosinophilic proteinaceous deposits in the respiratory epithelium of the maxilloturbinates margin. Examination of unit length labeling indices as a measure of growth showed significant increases in all treated groups of female mice. No evidence of exposure related lesions was found in the respiratory tract in the trachea, carina, bronchi, or lungs.

Mean Subjective Pathology Scores for Nasal Lesions in Female Mice at 13 Weeks

	<i>Glutaraldehyde</i>	<i>Intraepithelial neutrophils</i>	<i>Subepithelial neutrophils</i>	<i>Squamous metaplasia</i>
0 ppb		0	0.4	0
62.5 ppb		2.0	2.0	0
125 ppb		2.4	2.8	0
250 ppb		3.2	3.2	0
500 ppb		2.8	2.8	0.5
1000 ppb*		--	--	--

*Animals exposed to 1000 ppb died early in the experiment.

Greenspan *et al.* (1985) exposed male and female F-344 rats to 0, 0.3, 1.1 and 3.1 ppm glutaraldehyde and 0, 0.2, 0.63, and 2.1 ppm glutaraldehyde, respectively, in a 9-day study, and both sexes to 0, 21, 49, and 194 ppb glutaraldehyde in a 14 week study. Animal numbers were not specified. Exposures were conducted for 6 hours per day, 5 days per week. In the 9-day study, observations in the high and intermediate dose level groups included reduced body weight gain, inflammation of the nasal and olfactory mucosa, and sensory irritation. In the two highest doses of the 14-week study, statistically significant differences in body weight gain were observed as well as perinasal wetness. No histopathological indication of inflammation in olfactory or nasal mucosa was observed.

Mice were exposed to 0, 0.3, 1.0, and 2.6 ppm glutaraldehyde vapors for 6 hours/day for 4, 9, or 14 days (Zissu *et al.*, 1994). These mice were killed immediately after the exposure period. Other groups exposed to 1.0 ppm for 14 days were killed after recovery periods of 1, 2, and 4 weeks. After 4 days of exposure to the lowest dose, mice showed lesions in the respiratory epithelium of the septum, and the naso- and maxilloturbinates. After exposure to 1.0 ppm glutaraldehyde, lesions were still judged as severe after 2 weeks of recovery.

A study comparing the effects of intra-nasally instilled glutaraldehyde and formaldehyde on rat nasal epithelium found inflammation, epithelial degeneration, respiratory epithelial hypertrophy, and squamous metaplasia in treated animals (St. Clair *et al.*, 1990). Acute inhalation exposure to formaldehyde produced identical lesions. Ten-fold higher concentrations of instilled formaldehyde were required to produce the same effect as instilled glutaraldehyde.

In a chronic study, NTP (1998, 1999) exposed groups of 50 male and 50 female F344/N rats to 0, 250, 500, or 750 ppb glutaraldehyde vapor by inhalation for 6 h/day, 5 days/week, for 104 weeks. Survival of 500 and 750 ppb female rats was less than that of the chamber controls. Mean body weights of all exposed groups of male rats and 500 and 750 ppb female rats were generally less than those of the chamber controls. Increased incidences of nonneoplastic nasal lesions occurred primarily within the anterior section of the nose in 500 and 750 ppb rats and to a lesser extent in 250 ppb rats. The more significant lesions included hyperplasia and inflammation of the squamous and respiratory epithelia and squamous metaplasia of the respiratory epithelium. Thus 250 ppb (1000 $\mu\text{g}/\text{m}^3$) is a chronic LOAEL for rats.

In the same study NTP (1998, 1999) exposed groups of 50 male and 50 female B6C3F1 mice to 0, 62.5, 125, or 250 ppb glutaraldehyde vapor by inhalation for 6 h/day, 5 days/week, for 104

weeks. Survival of exposed mice was similar to that of the chamber controls. Mean body weights of female mice exposed to 250 ppb were generally less than those of the controls. The incidence of inflammation of the nose was marginally increased in 250 ppb females. Incidences of squamous metaplasia of the respiratory epithelium were increased in 250 ppb males and females and 125 ppb females. Incidences of hyaline degeneration of the respiratory epithelium were increased in all exposed groups of females. Thus 62.5 ppb was a chronic LOAEL for female mice.

Incidence of Nasal Lesions in Female Mice exposed for 104 weeks

	<i>Glutaraldehyde</i>	<i>Inflammation</i>	<i>Respiratory epithelium hyaline degeneration</i>	<i>Respiratory epithelium squamous metaplasia</i>
	0 ppb	6/50	16/50	7/50
	62.5 ppb	7/49	35/49	11/49
	125 ppb	13/50	32/50	16/50
	250 ppb	14/50	30/50	21/50

VI. Derivation of Chronic Reference Exposure Level (REL)

<i>Study</i>	NTP 1998, 1999
<i>Study population</i>	Male and female F344 rats and B6C3F1 mice (50/sex/group)
<i>Exposure method</i>	Continuous inhalation exposure (0, 62.5, 125, and 250 ppb in mice; 0, 250, 500, or 750 ppb in rats)
<i>Critical effects</i>	Respiratory epithelium squamous metaplasia
<i>LOAEL</i>	62.5 ppb (female mice)
<i>NOAEL</i>	Not observed
<i>BMC₀₅</i>	20.5 ppb
<i>Exposure continuity</i>	6 hr/day, 5 days/week
<i>Exposure duration</i>	104 weeks
<i>Equivalent continuous exposure</i>	3.7 ppb (20.5 x 6/24 x 5/7)
<i>Human equivalent concentration</i>	0.62 ppb (gas with extrathoracic respiratory effects, RGDR = 0.17, BW = 28 g, MV = 0.032 L/min, SA = 3 cm ²)
<i>LOAEL uncertainty factor</i>	not needed in BMC approach
<i>Subchronic uncertainty factor</i>	1
<i>Interspecies uncertainty factor</i>	3
<i>Intraspecies uncertainty factor</i>	10
<i>Cumulative uncertainty factor</i>	30
<i>Inhalation reference exposure level</i>	0.02 ppb (0.08 µg/m ³)

Several studies indicate that the upper respiratory tract is a target for the toxicity of glutaraldehyde from inhalation exposure. Reports of toxicity to humans show that exposure can

lead to occupational asthma as well as cause irritation of the eyes and nose with accompanying rhinitis. Likewise, animals exposed to glutaraldehyde by the inhalation route show evidence of respiratory irritation with the induction of lesions of the anterior nasal cavities upon long-term exposure (Gross *et al.*, 1994; Greenspan *et al.*, 1985; NTP, 1998, 1999). The NTP (1998, 1999) study yielded a chronic LOAEL for female mice of 62.5 ppb. Gross *et al.* (1994) showed neutrophilic infiltration in the olfactory epithelium in the lowest dose exposure group. (Female mice exposed to 62.5 ppb also showed subepithelial neutrophilic infiltration.) This level was taken to be the subchronic LOAEL. This effect on the nasal epithelium was demonstrated to be both concentration- and exposure duration-dependent.

A benchmark concentration was determined using EPA's version 1.20 BMC software and the dose-response data on respiratory epithelium squamous metaplasia in female mice. The quantal-linear model gave an MLE₀₅ of 31.24 ppb, a BMC₀₅ of 20.51 ppb, and a p value of 0.9471. With the benchmark approach no LOAEL UF is needed. The study was a lifetime study so the subchronic UF is 1. An interspecies UF of 3 rather than 10 was used since an RGDR adjustment had been made. The default intraspecies UF of 10 was used so that the total UF was 30. The resulting chronic REL for glutaraldehyde is 0.02 ppb (0.08 µg/m³).

For comparison with the proposed REL, the study of Gross *et al.* (1994) used 62.5 ppb continuous exposure. Multiplying by the RGDR of 0.17 and dividing by a cumulative uncertainty factor of 300 (3 for a LOAEL, 3 for subchronic, 3 for interspecies, and 10 for intraspecies) results in a REL of 0.035 ppb (0.1 µg/m³).

VII. Data Strengths and Limitations for Development of the REL

The major strength of the inhalation REL for glutaraldehyde is the availability of controlled exposure inhalation studies in multiple species at multiple exposure concentrations and with adequate histopathological analysis. Major areas of uncertainty are the lack of human data, the lack of reproductive and developmental toxicity studies, the lack of dermal sensitization studies, and the lack of observation of a NOAEL.

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*CHRONIC TOXICITY SUMMARY****n*-HEXANE***(normal hexane)***CAS Registry Number: 110-54-3****I. Chronic Toxicity Summary**

<i>Inhalation reference exposure level</i>	7000 µg/m³ (2000 ppb)
<i>Critical effect(s)</i>	Neurotoxicity; electrophysiological alterations in humans
<i>Hazard index target(s)</i>	Nervous system

II. Physical and Chemical Properties (HSDB, 1999)

<i>Description</i>	Colorless liquid, gas
<i>Molecular formula</i>	C ₆ H ₁₄
<i>Molecular weight</i>	86.10
<i>Density</i>	0.660 g/cm ³ @ 20° C
<i>Boiling point</i>	68.95°C
<i>Melting point</i>	-95.3°C
<i>Vapor pressure</i>	150 torr @ 25° C
<i>Solubility</i>	Insoluble in water; soluble in most organic solvents; very soluble in alcohol
<i>Conversion factor</i>	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 atrophy 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 noted 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.

B6C3F₁ 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.

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

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

VI. Derivation of Chronic Reference Exposure Level

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

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

The Sanagi study, which U.S. EPA used as the basis of its RfC, may overestimate hexane effect because of a confounding coexposure to acetone, which is known to potentiate hexane neuropathy. The minimum effective acetone inhalation concentration for potentiating hexane neuropathy is unclear, as studies (Ladefoged et al., 1994; Lam et al., 1991; Larsen et al., 1991) have used orally administered acetone. The minimum effective acetone inhalation dose for potentiation of carbon tetrachloride hepatotoxicity in male Sprague-Dawley rats was 2500 ppm over 4 hours (Charbonneau et al., 1986). A dose of 0.5% acetone in human drinking water is comparable, assuming equal absorption, to an inhalation concentration of approximately 1400 ppm ($0.5\% \text{ w/v} \times 2 \text{ L/day} \div 2 \text{ m}^3/\text{day} = 5 \text{ g/m}^3$; $5 \text{ g/m}^3 \times 1000 \text{ mg/g} \div 3.52 \text{ mg/m}^3 \text{ per ppm} = 1400 \text{ ppm}$). 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 $\mu\text{g}/\text{m}^3$ REL.

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

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

Study	Duration	Effect	LOAEL (ppm)	LOAEL (ppm) (TWA)	NOAEL (ppm)	NOAEL (ppm) (TWA)	total UF	REL (ppb)	REL (µg/m ³)
Sanagi <i>et al.</i> , 1980	6.2 years	decreased motor nerve conduction velocity; increased residual latency	58	20.7	Not observed		100 ^a	200	700
Chang <i>et al.</i> , 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	<i>Not observed</i>		300 ^b	300	1000

^a LOAEL uncertainty factor, 10; Intraspecies uncertainty factor, 10

^b LOAEL uncertainty factor, 10; Subchronic uncertainty factor, 3; Intraspecies uncertainty factor, 10

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

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

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

VII. Data Strengths and Limitations for Development of the REL

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

<i>Study</i>	<i>Species</i>	<i>Exposure concentration</i>	<i>Exposure regimen</i>	<i>TWA from NOAEL^a</i>	<i>TWA from LOAEL^a</i>
Sanagi <i>et al.</i> (1980)	Humans	58 ppm (mean)	10 m ³ /d, 5 d/wk, 6.2 yr (mean)	None	20.7 ppm
Chang <i>et al.</i> (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 <i>et al.</i> (1999)	F344 rats	0, 900, 3000, 9000 ppm	6 hr/d, 5 d/wk, 2 yr	None	161 ppm
Daughtrey <i>et al.</i> (1999)	B6C3F1 mice	0, 900, 3000, 9000 ppm	6 hr/d, 5 d/wk, 2 yr	None	161 ppm
Dunnick <i>et al.</i> (1989)	B6C3F1 mice	0, 500, 1000, 4000, 10,000 ppm	6 hr/d, 5 d/wk, 13 wk	89 ppm	179 ppm
Huang <i>et al.</i> (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 <i>et al.</i> (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 $\mu\text{g}/\text{m}^3$) 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 $\mu\text{g}/\text{m}^3$).

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CHRONIC TOXICITY SUMMARY

HYDRAZINE*(diamine; diamide; nitrogen hydride; levoxine)***CAS Registry Number: 302-01-2****I. Chronic Toxicity Summary**

<i>Inhalation reference exposure level</i>	0.2 µg/m³ (0.1 ppb)
<i>Critical effect(s)</i>	Amyloidosis of the liver and thyroid in hamsters
<i>Hazard index target(s)</i>	Alimentary system; endocrine system

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

<i>Description</i>	Colorless, oily liquid or white crystals
<i>Molecular formula</i>	N ₂ H ₄
<i>Molecular weight</i>	32.05 g/mol
<i>Boiling point</i>	113.5°C (Merck, 1983; CRC, 1994)
<i>Melting point</i>	2.0°C
<i>Vapor pressure</i>	14.4 torr @ 25°C
<i>Solubility</i>	Miscible with water, methyl-, ethyl-, isobutyl alcohols; slightly miscible with hydrocarbons; insoluble in chloroform, ether
<i>Conversion factor</i>	1.31 µg/m ³ per ppb at 25°C

III. Major Uses and Sources

Hydrazine is a highly reactive base and reducing agent. Its primary uses are as a high-energy rocket propellant, as a reactant in military fuel cells, in nickel plating, in the polymerization of urethane, for removal of halogens from wastewater, as an oxygen scavenger in boiler feedwater to inhibit corrosion, and in photographic development (Von Burg and Stout, 1991). Hydrazine was historically used experimentally as a therapeutic agent in the treatment of tuberculosis, sickle cell anemia, and non-specific chronic illnesses (Von Burg and Stout, 1991; Gold, 1987). The annual statewide industrial emissions from facilities reporting under the Air Toxics Hot Spots Act in California based on the most recent inventory were estimated to be 1664 pounds of hydrazine (CARB, 2000).

IV. Effects of Human Exposure

One person was occupationally exposed to hydrazine at unknown levels once per week for a period of 6 months (Sotaniemi *et al.*, 1971). The worker showed symptoms of conjunctivitis, tremors, and lethargy for 1-2 days following each exposure. Vomiting, fever, and diarrhea developed on the last day of exposure and progressed to abdominal pain and incoherence. The previously healthy 59-year old individual died three weeks after the last exposure. Evidence of tracheitis, bronchitis, heart muscle degeneration, and liver and kidney damage was found at autopsy. A single case report can not prove a cause and effect relationship between hydrazine exposures and the noted symptoms and death, but the repeated association between exposures and symptoms is highly suspicious. Liver toxicity is also associated with acute exposure to hydrazine.

The only epidemiological studies of human hydrazine exposures found involve workers in a hydrazine manufacturing plant (Wald *et al.*, 1984; Wald, 1985; Morris *et al.*, 1995). Workers were exposed to various durations of at least 6 months between 1945 and 1972 and have been followed through 1992. The studies are based on a review of medical records. Only 78 of 427 workers were believed to have had more than incidental exposure to hydrazine. Only cumulative mortality was reviewed. Health effects reported during or after hydrazine exposure were not examined. No increase in mortality was noted for lung cancer, other cancers, or causes other than cancer. However, these small studies have little power to detect increased mortality, and age of death was not examined. The authors reported that relative risks up to 3.5 could have gone undetected.

Dermal sensitization has also been reported from repeated contact with hydrazine (Van Ketal, 1964; Von Keilig and Speer, 1983; Wrangsjö and Martensson, 1986).

V. Effects of Animal Exposure

An inhalation study of the toxicity and carcinogenicity of hydrazine was conducted in cats, mice, hamsters, and dogs (Vernot *et al.*, 1985). Various animal groups were exposed 6 hours/day, 5 days/weeks for one year to concentrations of 0.05, 0.25, 1.0, and 5.0 ppm anhydrous hydrazine base. Exposed and controls groups were made up of the following animals: 100 Fischer 344 rats/sex at 0.05, 0.25, 1.0, and 5.0 ppm hydrazine plus 150 rats/sex as controls; 400 female C57BL/6 mice at 0.05, 0.25, and 1.0 ppm hydrazine plus 800 female mice as controls; 200 male Golden Syrian hamsters at 0.25, 1.0, and 5.0 ppm hydrazine plus 200 male hamsters as controls; 4 beagle dogs/sex at 0.25 and 1.0 ppm hydrazine plus 4 dogs/sex as controls. Animals were observed post-exposure for the following periods: 18 months for rats, 15 months for mice, 12 months for hamsters, and 38 months for dogs. Animals were observed hourly during the exposure period and daily in the post-exposure period.

No non-cancer toxic effects were observed in mice or dogs, with the exception of a single dog, exposed to 1.0 ppm hydrazine, which showed cyclic elevations in serum glutamic-pyruvic transaminase levels and, upon necropsy at 36 months post-exposure, showed liver effects described as “clusters of swollen hepatocytes that had highly vacuolated cytoplasm.” Of the

other species examined, hamsters showed toxicity at the lowest dose levels, particularly amyloidosis in various organs including liver, spleen, kidney, thyroid, and adrenal glands. An increased incidence of amyloidosis was seen at the lowest exposure level (0.25 ppm hydrazine) in the liver and thyroid (67/160 exposed vs. 42/180 control for the liver and 20/117 exposed vs. 9/155 control in the thyroid; $p \leq 0.01$ by Fisher's exact test). This effect was found to be dose related. The incidence of hemosiderosis of the liver was also significantly increased in all exposed groups. Significantly increased incidences of toxic effects observed in the 1.0 and 5.0 ppm hydrazine groups include amyloidosis of the spleen, kidney glomerulus, and adrenals glands, and lymphadenitis of the lymph nodes. Significantly increased toxic effects observed only in the highest dose group include amyloidosis of the kidney interstitium and thyroid, and senile atrophy of the testis. The authors note these effects appear to reflect accelerated changes commonly associated with aging in hamsters.

Incidence of Nonneoplastic Lesions in Male Hamsters (from Table 3 of Vernot *et al.*)

<i>Lesion</i>	<i>Control</i>	<i>0.25 ppm</i>	<i>1.0 ppm</i>	<i>5.0 ppm</i>
Liver				
Amyloidosis	42/180 (23)*	67/160 (42) ^a	68/148 (46) ^a	79/159 (50) ^a
Hemosiderosis	42/180 (23)	63/160 (39) ^a	77/148 (52) ^a	94/159 (59) ^a
Bile duct hyperplasia	14/180 (8)	31/160 (19) ^a	28/148 (19) ^a	44/159 (28) ^a
Biliary cyst	45/180 (25)	45/160 (28)	42/148 (28)	55/159 (35) ^b
Thyroid				
Amyloidosis	9/155 (6)	20/117 (17) ^a	11/127 (9)	22/137 (16) ^a
Adrenal				
Amyloidosis	38/177 (22)	49/199 (32) ^b	52/141 (37) ^a	76/153 (50) ^a

* Incidence of lesion (% of animals with lesion)

^a Incidence significantly greater than control, $p \leq 0.01$

^b Incidence significantly greater than control, $0.01 < p \leq 0.05$

In the hydrazine exposed rats, effects were observed in the respiratory tract of exposed animals. Specifically, squamous metaplasia of the larynx, trachea, and nasal epithelium (males only) was observed in the highest dose group (5.0 ppm hydrazine). Inflammation was also observed in the larynx and trachea of rats exposed to 5.0 ppm hydrazine. Increased incidence of focal cellular change of the liver was observed in female mice at 1.0 and 5.0 ppm hydrazine. Other effects with increased incidence only in the high dose group include hyperplastic lymph nodes in females, endometriosis, and inflammation of the uterine tube.

The toxic effects from inhalation of hydrazine over a six month period from both intermittent and continuous exposure scenarios were examined (Haun and Kinkead, 1973). Groups of 8 male beagle dogs, 4 female rhesus monkeys, 50 male Sprague-Dawley rats, and 40 female ICR rats per dose group were continuously exposed to 0.2 or 1.0 ppm hydrazine or intermittently (6 hours/day, 5 days/week) to 1.0 or 5.0 ppm hydrazine. A control group consisted of equal numbers of animals. The experimental design was such that each intermittent exposure group had a time-weighted-average matching continuous exposure group. Dose-related body weight reductions were observed in all treated groups as well as evidence of hepatic degeneration, fatty

deposition in the liver, central nervous system depression and lethargy, eye irritation, and anemia.

Toxic effects from the exposure of rats, mice, and dogs to airborne hydrazine at levels of 0, 4.6, or 14 ppm intermittently for 6 months were reported (Comstock *et al.*, 1954). Observed adverse effects included anorexia, irregular breathing, vomiting, fatigue, and emphysema in dogs; pulmonary congestion and emphysema in rats and mice; and lung and liver damage in rats.

Lymphoid bronchial hyperplasia was observed in guinea pigs exposed to 2-6 ppm hydrazine for 5 days/week for 19-47 days (Weatherby and Yard, 1955).

VI. Derivation of Chronic Reference Exposure Level (REL)

<i>Study</i>	Vernot <i>et al.</i> , 1985
<i>Study population</i>	Hamster
<i>Exposure method</i>	Inhalation of 0, 0.25, 1, and 5 ppm
<i>Critical effects</i>	Amyloidosis and hemosiderosis of the liver; thyroid amyloidosis
<i>LOAEL</i>	0.25 ppm
<i>NOAEL</i>	Not observed
<i>Exposure continuity</i>	6 hour/day, 5 days/week
<i>Exposure duration</i>	1 year
<i>Average experimental exposure</i>	0.045 ppm for LOAEL group (0.25 x 6/24 x 5/7)
<i>Human equivalent concentration</i>	0.045 ppm for LOAEL group (gas with systemic effects, based on RGDR = 1.0 using default assumption that lambda (a) = lambda (h))
<i>LOAEL uncertainty factor</i>	10 (low incidence above controls but serious adverse effects)
<i>Subchronic uncertainty factor</i>	1
<i>Interspecies uncertainty factor</i>	3
<i>Intraspecies uncertainty factor</i>	10
<i>Cumulative uncertainty factor</i>	300
<i>Inhalation reference exposure level</i>	0.0001 ppm (0.1 ppb, 0.0002 mg/m ³ , 0.2 µg/m ³)

Vernot *et al.* (1985) present a thorough examination of chronic health effects from inhalation exposure to hydrazine. This study was chosen for the development of the chronic reference exposure level because (1) it was conducted with an adequate number of animals, (2) the critical/sensitive adverse effect (degenerative change in the liver in hamsters) showed a dose-response relationship, and (3) the findings of this study support data found in studies by other groups.

This study shows a dose-related increase in the incidence of amyloidosis and hemosiderosis in hamsters intermittently exposed by inhalation to levels of hydrazine greater than 0.25 ppm. Other effects noted at 0.25 ppm included weight depression during exposure, mineralization of the kidney, and amyloidosis of the thyroid. Haun and Kinkead (1973) have also noted lesions of the

liver in dogs, monkeys, and mice exposed continuously to 0.2 ppm hydrazine for 6 months by inhalation. Comstock *et al.* (1954) observed liver damage in groups of rats exposed to hydrazine vapors. The single case report of hydrazine inhalation toxicity in humans showed necrosis and degeneration of the liver (Sotaniemi *et al.*, 1971).

VII. Data Strengths and Limitations for Development of the REL

The strengths of the inhalation REL for hydrazine include the availability of chronic inhalation exposure data from a well-conducted study with histopathological analysis. Major areas of uncertainty are the lack of adequate human exposure data, the lack of reproductive and developmental toxicity studies, and the lack of observation of a NOAEL in the key study.

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CHRONIC TOXICITY SUMMARY

HYDROGEN CHLORIDE*(Hydrochloric acid; anhydrous hydrogen chloride; muriatic acid)***CAS Registry Number: 7647-01-0****I. Chronic Reference Exposure Level**

<i>Inhalation reference exposure level</i>	9 µg/m³ (6 ppb)
<i>Critical effect(s)</i>	Hyperplasia of nasal mucosa, larynx, and trachea in rats
<i>Hazard index target(s)</i>	Respiratory system

II. Physical and Chemical Properties (HSDB, 1999)

<i>Description</i>	Colorless gas
<i>Molecular formula</i>	HCl
<i>Molecular weight</i>	36.46
<i>Density</i>	1.49 g/L @ 25° C
<i>Boiling point</i>	-84.9° C (HCl gas)
<i>Melting point</i>	-114.8° C (HCl gas)
<i>Solubility</i>	Soluble in water, alcohol, benzene, ether; insoluble in hydrocarbons
<i>Conversion factor</i>	1 ppm = 1.49 mg/m ³ at 25°C

III. Major Uses or Sources

Hydrogen chloride (HCl) is used in the manufacture of vinyl chloride, fertilizers, dyes, artificial silk, and pigments for paints. It is also used in electroplating, soap refining, and leather tanning. Other consumers of HCl include the photographic, textile and rubber industries (HSDB, 1999).

Hydrogen chloride is produced in large quantities during combustion of most materials and especially materials with a high chlorine content. Thus, HCl is a major product formed during the thermal decomposition of polyvinyl chloride, a commonly used plastic polymer (Burleigh-Flayer *et al.*, 1985). It is also released in large quantities during the test firing of some rocket and missile engines (Wohlslagel *et al.*, 1976). Since HCl is extremely hygroscopic, it generally exists as an aerosol in the ambient atmosphere. The annual statewide industrial emissions from facilities reporting under the Air Toxics Hot Spots Act in California based on the most recent inventory were estimated to be 2,570,888 pounds of HCl (CARB, 1999b).

IV. Effects of Human Exposure

Few reports are available on the effects of chronic HCl exposure on humans. Bleeding of the nose and gums and ulceration of the mucous membranes was observed following repeated occupational exposure to HCl mist at high but unquantified concentrations (Stokinger, 1981).

In another report, workers exposed to various mineral acids, including HCl, exhibited etching and erosion of the front teeth (Ten Bruggen Cate, 1968). Dental erosion was noted in 176 of 555 (32%) workers examined between 1962 and 1964, and progressive erosion was reported in 66 of 324 (20%) workers examined repeatedly. Rates of active erosion were highest (50%) in the most highly-exposed category (battery formation workers), intermediate (23%) in an intermediate-exposure category (picklers), and low (7%) in a low-exposure category (other processes). Grade 1 erosion (enamel loss) was noted in workers exposed for greater than 3 months; grade 2 erosion (loss of enamel and dentine) was noted after 2.5 to 5 years exposure; and grade 3 (loss of enamel and dentine with exposure of secondary dentine) was noted after six or more years of exposure.

V. Effects of Animal Exposure

Male Sprague-Dawley rats were exposed to 10 ppm HCl for 6 hours per day, 5 days per week over their lifetime (Sellakumar *et al.*, 1985). No differences in body weights or survival were observed between 99 exposed and 99 control animals. Increased incidences of hyperplasia of the nasal mucosa (62/99 vs. 51/99), larynx (22/99 vs. 2/99), and trachea (26/99 vs. 2/99) were observed in exposed rats compared to air-exposed controls.

A 90-day inhalation study using B6C3F1 mice and Sprague-Dawley and Fisher 344 rats exposed the animals (groups of 31 males and 31 females for each species and strain) to 10, 20, or 50 ppm HCl for 6 hours per day, 5 days per week over 90 days (Toxigenics, 1984). Several animals died during the study, though the deaths were not considered to be exposure related. A slight but significant decrease in body weight gain was reported in male and female mice and in male Fischer 344 rats in the high-exposure groups. No effect were noted in hematology, clinical chemistry, or urinalysis. Minimal or mild rhinitis was observed in both strains of rats. Concentration- and time-related lesions were noted in the anterior portion of the nasal cavity of exposed rats. Cheilitis, eosinophilic globules in the nasal epithelium and accumulation of macrophages in the peripheral tissues were observed in mice of all exposed groups. This study thus observed a LOAEL for both mice and rats of 10 ppm. The U.S. EPA considered this study supportive of the portal-of-entry effects observed at 10 ppm in the lifetime rat study (USEPA, 1999). Female rats (8-15/group) exposed to 302 ppm HCl for 1 hour either 12 days prior to mating or on day 9 of gestation exhibited severe dyspnea and cyanosis; the exposure was lethal to one-third of the exposed animals (Pavlova, 1976). Fetal mortality was significantly higher in rats exposed during pregnancy. Organ functional abnormalities observed in offspring exposed at 2-3 months of age were reported to be similar to those observed in the exposed dams.

Female rats were exposed to 302 ppm HCl for 1 hour prior to mating (GEOMET Technologies, 1981). Exposure killed 20 to 30% of the rats. In rats surviving 6 days after exposure, a decrease in blood oxygen saturation was reported, as were kidney, liver, and spleen effects. Estrus cycles

were also altered. In rats mated 12-16 days postexposure and killed on day 21 of pregnancy, a decrease in fetal weight, an increase in relative fetal lung weights, and reduced numbers of live fetuses were observed.

Derivation of Chronic Reference Exposure Level

<i>Study</i>	Sellakumar <i>et al.</i> , 1985
<i>Study population</i>	Sprague-Dawley rats (100 males)
<i>Exposure method</i>	Discontinuous whole-body inhalation (0 or 10 ppm)
<i>Critical effects</i>	Hyperplasia of the nasal mucosa, larynx and trachea
<i>LOAEL</i>	10 ppm
<i>NOAEL</i>	Not identified
<i>Exposure continuity</i>	6 hours per day, 5 days per week
<i>Average experimental exposure</i>	1.8 ppm for LOAEL group
<i>Human equivalent concentration</i>	0.57 ppm (gas with extrathoracic respiratory effects, RGDR = 0.32, based on rat MV _a = 0.33 L/min, MV _h = 13.8 L/min, SA _a (ET) = 15 cm ² ; Sa _h = 200 cm ³) (U.S. EPA, 1994)
<i>Exposure duration</i>	Lifetime
<i>LOAEL uncertainty factor</i>	3 (<30% incidence; mild effect)
<i>Subchronic uncertainty factor</i>	1
<i>Interspecies uncertainty factor</i>	3
<i>Intraspecies uncertainty factor</i>	10
<i>Cumulative uncertainty factor</i>	100
<i>Reference Concentration (RfC)</i>	0.006 ppm (6 ppb; 0.009 mg/m ³ ; 9 µg/m ³)

Both extrathoracic and tracheobronchial effects have been associated with exposures to hydrogen chloride. The REL was based on extrathoracic effects as humans are predicted to be relatively more susceptible to the effects of hydrogen chloride in that region. An intermediate LOAEL factor was used as the effects were both mild and occurring at a low incidence at the dose tested.

VII. Data Strengths and Limitations for Development of the REL

The USEPA based its RfC of 7 µg/m³ on the same study. U.S. EPA evaluated this RfC as having a low level of confidence because of (1) the use of only one dose; (2) limited toxicity evaluation; (3) the lack of reproductive toxicity data; and (4) the lack of chronic exposure studies (U.S. EPA, 1994). OEHHA agrees with this assessment. The database for chronic exposure to this common chemical is limited.

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CHRONIC TOXICITY SUMMARY

HYDROGEN CYANIDE*(Formonitrile; hydrocyanic acid; prussic acid)***CAS Registry Number: 74-90-8****I. Chronic Toxicity Summary**

<i>Inhalation reference exposure level</i>	9 $\mu\text{g}/\text{m}^3$ (8 ppb)
<i>Critical effect(s)</i>	CNS effects, thyroid enlargement, and hematological disorders in workers
<i>Hazard index target(s)</i>	Nervous system; endocrine system; cardiovascular system

II. Physical and Chemical Properties (HSDB, 1999)

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

III. Major Uses or Sources

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

Another common source of hydrogen cyanide is cigarette smoke. Levels in inhaled mainstream cigarette smoke range from 10 to 400 μg per cigarette (U.S. brands); 0.6% to 27% (w/w) of these mainstream levels are found in secondary or sidestream smoke (Fiskel *et al.*, 1981). The annual statewide industrial emissions from facilities reporting under the Air Toxics Hot Spots Act in

California based on the most recent inventory were estimated to be 188,665 pounds of hydrogen cyanide (CARB, 1999b).

IV. Effects of Human Exposure

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

El Ghawabi *et al.* (1975) studied 36 male electroplating workers in three Egyptian factories exposed to plating bath containing 3% copper cyanide, 3% sodium cyanide, and 1% sodium carbonate. Breathing zone cyanide concentrations ranged from 4.2 to 12.4 ppm (4.6 to 13.7 mg/m³), with means from 6.4 to 10.4 ppm (7.1 to 11.5 mg/m³), 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/m³) 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 syndrome 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 B₁₂ 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/m³) 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/m³ (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

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

The USEPA based its RfC of 3 µg/m³ 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:

<i>Study</i>	Jackson (1988)
<i>Study population</i>	Miniature swine
<i>Exposure method</i>	Daily oral administration of aqueous potassium cyanide
<i>Critical effects</i>	Behavioral effects; decreased blood T ₃ and T ₄
<i>LOAEL</i>	0.4 mg/kg-day
<i>NOAEL</i>	Not observed
<i>Exposure continuity</i>	Apparently 7 days per week
<i>Average exposure</i>	0.4 mg/kg-day (1.4 mg/m ³ for LOAEL group assuming 20 m ³ /day inhalation by a 70 kg person)
<i>Human equivalent concentration</i>	Not derived due to lack of species-specific data
<i>Exposure duration</i>	24 weeks
<i>LOAEL uncertainty factor</i>	3 (minimal effects at lowest dose)
<i>Subchronic uncertainty factor</i>	10 (based on assumed 27 year lifespan)
<i>Interspecies uncertainty factor</i>	10
<i>Intraspecies uncertainty factor</i>	10
<i>Cumulative uncertainty factor</i>	3,000
<i>Inhalation reference exposure level</i>	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/m³) similar to that reported by El Ghawabi (2.5 mg/m³). 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

<i>Inhalation reference exposure level</i>	10 µg/m³ (8 ppb)
<i>Critical effect(s)</i>	Nasal histological changes in B6C3F1 mice
<i>Hazard index target(s)</i>	Respiratory system

II. Physical and Chemical Properties (HSDB, 1999)

<i>Description</i>	Colorless gas
<i>Molecular formula</i>	H ₂ S
<i>Molecular weight</i>	34.08
<i>Density</i>	1.4 g/L @ 25° C (air = 1) (AIHA, 1991)
<i>Boiling point</i>	-60.7° C (CRC, 1994)
<i>Melting point</i>	-85.5° C (CRC, 1994)
<i>Vapor pressure</i>	15,600 Torr @ 25° C
<i>Solubility</i>	Soluble in water, hydrocarbon solvents, ether, and ethanol
<i>Odor threshold</i>	8.1 ppb (11 µg/m ³) (Amoore and Hautala, 1983)
<i>Odor description</i>	Resembles rotten eggs
<i>Conversion factor</i>	1 ppm = 1.4 mg/m ³ @ 25° C

III. Major Uses or Sources

Hydrogen sulfide (H₂S) 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 H₂S exist, there is inadequate occupational or epidemiological information for specific chronic effects in humans exposed to H₂S.

Bhambhani and Singh (1991) showed that 16 healthy subjects exposed for short durations to 5 ppm (7 mg/m³) H₂S 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/m³) H₂S caused coughing and throat irritation after 15 minutes.

In another study, ten asthmatic volunteer subjects were exposed to 2 ppm H₂S 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 FEV₁ 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 H₂S, 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 H₂S 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 unconsciousness. Four other workers were exposed chronically to H₂S and developed lacrimation, eye irritation, nausea, vomiting, headache, sore throat, and skin irritation but retained consciousness 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 unconsciousness 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/m³, respectively) H₂S for 6 hours/day, 5 days/week for 90 days (CIIT, 1983a,b). Measurements of neurological and hematological function revealed no abnormalities due to H₂S 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/m³).

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/m³, respectively) H₂S 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/m³) H₂S. 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/m³) 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/m³.)

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 H₂S 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/m³) for 4 hours resulted in mild perivascular edema (Lopez *et al.*, 1988).

A study by Saillenfait *et al.* (1989) investigated the developmental toxicity of H₂S 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 H₂S 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

<i>Study</i>	CIIT, 1983c
<i>Study population</i>	B6C3F1 mice (10-12 per group)
<i>Exposure method</i>	Discontinuous inhalation
<i>Critical effects</i>	Histopathological inflammatory changes in the nasal mucosa
<i>LOAEL</i>	80 ppm (112 mg/m ³)
<i>NOAEL</i>	30.5 ppm (42.5 mg/m ³)
<i>Exposure continuity</i>	6 hours/day, 5 days/week
<i>Exposure duration</i>	90 days
<i>Average experimental exposure</i>	5.4 ppm for NOAEL group (30.5 x 6/24 x 5/7)
<i>Human equivalent concentration</i>	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)
<i>LOAEL uncertainty factor</i>	1
<i>Subchronic uncertainty factor</i>	3
<i>Interspecies uncertainty factor</i>	3
<i>Intraspecies uncertainty factor</i>	10
<i>Cumulative uncertainty factor</i>	100
<i>Inhalation reference exposure level</i>	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 H₂S, 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 histopathological 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 $7 \mu\text{g}/\text{m}^3$, 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 $42 \mu\text{g}/\text{m}^3$ (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 $10 \mu\text{g}/\text{m}^3$ (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 $40 \mu\text{g}/\text{m}^3$ (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 $10 \mu\text{g}/\text{m}^3$ (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 $50 \mu\text{g}/\text{m}^3$, 50% would find the odor annoying.

On this basis, the proposed REL of $10 \mu\text{g}/\text{m}^3$ (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

ISOPHORONE

(1,1,3-trimethyl-3-cyclohexene-5-one; 3,5,5-trimethyl-2-cyclohexen-1-one; isoforon;
isoacetophorone)

CAS Registry Number: 78-59-1

I. Chronic Toxicity Summary

<i>Inhalation reference exposure level</i>	2,000 µg/m³ (400 ppb)
<i>Critical effect(s)</i>	Developmental effects (reduced crown-rump length of female rat fetuses); hepatocytomegaly and coagulative necrosis of the liver in mice
<i>Hazard index target(s)</i>	Development; liver

II. Chemical Property Summary (HSDB, 1995; CRC, 1994; CARB, 1997)

<i>Description</i>	Water-clear liquid with a peppermint-like odor
<i>Molecular formula</i>	C ₉ H ₁₄ O
<i>Molecular weight</i>	138.21 g/mol
<i>Boiling point</i>	215.2°C
<i>Melting point</i>	-8.1°C
<i>Vapor pressure</i>	0.44 torr at 25°C
<i>Solubility</i>	Slightly soluble in water (12,000 mg/L water at 25°C); miscible in organic solvents.
<i>Conversion factor</i>	5.65 µg/m ³ per ppb at 25°C

III. Major Uses and Sources

Isophorone is used extensively as a solvent in some printing inks, paints, lacquers, adhesives, vinyl resins, copolymers, coatings, finishes, and pesticides, in addition to being used as a chemical intermediate (HSDB, 1995). Since this compound has many different applications, release to the environment may originate from a wide variety of industrial sources including iron and steel manufacturers, manufacturers of photographic equipment and supplies, automobile tire plants, and printing operations. Coal-fired power plants may also emit isophorone to the air. Although it is mostly a man-made compound, isophorone has been found to occur naturally in cranberries (ATSDR, 1989). Occupational exposure may occur by inhalation or dermal contact. The annual statewide industrial emissions from facilities reporting under the Air Toxics Hot Spots Act in California based on the most recent inventory were estimated to be 2809 pounds of isophorone (CARB, 2000).

IV. Effects of Human Exposures

No information is available concerning long-term exposure or pharmacokinetics of isophorone in humans. In occupational monitoring studies, the time-weighted average concentration in breathing zones and workplace air of a screening plant ranged from 8.3-23 ppm and from 3.5-14.5 ppm, respectively (Samimi, 1982). Up to 25.7 ppm was detected in air of a silk screening printing plant in Pittsburgh, PA (Kominsky, 1983). The concentration in breathing zone samples from a decal manufacturing plant in Ridgefield, NJ was 0.7-14 ppm (Lee and Frederick, 1982). It was suspected that the reported eye and nose irritation of workers at the silk screening plant and at the decal manufacturing plant was the result of acute and subacute exposure to isophorone vapors.

Workers exposed to 5-8 ppm (28-45 mg/m³) of isophorone for one month complained of fatigue and malaise (NIOSH, 1978). When concentrations were reduced to 1-4 ppm, no adverse effects were reported. Acute exposure studies in humans (up to 400 ppm for 1 to 4 minutes) resulted in eye, nose and throat irritation, nausea, headache, and dizziness or faintness (Union Carbide, 1963). Inhalation exposure for 15 minutes to 10 ppm isophorone produced only mild effects in human subjects while 25 ppm produced irritation to eyes, nose, and throat (Silverman *et al.*, 1946).

V. Effects of Animal Exposures

Few reports have been published regarding the pharmacokinetics of isophorone in experimental animals. Isophorone was widely distributed in the major organs of the rat following 4 hour inhalation exposure to 400 ppm (ATSDR, 1989). Oral gavage of 4000 mg/kg body wt to rats and a rabbit also resulted in wide distribution of the chemical. The highest blood levels of isophorone were reached by 30 min in rabbits following oral gavage and had decreased dramatically by 21 hours, indicating rapid absorption and elimination of the chemical. Preliminary results of a pharmacokinetic study indicate that rats treated orally with ¹⁴C-isophorone excreted 93% of the radiolabel in the urine, expired air, and feces in 24 hours (ATSDR, 1989). The highest levels of ¹⁴C-isophorone were found in the liver, kidney, preputial gland, testes, brain, and lungs. Several metabolites were identified in the urine of orally dosed rats and rabbits, including 3-carboxy-5,5-dimethyl-2-cyclohexene-1-one, 3,5,5-trimethylcyclohexanol, and some glucuronide conjugates (Dutertre-Catella *et al.*, 1978). A portion of the chemical was excreted unchanged in expired air.

In an early inhalation study, 10 Wistar rats/group and 10 guinea pigs/group, all of mixed sex, were exposed to 0, 25, 50, 100, 200, or 500 ppm isophorone 8 hr/day, 5 days/week for 6 weeks (Smyth *et al.*, 1942). Increased mortality and reduced body weights were observed at 100 ppm and up in both species. However, eye and nose irritation was noted only at the highest dose. Minor changes in blood chemistry and histopathological changes in the kidney and lungs were noted in treated animals. However, later investigations determined that the isophorone used in this study was contaminated with appreciable amounts of compounds (Rowe and Wolf, 1963).

Therefore, some of the adverse effects (i.e., the lung lesions) may have been due to the contaminants. The accuracy of the concentration data in the 1942 study is also questionable.

No treatment-related histopathological lesions were found in lungs, livers, or kidneys of male and female rats exposed intermittently (6 hr/day, 5 days/week) to 37 ppm isophorone for 4 weeks compared to controls (Hazleton Labs, 1968; summarized by ATSDR, 1989). Histological examination was limited to 30% of the control and treated rats. Body weight gain, mean absolute liver weights, and mean liver-to-body weight ratios of treated rats were significantly reduced compared to controls. Slight variations in hematological findings were noted in treated rats (increased lymphocytes and hemoglobin content; decreased neutrophils) but were not considered different from controls.

Rats (10/sex) were exposed to 500 ppm isophorone 6 hr/day, 5 days/week for up to 6 months (Dutertre-Catella, 1976; summarized by ATSDR, 1989). Irritation of eyes and nasal mucosa was observed. One female and three males in the treatment group died during the study, which was considered to be a treatment-related effect. But no exposure-related histopathological lung or liver lesions were observed compared to controls. Dutertre-Catella (1976) also exposed rats and rabbits (number per group per sex not stated) to 250 ppm isophorone 6 hr/day, 5 days/week for 18 months (Dutertre-Catella, 1976). Irritation of eyes and nasal mucosa was observed in both species, but no deaths occurred in the treatment groups. Histopathological examination of the lungs and kidneys, urinalysis, and hematological analysis revealed no exposure-related changes in either species. However, cytoplasmic microvacuolization of hepatocytes was observed in both species (ATSDR, 1989).

In a 90-day feeding study, 20 CFE albino rats/group/sex were given isophorone in their diet at concentrations of 0, 750, 1500, or 3000 ppm. Four beagle dogs/group/sex received isophorone in gelatin capsules at concentrations of 0, 35, 75, or 150 mg/kg body wt-day (AME, 1972a,b). High dose rats exhibited slightly reduced weight gain compared to controls (8-10%) for most of the study. Average weight gain among the exposure groups of beagle dogs remained essentially unchanged during the entire study. Urinalysis, hematology, and clinical chemistry indices found no treatment-related effects in the animals at either the interim or final toxicological examinations. Gross pathology and a limited histopathological examination observed no treatment-related effects in either species. Data on isophorone purity and possible loss of isophorone from rat diet due to vaporization were not presented.

In the most comprehensive isophorone toxicity study to date, 50 F344/N rats/group/sex and 50 B6C3F1 mice/group/sex were administered 0, 250 or 500 mg isophorone/kg body wt 5 days/week by oral gavage (in corn oil) for 103 weeks (Bucher *et al.*, 1986; NTP, 1986). Clinical signs of toxicity were not seen during the length of the study. However, several deaths in male and female rats at the high dose occurred early in the study. A steep decline in survival rate of high dose male rats occurred after week 90. Male and female rats and female mice in the high dose group exhibited only a slight decrease in body weight (<10%) compared to controls. A 13-week range finding study for the 2-year study did not find compound-related lesions in the kidney (or any other organs) of rats and mice exposed up to 1000 mg/kg body wt-day. However, pathological examination of rats exposed to isophorone for 2 years revealed non-neoplastic lesions in the kidney. Increased mineralization of the collecting ducts in isophorone-exposed

male (but not female) rats was observed. This lesion was characterized by basophilic aggregates of mineral most often found in the medullary collecting ducts and occurred coincidentally with lesions of chronic nephropathy. Nephropathy was observed in almost half the female controls and nearly all the male controls. Isophorone exposure appeared to increase both the severity of nephropathy in low dose male rats and the incidence of nephropathy in dosed female rats, but the effects were not pronounced. However, the isophorone potentiation of nephropathy in rats may be due to 'male rat-specific nephropathy' and may not have any relevance to human exposure (Strasser *et al.* 1988). Other adverse effects in kidneys of isophorone-treated male rats include tubular cell hyperplasia (in a dose-related manner) and epithelial hyperplasia of the renal pelvis. In mice, an increased incidence of chronic focal inflammation was observed in the kidneys of males, but was not considered treatment-related. A dose-dependent increase in fatty metamorphosis occurred in the adrenal cortex of male rats, but the biological significance of this change is unknown. All isophorone-exposed male mice had an increased incidence of hepatocytomegaly and coagulative necrosis of the liver. However, treatment-related liver lesions were not observed in female mice. Increased incidence of hyperkeratosis of the forestomach was observed in dosed male and high dose female mice, but was probably not a relevant treatment-related effect.

Published studies on possible reproductive effects of isophorone are lacking. An unpublished inhalation study conducted by a commercial laboratory (Bio/dynamics, 1984b) studied possible teratogenicity due to isophorone in rats or mice at inhaled doses up to 115 ppm. Groups of 22 female rats and 22 female mice were exposed to 0, 25, 50, or 115 ppm isophorone (6 hr/day) on gestational days 6-15. Maternal toxicity in rats included dose-dependent alopecia and cervical/anogenital staining. Low body weights (7-8%) were occasionally observed in the 115 ppm group. In mice, maternal toxicity was confined to slightly decreased weight (7-8%) on one day in the 115 ppm group. No significant differences were found in uterine implantations, fetal toxicity, and external and internal malformations among the animals. However, a slight, but significant, growth retardation in the form of decreased crown-rump length was present among the high dose fetal rats. Also, a slight, but insignificant, increase in extra ribs and/or rudimentary ribs was seen in rat and mouse fetuses at the highest dose. In a pilot study for this developmental toxicity investigation (12 females/species), exencephaly was observed in 1 rat and 1 mouse undergoing late reabsorption and in 2 live rat fetuses from dams exposed to 150 ppm isophorone on gestational days 6-15 (Bio/dynamics, 1984a). Exencephaly was not observed at any dose level in the primary study.

Dutertre-Catella (1976) did not find adverse reproductive or developmental effects in rats exposed to 500 ppm isophorone (6 hr/day, 5 days/week) for 3 months before mating and throughout gestation (females only) as well. The pups were not examined for internal malformations so the study was incomplete for determination of developmental effects.

VI. Derivation of Chronic Reference Exposure Level (REL)

<i>Study</i>	Bio/dynamics 1984a,b
<i>Study population</i>	22 female mice/group, 22 female rats/group
<i>Exposure method</i>	Discontinuous whole body inhalation exposure during gestation (0, 25, 50, or 115 ppm)
<i>Critical effects</i>	Developmental effects (reduced crown-rump length of female rat fetuses); teratogenicity (exencephaly in fetal rats and mice) in range finding study at 150 ppm
<i>LOAEL</i>	115 ppm for reduced crown-rump length of female rat fetuses
<i>NOAEL</i>	50 ppm
<i>Exposure continuity</i>	6 hr/day during gestation
<i>Exposure duration</i>	Days 6-15 of gestation
<i>Average experimental exposure</i>	12.5 ppm (50 x 6/24)
<i>Human equivalent concentration</i>	12.5 ppm (gas with systemic effects, based on RGDR = 1.0 using default assumption that $\lambda(a) = \lambda(h)$)
<i>LOAEL uncertainty factor</i>	1
<i>Subchronic uncertainty factor</i>	1
<i>Interspecies uncertainty factor</i>	3
<i>Intraspecies uncertainty factor</i>	10
<i>Cumulative uncertainty factor</i>	30
<i>Inhalation reference exposure level</i>	0.4 ppm (400 ppb, 2 mg/m ³ , 2,000 µg/m ³)

The inhalation study by Bio/dynamics (1984a,b) presents data that indicate exposure during gestation may be the most sensitive indicator of non-neoplastic toxicity by isophorone. Exposure of pregnant rats to 115 ppm isophorone during gestation resulted in significant growth retardation of female rat fetuses (reduced crown-rump length). Exposure to 50 ppm isophorone, the NOAEL, produced no developmental effects. The authors had removed the two shortest female fetuses prior to statistical analysis. The result was that there was no significant difference in fetal growth retardation; therefore, this adverse effect is not significant. However, this selective culling before the statistical analysis is not scientifically appropriate in this case. In addition, the authors did not perform some of the scheduled fetal examinations. Otherwise, the growth retardation might have had even greater statistical significance. The pilot study (Bio/dynamics, 1984a) observed exencephaly in a few mouse and rat fetuses at 150 ppm. Exencephaly was not considered significant by the authors because it was not present in any fetuses of the primary study (Bio/dynamics, 1984b). However, exencephaly is included as a critical effect in this summary because it is considered a serious teratogenic effect that was present at a dose (150 ppm) only slightly higher than the LOAEL of the primary study (115 ppm). Alopecia of adult female rats was observed in many of the exposed animals. However, this effect may be considered more of an acute dermal irritation than a chronic effect. In addition, cervical and anogenital staining seen in many exposed rats is not considered a chronic 'adverse' effect.

For comparison with the proposed REL of 0.4 ppm, the inhalation LOAEL of 250 ppm for mild liver effects (Dutertre-Catella, 1976) in rats and rabbits intermittently exposed to isophorone for 18 months was used to estimate a REL. Use of a time adjustment ($6/24 \times 5/7$), an RGDR of 1, and a total UF of 100 (LOAEL to NOAEL = 3, interspecies = 3, and intraspecies = 10), also resulted in an estimated REL of 0.4 ppm. These results indicate that the REL will also protect against adverse liver effects.

While the toxicological significance of this liver effect observed by Dutertre-Catella (1976) is unknown, the NTP (1986) study observed an increased incidence of hepatocytomegaly and coagulative necrosis of the liver in treated male mice, but not in female mice and rats, orally gavaged with isophorone. Using 250 mg/kg-day as a LOAEL for mice and dividing by a total UF of 1000 (10 each for LOAEL to NOAEL, 10 for interspecies, and 10 for intraspecies) results in an oral REL of 0.25 mg/kg-day. Multiplying the oral REL by 3,500 $\mu\text{g}/\text{m}^3$ per mg/kg-day for route-to-route extrapolation results in a chronic inhalation REL estimate of 900 $\mu\text{g}/\text{m}^3$ (0.16 ppm), which is in good agreement with the REL developed from Dutertre-Catella (1976) and Biodynamics (1984a,b).

VII. Data Strengths and Limitations for Development of the REL

The strength of the database for isophorone is the consistent lack of relevant severe histopathological effects in the chronic inhalation study (Dutertre-Catella, 1976) and in the oral gavage study (NTP, 1986). Weaknesses of the database for isophorone include the lack of human exposure data, the lack of comprehensive long-term inhalation studies, and the lack of published peer-reviewed reproductive/developmental studies. The lack of human data may be due to isophorone's rather low potency for causing chronic, non-neoplastic, adverse effects. Inhalation of isophorone is a relevant route of exposure under occupational settings, but is most likely a minor route of exposure for the general population. Due to the insufficient characterization of the kidney and liver lesions in the oral gavage NTP study (Bucher *et al*, 1986; NTP, 1986) and the inhalation study (Dutertre-Catella, 1976), a comprehensive chronic study in rodent and non-rodent species would enhance the database for isophorone.

VIII. Potential for Differential Impacts on Children's Health

Since the REL is based on a developmental study, it is expected to be adequately protective of infants and children. However, there is no direct evidence in the literature to quantify a differential effect of isophorone in children relative to adults. Isophorone occurs in cranberries and thus presumably in cranberry juice, which is often mixed with other fruit juices. Children tend to consume more fruit juice. However, isophorone as a Hot Spot emission is unlikely to be a multimedia chemical, and there is no evidence to suggest that normal dietary levels of isophorone are associated with adverse health effects.

IX. References

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CHRONIC TOXICITY SUMMARY

ISOPROPANOL*(2-propanol; dimethylcarbinol; isopropyl alcohol)***CAS Registry Number: 67-63-0****I. Chronic Toxicity Summary**

<i>Inhalation reference exposure level</i>	7,000 $\mu\text{g}/\text{m}^3$ (3000 ppb)
<i>Critical effect(s)</i>	Kidney lesions in mice and rats; fetal growth retardation and developmental anomalies in rats
<i>Hazard index target(s)</i>	Kidney; development

II. Chemical Property Summary (HSDB, 1995)

<i>Description</i>	Colorless liquid at room temperature (25°C) with a pleasant odor. Slightly bitter taste.
<i>Molecular formula</i>	$\text{C}_3\text{H}_8\text{O}$
<i>Molecular Weight</i>	60.09
<i>Boiling point</i>	82.5°C
<i>Vapor Pressure</i>	44.0 torr at 25°C
<i>Solubility</i>	Miscible in water and most organic solvents; insoluble in salt solutions.
<i>Conversion factor</i>	1 ppb = 2.45 $\mu\text{g}/\text{m}^3$ at 25°C

III. Major Uses and Sources

Isopropanol is used as a solvent and in making many commercial products (HSDB, 1995). The annual production volume of isopropanol has been in excess of one billion pounds since 1956; it was ranked 50th among chemicals produced in the U.S. in 1994 (C&EN, 1995). Rubbing alcohol is a solution of 70% isopropanol in water. Specific uses and sources include: a component of antifreeze; a solvent for gums, shellac, essential oils, creosote and resins; extraction of alkaloids; component of quick drying oils and inks; component of denaturing alcohol; antiseptic for hand lotions; rubefacient; component of household products (after-shave lotions, cosmetics, etc.); the manufacture of acetone; deicing agent for liquid fuels; dehydrating agent and synthetic flavoring adjuvant. Isopropanol can enter the environment as emissions from its manufacture and use as a solvent. It naturally occurs as a plant volatile and is released during the microbial degradation of animal wastes. Human exposure will be both in occupational atmospheres and from use of consumer products containing isopropanol as a volatile solvent. An odor threshold has been estimated as 22 ppm (Amoore and Hautala, 1983), which is 7-fold higher than the chronic REL.

The annual statewide industrial emissions from facilities reporting under the Air Toxics Hot Spots Act in California based on the most recent inventory were estimated to be 525,826 pounds of isopropanol (CARB, 1999b).

IV. Effects of Human Exposures

Currently, there are no adequate chronic exposure data for isopropanol in humans. While isopropanol is not considered a dermal irritant, it is a defatting agent and can cause dermatitis with prolonged exposure to skin (IARC, 1977). A subacute study of daily oral intake of isopropanol (2.6 or 6.4 mg/kg body weight) by groups of 8 men for 6 weeks had no effect on blood cells, serum or urine and produced no subjective symptoms (Wills *et al.*, 1969). A pharmacokinetic study of men occupationally exposed to isopropyl alcohol revealed that uptake occurs readily via the inhalation route; acetone is the major metabolite (Brugnone *et al.*, 1983). Acetone was eliminated mainly by the lung but was also eliminated in the urine.

V. Effects of Animal Exposures

In metabolism studies with rats and mice, up to 92% of the administered dose (via i.v. or inhalation) of isopropanol was exhaled as acetone, CO₂ and the unmetabolized alcohol (Slauter *et al.*, 1994). Approximately 3-8% of the administered dose was excreted in urine as isopropanol, acetone, and a metabolite tentatively identified as isopropyl glucuronic acid. Isopropanol is readily absorbed from the GI tract and persists in the circulation longer than ethyl alcohol. Alcohol dehydrogenase oxidizes most isopropanol to acetone. Acetone may be further metabolized to acetate, formate, and finally CO₂. In another metabolism study, the amount of acetone in the blood stream was found to be directly related to the air concentration of isopropanol (Laham *et al.*, 1980). This finding indicated that the acetone metabolite could be used as a biochemical indicator of isopropanol exposure.

Subchronic studies by Guseinov and Abasov (1982) and Baikov *et al.* (1974) reported changes in certain hematologic and clinical chemistry parameters, as well as increases in some organ weights. But the Environmental Protection Agency deemed these studies insufficient to reasonably predict subchronic toxicity of isopropanol (Burleigh-Flayer *et al.*, 1994). Three different routes of exposure have been used by researchers for isopropanol toxicity studies: inhalation, oral gavage and presence in drinking water. The following subchronic and chronic studies exposed experimental animals to isopropanol by the inhalation route:

Toxicological and neurobehavioral endpoints were investigated in rats and mice following 13-week inhalation exposure (6 hr/day, 5 days/week) to 0, 100, 500, 1500 or 5000 ppm isopropanol (Burleigh-Flayer *et al.*, 1994). In rats, clinical signs observed following exposures included swollen periocular tissue (females) at the highest dose and perinasal encrustation (males) at 500 ppm and above. Narcosis was observed in a few animals of both species during exposure to 5000 ppm and possibly 1500 ppm as well. However, the animals became tolerant to the narcotic effects of isopropanol after week 2. No neurobehavioral changes were observed in any parameters of the functional observational battery. However, increased motor activity was noted

at week 9 of exposure in female rats of the 5000 ppm group. After an initial drop in body weight gain in the first week of exposure at the high dose (5000 ppm), rats in the 1500 and 5000 ppm groups had significant increases in body weight gain and/or body weight throughout most of the exposure period. But only the 5000 ppm group had greater than 10% body weight gain compared to controls. Increases in body weight and body weight gain greater than 10% were also noted in female mice in the 5000 ppm group. Consistent clinical pathology changes included an increase in mean corpuscular volume (rats; female mice) and mean corpuscular hemoglobin (male rats; female mice) at the 5000 ppm exposure level. Other changes noted include a slight anemia in rats at week 6 only and a slight dehydration in female mice at the end of the study. Relative liver weight in rats was elevated no more than 8% in the 5000 ppm groups. However, a 10 and 21% increase in relative liver weight was observed in female mice at 1500 and 5000 ppm, respectively. No gross lesions were observed in any organs. The only microscopic change observed was hyaline droplets within kidneys of all male rats. This change was not clearly concentration related, although it was most pronounced in the 5000 ppm group.

In a follow-up inhalation study spanning the lifetime of rats and mice, Burleigh-Flayer *et al.* (1997) exposed four groups of animals, each consisting of 75 CD-1 mice/sex and 75 Fischer 344 rats/sex, to 0, 500, 2500, or 5000 ppm isopropanol vapor. Of these, 55 mice/sex/group and 65 rats/sex/group were exposed 6 hr/day, 5 days/week for at least 78 weeks (mice) or 104 weeks (rats). Transient signs of narcosis were observed at the higher doses. Increased mortality and a decreased mean survival time (577 days versus 631 days for controls) were noted for male rats in the 5000 ppm group. Increases in body weight and/or body weight gain were observed for both sexes of mice and rats from the 2500 and 5000 ppm groups throughout the study. Concentration-related increases in absolute and relative liver weight were observed for male and female mice. In addition, increased absolute and/or relative liver and kidney weight were observed for male and/or female rats from the 2500- and 5000-ppm groups. Urinalysis and changes in urine chemistry, indicative of impaired kidney function (i.e. decreased osmolality and increased total protein, volume, and glucose), were noted for male rats in the 2500 ppm group and for male and female rats in the 5000 ppm group. At necropsy, the most significant noncancer lesions in rats were observed in the kidney, and were associated with an exacerbation of spontaneous chronic renal disease. The kidney lesions noted with increased severity and/or frequency included mineralization, tubular dilation, glomerulosclerosis, interstitial nephritis, interstitial fibrosis, hydronephrosis, and transitional cell hyperplasia. The authors considered chronic renal disease to be the main cause of death for male and female rats exposed to 5000 ppm and to account for much of the mortality observed for male rats exposed to 2500 ppm. Unlike the subchronic study, anemia was not observed in rats in the chronic study. In mice, an increased incidence of seminal vesicle enlargement was observed grossly in males in the 2500 and 5000 ppm groups. Microscopically, the lesions in mice included an increased incidence of ectasia (dilation) of the seminal vesicles for male mice in the 2500 and 5000 ppm groups, minimal renal tubular proteinosis for male and female mice from all isopropanol groups, and renal tubular dilation for female mice in the 5000-ppm group. The seminal vesicle effects did not have any associated inflammatory or degenerative changes. The enlargement may have been the result of either increased secretion or decreased evacuation of the secretory product by these glands. Microscopic evaluation of the livers of rats and mice revealed no exposure-related lesions. In a 13-week behavioral/neurotoxicity study by the same investigators, the reproducibility and reversibility of increased motor activity in isopropanol-exposed female Fischer 344 rats was

investigated (Burleigh-Flayer *et al.* 1998). Rats were exposed to 0 or 5000 ppm isopropanol for 6 hr/day, 5 days/week. Increased motor activity was characterized as the summation of ambulation, rearing and fine movements and was first observed 4 weeks following exposure to 5000 ppm isopropanol. Reversibility of this effect was observed 2 days following cessation of exposure in a subgroup of rats exposed to isopropanol for only 9 weeks. In the subgroup exposed for 13 weeks, reversal of the increased motor activity did not occur until 2 weeks following cessation of exposure. However, complete reversibility of the time versus activity profile, or habituation curve, was not noted until 42 days following exposure to isopropanol for 13 weeks.. Other effects included a significant increase in body weight and an increased incidence of swollen periocular tissue in isopropanol-exposed animals.

In a study conducted to investigate neurochemical and behavioural effects, 20 male Wistar rats/group were exposed to 0 or 300 ppm isopropanol 6 hr/day, 5 days/week for up to 21 weeks (Savolainen *et al.*, 1979). Enzyme activity of superoxide dismutase and azoreductase in cerebellar homogenate was decreased at week 20-21. Acid protease activity in glial cells was increased up to week 10. Open-field tests indicated sporadic changes in urination (10th week) and defecation (15th week). Isopropanol also appeared to depress caffeine stimulation activity at 15 weeks.

In a subchronic neurotoxicity study by Teramoto *et al.* (1993), motor and sensory nerve conduction velocity increased significantly following a 20-week exposure (8 hr/day, 5 days/week) of Jcl-Wistar rats to 8000 ppm isopropanol. Low dose (1000 ppm) exposure had no effect on conduction velocity. Conduction velocities returned to normal following the end of exposure. The sex of the rats in this study was not specified.

A developmental study in rats exposed pregnant dams (15/group) to 0, 3500, 7000 or 10,000 ppm isopropanol 7 hr/day on gestation days 1-19 (Nelson *et al.*, 1988). At the two highest exposure levels, feed intake (weeks 1 and 2 of exposure) and maternal body-weight gain were reduced. Narcosis was evident only at the 10,000 ppm level. Increased fetal resorptions and reduced fetal weights (59% of controls) occurred at the highest exposure level. Fetal weights were also significantly reduced (85% of controls) at 7000 ppm. A slight reduction in fetal weight (96% of controls) occurred at 3500 ppm but was significant in the sense that a dose-dependent relationship in fetal weight reduction was present across all exposed groups. Skeletal malformations (primarily rudimentary cervical ribs) were seen only in the presence of maternal toxicity at the two highest exposure levels. No detectable teratogenic effects were observed in the 3500 ppm group. The authors noted that the developmental effects at 3500 ppm were considered very slight, indicating that this exposure level is close to the LOAEL for isopropanol.

The following studies administered isopropyl alcohol to experimental animals by oral gavage:

In a developmental study, pregnant (VAF)CD(SD) rats (25/group) were gavaged with either 0, 400, 800 or 1200 mg/kg body wt-day of isopropanol daily on gestational days 6 through 15 (Tyl *et al.*, 1994). In the same study, pregnant New Zealand white rabbits (15/group) were dosed orally with either 0, 120, 240 or 480 mg/kg body wt-day of isopropanol daily during gestational days 6 through 18. In rats, fetal body weight exhibited a linear downward trend with increasing dose and was significantly lower at the highest dose compared to controls. However, the fetal

body weight differences at each dose level was less than 10% of controls. Maternal weight gain during gestation was significantly reduced at the highest dose level. In rabbits, maternal weight gain and food consumption was reduced during gestation at 480 mg/kg body wt-day. Four rabbits died after dosing at this level. No differences were observed in reproduction indices or in fetal development. No teratogenic effects were seen in either species.

In another developmental study performed to investigate neurotoxicity in rat pups, 64 time-mated Sprague-Dawley rats/group were administered 0, 200, 700 or 1200 mg/kg body wt-day isopropanol by oral gavage from gestational day 6 through postnatal day 21 (Bates *et al.*, 1994). One high-dose dam died on postnatal day 15, but there were no other clinical observations of effects on maternal weight, food consumption or gestation length. All fetal developmental indices were unaffected at the dose levels used. Developmental neurotoxicity, in the form of motor activity, auditory startle and active avoidance tests, was not found at any dose of isopropanol.

In a multi-generation study carried out to investigate potential reproductive and developmental effects of isopropanol, Sprague-Dawley rats were administered 0, 100, 500 or 1000 mg/kg body wt-day of isopropanol by oral gavage (Bevan *et al.*, 1995). P1 and P2 rats were dosed daily for 10 weeks prior to mating, throughout the mating, and during the gestation and lactation period for the F1 and F2 litters, respectively. In adult rats, centrilobular hepatocyte hypertrophy and increased relative liver weight (>10%) was observed in P2 males at 1000 mg/kg. A general increase in absolute and relative liver and kidney weights was observed (less than 10% in most cases) in treated animals in both P1 and P2 generations. However, with the exception of hepatocellular hypertrophy in P2 males, no histopathological effects relevant to human risk were present. Reproductive effects due to isopropanol were not seen at any dose level. Statistically significant reduction of body weights (5-12%) in F1 and F2 offspring and increased mortality (14%) in F1 offspring were observed at the 1000 mg/kg dose level.

The following toxicology studies administered isopropanol to experimental animals in drinking water:

In a study designed to investigate neurotoxicological effects, 22 male SPF rats/group were administered isopropanol in drinking water at concentrations of 0, 1, 2, 3, or 5% (w/v) for 12 weeks (Pilegaard and Ladefoged, 1993). Average daily intake of isopropanol was 0, 870, 1280, 1680 and 2520 mg/kg body wt, respectively. Water intake and body weights were consistently lower at the two highest doses. Relative weights of liver, kidney and adrenals were increased in a dose-dependent manner. However, histopathology revealed no treatment-related changes in organs other than the male rat-specific kidney lesions. Evidence of astrogliosis, in the form of increased glial fibrillary acidic protein in dorsal hippocampus, was not found in exposed rats.

In a 1-generation study, 10 Wistar-derived rats/sex/group were exposed to 0, 0.5, 1.25, 2.0, and 2.5% isopropanol in water for up to 18 weeks (USEPA/OTS, 1986). The doses are equivalent to 0, 325, 711, 1002, and 1176 mg/kg body wt-day, respectively, for males; to 0, 517, 1131, 1330, and 1335 mg/kg body wt-day, respectively, for females during the pre-mating phase; and to 0, 1167, 2561, 2825, and 2722 mg/kg body wt-day, respectively, in females during the post-partum phase. Exposure periods were: 70 days pre-mating, plus 15 days during mating, plus 42 days for

males; 21 days pre-mating plus 15 days during mating, plus 21 days gestation, plus 21 days rearing in females; and 21 days for the F₁ generation. At the highest two levels, body weights of males during the first two weeks were reduced and the body weights of females during the post-partum period were reduced. Water consumption and food ingestion were generally lower at the top three dose levels. The authors concluded that these effects were related to the unpalatability of drinking water containing isopropanol and not due to a toxic effect of the alcohol itself. Anemia was present in post-partum females. Red cell numbers were reduced in a dose-related manner at doses of 1.25% isopropanol or higher. Hematocrit was lower at the two highest doses while hemoglobin was lower at the highest dose. In males, mean cell volume was reduced at 1.25% isopropanol or higher. Absolute and relative liver and kidney weights were higher in most exposure groups at 2.0% or higher in both sexes, but no relevant pathology was seen. Absolute liver weight of females was also higher in the 1.25% group. Fetal weight gain was depressed in a dose-related fashion in the 1.25% and higher groups. Mean pup weights and pup survival were lower than controls at the two highest doses. Fewer pups were born per animal in the 2.5% exposure group. A teratogenic examination was not performed on the pups.

In a similar exposure study investigating the potential teratogenic effects of isopropanol, 20 pregnant Wistar-derived rats/group were exposed to 0, 0.5, 1.25 or 2.5% of the alcohol in drinking water (equivalent to 0, 596, 1242 and 1605 mg/kg body wt-day) during gestational days 6 to 16 (USEPA/OTS, 1992a,b). Water and feed consumption were reduced at the two highest doses while maternal body weight was significantly reduced at the highest dose. Fetal body weights were decreased in the two highest dose groups. Minor abnormalities and variants (reduced ossification of the skeleton) were present in fetuses of exposed groups in a dose-related manner. However, the authors concluded that the reduced fetal weights are probably a consequence of maternal growth retardation during the critical period of organogenesis. Similarly, the fetal abnormalities are probably due to small fetal size, related to slightly retarded development. Therefore, the study found no indication of teratogenesis.

A multi-generation study performed in 'white' rats also observed reduced body weights in F₁ offspring (Lehman *et al.*, 1945). Body weights of F₂ offspring were the same as controls. The adult rats had imbibed an average of 1.9 ml/kg (1470 mg/kg body wt) of isopropanol per day in drinking water 80 days prior to mating. No other developmental or reproductive effects were seen. In the same study, several dogs were given 4% isopropanol in drinking water for approximately 7 months. Histopathology at the end of exposure revealed a decrease in the number of nephrons with hydropic changes and necrosis of some of the tubular epithelium. Some capillary hemorrhages were also noted in the brains of two of the dogs. Average daily dose of isopropanol imbibed by the dogs could not be determined from data provided in the report.

VI. Derivation of Chronic Reference Exposure Level (REL)

<i>Study</i>	Burleigh-Flayer <i>et al.</i> (1997)
<i>Study population</i>	Rats and mice
<i>Exposure method</i>	Discontinuous whole-body inhalation (0, 504, 2,509 or 5,037 ppm)
<i>Critical effects</i>	Kidney lesions in mice and rats
<i>LOAEL</i>	2,509 ppm
<i>NOAEL</i>	504 ppm
<i>Exposure continuity</i>	6 hours/day, 5 days/week
<i>Exposure duration</i>	78 weeks in mice; 104 weeks in rats
<i>Average experimental exposure</i>	90 ppm for NOAEL group (500 x 6/24 x 5/7)
<i>Human equivalent concentration</i>	90 ppm for NOAEL group (gas with systemic effects, based on RGDR = 1.0 using default assumption that lambda (a) = lambda (h))
<i>LOAEL uncertainty factor</i>	1
<i>Subchronic uncertainty factor</i>	1
<i>Interspecies uncertainty factor</i>	3
<i>Intraspecies factor</i>	10
<i>Cumulative uncertainty factor</i>	30
<i>Inhalation reference exposure level</i>	3 ppm (3000 ppb, 7 mg/m ³ , 7000 µg/m ³)

The Burleigh-Flayer *et al.* (1997) study was selected because it was a chronic study, was recent, and was published in a respected, peer-reviewed journal. While numerous subchronic studies have been performed, this was the only study that conducted lifetime animal exposures. In addition, the chronic kidney effects observed in rats and mice were not seen in the subchronic studies, indicating that chronic exposure is necessary for development of these lesions.

The lesions observed in the kidneys of male rats in some of the studies described above is typical of a male rat-specific chronic renal disease and is not considered to be relevant to human risk assessment (Phillips and Cockrell, 1984; Beyer, 1992). However, the exacerbation of chronic renal disease in male and female rats, and the slight kidney damage observed in mice of both sexes following chronic isopropanol exposure indicates that the kidney is a sensitive indicator for nonneoplastic effects (Burleigh-Flayer *et al.*, 1997). Suggestive evidence also exists for kidney damage in dogs following subchronic exposure to isopropanol in drinking water (Lehman *et al.*, 1945).

Some isopropanol exposure studies noted increased liver and kidney weights in exposed animals but no observable relevant pathology. With particular relevance to the liver, this weight change may be considered to be more of a metabolic response rather than a toxic effect of the alcohol. The changes noted in the neurochemical and behavioural study by Savolainen *et al.* (1979) may have also been more of a metabolic response to the increased load of isopropanol. It is also possible that these changes reflected the development of tolerance. The changes in behavior

were small and unconvincing. This study would have benefited from additional dose levels to analyze for dose-response trends.

Other possible sensitive indicators of isopropanol toxicity include blood chemistry changes and reduced fetal body weights. However, the blood chemistry findings were conflicting among the various studies that investigated this endpoint. Reduced fetal weights at doses below maternal body weight reductions were minor (<10% compared to controls), but consistent, suggesting that reduced fetal weights are a manifestation of isopropanol developmental toxicity.

A comparative REL was calculated from the only reproduction/developmental study that utilized inhalation as the route of exposure (Nelson *et al.*, 1988). Exposure of pregnant rats to isopropanol during gestation caused dose-dependent reduction in fetal body weights across all treatment groups, resulting in a LOAEL of 3500 ppm (average measured concentration = 3510 ppm). A NOAEL was not observed for this effect. Skeletal malformations probably related to reduced fetal weight was observed at 7000 ppm and 10,000 ppm. The average exposure duration at the LOAEL for this study is 1024 ppm (7hr/24hr x 3510 ppm). Use of an RGDR of 1 and a cumulative uncertainty factor of 100 (3 for LOAEL to NOAEL, 3 for interspecies, and 10 for intraspecies) resulted in a REL of 10 ppm (25 mg/m³). Since the endpoint is a function of exposure only during gestation, no subchronic to chronic UF was used. This developmental REL is within an order of magnitude of the chronic REL for kidney lesions, and therefore, is also considered to be a critical effect.

The oral dose developmental studies by Tyl *et al.* (1994), Bevan *et al.* (1995), USEPA/OTS (1986), and USEPA/OTS (1992 a, b) provide supportive evidence that reduced fetal weights is a sensitive developmental endpoint. The USEPA/OTS (1992a,b) study provides supportive evidence for skeletal malformations in exposed rat fetuses.

VII. Data Strengths and Limitations for Development of the REL

Strengths of the database for isopropanol include availability of a well-conducted chronic study in two species, similar toxicological endpoints among different studies, and pharmacokinetic similarities between humans and experimental animals. Isopropanol is metabolized through a similar pathway to acetone and CO₂.

Weaknesses of the database for isopropanol include a lack of literature regarding chronic toxicity endpoints in humans. The deficiency of chronic toxicity cases in humans may be related to the relatively low chronic toxicity of isopropanol. Another weakness is that, while most developmental studies observed maternal and fetal effects, only one study was performed via the inhalation route.

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CHRONIC TOXICITY SUMMARY

MALEIC ANHYDRIDE*(2,5-furandione; cis-butenedioic anhydride; toxilic anhydride; maleic andride)***CAS Registry Number: 108-31-6****I. Chronic Toxicity Summary**

<i>Inhalation reference exposure level</i>	0.7 $\mu\text{g}/\text{m}^3$ (2.5 ppb)
<i>Critical effect(s)</i>	Neutrophilic infiltration of the nasal epithelium; irritation of the respiratory system in rats, hamsters and monkeys
<i>Hazard index target(s)</i>	Respiratory system

II. Chemical Property Summary (HSDB, 1995)

<i>Description</i>	Colorless or white solid
<i>Molecular formula</i>	$\text{C}_4\text{H}_2\text{O}_3$
<i>Molecular weight</i>	98.06 g/mol
<i>Boiling point</i>	202°C
<i>Melting point</i>	52.8°C
<i>Vapor pressure</i>	0.1 torr @ 25°C (AIHA, 1970)
<i>Solubility</i>	Soluble in water, ether, acetate, chloroform, dioxane; @ 25°C, 227 g/100 g acetone, 112 g/100 g ethyl acetate, 52.5 g/100 g chloroform, 50 g/100 g benzene, 23.4 g/100 g toluene, 19.4 g/100 g o-xylene, 0.6 g/100 g CCl_4 , 0.25 g/100 g ligroin
<i>Conversion factor</i>	4.0 $\mu\text{g}/\text{m}^3$ per ppb at 25°C

III. Major Uses and Sources

Maleic anhydride is used as a chemical intermediate in the synthesis of fumaric and tartaric acid, certain agricultural chemicals, resins in numerous products, dye intermediates, and pharmaceuticals (HSDB, 1995). It is also used as a co-monomer for unsaturated polyester resins, an ingredient in bonding agents used to manufacture plywood, a corrosion inhibitor, and a preservative in oils and fats. The annual statewide industrial emissions from facilities reporting under the Air Toxics Hot Spots Act in California based on the most recent inventory were estimated to be 7366 pounds of maleic anhydride (CARB, 2000).

IV. Effects of Human Exposure

In many occupational situations workers are exposed to mixtures of acid anhydrides, including maleic anhydride, phthalic anhydride, and trimellitic anhydride. For example, Barker *et al.* (1998) studied a cohort of 506 workers exposed to these anhydrides. In one factory, workers were exposed only to trimellitic anhydride, which has the lowest acceptable occupational exposure limit ($40 \mu\text{g}/\text{m}^3$) of the three anhydrides. In that factory there was an increased prevalence of sensitization to acid anhydride and work related respiratory symptoms with increasing full shift exposure even extending down to levels below the current occupational standard. However, none of the workplaces had exposure only to maleic anhydride and a dose-response relationship was not seen with mixed exposures.

The following reports involve exposure only to maleic anhydride.

There are several case reports describing asthmatic responses possibly resulting from exposure to maleic anhydride. An individual showed an acute asthmatic reaction after exposure to dust containing maleic anhydride (Lee *et al.*, 1991). Workplace concentrations of maleic anhydride were $0.83 \text{ mg}/\text{m}^3$ in the inspirable particulate mass and $0.17 \text{ mg}/\text{m}^3$ in the respirable particulate mass. Bronchial provocation testing was performed with phthalic anhydride, lactose, and maleic anhydride. Exposure of this individual to maleic anhydride (by bronchial provocation testing) at $0.83 \text{ mg}/\text{m}^3$ and $0.09 \text{ mg}/\text{m}^3$ in inspirable and respirable particulate mass, respectively, showed a response of cough, rhinitis, and tearing within two minutes. Within 30 minutes, rales developed in both lungs and peak flow rate decreased 55%.

An individual occupationally exposed to maleic anhydride developed wheezing and dyspnea upon exposure (Gannon *et al.*, 1992). After a period without exposure, two re-exposures both resulted in episodes of severe hemolytic anemia. There was no evidence of pulmonary hemorrhage. Radioallergosorbent testing showed specific IgE antibodies against human serum albumin conjugates with maleic anhydride, phthalic anhydride, and trimellitic anhydride, but not with tetrachlorophthalic anhydride. A critique of the Gannon *et al.* (1992) study by Jackson and Jones (1993) questions the relationship of maleic anhydride exposure to the onset of the anemia, since there were extended periods of exposure to maleic anhydride before symptoms appeared.

Another case report described occupational asthma due to exposure to maleic anhydride (Guerin *et al.*, 1980).

Humans exposed to maleic anhydride showed respiratory tract and eye irritation at concentrations of 0.25 to 0.38 ppm (1 to $1.6 \text{ mg}/\text{m}^3$) maleic anhydride (Grigor'eva, 1964). No irritation was reported at 0.22 ppm maleic anhydride.

V. Effects of Animal Exposure

Short *et al.* (1988) chronically exposed CD rats (15/sex/group), Engle hamsters (15/sex/group), and rhesus monkeys (3/sex/group) to maleic anhydride by inhalation. Four groups of each species were exposed to concentrations of 0, 1.1, 3.3, or $9.8 \text{ mg}/\text{m}^3$ maleic anhydride for 6

hours/day, 5 days/week, for 6 months in stainless steel and glass inhalation chambers. Solid maleic anhydride was heated to 53°C to generate vapors, which were then mixed with a stream of nitrogen. Chamber target levels were monitored by gas chromatography as total maleic (maleic anhydride plus maleic acid). No exposure-related increase in mortality occurred. Of the species examined, only rats showed significant changes in body weight during the course of the experiment, with reductions among males in the high-dose groups after exposure day 40 and a transient weight reduction from days 78-127 in the mid-dose group. All species exposed to any level of maleic anhydride showed signs of irritation of the nose and eyes, with nasal discharge, dyspnea, and sneezing reported frequently. No exposure-related eye abnormalities were reported. The severity of symptoms was reported to increase with increased dose. No dose-related effects were observed in hematological parameters, clinical chemistry, or urinalysis. No effects on pulmonary function in monkeys were observed. Dose-related increases in the incidence of hyperplastic change in the nasal epithelium occurred in rats in all exposed groups, and in hamsters in the mid- and high-dose groups. Neutrophilic infiltration of the epithelium of the nasal tissue was observed in all species examined at all exposure levels. All changes in the nasal tissues were judged to be reversible. The only other significant histopathological observation was slight hemosiderin pigmentation in the spleens of female rats in the high-dose group.

Incidence of epithelial hyperplasia of the nasal mucosa in animals from Short *et al.* (1988)

Maleic anhydride (mg/m ³)	0	0	1.1	1.1	3.3	3.3	9.8	9.8
Pathology grade	Trace	Mild	Trace	Mild	Trace	Mild	Trace	Mild
Rat								
Male	0/15	0/15	2/15	6/15	1/15	14/15	0/15	12/15
Female	0/15	0/15	6/15	5/15	4/15	10/15	0/15	14/15
Combined		0/30		11/30		24/30		26/30
Hamster								
Male	0/15	0/15	0/15	0/15	0/15	5/15	0/15	8/15
Female	0/15	0/15	0/15	0/15	4/15	4/15	1/15	4/15
Combined		0/30		0/30		9/30		12/30
Monkey								
Male	0/3	0/3	0/3	0/3	0/3	0/3	0/3	0/3
Female	0/3	0/3	0/3	0/3	0/3	0/3	0/3	0/3
Combined		0/6		0/6		0/6		0/6

The teratogenicity and multigeneration reproductive toxicity of maleic anhydride were also investigated (Short *et al.*, 1986). To evaluate teratogenicity, pregnant CD rats were treated orally with maleic anhydride in corn oil at concentrations of 0, 30, 90, or 140 mg/kg-day from gestational days 6-15. Animals were necropsied on gestational day 20. No statistically significant dose-related effects were observed in maternal weight gain, implantation, fetal viability, post-implantation loss, fetal weight, or malformations. Groups of 10 male rats and 20 female rats/group (F₀ animals) were orally treated with 0, 20, 55, or 150 mg/kg-day maleic anhydride in corn oil to study multigeneration reproductive toxicity. Animals within the same dose group were bred together after 80 days of treatment to produce two F₁ generation animals (F_{1a} and F_{1b}) and animals from the F₁ generation were interbred to produce two F₂ generation animals (F_{2a} and F_{2b}). A significant increase in mortality was observed among both F₀ and F₁

generation animals in the high-dose group. Total body weight was significantly reduced in animals in the high-dose group at Week 11 of exposure for the F₀ generation males and females and at Week 30 of exposure in the F₁ generation males. No consistent pattern of dose- or treatment-related effect on fertility, litter size, or pup survival was observed. Examination of F₀ animals showed necrosis of the renal cortex in the high-dose group (60% of males and 15% of females). Absolute kidney weights were significantly increased in F₁ females in the low- and mid-dose groups, although there was no histological correlate. No changes in organ weight or histology were observed in the F₂ generation animals.

VI. Derivation of Chronic Reference Exposure Level (REL)

<i>Study</i>	Short <i>et al.</i> , 1988
<i>Study population</i>	Rats (15/sex/group), hamsters (15/sex/group), monkeys (3/sex/group)
<i>Exposure method</i>	Discontinuous inhalation exposure (0, 1.1, 3.3, or 9.8 mg/m ³)
<i>Critical effects</i>	Neutrophilic infiltration of the nasal epithelium; epithelial hyperplasia; respiratory irritation
<i>LOAEL</i>	1.1 mg/m ³
<i>NOAEL</i>	Not observed in rats
<i>BMC₀₅</i>	0.12 mg/m ³ for mild epithelial hyperplasia in rats (males and females combined)
<i>Exposure continuity</i>	6 hr/day, 5 days/week
<i>Exposure duration</i>	6 months
<i>Average experimental exposure</i>	21 µg/m ³ for the BMC ₀₅ (0.12 x 6/24 x 5/7 x 1000)
<i>Human equivalent concentration</i>	21 µg/m ³ for the BMC ₀₅ (Due to the lack of aerosol particle size data for the critical study, a human equivalent concentration could not be developed using recommended methods of inhalation dosimetry.)
<i>LOAEL uncertainty factor</i>	not needed in benchmark approach
<i>Subchronic uncertainty factor</i>	1
<i>Interspecies uncertainty factor</i>	3 (see below)
<i>Intraspecies uncertainty factor</i>	10
<i>Cumulative uncertainty factor</i>	30
<i>Inhalation reference exposure level</i>	0.7 µg/m ³ 0.2 ppb)

Short *et al.* (1988) examined the toxicity of maleic anhydride to rats, hamsters, and monkeys by the inhalation route of exposure. Dose- and exposure related effects, although mild and reversible, were observed at all exposure levels. Specifically, exposure to maleic anhydride vapors resulted in hyperplastic change in the nasal epithelium of rats and hamsters (obligate nose breathers). Neutrophilic infiltration of the nasal epithelium was observed in all three species at all levels of exposure. All species also showed signs of irritation at all exposure levels. The observation that acute maleic anhydride is a strong respiratory irritant to humans (ACGIH, 1992)

suggests that this is a valid endpoint of toxicity to humans as well. Human exposure at levels as low as $\sim 1 \text{ mg/m}^3$ appears to trigger acute asthmatic reactions in sensitive individuals (Lee *et al.*, 1991). The histological changes observed by Short *et al.* occurring as a result of inhalation exposure to a known strong irritant such as maleic anhydride are considered to be the adverse effect of repetitive acute exposures, rather than a chronic response, in the development of the REL.

The chronic REL was developed using the benchmark approach. The gamma model in the U.S. EPA's BMDS software yielded a BMC_{05} of 0.12 mg/m^3 for mild epithelial hyperplasia in male and female rats combined. Because of the similarities among species and the inclusion of monkeys in the study, an interspecies uncertainty factor of 3, rather than 10, was used. Although there is no evidence of a toxic response similar to the development of asthma in animals, the 1.1 mg/m^3 LOAEL from the animal studies of Short *et al.* (1988) results in a REL of $0.7 \text{ }\mu\text{g/m}^3$ which should protect asthmatics from maleic and other anhydrides.

VII. Data Strengths and Limitations for Development of the REL

The major strengths of the REL for maleic anhydride are the availability of multiple-species, multiple-dose subchronic inhalation studies, and the observation of a mild effect LOAEL. The major uncertainties are the lack of human data and the lack of a NOAEL observation.

VIII. Potential for Differential Impacts on Children's Health

Minimal teratogenic and reproductive adverse effects were seen at the lowest oral dose of maleic anhydride (20 mg/kg-day), given to rats during gestation (Short *et al.*, 1986). This dose is equivalent to a person inhaling 70 mg/m^3 . Thus the chronic REL of $0.7 \text{ }\mu\text{g/m}^3$ should protect children. Maleic anhydride is a respiratory irritant and an inducer of asthma. Exacerbation of asthma has a more severe impact on children than on adults. However, there is no direct evidence in the literature to quantify a differential effect of maleic anhydride in children.

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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**

<i>Inhalation reference exposure level</i>	4,000 $\mu\text{g}/\text{m}^3$ (3,000 ppb)
<i>Critical effect(s)</i>	Increased incidence of abnormal cervical ribs, cleft palate, and exencephaly in mice
<i>Hazard index target(s)</i>	Teratogenicity

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

<i>Description</i>	Colorless liquid
<i>Molecular formula</i>	CH_3OH
<i>Molecular weight</i>	32.04 g/mol
<i>Boiling point</i>	64.6°C
<i>Melting point</i>	-97.6°C
<i>Vapor pressure</i>	92 torr at 20°C
<i>Solubility</i>	Methanol is miscible with water, ethanol, ether and many other organic solvents.
<i>Conversion factor</i>	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/m³ (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/m³.

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/m³) to 375 ppm (490 mg/m³).

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

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

Ubaydullayev (1968) exposed 3 to 6 subjects to methanol vapor for short durations (40 minutes for some subjects and others for an unspecified amount of time). Electrical brain cortex reflex activity was significantly altered upon exposures to 1.17 mg/m³ (0.89 ppm) or 1.46 mg/m³ (1.11 ppm). No effect was observed at 1.01 mg/m³ (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/m³ (520 ppm) to 6650 mg/m³ (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/m³ for 6 to 8 hours per day for either 1 day or 1, 2, 4 or 6 weeks resulted in a significant reduction in testosterone levels (Cameron *et al.*, 1984; Cameron *et al.*, 1985).

Ubaydullayev (1968) exposed rats (15 per group) to 0, 0.57, or 5.31 mg/m³ 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/m³ group. Effects observed in the 5.31 mg/m³ 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 encephalocele 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)

<i>Study</i>	Rogers <i>et al.</i> (1993)
<i>Study population</i>	Pregnant mice
<i>Exposure method</i>	Discontinuous inhalation, 7 hours/day on days 6-15 of gestation
<i>Critical effects</i>	Abnormal cervical ribs, exencephaly, cleft palate
<i>LOAEL</i>	5000 ppm
<i>NOAEL</i>	1000 ppm
<i>Benchmark Concentration (BMC₀₅)</i>	305 ppm
<i>Exposure continuity</i>	7 hr/day
<i>Exposure duration</i>	10 days
<i>Average experimental exposure</i>	89 ppm at BMC ₀₅ (305 ppm x 7/24)
<i>Human equivalent concentration</i>	89 ppm at BMC ₀₅ (gas with systemic effects, based on RGDR = 1.0 using default assumption that lambda (a) = lambda (h))
<i>Subchronic uncertainty factor</i>	1 (see below)
<i>LOAEL uncertainty factor</i>	1
<i>Interspecies uncertainty factor</i>	3
<i>Intraspecies uncertainty factor</i>	10
<i>Cumulative uncertainty factor</i>	30
<i>Inhalation reference exposure level</i>	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 MLE₀₅ and BMC₀₅ for cervical ribs were 824 ppm (1079 mg/m³) and 305 ppm (400 mg/m³), 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

METHYL BROMIDE*(bromomethane; monobromomethane)***CAS Registry Number: 74-83-9****I. Chronic Toxicity Summary**

<i>Inhalation reference exposure level</i>	5 $\mu\text{g}/\text{m}^3$ (1 ppb)
<i>Critical effect(s)</i>	Histological lesions of the olfactory epithelium of the nasal cavity in rats
<i>Hazard index target(s)</i>	Respiratory system; nervous system; development

II. Physical and Chemical Properties (HSDB, 1994)

<i>Description</i>	Colorless gas
<i>Molecular formula</i>	CH_3Br
<i>Molecular weight</i>	94.95 g/mol
<i>Density</i>	3.89 g/L @ 25°C
<i>Boiling point</i>	3.6°C
<i>Vapor pressure</i>	1420 torr @ 20°C
<i>Solubility</i>	Soluble in ethanol, benzene, carbon disulfide, and 1.75% (w/w) in water
<i>Odor threshold</i>	20.6 ppm
<i>Odor description</i>	Sweetish odor
<i>Metabolites</i>	Methanol, bromide, 5-methylcysteine
<i>Conversion factor</i>	1 ppm = 3.89 mg/m^3 @ 25°C

III. Major Uses and Sources

Methyl bromide (MeBr) was used historically as an industrial fire extinguishing agent and was introduced in the U.S. from Europe in the 1920s. Current uses of MeBr include the fumigation of homes and other structures for termites and other pests. Methyl bromide is also used to fumigate soil before planting and fruits and vegetables after harvest. In 1981, 6.3 million pounds of MeBr were reportedly used in California (Alexeeff and Kilgore, 1983). By 1991, its use had grown to 18.7 million pounds in the state (Cal/EPA, 1993). The annual statewide industrial emissions from facilities reporting under the Air Toxics Hot Spots Act in California, based on the most recent inventory, were estimated to be 75,575 pounds of methyl bromide (CARB, 1999). This does not include emissions of methyl bromide during its use as a pesticide.

IV. Effects of Human Exposure

Workers (n = 32) exposed to MeBr during fumigation of soil or structures were compared to a referent group of 29 workers not exposed to MeBr, but exposed to other fumigants (Anger *et al.*, 1986). Exposures to MeBr were not quantified. It was found that workers exposed to MeBr had a higher rate of neurological symptoms and performed less well on several behavioral tests. Several confounding factors were present in this study, including lack of adjustments for age, alcohol consumption, prescription medication, illegal drugs, education, or ethnic group between the exposed and the referent groups.

V. Effects of Animal Exposure

The first experimental animal study on repeated MeBr exposures was carried out and reported by Irish and associates (1940). In this study, rats (135 per group), rabbits (104 per group), or female rhesus monkeys (13 per group) were exposed to 0, 17, 33, 66, 100, or 220 ppm (0, 66, 128, 256, 388, or 853 mg/m³) 7-8 hours/day, 5 days/week for 6 months or until the majority of the animals exhibited severe signs of toxicity. Mortality was seen in rats, guinea pigs, and monkeys at 100 ppm. Rabbits began to die at 33 ppm. Severe effects, including paralysis, were seen after exposure to 66 ppm in rabbits and monkeys. None of the species exhibited adverse effects after exposure to 17 ppm.

Kato and associates (1986) observed focal lesions in the brain and heart in rats (10-12 per group) after inhalation of 150 ppm (585 mg/m³) MeBr 4 hours/day, 5 days/week for 11 weeks. In another experiment, rats were exposed to 0, 200, 300, or 400 ppm (0, 777, 1160, or 1550 mg/m³) MeBr 4 hours/day, 5 days/week for 6 weeks. In this experiment, rats exposed to any concentration of MeBr exhibited coronary lesions, and exposures of 300 ppm or greater resulted in neurological dysfunction, including ataxia and paralysis. Testicular atrophy was noted in 6 of the 8 animals exposed to 400 ppm.

Anger *et al.* (1981) determined that rabbits are more sensitive than rats to neurotoxicity of MeBr. In this study, rats or rabbits were exposed to 0 or 65 ppm (0 or 254 mg/m³) MeBr for 7.5 hours/day, 4 days/week, for 4 weeks. Nerve conduction velocity and eyeblink reflex were impaired in the rabbits but not rats exposed to 65 ppm MeBr. Similarly, rats did not exhibit neurological signs after exposure to 55 ppm (215 mg/m³) MeBr for 36 weeks. Rabbits exposed to 26.6 ppm (104 mg/m³) did not display any neurological effects after 8 months exposure (Russo *et al.*, 1984).

In the studies of Reuzel and associates (1987, 1991), groups of 50 male and 60 female Wistar rats were exposed to 0, 3, 30, or 90 ppm methyl bromide (98.8%) for 6 hours per day, 5 days per week. Three groups of animals (10/sex/exposure level) were killed for observations at 14, 53, and 105 weeks of exposure. Body weight, hematology, clinical chemistry, and urinalyses were examined throughout the experiment in addition to histopathology and organ weights at time of necropsy. Exposures of males and females to 90 ppm resulted in reduced body weight. Exposure to 90 ppm also resulted in significant lesions in the heart in the form of cartilaginous metaplasia and thrombus in the males, and myocardial degeneration and thrombus in the

females. Exposure of males to 30 or 90 ppm resulted in a decrease in relative kidney weight. Histological changes in the nose, heart, esophagus, and forestomach were the principal effects of methyl bromide toxicity. At the lowest concentration (3 ppm), very slight degenerative changes in the nasal epithelium, and olfactory basal cell hyperplasia were noted in both sexes at 29 months. Based on this study, a LOAEL of 3 ppm (11.7 mg/m³) was determined.

The National Toxicology Program (NTP) conducted a 13-week and a chronic study on the toxicology and carcinogenesis of methyl bromide in rats and mice (NTP, 1990). In the 13-week study, 18 rats/sex/group were exposed to 0, 30, 60, or 120 ppm (0, 117, 233, or 466 mg/m³) MeBr 6 hours/day, 5 days/week. The mice were exposed to 0, 10, 20, 40, 80, or 120 ppm (0, 39, 78, 155, 311, or 466 mg/m³) 6 hours/day, 5 days/week. Hematological parameters and selected organ weights were measured in both species, in addition to histopathological changes. Pseudocholinesterase activity and neurobehavioral tests were conducted in the mice. Serious effects, including 58% body weight loss, 17% mortality and severe curling and crossing of the hindlimbs were observed in mice exposed to 120 ppm MeBr. Exposure of males to 40 ppm or higher resulted in significant effects on several hematological parameters, including decreased mean cell hemoglobin and increased red blood cell count. The only exposure-related histological effect was olfactory epithelial dysplasia and cysts in the rats of both sexes exposed to 120 ppm.

A 6-week study in rats and mice (5 animals/sex/group) exposed to 0 or 160 ppm (0 or 624 mg/m³) showed high mortality rates, loss in body weight and histological changes in multiple organ systems including brain, kidney, nasal cavity, heart, adrenal gland, liver, and testes (NTP, 1990).

An exposure of mice (86 animals/group) to 0, 10, 33, or 100 ppm (0, 38.8, 128, or 388 mg/m³) MeBr for 6 hours/day, 5 days/week, for 103 weeks was also conducted by NTP (1990). In this study, high mortality rates in both males and females in the 100 ppm group resulted in a discontinuation of exposure after 20 weeks. A low incidence of sternal dysplasia and a significant decrease in locomotor activity were noted in the 10 ppm group.

A 5-day exposure of rats (10 animals/group) to 0, 90, 175, 250, or 325 ppm (0, 350, 680, 971, or 1260 mg/m³) resulted in lesions in the nasal olfactory sensory cells, the cerebellum and adrenal gland beginning at 175 ppm (Hurt et al., 1987). Hurt and Working (1988) later observed severe histological damage to the nasal epithelium following a single exposure to 90 or 200 ppm (351 or 780 mg/m³) MeBr. Olfactory function, measured by the ability to locate buried food, was impaired at the 200 ppm exposure. In this study, reduced testosterone and testicular glutathione levels were observed in the male rats exposed to 200 ppm, but no effects on spermatogenesis, sperm quality, or testes histopathology were noted.

Sikov *et al.* (1981) examined the teratogenic potential of MeBr in rats and rabbits exposed to 0, 20, or 70 ppm (0, 78, or 272 mg/m³) 7 hours/day, 5 days/week for 3 weeks during days 1-19 (rats) or 1-24 (rabbits) of gestation. No maternal or fetal effects were observed in the rats, however, severe maternal neurotoxic effects were observed in the rabbits that resulted in 24/25 deaths. In this study, no significant maternal or fetal effects were observed at a concentration of 20 ppm.

Another developmental toxicity study was conducted in rabbits by Breslin *et al.* (1990). In this study, rabbits were exposed to 0, 20, 40, or 80 ppm (0, 78, 156, or 312 mg/m³) MeBr for 6 hours/day on gestation days 6-19. Maternal toxicity was observed at 80 ppm and included reduced body weight gain and signs of neurotoxicity. In addition to the maternal effects observed, a significant increase in incidence of gall bladder agenesis and fused sternbrae were observed in the offspring exposed to 80 ppm. No adverse effects were observed at 40 ppm or lower concentrations.

A 2-generation reproduction and developmental toxicity study on MeBr in rats was conducted by American Biogenics Corporation (1986). Groups of rats (25/sex/concentration) were exposed to 0, 3, 30, or 90 ppm (0, 12, 117, or 350 mg/m³) MeBr 6 hours/day, 5 days/week during pre-mating, gestation, and lactation through 2 generations. Significant decreases in body weight during the pre-mating period and at the end of the study were observed in the males exposed to 90 ppm. Although some adult organ weights were affected in the 90-ppm group, there was no evidence of histopathology in these organs. Neonatal body weights were decreased by exposure to 30 ppm. There was a decreased cerebral cortex width in the 90 ppm F₁ group, reduced brain weight in 30 ppm F₁ females, and reduced fertility in the 30 and 90 ppm F_{2b} groups.

VI. Derivation of Chronic Reference Exposure Level

<i>Study</i>	Reuzel <i>et al.</i> , 1987; 1991
<i>Study population</i>	Male and female Wistar rats (50 and 60 per group, respectively)
<i>Exposure method</i>	Discontinuous inhalation exposures (0, 3, 30, or 90 ppm) over 29 months
<i>Critical effects</i>	Basal cell hyperplasia of the olfactory epithelium of the nasal cavity
<i>LOAEL</i>	3 ppm
<i>NOAEL</i>	Not observed
<i>Exposure continuity</i>	6 hr/day, 5 days/week
<i>Exposure duration</i>	29 months
<i>Average experimental exposure</i>	0.54 ppm for the LOAEL group
<i>Human equivalent concentration</i>	0.12 ppm for the LOAEL group (gas with extrathoracic respiratory effects, RGDR = 0.23, based on MV = 0.03 m ³ /min, SA = 11.6 cm ²)
<i>LOAEL uncertainty factor</i>	3 (20% extra risk of a mild effect)
<i>Subchronic uncertainty factor</i>	1
<i>Interspecies uncertainty factor</i>	3
<i>Intraspecies uncertainty factor</i>	10
<i>Cumulative uncertainty factor</i>	100
<i>Inhalation reference exposure level</i>	0.001 ppm (1 ppb, 0.005 mg/m ³ , 5 µg/m ³)

The chronic REL for methyl bromide is also the U.S EPA RfC.

VII. Data Strengths and Limitations for Development of the REL

The major strengths of the REL for methyl bromide are the use of a comprehensive, long-term, multiple dose study with large sample sizes, and the availability of supporting data including long-term studies in other species and reproductive and developmental studies. The major uncertainties are the lack of human data and the lack of a NOAEL observation for the critical effect.

The California Department of Pesticide Regulation used a different approach that adjusts for respiration rate differences between humans and animals and which uses 10-fold uncertainty factors for interspecies differences, for intraspecies variability, and for a LOAEL to NOAEL extrapolation. Applying these factors to the same 3 ppm LOAEL results in a level for children and adults of 1 and 2 ppb (4 and 8 $\mu\text{g}/\text{m}^3$), respectively.

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CHRONIC TOXICITY SUMMARY

METHYL CHLOROFORM*(1,1,1-trichloroethane, methyltrichloromethane)***CAS Registry Number: 71-55-6****I. Chronic Toxicity Summary**

<i>Inhalation reference exposure level</i>	1,000 µg/m³ (200 ppb)
<i>Critical effect(s)</i>	Astrogliosis in the sensorimotor cortex (brain) of gerbils
<i>Hazard index target(s)</i>	Nervous system

II. Chemical Property Summary (HSDB, 1999)

<i>Description</i>	Colorless liquid
<i>Molecular formula</i>	C ₂ H ₃ Cl ₃
<i>Molecular weight</i>	133.42 g/mol
<i>Density</i>	1.3376 g/cm ³ @ 20° C
<i>Boiling point</i>	74.1° C
<i>Melting point</i>	-30.4° C
<i>Vapor pressure</i>	127 torr @ 25° C
<i>Solubility</i>	Soluble in acetone, benzene, methanol, carbon tetrachloride
<i>Conversion factor</i>	5.47 µg/m ³ per ppb at 25°C

III. Major Uses and Sources

Methyl chloroform is used as a solvent for adhesives and for metal degreasing (ACGIH, 1992). It is also used in the manufacture of vinylidene chloride and in textile processing and dry cleaning. The annual statewide industrial emissions from facilities reporting under the Air Toxics Hot Spots Act in California, based on the most recent inventory, were estimated to be 25,316,458 pounds of methyl chloroform (CARB, 1999a). Statewide monitored median and mean concentrations of methyl chloroform have been generally declining; decreasing from 0.8 or 1.71 ppb in 1990 to 0.12 or 0.30 ppb in 1996 (CARB, 1999b).

IV. Effects of Human Exposure

A 44-year old woman was diagnosed with peripheral neuropathy following 18 months of occupational exposure to methyl chloroform in a solvent bath (House *et al.*, 1994). There was no identified exposure to agents known to cause peripheral neuropathy, such as n-hexane or

trichloroethylene. The worker reported that she wore protective gloves and a respirator, both of which frequently leaked. Seven months following removal from exposure, the worker showed improved nerve conduction.

Other case reports have identified the nervous system as a target of methyl chloroform toxicity in similar exposure scenarios. Three workers developed distal sensory neuropathy after working with methyl chloroform in a degreasing operation with repeated dermal exposure (Liss, 1988; Howse *et al.*, 1989). Changes were observed in nerve conduction in the upper extremities accompanied by both axonopathy and myelopathy.

Twenty-eight workers with chronic exposure to high (but unquantified) concentrations of 1,1,1-trichloroethane had significant deficits in memory, intermediate memory, rhythm, and speed based on the Luria-Nebraska Neuropsychological Battery (Kelafant *et al.*, 1994). Deficits in vestibular, somatosensory, and ocular components of balance were noted.

A 13-year-old male died after intentional inhalation of 1,1,1-trichloroethane (Winek *et al.*, 1997). Autopsy findings included tissue congestion of lung, liver and kidney.

Cardiac arrhythmia resulting from heightened cardiac sensitivity to epinephrine has been reported in several case reports of high acute inhalation exposures to methyl chloroform (ATSDR, 1990). There are case reports of arrhythmias persisting for two weeks or more after cessation of exposure to methyl chloroform (McLeod *et al.*, 1987).

An epidemiological study of workers chronically exposed to low levels of methyl chloroform (<250 ppm) found no changes in blood pressure, heart rate, or electrocardiogram (Kramer *et al.*, 1978). This study consisted of 151 workers who had been exposed for more than one year. No neurophysiological testing was done.

Another study of 22 female workers exposed to methyl chloroform (plus 7 unexposed control workers) at concentrations ranging from 110-345 ppm in air for a mean of 6.7 years failed to identify neurotoxicity resulting from methyl chloroform exposure (Maroni *et al.*, 1977). The examination included evaluation for neurologic symptoms, changes in nerve conduction, and psychomotor tests.

Liver disease was observed in a worker exposed to methyl chloroform in a clothing factory screen printing room (Cohen and Frank, 1994). The worker was exposed for a total of 4 years before occupational exposure was identified as the cause of the liver disease. The worker sprayed an adhesive (containing 65% methyl chloroform, 25% propane and dimethyl ether, and 10% inert ingredients) during which the worker reported often feeling dizzy or intoxicated. Three months following removal of the worker from exposure, liver function tests, although still abnormal, were significantly improved. Other case reports support these findings (Hodgson *et al.*, 1989; Halevy *et al.*, 1980).

Six male volunteers were exposed to 35 and 350 ppm methyl chloroform for 6-hours on two separate occasions (Nolan *et al.*, 1984). Absorption was determined to be 25% of the inhaled dose. Of the absorbed dose, 91% was excreted unchanged in the expired air. Although the odor

was perceptible for the duration of the exposure, no subjective symptoms were reported by the volunteers.

V. Effects of Animal Exposure

Gerbils (4/sex/dose plus 24 sex-matched control animals) were continuously exposed to 70, 210, or 1000 ppm methyl chloroform for 3 months (Rosengren *et al.*, 1985). A 4-month (solvent-free) recovery period following exposure was included to evaluate “lasting or permanent changes.” Body weights were not changed significantly as a result of exposure. Brain weights in the animals in the 1000 ppm dose group were significantly decreased. Fibrillary astrocytes are formed in the brain in response to injury. Brain injury in methyl chloroform exposed gerbils was evaluated by detection of glial fibrillary acidic (GFA) protein, the main protein subunit of astroglial filaments. Increased levels of GFA protein were detected in the sensorimotor cerebral cortex of animals exposed to 210 or 1000 ppm methyl chloroform.

A later study in gerbils examined the effects of a 3-month continuous exposure to 70 ppm methyl chloroform followed by a 4-month recovery period (Karlsson *et al.*, 1987). DNA content was significantly decreased in three areas of the brain: posterior cerebellar hemisphere, anterior cerebellar vermis, and hippocampus. The authors contended that depressions in DNA content reflect decreased cell density.

No evidence of peripheral neuropathy or other neurotoxicity was detected in rats exposed to 200, 620, or 2000 ppm methyl chloroform 6 hours per day, 5 days per week for 13 weeks (Mattson *et al.*, 1993). The study included a functional observational test battery and measured visual, somatosensory, auditory and caudal nerve-evoked potentials. Histopathology of the brain, spinal cord, peripheral nerves and limb muscles was also examined at the end of the 13-week exposure.

Forty percent of all mice continuously exposed to 1000 ppm methyl chloroform for 14 weeks exhibited evidence of hepatocellular necrosis (McNutt *et al.*, 1975). A statistically significant increase in liver weight per body mass was observed throughout the study. Electron microscopy revealed accumulation of triglyceride droplets in the centrilobular hepatocytes following one week of exposure to 1000 ppm methyl chloroform. After 4 weeks of exposure, cytoplasmic alterations in centrilobular hepatocytes included a loss of polyribosomes and increased smooth endoplasmic reticulum. Similar changes observed occasionally in hepatocytes from mice exposed to 250 ppm were not as dramatic.

Mild hepatocellular changes were observed in rats exposed to 1500 ppm methyl chloroform 6 hours per day, 5 days per week for 6, 12, and 18 months (Quast *et al.*, 1988). At 24 months, these slight effects were no longer discernible due to confounding geriatric changes. No hepatocellular changes or other adverse effects were observed in rats exposed to 150 or 500 ppm methyl chloroform for up to 24 months.

The developmental toxicity of inhaled methyl chloroform was studied in CD-1 mice. Mice were exposed on gestation days 12 through 17 to either 2000 ppm methyl chloroform for 17 hours per day or 8000 ppm methyl chloroform for 1 hour three times per day (Jones *et al.*, 1996). There

were no effects on pregnancy outcome, but exposed pups has reduced weight gain, had poorer results on motor coordination tests and showed delays in negative geotaxis (orienting towards the top of a sloped screen).

VI. Derivation of Chronic Reference Exposure Level (REL)

<i>Study</i>	Rosengren <i>et al.</i> (1985)
<i>Study population</i>	Mongolian gerbils (4/sex/dose)
<i>Exposure method</i>	Whole-body inhalation exposure
<i>Critical effects</i>	Astrogliosis in the sensorimotor cerebral cortex
<i>LOAEL</i>	210 ppm
<i>NOAEL</i>	70 ppm
<i>Exposure continuity</i>	Continuous
<i>Average experimental exposure</i>	70 ppm for NOAEL group
<i>Human equivalent concentration</i>	Not derived (species-specific data for gerbils unavailable to validate assumption of RGDR=1)
<i>Exposure duration</i>	3 months
<i>LOAEL uncertainty factor</i>	1
<i>Subchronic uncertainty factor</i>	3
<i>Interspecies uncertainty factor</i>	10
<i>Intraspecies uncertainty factor</i>	10
<i>Cumulative uncertainty factor</i>	300
<i>Inhalation reference exposure level</i>	0.2 ppm (200 ppb; 1 mg/m ³ ; 1,000 µg/m ³)

VII. Data Strengths and Limitations for Development of the REL

Case reports indicate that the nervous system and the liver are targets of the toxicity of methyl chloroform (House *et al.*, 1994; Liss, 1988; Howse *et al.*, 1989; Cohen and Frank, 1994). The largest of the epidemiological studies (Kramer *et al.*, 1978; Maroni *et al.*, 1977), however, did not identify adverse effects as a result of chronic methyl chloroform exposure. The Kramer *et al.* (1978) study limited its evaluation to changes in blood pressure, heart rate, or electrocardiogram and exposure levels were only characterized as less than 250 ppm. Maroni *et al.* (1977) conducted their study among 22 women exposed occupationally to methyl chloroform levels as low as 110 ppm. Although the subjects were evaluated specifically for signs of neurotoxicity, the small sample size limits conclusions that can be drawn from their failure to identify adverse effects in this population. If no effects are associated with the exposures in the 2 studies (Kramer *et al.*, 1978; Maroni *et al.*, 1977), the REL predicted would be approximately 3 ppm.

Data from animal studies generally support the findings of the case reports from human exposures. Both neurotoxicity and hepatotoxicity have been identified among animals exposed by inhalation to methyl chloroform. The adverse effect observed at the lowest level in these studies was the development of astrogliosis in the brains of gerbils exposed for 3 months to 210 ppm methyl chloroform (Rosengren *et al.*, 1985). A no-observed-adverse-effect-level (NOAEL)

in this study was 70 ppm methyl chloroform. A subsequent study identified a more subtle change in the brains of gerbils exposed similarly to 70 ppm methyl chloroform, with slightly decreased DNA content found in several discrete brain regions of exposed animals. However, the relationship between tissue DNA content and cell density as an indication of adverse effect in the brain was considered too tenuous for the development of a guidance value for chronic exposure to methyl chloroform.

The major strengths of the REL for methyl chloroform are the observation of the NOAEL and the continuous subchronic exposure regimen. The major uncertainties are the lack of human exposure data, the lack of dose-response information, and the lack of comprehensive multi-organ effects data.

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CHRONIC TOXICITY SUMMARY

METHYL ISOCYANATE(MIC, $CH_3-N=C=O$)**CAS Registry Number: 624-83-9****I. Chronic Toxicity Summary**

<i>Inhalation reference exposure level</i>	1 $\mu\text{g}/\text{m}^3$ (0.5 ppb)
<i>Critical effects(s)</i>	Decreased weight gain and lung pathology at cessation of exposure in rats
<i>Hazard index target(s)</i>	Respiratory system; reproductive system

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

<i>Description</i>	Colorless liquid
<i>Molecular formula</i>	C_2H_3NO
<i>Molecular weight</i>	57.06 g/mol
<i>Boiling point</i>	39.5°C
<i>Melting point</i>	-45°C
<i>Vapor pressure</i>	348 torr @ 20°C, 600 torr @ 30°C (Varma and Guest, 1993)
<i>Solubility</i>	10 percent in water @ 15°C
<i>Conversion factor</i>	2.3 $\mu\text{g}/\text{m}^3$ per ppb at 25°C

III. Major Uses and Sources (Dave, 1985; U.S. EPA, 1986; HSDB, 1995)

Methylisocyanate (MIC) is prepared industrially by reacting methylamine with phosgene, oxidizing monomethylformamide at high temperatures ($\geq 550^\circ\text{C}$), or heating metal methylisocyanates. Because of its high reactivity, MIC is used as an intermediate in organic synthesis, most notably in the production of carbamate based pesticides. Tobacco smoke from some brands of cigarettes also contains MIC (about 4 μg per cigarette). Workers exposed to the MIC 8-hour threshold limit value of 0.02 ppm (46 $\mu\text{g}/\text{m}^3$) are exposed to approximately 460 μg MIC in a workday. Based on the most recent inventory, the annual statewide industrial emissions from facilities reporting under the Air Toxics Hot Spots Act in California were negligible (CARB, 2000). This does not include estimates of emissions of breakdown products from the use of metam sodium in agricultural applications. Use of metam sodium averaged 15,400,000 pounds/year from 1995 to 1999.

IV. Effects of Human Exposure

Although occupational exposures to MIC have been documented (Varma, 1986), few known exposures to the general public have occurred. A major exposure occurred in Bhopal, India in December 1984. Because of the sudden, short-term release (30-45 minutes), no measurements occurred, but the air concentration was estimated as 13 ppm (Dave, 1985) to 100 ppm (Varma, 1986).

The chemical identity of the ultimate toxicant has not been unequivocally determined and may consist of more than one chemical species. Although the chemistry of MIC suggests that hydrolysis to methylamine and dimethylurea is rapid, such hydrolysis in moist air is probably slow, and the reaction with photochemically produced hydroxyl radical is also slow (chemical $T_{1/2}$ about 3 months) (U.S. EPA, 1986). Brown *et al.* (1987) have shown that the alkylisocyanates (e.g., MIC) are relatively resistant (compared to the arylisocyanates) to hydrolysis in water. Hence, despite the high water reactivity of MIC, this compound could possibly persist in the environment for many days after an initial release.

Within 5 days of the initial exposure to MIC at Bhopal, more than 2,000 deaths occurred (Dave, 1985), while 4,000 more deaths were documented during the following decade (Lepkowski, 1994). The initial symptoms among the population living near the MIC plant were irritation and difficulty in breathing (Varma, 1986). Blindness occurred in more than 10,000 exposed persons but later resolved in most cases (Andersson *et al.*, 1990). The acute damage that led to death was mainly to the respiratory system, most likely pulmonary edema, bronchospasm, and electrolyte imbalance (Varma, 1986). However extrapulmonary damage, including tissue anoxia, gastrointestinal symptoms, and muscular weakness, were also observed (Dave, 1985). Within a year of the exposure, survivors continued to exhibit damage to the lung and eyes. Fibrosis of the lungs was seen in 30 percent of this group (Dave, 1985).

Reproductive toxicity was observed among women exposed to MIC in Bhopal. Varma (1987) reported 43 percent unsuccessful pregnancies among 865 women who were pregnant at the time of the MIC release. Among the live births, 14 percent of the infants died within 30 days, whereas a death rate of only 3 percent for the same interval was recorded 2 years prior to the release. Bhandari *et al.* (1990) reported increased spontaneous abortions and neonatal deaths among exposed women who were pregnant at the time of exposure compared to a control group in another city. In the latter study, stillbirths and congenital malformations were similar in the exposed and non-exposed groups.

Non-reproductive, non-pulmonary responses were evident in a group of exposed Bhopal residents, 3-years following exposure to the MIC vapors. Loss of vision and loss of visual acuity were more prominent among exposed residents than among unexposed people, and the losses appeared to be dose-dependent (Andersson *et al.*, 1990). In this study, the surrogate for dose was extent of early deaths in a housing cluster. Similarly, cataracts were reported more often among the exposed than among the unexposed group.

The lesions associated with lung damage may be expressed as pulmonary edema for immediate effects (Varma, 1986), and lesions associated with the bronchoalveolar area for long-term effects

(Dave, 1985, Varma, 1986). Vijayan *et al.* (1995) studied cellular components of bronchoalveolar lavage (BAL) and pulmonary function in Bhopal patients 1.3, 2.7, and 5.1 years after exposure to MIC. All had lived within 3-miles of the factory and all experienced acute respiratory and ophthalmic symptoms on the day of exposure. All were experiencing continued respiratory symptoms. Among the exposed people, decrements in forced vital capacity and forced expiratory volume (at 1-minute) were observed. In general, the decrements ranged from 12 - 21 percent of predicted values, whereas the control group exhibited decrements of 2 - 4 percent of the expected values. Analysis of the BAL revealed increases in total cells (all exposed groups), increased absolute numbers of macrophages (all exposure groups), decreased percentage of lymphocytes (2.7 and 5.1 year groups), and increased numbers and percentage of neutrophils (5.1 year group). These cell types are involved, through the secretion of various factors, in inflammatory and immunologic processes in the lung (Reiser and Last, 1986). The Vijayan *et al.* (1995) study thus suggests long term damage to lung parenchyma among people who survived the initial acute effects of MIC exposure.

In summary, humans exposed acutely by inhalation to MIC may experience long-term (as well as immediate) damage to pulmonary and extrapulmonary systems. The lung is probably the critical target organ for long-term effects from acute exposure, although adverse effects on other organs (e.g., eye, reproductive, and gastrointestinal) also exist. The late responses to the acute exposure suggest an immunological component, which could involve several systems including lung, eye, liver, and kidney. The chemical identity of the ultimate toxicant is unknown and may be more than one compound.

Avashia *et al.* (1996) assessed pulmonary effects from long-term, low-level MIC for more than 400 workers at a large chemical facility. Serial pulmonary function data, cigarette smoking histories, and industrial-hygiene measurements were available. Jobs were classified according to level of MIC exposure as none, low, moderate or high. Where work records were incomplete, exposures were based on the ratings of supervisors and coworkers. The frequency of pulmonary impairment was evaluated for the assumed four levels of exposure. No specific or consistent pulmonary impairment was evident. Unfortunately the report gave no quantitative classification of low, moderate or high MIC levels.

V. Effects of Animal Exposure

Experimental animal studies have been designed to address the experiences of the victims of the Bhopal disaster, in which the exposure has been described as acute because of the short duration (30-45 min). No studies were found that described exposure duration greater than 10 days. However, a chronic component to MIC exposure may exist as a result of slower rates of hydrolysis in air (compared to water), the presence of carbamylated hemoglobin in MIC-exposed people, and the change from edematous to inflammatory and/or fibrotic lesions with time. Further, a glutathione-dependent reversible MIC transport system has been suggested in experimental animals (see below).

MIC is absorbed through the respiratory tract and distributed to non-respiratory organs in experimental animals. In an acute (30 min) inhalation exposure to a dose of ^{14}C -MIC (labeled in

the isocyanate moiety) equivalent to one-LC₅₀ (23 mg/L), rats accumulated protein-bound radioactivity (including carbamylated proteins) in brain, liver, kidney, and lung, but not in blood (Bhattacharya *et al.*, 1988). Ferguson *et al.* (1988) exposed guinea pigs by inhalation to 0.47 ppm ¹⁴C-MIC (methyl group) for 6-hours. At the end of exposure, the label was found in arterial and venous blood, bile, and urine. At 2.7 days post-exposure, the label decreased to 2-7 percent. MIC was retained in the nasal-laryngeal area of the guinea pigs.

MIC, like reactive isocyanates in general, can react with biological molecules containing amino, alcohol, or sulfhydryl groups, as well as with water. While hydrolysis in an aqueous environment, such as the lung, is theoretically possible, measurements show that alkyl isocyanates are relatively resistant (compared to arylisocyanates) to such hydrolysis (Brown *et al.* 1987). The absence of a role for MIC hydrolytic products, methylamine (MA) or dimethylurea (DMU), is also suggested by the work of Jeevaratnam and Sriramachari (1994) and Sriramachari *et al.* (1994). Inhalation (30 min) or subcutaneous exposure of rats to either hydrolytic product at levels equivalent to the LC₅₀ or LD₅₀ did not result in death. Similarly, neither methylamine nor dimethylurea duplicated the acute effects of respiratory necrosis and congestion. However, exposure to these hydrolytic products did lead to interstitial pneumonitis, an observation that suggests MA and/or DMU could lead to subsequent inflammatory responses if sufficient amounts are present.

A role for methylamine in reproductive/developmental toxicity was investigated by Guest and Varma (1991). In a mouse study, pregnant dams were exposed to varying doses (intraperitoneal) of methylamine (as well as the di- and trimethyl compounds). Reproductive toxicity was not observed for methylamines. However, in cultured embryo experiments, decrements in crown-rump length, yolk-sac diameter, head length, and embryo survival were observed. The concentrations were high (>0.75 mM) and the interpretation of the biological activity of methylamine in terms of inhalation exposure is difficult.

MIC is a carbamylating intermediate; this is the basis for its use in the manufacture of carbamate based pesticides. In the same way, MIC should react with the appropriate functional groups of proteins, peptides, and nucleic acids. However, *in vitro* studies with cholinesterases show that such a reaction is not efficient (Brown *et al.*, 1987), an observation which may be explained by the presence of protonated amino groups at physiological pH (Baillie and Slatter, 1991).

A transport system for MIC via reduced glutathione (GSH) has been suggested by the discovery of the MIC-adduct, S-(N-methylcarbamoyl)glutathione (SMG), in the bile and the MIC-adduct of N-acetylcysteine (mercapturic acid, AMCC) in the urine of rats exposed to MIC by non-inhalation routes (Pearson *et al.*, 1990; Slatter *et al.*, 1991). The reaction of MIC with GSH and with cysteine is reversible, and can provide a source of free MIC in the tissues (Baillie and Slatter, 1991). Similar studies in experimental animals exposed to MIC by inhalation have not been reported. However, humans exposed by inhalation to N,N-dimethylformamide (H-C(=O)-N(CH₃)₂) excrete AMCC in urine (Mraz and Nohova, 1992). Hence a reversible MIC-transport system in animals, including humans, is possible, and the presence of high levels of GSH in human lavage fluid (Cantin *et al.*, 1987) would permit the initiation of this mechanism.

The toxicity of the adduct SMG was tested in mouse embryo culture (Guest *et al.*, 1992). Mouse embryos, at day 8 of gestation in vivo, were removed from their dams and cultured in the presence (and absence) of SMG. Dose-dependent (0.25 - 2 mM) decrements were observed for yolk sac diameter, crown-rump length, somite number, and protein content. Delayed DNA synthesis in the embryos and in yolk-sacs occurred in the presence of 0.25 mM SMG. Similar to the results obtained with methylamine, the SMG concentrations were high and the exposures were not by inhalation. However, the data show that a MIC metabolite, SMG, has toxic properties. In the presence of GSH (1 or 3 mM), the extent of the SMG-dependent toxicities was decreased. Such data demonstrate the reversibility of the binding between MIC and GSH.

Three inhalation studies were identified in which experimental animals were exposed to more than one dose of MIC. Among these studies, two used exposure durations for more than one day (Dodd and Fowler, 1986; Mitsumori *et al.*, 1987). Rats and mice were exposed by inhalation to 0, 1.1, and 2.8 (female) or 3.0 (male) ppm MIC for 6 hr/day for a total of 4 days, and then followed during a 91-day post-exposure interval (Mitsumori *et al.*, 1987). Among the rats, post-exposure deaths occurred by 49 days (male) and 14 days (female) at the high dose. Among the mice, only 1 male mouse died at 16 days post-exposure. Reduced weight gain was observed among the female and male rats in the high dose group, prior to death, although the absolute weights were not different from the unexposed rats one day before the end of exposure. Among the mice, a slowed weight gain was observed at 3- and 6-days post exposure (male) and 1 day post exposure (female) at the high dose, but normal weight gain returned by 1 week following cessation of exposure. At 7 days post-exposure, microscopic changes were observed in the respiratory system among the high dose rats of both sexes. Between 8- and 27 days post-exposure, increased lesions in the respiratory tract and also in liver, thymus, spleen, heart, and brain were observed at the high dose. Similar lesions were not observed in rats exposed to 1.1 ppm MIC and followed to the 8-27 day post-exposure. Among survivors, the incidence of lesions decreased to control values by 91 days. Among the mice, treatment related changes in the respiratory tract were observed at the high dose at 7 days post-exposure. Between 28 and 91 days, the lesions associated with the upper respiratory tract disappeared, whereas those associated with the major bronchi remained, although somewhat attenuated. These data suggest that the rat is more sensitive than the mouse to the effects of MIC. A LOAEL of 2.9 ppm is indicated, based on post-exposure decreased weight gain and respiratory tract changes in rats.

Dodd and Fowler (1986) exposed rats to 0, 0.15, 0.6, and 3.1 ppm MIC for two 4-day sessions at 6-hours/day and examined the animals within 1-day following exposure. The 2-cycle exposure included a 2-day recess from exposure. No deaths occurred at any MIC concentration during the exposure. Lesser weight gain occurred for rats in the 3.1 ppm groups, whereas weight among the rats in the 0.15 and 0.6 ppm MIC groups was indistinguishable from the air-exposed control animals. On exposure days 3 and 8, mean food consumption values in the high dose group were below those for the non-exposed group. At the time of termination, male rats exposed to 3.1 ppm MIC exhibited a 38 percent increase in hemoglobin concentration and a 26 percent decrease ($p < 0.001$) in oxygen saturation, compared to the unexposed rats ($p < 0.001$). Such changes were not observed for the female rats exposed to 3.1 ppm or for rats of either sex exposed to 0.15 or 0.6 ppm MIC. Absolute lung weights increased ($p < 0.001$) in both sexes after exposure to 3.1 ppm, compared to the control rats. Decreases in liver, kidney and testes absolute weights were observed in this exposure group, but the authors interpreted these data as a reflection of the body

weight losses. No weight changes were observed in rats exposed to 0.15 or 0.60 ppm MIC. Gross and microscopic lesions were observed in rats (female and male) exposed to 3.1 ppm, but not in rats exposed to 0, 0.15, or 0.6 ppm MIC. The microscopic lesions occurred in the respiratory tract and consisted of inflammation, epithelial necrosis, squamous metaplasia, and epithelial hyperplasia. These lesions extended into the bronchioles. These data suggest a NOAEL of 0.6 ppm MIC, based on weight gain loss, absolute lung weight, and lung histopathology in rats, immediately following cessation of exposure.

Post-exposure changes in lung pathology also occurred in the rats surviving 3.1 ppm in the Dodd and Fowler (1986) study. The early lesions associated with inflammation, epithelial necrosis, squamous metaplasia, and epithelial hyperplasia extending to the bronchioles either decreased in severity or receded toward the upper respiratory tract by 85-days post-exposure. In males, the intraluminal and submucosal fibroplasia changed in appearance during this interval, due in part to the maturation of fibrous tissue. Mucous plugs were also seen in the terminal bronchioles and alveoli in some rats. The importance of this observation is the progressive character of MIC induced lung disease. Such progression may be difficult to follow at lower doses, if the times involved are of insufficient duration.

Sethi *et al.* (1989) exposed rats by inhalation to 0, 0.21, 0.26, and 0.35 ppm MIC for 6 days at 0.5 hr/day. Statistical evaluation was not presented. No post-exposure deaths were reported, although lethality was recorded for rats exposed to 3.5 and 35 ppm for only 10 minutes. Following the 0.5 hr \times 6-day exposure, the weight gain declined in proportion to the exposure dose. At the lowest dose (0.21 ppm) the weight gain was 111 g after 91 days post-exposure, compared to a weight gain of 218 g during the same interval among the non-exposed rats. The absolute weights of the rats at the end of the exposure were not given. According to the narrative, inflammatory lesions of bronchopulmonary tissue were present; their extent increased with dose. A dose-response increase in markers of lung infection was present and suggests that the MIC exposed rats were more prone to infectious agents than were the unexposed animals. Non-specific lesions in liver and kidneys were also observed and appeared to be dose dependent, but the authors suggested that these effects could be a result of the lung infections.

Fetotoxicity was observed in two experimental animal studies (Schwetz *et al.*, 1987; Varma, 1987). Among female mice exposed to 0, 1, or 3 ppm MIC during gestation days 14 - 17 for 6 hr/day, an increased incidence of fetal deaths was observed at 1 ppm (Schwetz *et al.*, 1987). At 3 ppm, the average number of pups/litter decreased relative to the air-exposed controls. The dams were unaffected in terms of survival, body weight, or length of gestation. Non-gestational exposure (6 hr/day, 4 days) did not affect the number of pregnancies or the live litter sizes, suggesting that the fetotoxic effect may be specific to the female reproductive tract rather than a general attribute of systemic toxicity. Similarly, female mice exposed for 3 hours on gestation day 8 to 0, 2, 6, 9 or 15 ppm MIC gave birth to pups with decreased body weights at the lowest dose, although a good dose-response was not observed (Varma, 1987). At 9 or 15 ppm MIC, the surviving dams lost 75 - 80 percent of their fetuses. Maternal mortality and decreased skeletal lengths were also observed at 9 and 15 ppm. A distinction between maternally induced fetotoxicity and a direct effect on fetal health could not be made. Because the inhalation exposure to the dams occurred for only 3 hrs on one day, a chronic LOAEL is not suggested. Exposure of male rats to one dose of 3.2 mg/L for 8 minutes resulted in a 21 percent fertility rate

among the cohabited female rats within the day 8-14 period post-exposure compared to a fertility rate of 40% for controls; however, the rates increased after 15 days post-exposure (Agarwal and Bose, 1992). There was no evidence of fetotoxicity among the dams impregnated by the MIC-exposed male rats. Exposure of male and female mice to 0, 1, or 3 ppm MIC did not result in altered body weights, fertility, or litter size (Schwetz *et al.*, 1987). The results suggest that exposures to MIC at doses that are not toxic to adult male or female (pregestational) mice or rats do not result in adverse reproductive outcomes.

VI. Derivation of Chronic Reference Exposure Level (REL)

<i>Study</i>	Dodd and Fowler (1986)
<i>Study populations</i>	F344 rats
<i>Exposure method</i>	Inhalation (0, 0.15, 0.6, or 3.1 ppm)
<i>Critical effects</i>	Decreased weight gain and lung pathology immediately after cessation of exposure
<i>LOAEL</i>	3.1 ppm
<i>NOAEL</i>	0.6 ppm
<i>Exposure continuity</i>	6 hours/day, 8 days/10 day experiment (2-cycles, with one 2-day recess from exposure)
<i>Exposure duration</i>	10 days
<i>Average experimental exposure</i>	0.12 ppm for the NOAEL group (0.6 x 8/10 x 6/24)
<i>Human equivalent factor</i>	0.15 ppm for the NOAEL group (gas with pulmonary respiratory effects, RGDR = 1.23, based on BW = 152 g, MV = 0.12 L/min, SA = 225 cm ²)
<i>LOAEL uncertainty factor</i>	1
<i>Subchronic uncertainty factor</i>	10
<i>Interspecies uncertainty factor</i>	3
<i>Intraspecies uncertainty factor</i>	10
<i>Cumulative uncertainty factor</i>	300
<i>Inhalation reference exposure level</i>	0.5 ppb (1 µg/m ³)

Although the exposure was for only 10 days, the Dodd and Fowler (1986) study includes the longest exposure duration of the available investigations and also uses some of the lower exposure levels (down to 0.15 ppm). The microscopic findings of the respiratory tract were statistically analyzed, although an observation of the tabulated data at the four doses (0, 0.15, 0.6, and 3.1 ppm) clearly shows a NOAEL of 0.6 ppm. Other endpoints with the same NOAEL were increased hemoglobin and increased absolute lung weights. The symptomatic ramifications of the increased hemoglobin are unknown, although similar increases were reported for humans exposed to MIC in Bhopal (Srivastava *et al.*, 1988). The lung weight gain may be a reflection of the pathological changes seen in the microscopic studies.

Decreased body weight gain was also seen in the experimental 4 day rat inhalation study of Mitsumori *et al.* (1987) (NOAEL = 1.1 ppm), except that the decrease in the latter study did not

occur until 1 and 3 days (female and male, respectively) post-exposure. The apparent discrepancy could be explained, in part, on the basis of the length of exposure, which was twice as long in the Dodd and Fowler (1986) study. However, the weight gain loss in the Dodd and Fowler (1986) study was initiated within one day of the start of exposure.

The MIC chronic REL of 0.5 ppb is based on endpoints observed within 1 day of cessation of exposure. Post-exposure evaluation showed that, at a higher exposure level (3.1 ppm), progressive changes, including death, occurred. Post-exposure observations, however, were not reported at the 0.15 and 0.6 ppm MIC levels. The attribute of delayed MIC inhalation toxicity has also been observed in other experimental animals studies (Dodd and Fowler, 1986, Mitsumori *et al.*, 1987). In the case of the human MIC exposure in Bhopal, India, death did not occur during the immediate 30 - 45 minute exposure, but exhibited a lag phase. A few deaths occurred during the first few hours, the maximum occurred at 2 - 3 days, and by the end of a week about 2500 deaths were documented (Dave, 1985; Varma, 1986; Varma and Guest, 1993), although Varma (1986) suggests that the immediate number may be closer to 5,000. One report suggests that during the intervening decade as many as 6,000 deaths may be attributed to the initial exposure in Bhopal (Lepkowski, 1994). Such information suggests that the presence of an adverse effect at the NOAEL of 0.6 ppm (Dodd and Fowler, 1986) might be possible if the rats were observed during an extended post-exposure interval. Experimental evidence is needed to test this hypothesis.

Only one study was identified in which post-exposure observations were made on experimental animals exposed subchronically by inhalation to multiple doses of MIC. Mitsumori *et al.* (1987) exposed rats to 0, 1.1, and 2.8 (females) or 3.0 (males) ppm MIC for 6 hr/day for 4 days and observed the rats for 91 days. No deaths and no weight gain loss (in contrast to Dodd and Fowler, 1986) were present until the post-exposure period and were mainly observed in animals exposed at the high dose. Using a NOAEL of 1.1 ppm MIC, a chronic REL of 1.1 ppb ($2.6 \mu\text{g}/\text{m}^3$) was derived. The REL based on the Mitsumori *et al.* (1987) study is similar to the REL based on immediate effects (Dodd and Fowler, 1986), and may indicate that the time of occurrence of exposure related effects may not be as important as the MIC air concentration.

VII. Data Strengths and Limitations for Development of the REL

The strengths of the inhalation REL for MIC include the availability of controlled exposure inhalation studies in multiple species at multiple exposure concentrations and with adequate histopathological analysis and the observation of a NOAEL. Major areas of uncertainty are the lack of adequate human exposure data and the lack of chronic inhalation exposure studies.

VIII. Potential for Differential Impacts on Children's Health

Since exposures to MIC at levels that are not toxic to adult male or female (pregestational) mice or rats do not result in adverse reproductive outcomes, the chronic REL of $1 \mu\text{g}/\text{m}^3$ should adequately protect infants and children. MIC is a respiratory irritant and the developing respiratory system is more sensitive than that of adults. However, there is no direct evidence in the literature to quantify a differential effect of MIC on the respiratory system of infants and children.

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CHRONIC TOXICITY SUMMARY

METHYL *t*-BUTYL ETHER

(MTBE; 2-methoxy-2-methylpropane; tert-butyl methyl ether;
methyl 1,1-dimethyl ether)

CAS Registry Number: 1634-04-4

I. Chronic Toxicity Summary

<i>Inhalation reference exposure level</i>	8000 µg/m³ (2000 ppb)
<i>Critical effect(s)</i>	Nephrotoxicity, prostration, periocular swelling in Fischer 344 rats
<i>Hazard index target(s)</i>	Kidney; eyes; alimentary system

II. Physical and Chemical Properties (HSDB, 1994)

<i>Description</i>	Colorless liquid
<i>Molecular formula</i>	C ₅ H ₁₂ O
<i>Molecular weight</i>	88.15 g/mol
<i>Density</i>	0.7405 g/cm ³ @ 20°C
<i>Boiling point</i>	55.2°C @ 760 mm Hg
<i>Vapor pressure</i>	245 torr @ 20°C
<i>Solubility</i>	Soluble in alcohol, ether, and 5% soluble in water
<i>Conversion factor</i>	1 ppm = 3.61 mg/m ³ @ 25° C; 3.67 mg/m ³ @ 20° C

III. Major Uses or Sources

Methyl t-butyl ether (MTBE) is used as a gasoline additive to improve octane ratings and reduce emissions of some pollutants, in industry to improve miscibility of solvents, and in clinical medicine to dissolve cholesterol gall stones (Yoshikawa *et al.*, 1994). The annual statewide industrial emissions from facilities reporting under the Air Toxics Hot Spots Act in California, based on the most recent inventory, were estimated to be 215,182 pounds of MTBE (CARB, 1999).

IV. Effects of Human Exposure

Gasoline (with 10% MTBE) tanker drivers reported significantly higher fatigue at the end of the work week than before the work week, and those with longer exposure to gasoline with MTBE during the work week reported significantly higher fatigue than drivers with shorter exposure (Hakkola *et al.*, 1997). 20% of drivers reported symptoms such as headache, dizziness, nausea,

and dyspnoea at the end of work week. No human chronic toxicity or chronic epidemiology information for MTBE without coexposure to gasoline was found.

Ten healthy male volunteers undergoing light physical work were exposed to 5, 25, and 50 ppm MTBE vapor for 2 hours (Nihlen *et al.*, 1998). While a solvent smell was noted at these concentrations, there were no consistent concentration-related effects on reported ocular or nasal irritation. The blockage index (a measure of nasal airway resistance) increased significantly after exposure but was not correlated with exposure concentration.

V. Effects of Animal Exposure

Male and female rats (50/sex/group) were exposed by inhalation for 6 hours/day, 5 days/week to mean concentrations of 0, 403, 3023, or 7977 ppm (0, 1453, 10,900, or 28,760 mg/m³) MTBE for 24 months (Chun *et al.*, 1992). Clinical signs, hematology, body weights and food consumption were monitored. Necropsy included measurements of organ weights and histopathology. Corticosterone levels were measured on 10 animals prior to sacrifice. Serum enzymes were not monitored. The NOAEL for several endpoints, including non-alpha-2μ-globulin induced nephrotoxicity, increased relative liver and kidney weights and prostration in females, and periorbital swelling in both sexes was 403 ppm (1453 mg/m³).

Mice were exposed for 6 hours/day, 5 days/week for 18 months to MTBE concentrations of 0, 402, 3014, or 7973 ppm (0, 111, 835, or 2208 mg/m³) (Burleigh-Flayer *et al.*, 1992). The mice exposed to the highest concentration (7973 ppm) all exhibited ataxia. Prostration was also noted in 8 of 50 animals in this group. Liver weights were elevated in a concentration-dependent manner in the female mice but this change was not significant at the lowest concentration (402 ppm). Kidney weights were elevated in the female mice exposed to 7973 ppm. At the highest concentration, a significant increase in hepatocellular hypertrophy and adrenal gland weight was detected in the male mice. Spleen weights were increased in the females exposed to the highest concentration.

Moser *et al.* (1998) exposed female B6C3F₁ mice to 7924 ppm (2195 mg/m³) MTBE for 4 months, or 7919 ppm (2194 mg/m³) MTBE for 8 months; controls received plain air. Body weight increases for control and MTBE-exposed mice, respectively, were 57% and 37% at 4 months and 79% and 45% at 8 months: the reduced weight gain in MTBE-exposed mice was significantly different from the controls at both time points. In MTBE-exposed mice, mean uterine weight was 83% reduced relative to controls at 4 and 8 months. Ovary weight was also reduced in exposed mice, the mean weight being 55% of control at 4 months and 51% of control at 8 months. Pituitary weights were decreased by 44% and 31% at 4 and 8 months, relative to controls. Disturbances of the estrus cycle and histological changes in the reproductive organs were also noted. Although the changes in organ weights and histology were suggestive of an anti-estrogenic effect of MTBE, serum estrogen levels were unaffected. No changes in estrogen receptor (ER) immunoreactivity in reproductive system tissues were observed. Experiments *in vitro* failed to demonstrate any inhibition of estradiol binding to ER by MTBE or its metabolites. No inhibition of ER by MTBE was detected, nor was there any inhibition of the induction of ER by estradiol. The authors concluded that the apparent anti-estrogenic effects of MTBE were not

mediated via the ER, and drew a parallel with the anti-estrogenic effects of dioxins and chlorinated biphenyls.

Tests for histopathology in the respiratory tract, plasma corticosterone levels, motor activity and neurobehavioral endpoints were performed in rats exposed to MTBE at concentrations of 0, 797, 3920, or 8043 ppm (0, 2877, 14151, or 29035 mg/m³), 6 hours/day, 5 days/week for 13 weeks (Dodd and Kintigh, 1989). Of these endpoints, the most significant finding was an elevation in plasma corticosterone in the high dose group. This finding was consistent with the elevated adrenal weights reported by Burleigh-Flayer *et al.* (1992). A clear dose-response for neurotoxic effects in these rats was not established. Biles *et al.* (1987) reported a NOAEL of 300 ppm (1083 mg/m³) MTBE for decreased pup viability in rats exposed for 6 hours/day, 5 days/week for a total of 16 weeks. Animals exposed to 1240 ppm (4470 mg/m³) or 2860 ppm (10,311 mg/m³) MTBE exhibited slightly decreased pup survival.

Neeper-Bradley (1991) exposed rats to 0, 402, 3019, or 8007 ppm (0, 111, 836, and 2218 mg/m³) MTBE over 2 generations. Exposures were for 6 hours/day, 5 days/week during the prebreeding period, and for 7 hours/day, 5 days/week during gestation and lactation. Parental effects of MTBE exposure were observed, including ataxia, blepharospasm, lack of startle reflex, and increased relative liver weights (F1 generation only). There were no histological changes in the organs from either parental generation. Reduced body weights were observed in the F1 and F2 pups at the 3019 and 8007 ppm concentrations. Reduced survivability to postnatal day 4 was observed in the 8007 ppm group. No adverse effects were noted at the 403 ppm (111 mg/m³) concentration.

In a developmental and reproductive toxicity study, Conaway and associates (1985) found no significant increases in maternal or fetal toxicity, nor in pregnancy rates or in any gross toxicologic parameter tested with pregnant rats or mice exposed during gestation to concentrations of MTBE up to 3300 ppm (11,897 mg/m³).

Maternal toxicity, in the form of hypoactivity and ataxia, was observed in pregnant mice exposed during gestation to 4076 ppm (14,690 mg/m³) MTBE (Bushy Run Research Center, 1989a). Significant reductions in food intake and body weight gain were observed in dams exposed to 8153 ppm (29,390 mg/m³). Fetal body weight was significantly reduced in the 4076 ppm group, and there were significant increases in the incidences of skeletal variations and unossified phalanges in the 4076 and 8153 ppm groups. Pregnant rabbits exposed to similar concentrations during gestation showed no significant maternal or fetal toxicity or developmental toxicity up to a concentration of 8021 ppm (28,918 mg/m³) (Bushy Run Research Center, 1989b).

VI. Derivation of Chronic Reference Exposure Level

<i>Study</i>	Chun <i>et al.</i> , 1992; Bird 1997
<i>Study population</i>	Male and female rats (50 per sex/group)
<i>Exposure method</i>	Discontinuous whole-body inhalation exposures (0, 403, 3023, or 7977 ppm)
<i>Critical effects</i>	Nephrotoxicity, increased liver and kidney weight, prostration and periocular swelling
<i>LOAEL</i>	3023 ppm
<i>NOAEL</i>	403 ppm
<i>Exposure continuity</i>	6 hours per day, 5 days per week
<i>Exposure duration</i>	24 months
<i>Average experimental exposure</i>	72 ppm for the NOAEL group
<i>Human equivalent concentration</i>	72 ppm for the NOAEL group (gas with systemic effects, based on RGDR = 1.0 using default assumption that $\lambda(a) = \lambda(h)$)
<i>LOAEL uncertainty factor</i>	1
<i>Subchronic uncertainty factor</i>	1
<i>Interspecies uncertainty factor</i>	3
<i>Intraspecies uncertainty factor</i>	10
<i>Cumulative uncertainty factor</i>	30
<i>Inhalation reference exposure level</i>	2 ppm (2000 ppb, 8 mg/m ³ , 8000 µg/m ³)

The USEPA (1995) based its RfC of 3000 µg/m³ on the same study but included a Modifying Factor (MF) of 3 for database deficiencies. The criteria for use of modifying factors are not well specified by U.S. EPA. Such modifying factors were not used by OEHHA.

VII. Data Strengths and Limitations for Development of the REL

The major strengths of the REL for MTBE are the use of a comprehensive, long-term multiple dose study with large sample sizes and the observation of a NOAEL. The major uncertainty is the lack of human data.

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CHRONIC TOXICITY SUMMARY

METHYLENE CHLORIDE*(dichloromethane, methylene dichloride)***CAS Registry Number: 75-09-2****I. Chronic Toxicity Summary**

<i>Inhalation reference exposure level</i>	400 µg/m³ (100 ppb)
<i>Critical effect(s)</i>	Carboxyhemoglobin formation above 2% in human workers
<i>Hazard index target(s)</i>	Cardiovascular system; nervous system

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

<i>Description</i>	Colorless liquid
<i>Molecular formula</i>	CH ₂ Cl ₂
<i>Molecular weight</i>	84.93
<i>Density</i>	1.32 g/cm ³ @ 20° C (ACGIH, 1991)
<i>Boiling point</i>	39.75° C
<i>Vapor pressure</i>	400 torr @ 24.1° C
<i>Solubility</i>	Miscible with most organic solvents, slightly soluble in water (ACGIH, 1991)
<i>Conversion factor</i>	1 ppm = 3.47 mg/m ³ @ 25° C

III. Major Uses and Sources

Methylene chloride (MC) is used in paint and varnish remover, in aerosols as a cosolvent or vapor pressure depressant, and in solvent degreasing and metal cleaning. It is also used in plastics processing and in extraction of fats and oils from food products (HSDB, 1999). The annual statewide industrial emissions from facilities reporting under the Air Toxics Hot Spots Act in California, based on the most recent inventory, were estimated to be 3,504,271 pounds of methylene chloride (CARB, 1999a). Both mean and maximum monitored ambient methylene chloride concentrations have decreased slightly between 1990 and 1996 (CARB, 1999b). Median and maximum concentrations were 1.09 and 11 ppb in 1990 and 0.66 and 5.6 ppb in 1996.

IV. Effects of Human Exposure

Effects of a controlled 2-hour inhalation exposure to MC included CNS depression at concentrations of 1000 ppm (3500 mg/m³) or more and increased blood carboxyhemoglobin

(COHb) content at lower concentrations (500 ppm) due to metabolism of MC to carbon monoxide (Stewart *et al.*, 1972). High levels of COHb can be found in the blood hours after exposure to methylene chloride, due to its partitioning into fat and its slow release into circulation with subsequent metabolism, leading to formation of carbon monoxide (Engstrom and Bjurstrom, 1977). In situations of chronic exposure, carbon monoxide toxicity is also of concern. Barrowcliff (1978) documented the case of an adult male who developed an unsteady gait, a peculiar dysarthria and a loss of memory. The man had worked with 15-50 liters of methylene chloride daily for 3 years in a poorly ventilated room while cleansing road materials. No natural disease could be found to explain his conditions and the effects were attributed to chronic carbon monoxide poisoning.

Twelve women volunteer subjects were exposed to 0, 300, or 800 ppm methylene chloride for 4 hours (Fodor and Winneke, 1971). Neurobehavioral vigilance was measured by auditory discrimination of intensity of certain sound pulses against a background of continuous white noise. A significant interactive effect between methylene chloride concentration and duration of exposure using 2-way ANOVA ($p < 0.01$) was found.

Human erythrocytes enzymatically convert methylene chloride to formaldehyde in cell-culture experiments (Hallier *et al.*, 1994).

A subacute controlled exposure of eleven resting non-smokers to methylene chloride was conducted by DiVincenzo and Kaplan (1981a). The eleven subjects were exposed to 50, 100, 150, or 200 ppm methylene chloride for 7.5 hours on 5 consecutive days. Exposure to all concentrations led to dose-dependent elevation in COHb concentrations in the blood and elevated exhaled CO. The peak blood COHb saturations were 1.9, 3.4, 5.3, and 6.8%, respectively, for the 50, 100, 150, and 200 ppm groups.

Divincenzo and Kaplan (1981a) also measured COHb percentage in the blood of workers occupationally exposed to methylene chloride and a group of workers not exposed to methylene chloride. The 19 workers exposed to methylene chloride had mean blood COHb concentrations of 2.3% in the morning and 3.9% at the end of the work-shift. Ambient concentrations in the workplace were estimated from 57 samples, which ranged from 0 to 250 ppm, with a mean concentration of 40 ppm. Three exposed workers also wore monitors to estimate personal exposures. The time-weighted average exposure for these workers was 33 ppm. Controls (8 subjects) had significantly lower mean blood COHb concentrations of 0.8% in the AM and 1.3% in the PM compared with the exposed workers. The length of employment of the exposed workers was not given.

A companion study by DiVincenzo and Kaplan (1981b) showed that smoking and methylene chloride exposure result in an additive effect on COHb levels compared with levels in non-smokers. Similarly, light, moderate or heavy exercise workloads resulted in higher COHb levels.

Soden *et al.* (1996) showed a dose-response increase in carboxyhemoglobin levels in non-smokers with increasing methylene chloride exposure in workers involved in triacetate fiber production. Carboxyhemoglobin levels ranged from 1.77% to 4% from exposures ranging from 6.5 to 89.7 ppm, respectively. The number of employees in the study was not reported.

Although animal studies have shown COHb-induced cardiovascular effects following MC exposure (Aviado *et al.*, 1977), no data exist on this outcome in humans. However, studies of men with coronary artery disease and exercise-induced angina report a decrease in time to onset of exercise-induced angina following exposure to carbon monoxide (CO) at concentrations sufficient to result in blood COHb levels of about 2% (Kleinman *et al.*, 1989; Allred *et al.*, 1989). A physiologically based pharmacokinetic model of MC and CO estimated that a 1-hour exposure to 340 ppm (1200 mg/m³) MC at a ventilation rate of 9 liters/min would result in a peak blood COHb level of 2% (Andersen *et al.*, 1991; Reitz, 1994). The California Ambient Air Quality Standard for CO is based on a blood COHb level of 2% (CARB, 1982).

An epidemiological study of 751 male workers in the Eastman Kodak Company exposed to daily 8-hour time-weighted average concentrations of 30-125 ppm methylene chloride for up to 30 years was conducted by Friedlander and associates (1978). A control group of workers in production but not exposed to methylene chloride was used together with New York state cause and age-specific mortality rates. The follow-up period for these workers was 13 years, with 97% success. The studies did not indicate any increase in risk of death from circulatory disease, cancer, or other causes due to methylene chloride exposure.

A study of female pharmaceutical workers in eight different factories exposed to a variety of organic solvents indicated that solvent exposure, and particularly methylene chloride exposure, resulted in an increase in spontaneous abortions (Taskinen *et al.*, 1986). In all, 1795 pregnancies were followed, with 142 spontaneous abortions occurring. The odds ratio for methylene chloride exposure was 1.0 to 5.7 (average = 2.3; $p < 0.06$). There was a significant effect of exposure to 4 or more solvents, compared with age-matched controls ($p < 0.05$). The concentrations of MC were not reported in the study.

The U.S. Occupational Safety and Health Administration reduced its permissible exposure limits (PEL) for MC from 500 ppm to 25 ppm in 1997 (U.S. CFR, 1997).

V. Effects of Animal Exposure

Nitschke *et al.* (1988) found that a 2-year exposure to 0, 50, 200, or 500 ppm MC for 6 hours/day, 5 days/week resulted in significant histopathologic lesions in the livers of rats exposed to 500 ppm. No significant adverse effects were observed at 200 ppm or lower. The predominant hepatocellular lesion was fatty vacuolization of hepatocytes.

Female B6C3F1 mice inhaling 2000 ppm MC for 1 to 26 weeks had 40 to 60% lower cell turnover rates of bronchiolar cells compared with controls (Kanno *et al.*, 1993). At this concentration no observable pathological changes were found in the lungs of MC exposed animals.

A continuous exposure of mice (16 per group) to 100 ppm MC for 1, 2, 3, 4 or 10 weeks resulted in significant elevation in liver triglycerides beginning at 2 weeks and lasting throughout the 10-week period (Weinstein and Diamond, 1972). Liver/body weight ratios were unaffected at any

time point. After 1 week, small fat droplets were apparent in centrilobular hepatocytes and a decrease in hepatic glycogen was also noted. Necrosis was not observed during the 10-week period, but fat droplet size increased and glycogen depletion persisted.

Male and female Sprague-Dawley rats and Golden Syrian hamsters inhaled methylene chloride (0, 500, 1500, or 3500 ppm) for 6 hr per day, 5 days a week over 2 years (Burek *et al.*, 1984). The groups consisted of 129 rats per sex per concentration, and 107 to 109 hamsters per sex per concentration. Females rats inhaling 3500 ppm had an increased mortality rate while female hamsters inhaling 1500 or 3500 ppm had decreased mortality rates. Slight histopathological findings were noted in livers of rats exposed to 500, 1500, or 3500 ppm MC. Decreased amyloidosis was also found in livers and other organs of hamsters at each of the three MC concentrations. Overall, effects were more potent in rats compared with hamsters, which had fewer spontaneous age-related changes, decreased mortality (at least for females), and evidence of specific target organ toxicity was weak. Carboxyhemoglobin values were elevated in both rats and hamsters exposed to 500 ppm or more of MC, with the percentage increase greater in hamsters than in rats.

Monkeys were observed to be more susceptible subjects for methylene chloride induced COHb than dogs upon 14-week subchronic continuous exposure to 25 or 100 ppm (Haun *et al.*, 1972). At 25 ppm, approximately 1.5% COHb was reached in the 4 monkeys, compared to approximately 0.5% in 16 dogs. Monkeys exposed to 100 ppm MC had COHb levels of approximately 4% compared with 2% in the dogs.

Oral ethanol pretreatment in rats has been shown to suppress the COHb formation characteristic of methylene chloride exposure through inhibition of biotransformation of methylene chloride (Glatzel *et al.*, 1987).

Gerbils (10/sex per group; 60 controls) exposed continuously to MC concentrations of 210, 350, or 700 ppm for a period of 3 months, with a 4-month follow-up period, showed irreversible cellular and biochemical changes in brain (Rosengren *et al.*, 1986). A high mortality rate (19/20) was observed in the 700 ppm group, and this exposure was terminated after 7 weeks. The gerbils exposed to 350 ppm also had a high mortality rate (9/20) and this exposure was terminated after 10 weeks. The gerbils exposed to 210 ppm had no premature mortality and the exposure continued for the full 3 months. Four months after termination of exposure, the animals in the 350 and 210 ppm groups had significantly decreased brain DNA content in the hippocampus. The 350 ppm group exhibited elevated astroglial proteins in the frontal and sensory motor cerebral cortex, consistent with astrogliosis in these regions. In addition, the gerbils exposed to 350 ppm MC had significantly decreased DNA in the cerebellar hemispheres. Complimentary studies by these investigators showed that the formation of carboxyhemoglobin did not increase in gerbils between the 210 and 350 ppm exposures, indicating that the metabolism of MC to CO is saturable at concentrations below those in the study. On the other hand, the neurotoxic brain biochemical alterations were significantly greater in gerbils exposed to 350 ppm as compared with the 210 ppm group, implying that carboxyhemoglobin induced cerebral hypoxia is not the major cause of MC-induced neurotoxicity in the brain.

Rats (50 per sex per group) were exposed to 0, 1000, 2000, or 4000 ppm methylene chloride 6 hours/day, 5 days/week for 102 weeks (NTP, 1986). Both sexes exhibited hemosiderin

pigmentation in the liver in a dose-dependent fashion, beginning with the 1000 ppm concentration. Squamous metaplasia of the nasal cavity was observed in female rats, and thyroid C-cell hyperplasia was observed in males exposed to 2000 ppm or greater. Kidney tubule degeneration (not otherwise specified) was increased at all exposure levels.

Mice (50 per sex per group) exposed to 0, 2000, or 4000 ppm methylene chloride 6 hours/day, 5 days/week for 102 weeks showed increased incidence of liver cytologic degeneration and splenic atrophy at 4000 ppm (males) (NTP, 1986). Male and female mice also had an increased incidence of kidney tubule casts (not otherwise specified) at 2000 ppm or greater, and significant testicular atrophy was observed in males at 4000 ppm. Female mice showed cytologic degeneration in the liver at 2000 ppm or greater, and ovarian atrophy at 2000 ppm or greater.

A six month exposure to 5000 ppm MC of 8 guinea pigs for 7 hours/day, 5 days/week resulted in 3 deaths; 2 showed moderate centrilobular fatty degeneration of the liver and extensive pneumonia at necropsy (Heppel *et al.*, 1944). None of the 14 control animals died. Food consumption and body weight were lower in the exposed guinea pigs, compared with control pigs. One out of 12 rats died at this concentration, and the liver histology in this animal revealed multiple thrombi in renal vessels, associated with marked cortical infarction. By comparison, dogs and rabbits showed no signs of illness, nor were blood pressure or hematological values altered at the 5000 ppm concentration. At 10,000 ppm, 2 of 4 dogs showed moderate centrilobular congestion, narrowing of liver cell cords, and slight to moderate fatty degeneration. One of 2 monkeys revealed disseminated tuberculosis lesions, but no other histological alterations. Four out of 6 guinea pigs had moderate fatty degeneration of the liver at this concentration.

The offspring of rats (10 dams per group) exposed during gestation to 0 or 4500 ppm methylene chloride exhibited altered rates of behavioral habituation to novel environments (Bornschein *et al.*, 1980). This effect was observed beginning at 10 days of age but was still demonstrable in rats 150 days old. The authors concluded that elevated maternal COHb could have been a contributing factor in the developmental impairment.

In a study of the effects of methylene chloride on estrous cycle and serum prolactin, groups of 15 female rats were exposed to 0 or 3500 ppm for 6 hours/day for 15 to 19 consecutive days (Breslin and Landry, 1986). Males (15 per group) were exposed for 5 hours/day for 5 consecutive days. Female rats exhibited decreased body weight and increases in the estrous cycle duration and in serum prolactin. Males did not show any significant effects on serum prolactin from methylene chloride exposure.

Pregnant mice and rats were exposed to 0 or 1250 ppm MC 7 hours/day, on days 6 through 15 of gestation (Schwetz *et al.*, 1975). Significantly elevated absolute liver weights were seen in maternal animals from both species. In addition, significantly increased incidences of delayed ossification of the sternbrae were seen in both species, compared to controls.

Methylene chloride exposure of female rats before or during gestation to 4500 ppm resulted in elevated maternal liver weights and decreased birth weights of the offspring, but no terata or skeletal/soft tissue anomalies (Hardin and Manson, 1980).

A 2-generation reproduction test was conducted by Dow Chemical Company (Nitschke *et al.*, 1985) which showed no significant reproductive or developmental effects in rats exposed to 0, 100, 500, or 1500 ppm MC 6 hours/day, 5 days/week, for 14 weeks. The exposure conditions were identical for the F₀ and F₁ generations.

VI. Derivation of Chronic Reference Exposure Level (REL)

<i>Study</i>	DiVincenzo and Kaplan (1981a)
<i>Study population</i>	19 workers, 8 controls
<i>Exposure method</i>	Occupational inhalation exposure
<i>Critical effects</i>	Significantly elevated carboxyhemoglobin levels (> 2%)
<i>LOAEL</i>	40 ppm (ambient workplace exposures averaged 40 ppm with a range of 0 to 250 ppm); controls = 0 ppm
<i>NOAEL</i>	Not observed
<i>Exposure continuity</i>	8 hours/day, 5 days/week
<i>Exposure duration</i>	Length of employment unspecified
Average occupational exposure	14 ppm for LOAEL group (40 x 10/20 x 5/7)
<i>LOAEL uncertainty factor</i>	10
<i>Subchronic uncertainty factor</i>	1 (see following text for explanation)
<i>Interspecies uncertainty factor</i>	1
<i>Intraspecies uncertainty factor</i>	10
<i>Cumulative uncertainty factor</i>	100
<i>Inhalation reference exposure level</i>	0.1 ppm (100 pbb; 0.4 mg/m ³ ; 400 µg/m ³)

Workers were exposed to average measured concentrations of 40 ppm during the workday, and the personal monitors on 3 of the subjects indicated a 8-hour time-weighted average of 33 ppm over a 2-week period. The average COHb levels were 3.9% at the end of the work-shift. Elevated carboxyhemoglobin concentrations of above 2% are considered to aggravate angina in some individuals (CARB, 1982). In effect, 2% COHb can be considered a NOAEL for aggravation of angina. Therefore, the 33 ppm concentration was considered a LOAEL for the formation of greater than 2% COHb. The duration of the employment period was not specified. However, in the DiVincenzo and Kaplan (1981a) study, the levels of COHb did not appear to increase over a period of 5 days in experimental exposures using volunteers, therefore an uncertainty factor for subchronic exposure was not necessary. A number of factors contribute to the uncertainty in determining the degree of sensitivity to methylene chloride, including activity level, metabolic enzyme activity, age, and background COHb status (e.g., from smoking, etc.).

The subchronic study by Haun *et al.* (1972) with monkeys reported a NOAEL of 25 ppm and a LOAEL of 100 ppm for 2% COHb formation following a 14-week exposure. These results are consistent with the LOAEL reported in the DiVincenzo and Kaplan study. However, the human occupational study likely contains less uncertainty, since the toxicokinetics of the effect,

including rate of formation of CO and thus COHb is metabolism-dependent, resulting in considerable potential interspecies differences.

The study in hamsters by Burek *et al.* (1984) showed a LOAEL for elevated carboxyhemoglobin of 500 ppm. A time-weight average exposure and HEC of 89 ppm was calculated. Using a 10-fold LOAEL uncertainty factor, a 3-fold interspecies uncertainty factor for residual uncertainty not accounted for in the HEC calculation, and a 10-fold intraspecies uncertainty factor, a REL of 300 ppb or 1000 $\mu\text{g}/\text{m}^3$ was derived. Thus, the REL derived from the best available animal study is comparable to the 400 $\mu\text{g}/\text{m}^3$ REL derived from the best-available human study.

VII. Data Strengths and Limitations for Development of the REL

The major strength of the key study (DiVincenzo and Kaplan, 1981a) used to derive the REL for methylene chloride is that human health effects were observed. The major uncertainties from the key study itself are the lack of a NOAEL observation, the difficulty in estimating exposures, and the discontinuous and variable nature of the exposures.

The health effects database for methylene chloride includes, in addition to an adequate study of human occupational exposures (DiVincenzo and Kaplan, 1981a), an adequate lifetime inhalation exposure study in 2 species of laboratory animals (Burek *et al.*, 1984). The REL values derived from these studies (400 $\mu\text{g}/\text{m}^3$ vs. 1,000 $\mu\text{g}/\text{m}^3$) are comparable. That both the human and animal studies measured the same endpoint and arrived at similar conclusions is a circumstance that is rarely found but one that considerably increases the weight of evidence from which the REL was derived. The two studies complement each other, as the animal study involved controlled, measured exposures over a lifetime but introduces the uncertainty of predicting human health effects from animal observations, and the human study involved poorly characterized human exposures but lacks the uncertainty inherent in interspecies extrapolation.

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CHRONIC TOXICITY SUMMARY

4,4'-METHYLENE DIANILINE

(MDA; 4,4'-diaminodiphenylmethane; 4,4'-diphenylmethanediamine; DAPM; dianilinemethane)

CAS Registry Number: 101-77-9

I. Chronic Toxicity Summary

<i>Inhalation reference exposure level</i>	20 µg/m³ (2 ppb)
<i>Critical effect(s)</i>	Ocular toxicity to the retinas of guinea pigs
<i>Hazard index target(s)</i>	Eyes; alimentary system (hepatotoxicity)

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

<i>Description</i>	Colorless to pale yellow flakes; tan
<i>Molecular formula</i>	C ₁₃ H ₁₄ N ₂
<i>Molecular weight</i>	198.3 g/mol
<i>Boiling point</i>	398-399°C
<i>Melting point</i>	92.5°C
<i>Vapor pressure</i>	1 torr @ 197°C
<i>Solubility</i>	Soluble in alcohol, benzene, ether; 273 g/100 g acetone; 0.1 g/100 g water @ 25°C
<i>Conversion factor</i>	8.1 µg/m ³ per ppb at 25°C

III. Major Uses and Sources

4,4'-Methylene dianiline (MDA) is synthesized by the reaction of aniline with formaldehyde. MDA's major uses are as a chemical intermediate in the synthesis of certain isocyanates and polyurethane polymers, as a corrosion inhibitor, in the preparation of azo dyes, as a rubber preservative, and in the curing of epoxy resins and neoprene (HSDB, 1995; ACGIH, 1992). The annual statewide industrial emissions from facilities reporting under the Air Toxics Hot Spots Act in California based on the most recent inventory were estimated to be 1133 pounds of MDA (and its dichloride) (CARB, 2000).

IV. Effects of Human Exposure

Several cases of human exposure to MDA have identified the compound as a hepatotoxicant which produces cholestatic jaundice (Kopelman *et al.*, 1966; McGill and Motto, 1974; Williams *et al.*, 1974; Bastian, 1984). Bastian (1984) described cases of acute hepatic illness in four workers exposed from laying floors using an epoxy resin base, which contained MDA as a curing agent. The workers were exposed via fumes and dusts in the air as well from hand

contact with powder and had worked with epoxy resins for periods ranging from one to 12 years. The level of exposure was not quantified. The workers initially reported to the hospital with symptoms of abdominal pain three days after the most recent exposure and all were discharged within four days. Two workers continued to show severe symptoms five days after the onset, with abdominal pain, jaundice, a tender liver, nausea, dyspnea, and muscular pain. Plasma bilirubin, alkaline phosphatase, and aspartate aminotransferase levels were elevated. Some symptoms did not subside until two months after the onset. One worker, after another exposure, experienced nausea, abdominal pain, and muscular pain. A second worker reported further symptoms of headache, tiredness, and decreased libido.

Williams *et al.* (1974) reported symptoms in 6 of approximately 300 workers exposed to MDA by surface coating concrete walls with epoxy resins. Exposure probably occurred by inhalation, ingestion, and skin contact as a result of mixing powder containing MDA. Symptoms of clinical hepatitis in the 6 workers appeared two days to two weeks after beginning work; five of the six had elevated bilirubin levels, and one liver biopsy showed bile stasis. All the workers recovered completely after an unspecified time.

McGill and Motto (1974) described hepatitis among 13 men who, over the course of 6 years, were occupationally exposed to MDA in the blending of epoxy resins used in the manufacture of insulating material. Among the 13 patients showing symptoms, all reported weakness, jaundice, and dark urine; 11 reported abdominal pain, nausea or vomiting, and anorexia; and over half reported fever, chills and/or headache. All the workers recovered within a 10 week period. After the first cases of hepatitis occurred, air sampling showed initial levels of MDA to be 0.1 ppm in the work area. After additional cases of hepatitis occurred, measures were taken to reduce worker exposure, and air levels were reduced to as low as 0.0064 ppm. The authors concluded that percutaneous absorption was the likely major route of exposure in light of the fact that cases occurred in spite of measures taken to reduce air levels and there was evidence that significant hand contact with the compound occurred during the workday. Since the symptoms appeared within one to 18 days after “working intensively” with the compound and exposure routes were not clearly established, quantitation of exposure levels was considered difficult.

The most well-known incident of MDA toxicity to humans resulted from ingestion of bread made with flour contaminated with MDA during transport (Kopelman *et al.*, 1966a). Eighty-four persons showed symptoms of abdominal pain and some degree of jaundice. All patients had elevated serum alkaline phosphatase and glutamic oxaloacetic transaminase levels. Seventeen had serum bilirubin levels over 5 mg/100 ml. Liver biopsy was performed on 8 persons and evaluated in a separate study (Kopelman *et al.*, 1966b). The primary finding was an unusual lesion described during the early course of the disease as portal zone cholangitis and later as centrilobular cholestasis with necrosis. The initial study reported that all but 2 patients had complete recovery within several weeks. However, a two year follow-up study of 14 individuals showed that 10 still had symptoms of some severity 7 to 23 months after initial onset including food intolerance, gastrointestinal disturbances, fatigue, and visual disturbances (Kopelman, 1968).

Human effects other than hepatotoxicity have been described including several cases of contact dermatitis and skin sensitization (LeVine, 1983; Van Joost *et al.*, 1987; de Pablo *et al.*, 1992;

Bruynzeel and van der Wegen-Keijser, 1993). A case report of a man exposed to MDA with potassium carbonate and γ -butyrolactone by accidental ingestion has been described (Roy *et al.*, 1985). In addition to hepatitis and abnormal liver function, which persisted over 18 months, the patient developed a progressively worsening retinopathy described as a “malfunction of the retinal pigment epithelium” accompanied by diminished visual acuity. The patient improved after approximately 3 months, but after examination at 18 months had not completely recovered.

Another report described the development of acute cardiomyopathy in addition to hepatitis in a worker exposed to a large quantity of MDA dust as the result of air filtration malfunction (Brooks *et al.*, 1979). The patient showed an abnormal ECG and an elevated cardiac LDH isoenzyme profile, which returned to normal within one month of onset.

V. Effects of Animal Exposure

The carcinogenicity of MDA was investigated in F344/N rats and B6C3F₁ mice (50/sex/dose group) administered in the drinking water at concentrations of 0, 150, and 300 ppm MDA (dihydrochloride) for 103 weeks (Lamb *et al.*, 1986). A 14-day range finding study was also conducted with 5 animal/sex/species/dose group, with exposure levels of 0, 200, 400, 800, 1600, and 3200 ppm MDA. A 13-week subchronic study was conducted with 10 animals/sex/species/dose group and exposure levels of 0, 25 (mice), 50, 100, 200, 400, and 800 (rats) ppm MDA. Using body weight and drinking water values from the study, low and high daily doses in the chronic study were calculated to be 9 and 16 mg/kg-day for male rats, 10 and 19 for female rats, 25 and 57 for male mice, and 19 and 43 for female mice. In the chronic study, survival was reduced among male mice treated with 300 ppm MDA. Final mean body weights were reduced in the 300 ppm dose group of female rats (-9%), male mice (-13%), and female mice (-16%). Among rats, non-cancer effects included follicular cysts and follicular-cell hyperplasia of the thyroid (significantly increased incidence in high-dose females; $p < 0.05$ by Fisher's exact test). In the liver, the incidence of fatty and focal cellular change was elevated in low-dose male and female rats and also in high dose male rats. Incidence of unspecified dilatation of the liver was also elevated in high-dose male rats. Increased incidence of kidney mineralization was found in male rats treated with 300 ppm MDA. Among mice, incidence of liver degeneration was elevated in males in both treatment groups and females in the high-dose group ($p < 0.01$ by Fisher's exact test). Incidence of kidney nephropathy was increased in male and female mice in both treatment groups and mineralization of the renal papilla was increased in both sexes in the high-dose group ($p < 0.01$). From the 13-week study, the authors noted thyroid and bile duct effects in rats at 800 ppm MDA in water and in mice at 400 ppm MDA in water.

Albino and pigmented guinea pigs were exposed to aerosols of methylene dianiline in polyethylene glycol 200 (PEG) in nose-only exposure chambers (Leong *et al.*, 1987). Animals (8 of each strain) were exposed to a time-weighted average aerosol concentration of 0.44 g MDA/m³ in air for 4 hours/day, 5 days/week for 2 weeks. Eight control animals were neither exposed to aerosol nor placed in the exposure chamber. Two weeks after the exposure period, animals were evaluated for dermal sensitization and irritation by challenge with 0.05 ml of 0, 2, 20, and 200 mg MDA/ml in PEG for up to 24 hours. No evidence of dermal irritation or

sensitivity was found. Subsequently, the animals were also examined for pulmonary sensitization by challenge with aerosols containing 0.01 and 0.05 ml of 200 mg MDA/ml PEG. Lung insufflation pressures were measured as an indication of changes in lung distensibility. No evidence of pulmonary sensitization was found. After the pulmonary challenge, the animals were examined histopathologically, with emphasis on eye, lung, liver, and kidney toxicity. Ocular toxicity ranging from mild to more severe was observed in all MDA-treated animals, but in none of the control animals. Pigmented animals did not differ in sensitivity or effect compared to albino animals. Mild lesions were described as “retraction and thickening of the outer segments of the photoreceptor cells” while more severe effects included swelling “through the inner segments of the photoreceptor cells to the outer nuclear layer.” Some evidence of inflammatory cell infiltration was also noted and the pigmented epithelial layer was also degenerated. The authors conclude that the effects were attributable to MDA because no retinal lesions have been associated with exposure to the PEG vehicle. Furthermore, the inhalation exposures to MDA are the likely cause rather than the dermal and lung sensitization study exposures because these subsequent studies were conducted on control as well as treated animals. Pulmonary granulomas consisting of “an aggregate of macrophages surrounded by a thin mantle of lymphocytes” were found in 7 of the 16 MDA-exposed animals and one of the 8 control animals (level of significance was not stated). Treated and control animals had a high background incidence of pulmonary lesions including slight to mild bronchitis. No liver or kidney effects were detected in treated animals.

Nine purebred beagle dogs were treated orally (by capsule) with 70 mg “crude” (4 dogs) or “purified” (5 dogs) MDA in corn oil three days per week for a period ranging from approximately 3 to 7 years (Deichmann *et al.*, 1978). No concurrent controls were included since untreated animals were regularly maintained in the laboratory. After 2 years, cystoscopic examination was performed at 15-month intervals. After 4½ years, clinical chemistry tests were performed at 4 month intervals on 3 dogs from each group. Microscopic examination of urinary bladder, liver, heart, ovaries, uterus, and lymph nodes was performed on moribund animals or at the end of the experimental period (7 years, 2 months). Liver toxicity was noted in all the treated animals. Effects were described as fatty change, cell degeneration and necrosis, and lymphoid cell infiltration. One dog from each treatment group died from the toxic effect on the liver. The kidneys of four treated animals (two from each group) showed toxic effects including granuloma, glomerular nephritis, and congestion with cloudy swelling. Two dogs treated with “purified” and one dog treated with “crude” MDA showed toxicity to the spleen described as hemosiderosis and swelling with lymphocyte infiltration.

Wistar rats (5/sex/dose) were treated orally with 0, 0.0083, and 0.083 g MDA/kg body weight in propylene glycol daily for 12 weeks (Pludro *et al.*, 1969). Doses were 1% and 10% of the experimentally determined median lethal dose. No significant changes in body weight or hematological parameters were found, although serum albumin, β -globulin, and γ -globulin were elevated in animals in the 0.083 mg/kg dose group. The livers of all the animals in the high dose group showed signs of degeneration, including atrophy of the parenchyma and stromal hyperplasia in the portal areas. Also in this dose group, all animals showed hypertrophy of the lymphatic nodules of the spleen. In the low dose group, one animal showed a liver lesion and one a lesion in the spleen.

Schoental (1968) treated rats (8/sex) with MDA in 25% aqueous ethanol by stomach tube. Rats were given 20 mg doses a total of 2-5 times over several weeks up to 7½ months (frequency not specified). Animals showed necrosis of the liver and kidney and congestion and edema of the lungs.

Visual toxicity was reported in 15 cats treated perorally with 25-100 mg MDA/kg body weight in a 1% aqueous suspension (Schilling von Canstatt *et al.*, 1966). In four animals treated once with 100 mg/kg, no blindness was reported. In all the other treated animals (four with one dose of 100 mg/kg, two with one dose of 150 mg/kg, and two with three doses of 25 mg/kg and 3 doses of 50 mg/kg), blindness occurred within 8 days. Three of the eight recovered sight within 4 days. Two other treated animals were examined microscopically, one treated with 25 and then 50 mg/kg and one treated once with 200 mg/kg. The first was examined after 7 days and showed signs of granular degeneration of the rods and cones with some proliferation of the pigmented epithelium. The second was examined after 4¼ years and showed atrophy of the retinal neuroepithelium. The authors noted that no visual disturbances were found in other MDA treated experimental animals, including dog, rabbit, guinea pig, and rat.

VI. Derivation of Chronic Reference Exposure Level (REL)

<i>Study</i>	Leong <i>et al.</i> , 1987
<i>Study population</i>	Guinea pigs
<i>Exposure method</i>	Discontinuous inhalation exposure (nose only) of aerosols
<i>Critical effects</i>	Degeneration of retinal epithelium
<i>LOAEL</i>	440 mg/m ³ (54 ppm)
<i>NOAEL</i>	Not observed
<i>Exposure continuity</i>	4 hours/day, 5 days/week
<i>Exposure duration</i>	2 weeks
<i>Average experimental exposure</i>	52 mg/m ³ for LOAEL group (440 x 4/24 x 5/7) (6.4 ppm)
<i>Human equivalent concentration</i>	52 mg/m ³ using the default assumption of RGDR = 1 for a gas with systemic effects
<i>LOAEL uncertainty factor</i>	10 (incidence = 100%)
<i>Subchronic uncertainty factor</i>	10
<i>Interspecies uncertainty factor</i>	3
<i>Intraspecies uncertainty factor</i>	10
<i>Cumulative uncertainty factor</i>	3,000
<i>Inhalation reference exposure level</i>	0.02 mg/m ³ (20 µg/m ³ ; 0.002 ppm; 2 ppb)

Two specific types of toxicity have been associated with exposure to MDA: hepatotoxicity and ocular toxicity. Several studies have demonstrated hepatotoxicity in experimental animals. The best study of long term toxicity of MDA was the report by Lamb *et al.* (1986). In addition to addressing the carcinogenicity of MDA, Lamb described non-cancer health effects, which resulted from lifetime exposure of two species, rats and mice, to MDA at two concentrations in the drinking water. The 150 ppm dose level was a LOAEL for fatty change and focal cellular

change to the livers of male and female rats as well as for liver degeneration in male mice. The corresponding effects were also observed in high-dose male rats and male mice. Nephropathy was observed in mice of both sexes at the 150 and 300 ppm. There is abundant evidence from both human and animal studies that MDA is hepatotoxic. Bastian (1984), Williams *et al.* (1974), and McGill and Motto (1974) reported hepatitis in people exposed by inhalation and dermal absorption routes. Kopelman *et al.* (1966a,b) demonstrated human hepatotoxicity from exposure by the oral route. However, limited data detailing exposure levels associated with adverse health effects in humans preclude the development of a chronic REL from studies in humans.

The other toxic effect of potential concern from MDA exposure is ocular toxicity. Leong *et al.* (1987) reported damage to the retinas of guinea pigs exposed for 2 weeks to MDA aerosols (0.44 g/m³ for 4 hr/day, 5 days/week; average experimental exposure = 52 mg/m³) by inhalation. Schilling von Canstatt *et al.* (1966) also reported blindness in cats treated orally with MDA. A single case of retinopathy and visual toxicity in humans was reported in a man who accidentally ingested MDA with potassium carbonate and γ -butyrolactone. The Leong *et al.* (1986) study was selected for the development of the chronic REL because, although conducted for a relatively short period of time, the study appears to address the most sensitive endpoint of toxicity by the most appropriate route of exposure (inhalation). The studies, which established the hepatotoxicity of MDA, were conducted by the oral route of exposure.

As a comparison with the proposed REL, the study by Lamb *et al.* (1986) found a LOAEL of 9 mg/kg-day for liver changes in male rats. Use of a LOAEL UF of 3, an interspecies UF of 10, and an intraspecies UF of 10 results in an oral chronic REL of 0.03 mg/kg-day. Use of route-to-route extrapolation with the assumption that a 70 kg person breathes 20 m³ of air per day leads to an inhalation chronic REL estimate of 100 μ g/m³. The proposed chronic REL based on Leong *et al.* (1987) is lower by a factor of 5 than that obtained by using Lamb *et al.* (1986) and should be protective of hepatotoxicity.

VII. Data Strengths and Limitations for Development of the REL

The strengths of the inhalation REL for 4,4'-methylene dianiline include the availability of a controlled exposure inhalation study. Major areas of uncertainty are the lack of adequate human exposure data, the lack of chronic inhalation exposure studies, the lack of reproductive and developmental toxicity studies, and the lack of observation of a NOAEL. In addition the test animals were under additional stress due to the restraint used to obtain nose-only exposure, while the control animals were not restrained. Liver toxicity has been included as a potential critical effect because of uncertainty regarding the relative potency of this compound in causing liver toxicity in different species by different routes of exposure.

When assessing the health effects of methylene dianiline, its carcinogenicity must also be assessed.

VIII. Potential for Differential Impacts on Children's Health

No evidence to support a differential effect of methylene dianiline on infants and children was found in the literature.

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CHRONIC TOXICITY SUMMARY

METHYLENE DIPHENYL ISOCYANATE*(diphenylmethane diisocyanate)***CAS Registry Number: 101-68-8****I. Chronic Reference Exposure Level**

<i>Inhalation reference exposure level</i>	0.7 µg/m³
<i>Critical effect(s)</i>	Hyperplasia of the olfactory epithelium in rats
<i>Hazard index target(s)</i>	Respiratory system

II. Physical and Chemical Properties (HSDB, 1995)

<i>Description</i>	Light yellow solid
<i>Molecular formula</i>	C ₁₅ H ₁₀ N ₂ O ₂ (monomer)
<i>Molecular weight</i>	Variable (monomer = 250.27 g/mol)
<i>Density</i>	1.197 g/cm ³ @ 70°C (monomer)
<i>Boiling point</i>	196°C (monomer)
<i>Melting point</i>	37°C (monomer)
<i>Vapor pressure</i>	0.001 torr @ 40°C (monomer)
<i>Solubility</i>	Soluble in acetone, benzene, kerosene, and nitrobenzene (monomer)
<i>Conversion factor</i>	Monomer: 1 ppm = 10.2 mg/m ³ at 25°C; Not applicable for polymer

III. Major Uses or Sources

Methylene diphenyl isocyanate (MDI) is used for bonding rubber to nylon. MDI is also used in the manufacture of lacquer coatings and in the production of polyurethane resins and spandex fibers (HSDB, 1995). It is often handled in a partially polymerized form ("MDI polymer"), which has a much lower vapor pressure than the monomer. The annual statewide industrial emissions from facilities reporting under the Air Toxics Hot Spots Act in California based on the most recent inventory were estimated to be 30,398 pounds of MDI (CARB, 2000).

IV. Effects of Human Exposure

A 5-year occupational study of 107 workers from a polyurethane plastic manufacturing plant examined pulmonary function, respiratory symptoms, and smoking habits (Musk *et al.*, 1982, 1985). No significant changes in pulmonary function or respiratory symptoms were observed

when controlled for smoking. Mean MDI concentrations measured ranged from 0.0003 to 0.0006 ppm.

Significantly increased prevalence of asthma in female workers and of chronic bronchitis in male and female workers was observed following occupational exposure to low levels of MDI (<0.02 ppm) (Pham *et al.*, 1988). Workers from two plants were grouped by job classification and evaluated in this study conducted in 1976; workers were grouped as unexposed (62 men, 21 women), indirectly exposed (61 men, 56 women), or directly exposed (91 men, 27 women). Further characterization of the exposure groups was not presented. Decrements in pulmonary function (measured by VC, FEV₁ and single-breath carbon monoxide diffusion tests) were observed in men in the direct and indirect exposure groups: decrements in men with a history of direct exposure to MDI were statistically significant. Workers were also grouped by duration of occupational exposure (<20 months, 20-60 months, >60 months). Workers with known (direct or indirect) occupational exposure to MDI for greater than 60 months exhibited statistically significant decrements in pulmonary function tests. The follow-up examination of this study describes data from male workers only. At the time of the 5-year follow-up, air levels had been reduced to below the maximum allowed air concentration of 0.005 ppm by a modification of the ventilation system. Statistically significant decrements in pulmonary function were observed again in workers with known direct occupational exposure to MDI. Workers who were exposed at the time of the 1976 study but had since been removed from exposure did not exhibit decrements in pulmonary function, leading the authors to conclude that the effects of low-level exposure to MDI are to some extent reversible. Flaws in study design, including lack of exposure characterization, attrition, and inclusion of asthmatics in cohorts, preclude a quantitative assessment of MDI exposure on lung function.

An epidemiologic study of foundry workers reported more respiratory symptoms and significantly lower mean FEV₁ and maximum mid-expiratory flow at 25-75% in exposed workers compared to controls (Johnson *et al.*, 1985). However, MDI-exposed workers also had unquantified exposure to silica, metal dust, phenol formaldehyde, and a pyridine derivative precluding the evaluation of respiratory effects resulting from MDI exposure.

A worker with 5 years occupational exposure and suspected MDI hypersensitivity was exposed continuously in a controlled chamber to 5 ppb for 15 minutes, then 10 ppb for 30 minutes, and 20 ppb for 15 minutes (Marczynski *et al.*, 1992). The worker had not been exposed to MDI in the workplace for 5 days prior to the test challenge. Exposure to MDI resulted in an immediate, moderate, asthmatic reaction associated with significant hypoxemia.

IgG antibodies recognizing MDI-human serum albumin conjugates were detected in 4 of 5 MDI-exposed workers (Aul *et al.*, 1999). The levels of specific IgG antibodies were more elevated with polymeric MDI compared with monomeric MDI.

A workplace death of a 39-year-old foundry worker was ascribed to occupational asthma induced by MDI exposure (Carnio *et al.*, 1997). Postmortem pulmonary findings included epithelial desquamation, mucosal eosinophilic/neutrophilic infiltration, bronchial vessel dilatation, and edema and hypertrophy of smooth muscle.

V. Effects of Animal Exposure

Rats were exposed to 0.2, 1.0, and 6.0 mg/m³ aerosolized MDI polymer 6 hours per day, 5 days per week for 24 months (Reuzel *et al.*, 1990; 1994). Statistically significant increased incidences of basal cell hyperplasia, olfactory epithelial degeneration, alveolar duct epithelialization, localized alveolar bronchiolization, and adenomas were observed in male and female rats exposed to 6.0 mg/m³ MDI. An accumulation of macrophages with yellow pigment was also noted in the lungs and mediastinal lymph nodes. Male rats exposed to this concentration also exhibited a statistically significant increase in the incidence of Bowman's gland hyperplasia. Male rats exposed to 1 mg/m³ MDI also exhibited statistically significant increased incidences of basal cell hyperplasia and Bowman's gland hyperplasia. An accumulation of macrophages with yellow pigment was observed in the lungs of female rats and the lungs and mediastinal lymph nodes of male rats exposed to 1 mg/m³. No adverse effects were noted in rats exposed to 0.2 mg/m³ MDI.

Hyperplasia of the olfactory epithelium with MDI exposure (Reuzel *et al.*, 1990; 1994)

Concentration (mg/m ³)	Males			Females			Combined		
	Responders	N	Incidence	Responders	N	Incidence	Responders	N	Incidence
0	14	60	0.23	4	60	0.067	18	120	0.15
0.2	13	60	0.22	8	60	0.13	21	120	0.18
1	26	60	0.43	8	60	0.13	34	120	0.28
6	32	60	0.53	49	59	0.83	81	119	0.68

Guinea pigs were exposed to 2 ppm MDI 3 hours per day for 5 days (Aizicovici *et al.*, 1990). Qualitative immunostaining techniques indicated that MDI was localized in the respiratory tract. The spleen, lymph nodes, and thymus had very little staining. However, another study exposed guinea pigs to 4 ppb radiolabelled toluene diisocyanate (TDI) for 1-hour and found measurable radioactivity in extrathoracic tissues and body fluids (Kennedy *et al.*, 1989). Therefore, there is a possibility that MDI may be transported to sites other than the respiratory tract, such as the ovaries and testes, following inhalation exposure.

Gravid Wistar rats, Crl:(WI)BR, were exposed by whole-body inhalation to clean air (control) and to 1, 3, and 9 mg/m³ MDI, respectively, for 6 hr per day from days 6 to 15 post conception (Buschmann *et al.*, 1996). Rats were killed on day 20. The lung weights in the high-dose group were significantly increased compared to the sham-treated control animals. Treatment did not influence any other maternal and/or fetal parameters investigated (including maternal weight gain, number of corpora lutea, implantation sites, pre- and postimplantation loss, fetal and placental weights, gross and visceral anomalies, and degree of ossification). A slight but

significant increase in litters with fetuses displaying asymmetric sternebra(e) was observed after treatment with the highest dose. Although the relevance of an increase of this minor anomaly in doses which maternal toxicity is limited and within the limits of biological variability, a substance-induced effect in the high-dose group cannot be excluded with certainty. Thus, the authors reported a NOAEL of 3 mg/m³ for embryotoxic effects.

VI. Derivation of Chronic Reference Exposure Level (REL)

<i>Study</i>	Reuzel <i>et al.</i> , 1990; 1994
<i>Study population</i>	Rats
<i>Exposure method</i>	Inhalation of polymeric aerosolized MDI (0, 0.2, 1.0, and 6.0 mg/m ³)
<i>Critical effects</i>	Hyperplasia of the olfactory epithelium
<i>LOAEL</i>	1 mg/m ³
<i>NOAEL</i>	0.2 mg/m ³
<i>Benchmark Concentration (BMC₀₅)</i>	0.25 mg/m ³ (95% lower confidence limit on concentration for a 5% incidence of response based on analysis of the combined male and female data with a linear model, the best-fitting of 6 models examined, p = 0.99)
<i>Study continuity</i>	6 hours per day, 5 days per week
<i>Study duration</i>	24 months
<i>Average experimental exposure</i>	0.046 mg/m ³ for BMC ₀₅ group (0.25 x 6/24 x 5/7)
<i>Human equivalent concentration</i>	0.020 mg/m ³ for BMC ₀₅ group (particle with extrathoracic respiratory effects, RDDR = 0.453, based on MMAD = 0.68 µm and sigma g = 2.93)
<i>LOAEL uncertainty factor</i>	1
<i>Subchronic uncertainty factor</i>	1
<i>Interspecies uncertainty factor</i>	3
<i>Intraspecies uncertainty factor</i>	10
<i>Cumulative uncertainty factor</i>	30
<i>Inhalation reference concentration</i>	0.7 µg/m ³

The data of Reuzel *et al.* (1990, 1994) were examined with six quantal dose-response models (linear, log-normal, Weibull, logistic, quadratic, gamma) using USEPA BMDS 1.2. All models except the quadratic gave a good fit to the combined male and female data set. The linear model was selected as the best-fitting model. Possible differences between male and female susceptibility are suggested by the gender-specific data, although the significance of these differences is uncertain.

USEPA used the same two studies and a BMC₁₀ approach to develop an RfC of 0.6 µg/m³. Since USEPA used a 3-fold database uncertainty factor, their BMC₁₀-based RfC is comparable to the BMC₀₅-based OEHHA REL.

VII. Data Strengths and Limitations for Development of the REL

Strengths of the REL for MDI include the use of a well-conducted, long-term inhalation study, the observation of a NOAEL, and the estimation of a benchmark concentration. A limitation of the REL is that it is based on data on exposures to MDI “polymer” which actually contains nearly 50% monomer. Monomers may in some cases be more toxic than polymers. Thus, effects of pure monomeric MDI may occur at concentrations somewhat lower than observed in the reported study on MDI polymer. However, the capacity of MDI polymer to induce immunologic sensitization is greater than that of MDI monomer (Aul *et al.*, 1999). The relative potential of MDI monomer and polymer to induce hyperplasia of the olfactory epithelium is unknown.

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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

<i>Inhalation reference exposure level</i>	9 µg/m³ (2 ppb)
<i>Critical effect(s)</i>	Respiratory effects (nasal inflammation, olfactory epithelial metaplasia, respiratory epithelial hyperplasia) in mice
<i>Hazard index target(s)</i>	Respiratory system, blood systems

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

<i>Description</i>	White crystalline powder; odor of mothballs
<i>Molecular formula</i>	C ₁₀ H ₈
<i>Molecular weight</i>	128.6 g/mol
<i>Density</i>	4.42 g/cm ³ @ 20°C
<i>Boiling point</i>	218°C
<i>Melting point</i>	80.5 °C
<i>Vapor pressure</i>	0.078 Torr @ 25°C (Sonnenfeld <i>et al.</i> , 1983); 0.10 Torr @ 27°C (CRC, 1994)
<i>Conversion factor</i>	5.26 µg/m ³ per ppb at 25°C

III. Major Uses or Sources

Naphthalene is a natural constituent of coal tar (approximately 11%) (HSDB, 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 ($105 \mu\text{g}/\text{m}^3$). 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).

	<i>0 ppm</i>	<i>10 ppm</i>	<i>30 ppm</i>
<i>Nasal inflammation</i>	3/139	34/134	108/270
<i>Olfactory epithelial metaplasia</i>	0/139	131/134	269/270
<i>Respiratory epithelial hyperplasia</i>	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 through 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)

<i>Study</i>	NTP (1992)
<i>Study population</i>	B6C3F1 mice (75 or 150/group/sex)
<i>Exposure method</i>	Discontinuous whole-body inhalation exposures to 0, 10, or 30 ppm naphthalene vapor
<i>Critical effects</i>	Nasal inflammation, olfactory epithelial metaplasia, and respiratory epithelial hyperplasia
<i>LOAEL</i>	10 ppm (96% incidence for males and 100% incidence for females)
<i>NOAEL</i>	Not observed
<i>Exposure continuity</i>	6 hours/day for 5 days/week
<i>Average experimental exposure</i>	1.8 ppm (10 ppm x 6/24 x 5/7) for LOAEL group
<i>Exposure duration</i>	104 weeks
<i>Subchronic uncertainty factor</i>	1
<i>LOAEL uncertainty factor</i>	10
<i>Interspecies uncertainty factor</i>	10 (see below)
<i>Intraspecies uncertainty factor</i>	10
<i>Cumulative uncertainty factor</i>	1000
<i>Inhalation reference exposure level</i>	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/m³ 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

NICKEL AND NICKEL COMPOUNDS: NICKEL OXIDE

<i>Molecular Formula</i>	<i>Molecular Weight</i>	<i>Synonyms</i>	<i>CAS Registry Number</i>
Ni	59	elemental nickel	7440-02-0
NiO	74.69	nickel oxide	1313-99-1
NiCl ₂	129.6	nickel chloride nickel dichloride	7718-54-9
NiSO ₄	154.75	nickel sulfate nickelous sulfate	7786-81-4
NiCO ₃	118.7	nickel carbonate carbonic acid nickel salt	3333-67-3
Ni ₃ S ₂	240.19	nickel subsulfide trinickel disulfide heazlewoodite	12035-72-2

I. Chronic Toxicity Summary

A. Nickel and Nickel Compounds (except nickel oxide)

<i>Inhalation reference exposure level</i>	0.05 µg Ni/m³
<i>Critical effect(s)</i>	Lung, nasal epithelial and lymphatic pathology in male and female rats
<i>Hazard index target(s)</i>	Respiratory system; hematopoietic system

B. Nickel Oxide

<i>Inhalation reference exposure level</i>	0.10 µg Ni/m³
<i>Critical effect(s)</i>	Lung and lymphatic pathology in male and female rats
<i>Hazard index target(s)</i>	Respiratory system; hematopoietic system

II. Physical and Chemical Properties (from HSDB, 1995)

<i>Description</i>	Ni metal: Silvery metal; NiCl ₂ : deliquescent crystals (U.S.EPA, 1985)
<i>Molecular formula</i>	See above
<i>Molecular weight</i>	See above
<i>Density</i>	8.9 g/cm ³ @ 20°C (Ni)
<i>Boiling point</i>	2730°C (Ni)
<i>Vapor pressure</i>	Not applicable
<i>Solubility</i>	Elemental nickel, nickel subsulfide, and nickel oxide are insoluble in water, but are soluble in dilute nitric, hydrochloric, and sulfuric acids. The chloride and sulfate forms of nickel are water soluble.
<i>Conversion factor</i>	Not applicable for fumes and dusts

III. Major Uses and Sources

The most common airborne exposures to nickel compounds are to insoluble nickel compounds such as elemental nickel, nickel sulfide, and the nickel oxides from dusts and fumes. Contributions to nickel in the ambient air are made by combustion of fossil fuels, nickel plating, and other metallurgical processes. The most common oxidation state of nickel is the divalent (Ni²⁺) form (U.S.EPA, 1985). Elemental nickel is a malleable, silvery-white metal that is highly resistant to strong alkali. Because of its corrosion resistance, nickel is used in the production of stainless steel, permanent magnets, and other alloys that require resistance to extremes of temperature or stress (U.S.EPA, 1985). Nickel is also used in electroplating baths, batteries, textile dyes, and catalysts (U.S.EPA, 1985). Nickel dust or powder is flammable (CDTSC, 1985). Due to its unique toxicological and physico-chemical properties, nickel carbonyl is not included in this summary. The annual statewide industrial emissions from facilities reporting under the Air Toxics Hot Spots Act in California, based on the most recent inventory, were estimated to be 110,334 pounds of nickel (CARB, 1999).

IV. Effects of Human Exposure

Several studies have indicated that occupational inhalation exposure to nickel aerosols can result in development of asthma specific to nickel. Davies (1986) found 3 cases of asthma among 53 nickel-plating workers without a history of asthma prior to employment. Novey *et al.* (1983) described biphasic metal-specific bronchial responses in an individual metal-plating worker exposed to nickel and chromium salts. In another case, immunological studies conducted in a 24-year old man showed nickel-specific antibodies in the serum after several weeks of working in a nickel-plating shop using nickel sulfate (McConnell *et al.*, 1973). Dermatitis was observed on exposed areas of his skin, and pulmonary function, measured by FEV₁ with and without isoproterenol challenge, was significantly impaired compared with a control subject and normal

values. Dyspnea, non-productive cough, chest-tightness, and wheezing were reported as symptoms by this worker during the work period.

A group of 7 metal plating workers with occupational asthma were evaluated for atopy and pulmonary function challenge in response to inhalational challenge with nickel and other metals (Cirla *et al.*, 1985). Three of the asthmatics tested positive for the presence of nickel-specific IgE antibodies. Positive reactions to skin testing with nickel were found in 3 of the asthmatic workers who also had dermatitis. Six out of the 7 asthmatics exhibited significantly decreased FEV₁ (> 15%) when exposed to 0.3 mg/m³ nickel sulfate for 30 minutes. Control challenges with other metal salts did not reveal similar deficits in FEV₁.

Although asthma has been described in the above studies, occupational inhalation of nickel dusts has not been found to be associated with pulmonary fibrosis (Muir *et al.*, 1993). An occupational epidemiology report by Broder *et al.* (1989) found no significant effects on pulmonary function in relation to nickel exposure in a nickel smelter, however a healthy worker effect was observed in this study.

V. Effects of Animal Exposure

Early studies on the chronic non-cancer effects of metallic nickel dust were complicated by early mortality and cancer in guinea pigs and rats (Hueper, 1958).

A 2-year inhalation study of nickel oxide in rats and mice (65 per sex, per group) was conducted by the National Toxicology Program (NTP, 1994a). In the first study, rats were exposed to 0, 0.62, 1.25, or 2.5 mg nickel oxide/m³ (0, 0.5, 1.0, or 2.0 mg Ni/m³) 6 hours/day, 5 days/week for 104 weeks. In addition to the carcinogenic effects of nickel oxide, a number of non-cancerous lesions were observed, particularly in the lungs. The incidence of inflammatory pigmentation in the alveoli was significantly greater in all exposed groups, compared to controls. The severity of the lesions reportedly increased with increasing exposure. Atypical alveolar hyperplasia was also seen in all exposed groups. Lymphoid hyperplasia in the bronchial lymph nodes was observed in males and females exposed to 1 mg Ni/m³ or greater at 7 and 15 months and the incidence generally increased with increasing concentration at the end of the 2-year study. Females had an increased incidence of adrenal medullary hyperplasia at all exposures of nickel oxide. Body weights were significantly lower in the groups exposed to 2.0 mg Ni/m³ for both sexes, and in males exposed to 1.0 mg Ni/m³.

A companion study on nickel oxide in mice conducted by NTP showed similar lung inflammatory changes as seen in the rats, in addition to pigmentation of the alveolar region at all exposure concentrations, compared with controls (NTP, 1994a). The mice were exposed to 0, 1.0, 2.0, or 3.9 mg Ni/m³. Bronchial lymph-node hyperplasia was also evident in all nickel-exposed animals. Body weights were slightly but significantly lower in the 3.9 mg Ni/m³ group, compared with controls.

A continuous exposure of rats (20 - 40 per group) to 0, 60, or 200 µg Ni/m³ as nickel oxide for 2 years resulted in severe pulmonary damage and premature mortality so that carcinogenesis could not be evaluated (Glaser *et al.*, 1986). Pulmonary alveolar proteinosis and septal fibrosis were

observed in the animals exposed to nickel. Only 1 rat per group survived the nickel exposures to the end of the experiment.

A 2-year study on the effects of nickel subsulfide in rats and mice was conducted by NTP (1994b). Rats (52-53 per sex per group) were exposed to 0, 0.15, or 1 mg $\text{Ni}_3\text{S}_2/\text{m}^3$ (0, 0.11, or 0.73 mg Ni/m^3) for 6 hours/day, 5 days/week for 104 weeks. Body weights were lowered in rats exposed to 0.73 mg Ni/m^3 compared with controls. Lung inflammation, alveolar hyperplasia, macrophage hyperplasia, and pulmonary fibrosis were observed with a significantly increased incidence at both nickel concentrations. Female rats exposed to nickel had significantly increased adrenal medullary hyperplasia. In addition to the pulmonary lesions, nasal inflammation and olfactory epithelial atrophy was observed in both sexes exposed to 0.73 mg Ni/m^3 .

In the second phase of the NTP study (NTP, 1994b), mice were exposed to 0, 0.6, or 1.2 mg $\text{Ni}_3\text{S}_2/\text{m}^3$ (0, 0.44, or 0.88 mg Ni/m^3) for 6 hours/day, 5 days/week for 104 weeks. The same pathological lesions were observed in the lung and nasal passages as in the rats in the above study. These lesions were evident at both the 0.44 mg Ni/m^3 and the 0.88 mg Ni/m^3 concentrations. The adrenal medullary hyperplasia seen in female rats was not observed in the mice.

An exposure of rats to either 0 or 0.97 mg $\text{Ni}_3\text{S}_2/\text{m}^3$ (0 or 0.71 mg Ni/m^3) for 6 hours/day, 5 days/week for 78-80 weeks resulted in decreased body weight, hyperplasia, metaplasia, and neoplasia in the lungs due to Ni (Ottolenghi *et al.*, 1974).

The NTP (1994c) studied the chronic non-cancer and carcinogenic effects of nickel sulfate hexahydrate on rats and mice. Rats were exposed to 0, 0.12, 0.25, or 0.5 mg NiSO_4/m^3 (0, 0.03, 0.06, or 0.11 mg Ni/m^3) for 6 hours/day, 5 days/week for 104 weeks. Chronic effects of nickel exposure in rats included inflammatory lesions in the lung, lung macrophage hyperplasia, alveolar proteinosis, and fibrosis, in addition to bronchial lymph node hyperplasia and nasal epithelial atrophy. The above effects were seen at exposures of 0.06 mg Ni/m^3 or greater.

Mice were exposed to a similar regimen that included 0, 0.06, 0.11, and 0.22 mg Ni/m^3 as nickel sulfate hexahydrate (NTP, 1994c). Similar pulmonary, lymphatic and nasal changes were observed in the mice as with the rats. Fibrosis was not reported, but an increased incidence of interstitial infiltration and alveolar proteinosis were observed at exposures of 0.11 mg Ni/m^3 or greater. No clinical findings or hematological effects were observed, but body weights were significantly depressed in all groups of nickel-exposed female mice. The body weights of males were reduced only in the group exposed to 0.22 mg Ni/m^3 .

Rats and mice (10 per group) were exposed to nickel sulfate, nickel subsulfide, or nickel oxide 6 hours/day, 5 days/week, for 13 weeks (Dunnick *et al.*, 1989). Exposure-related increases in lung weight and histological lesions were observed in both species for all nickel exposures. Histological lesions included inflammatory changes, fibrosis, and alveolar macrophage hyperplasia. Nasal lesions were also observed in animals treated with nickel sulfate or nickel subsulfide. Lung weight changes were observed at exposures of 0.05 mg Ni/m^3 or greater in female rats. Macrophage hyperplasia in the alveolar region was observed at concentrations as

low as 0.02 mg Ni/m³. Additional inflammatory lesions in the lungs were observed at 0.1 mg Ni/m³.

A similar study by Haley *et al.* (1990) found that exposure of mice to nickel sulfate, nickel subsulfide, or nickel oxide resulted in various immunological effects. Mice were exposed to 0, 0.11, 0.45, or 1.8 mg Ni/m³ as Ni₃S₂; 0.47, 2.0, or 7.9 mg Ni/m³ as NiO; and 0.027, 0.11, and 0.45 mg Ni/m³ as NiSO₄ for 6 hours/day, 5 days/week for 13 weeks. Nickel exposures consistently decreased splenic antibody-forming cell (AFC) responses, with significant decreases occurring at 1.8 mg Ni/m³ as nickel subsulfide. In contrast, AFC responses in the lung-associated lymph nodes were consistently increased, indicating a possible indirect influence of inflammatory mediators released in the lung on local lymph nodes.

Rabbits (8 nickel exposed and 8 controls) exposed to 0.24 mg Ni/m³ as nickel chloride 6 hours/day, 5 days/week for 4 weeks exhibited significantly decreased macrophage lysozyme activity in pulmonary lavage fluid and in macrophage cultures, compared with control animals (Lundborg and Camner, 1984). Similar exposures of rabbits to chlorides of cadmium, cobalt, or copper did not reduce lysozyme activity.

VI. Derivation of Chronic Reference Exposure Level (REL)

A. Nickel and Nickel Compounds (except nickel oxide)

<i>Study</i>	National Toxicology Program, 1994c
<i>Study population</i>	Male and female F344/N rats (52-53 per group)
<i>Exposure method</i>	Discontinuous inhalation
<i>Critical effects</i>	Pathological changes in lung, lymph nodes, and nasal epithelium: (1) active pulmonary inflammation, (2) macrophage hyperplasia, (3) alveolar proteinosis, (4) fibrosis, (5) lymph node hyperplasia, (6) olfactory epithelial atrophy
<i>LOAEL</i>	60 µg Ni/m ³ (as nickel sulfate hexahydrate)
<i>NOAEL</i>	30 µg Ni/m ³
<i>Exposure continuity</i>	6 hours/day, 5 days/week
<i>Exposure duration</i>	104 weeks
<i>Average experimental exposure</i>	5.4 µg Ni/m ³ for NOAEL group (30 x 6/24 x 5/7)
<i>Human equivalent concentration</i>	1.6 µg Ni/m ³ for NOAEL group males (particulate with respiratory effects, RDDR = 0.29 based on MMAD = 2.5, sigma g = 1.26, male rat body weight = 380 g, SA(PU) = 0.34 m ² , DEP(PU) = 0.024)
<i>LOAEL uncertainty factor</i>	1
<i>Subchronic uncertainty factor</i>	1
<i>Interspecies uncertainty factor</i>	3
<i>Intraspecies uncertainty factor</i>	10
<i>Cumulative uncertainty factor</i>	30
<i>Inhalation reference exposure level</i>	0.05 µg Ni/m ³

Nickel Oxide

<i>Study</i>	National Toxicology Program, 1994c
<i>Study population</i>	Male and female F344/N rats (52-53 per group)
<i>Exposure method</i>	Discontinuous inhalation
<i>Critical effects</i>	Pathological changes in lung and lymph nodes: (1) active pulmonary inflammation, (2) lymph node hyperplasia Adrenal medullary hyperplasia (females)
<i>LOAEL</i>	500 $\mu\text{g Ni/m}^3$
<i>NOAEL</i>	Not observed
<i>Exposure continuity</i>	6 hours/day, 5 days/week
<i>Exposure duration</i>	104 weeks
<i>Average experimental exposure</i>	89.5 $\mu\text{g Ni/m}^3$ for LOAEL group (500 x 6/24 x 5/7)
<i>Human equivalent concentration</i>	30 $\mu\text{g Ni/m}^3$ for LOAEL group males (particulate with respiratory effects, RDDR = 0.29 based on MMAD = 2.5, sigma g = 1.26, male rat body weight = 380 g, SA(PU) = 0.34 m^2 , DEP(PU) = 0.024)
<i>LOAEL uncertainty factor</i>	10
<i>Subchronic uncertainty factor</i>	1
<i>Interspecies uncertainty factor</i>	3
<i>Intraspecies uncertainty factor</i>	10
<i>Cumulative uncertainty factor</i>	300
<i>Inhalation reference exposure level</i>	0.10 $\mu\text{g Ni/m}^3$

The studies conducted by NTP (1994 a,b, & c) all showed similar non-carcinogenic effects in rats and mice, regardless of the form of nickel administered. It therefore appears that soluble and insoluble forms of nickel cause similar effects in rodents. The human epidemiological literature predominantly describes cancer mortality rates from occupational exposures to nickel compounds, but does not specifically examine non-cancer effects. However, it is clear from many case reports that allergies and dermatitis can occur in exposed workers. Hypersensitive reactions to nickel have not been quantitatively studied in humans or animals, therefore it is not possible to develop an REL based on immunological hypersensitivity at the present time. A host of subacute and subchronic animal studies have shown nickel to affect certain immunological responses unrelated to hypersensitivity, but the applicability of these results to chronic human exposures and responses involves considerable uncertainty. Furthermore, data show that nickel may precipitate onset of asthma in occupational settings.

The results of the NTP studies and these dose response analyses support the speciation of nickel oxide for noncancer effects. The health effects data for nickel oxide indicate that its adverse pulmonary effects were less severe (absence of fibrosis, lower chronic lung inflammation severity scores) at higher doses than the pulmonary effects observed for nickel sulfate and nickel

sub sulfide. The higher chronic REL value for nickel oxide of $0.1 \mu\text{g}/\text{m}^3$ reflects these dose response differences. Furthermore, while it is based upon a LOAEL, the lower severity of the adverse health effects at the LOAEL mitigates some of the uncertainty associated with use of a LOAEL rather than a NOAEL. OEHHA therefore concludes that $0.1 \mu\text{g}/\text{m}^3$ is an appropriate REL for nickel oxide. However, in setting inhalation exposure RELs for groups of compounds, OEHHA uses the most sensitive strain, species, sex, chronic endpoint, and agent for each group of substances. Therefore, as the pulmonary toxicity of the relatively insoluble nickel subsulfide is greater than that of nickel oxide and closer to that of nickel sulfate, OEHHA proposes to use the chronic REL derived from nickel sulfate for all other nickel compounds.

VII. Data Strengths and Limitations for Development of the REL

The strengths of the inhalation REL include the availability of controlled lifetime exposure inhalation studies in multiple species at multiple exposure concentrations and with adequate histopathological analysis and the observation of a NOAEL. The major areas of uncertainty are the lack of adequate human exposure data and the lack of lifetime toxicity studies in any non-rodent species.

In addition to being inhaled, airborne nickel can settle onto crops and soil and enter the body by ingestion. Thus an oral chronic reference exposure level for nickel is also required.

Derivation of Oral Chronic Reference Exposure Level

<i>Study</i>	Ambrose <i>et al.</i> , 1976
<i>Study population</i>	Rats
<i>Exposure method</i>	Diet
<i>Critical effects</i>	Decreased body and organ weights
<i>LOAEL</i>	1000 ppm (50 mg/kg-day)
<i>NOAEL</i>	100 ppm (5 mg/kg-day)
<i>Exposure continuity</i>	Continuous
<i>Exposure duration</i>	Lifetime
<i>Average exposure</i>	5 mg/kg-day
<i>Human equivalent concentration</i>	5 mg/kg-day
<i>LOAEL uncertainty factor</i>	1
<i>Subchronic uncertainty factor</i>	1
<i>Interspecies uncertainty factor</i>	10
<i>Intraspecies uncertainty factor</i>	10
<i>Cumulative uncertainty factor</i>	300
<i>Oral reference exposure level</i>	0.05 mg/kg-day

The oral REL for nickel used the same study used for the U.S. EPA's oral Reference Dose (RfD). U.S. EPA assumed that rat consumption of 1 ppm Ni in the feed resulted in a dose of 0.05 mg/kg/day. An uncertainty factor of 10 was used for interspecies extrapolation and another of 10 to protect sensitive human populations. An additional uncertainty factor of 3 was used by U.S. EPA to account for inadequacies in reproductive studies of nickel. OEHHA has not used such special uncertainty or modifying factor because the criteria for their use are not well presented.

In addition there is an extensive toxicologic database on nickel in general which includes studies on reproductive effects.

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CHRONIC TOXICITY SUMMARY

PHENOL*(Carbolic acid, phenylic acid, phenyl hydroxide)***CAS Registry Number: 108-95-2****I. Chronic Toxicity Summary**

<i>Inhalation reference exposure level</i>	200 µg/m³ (50 ppb)
<i>Critical effect(s)</i>	Twitching, muscle tremors, neurological impairment; elevated serum liver enzymes in rats
<i>Hazard index target(s)</i>	Alimentary system; circulatory system; kidney; nervous system

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

<i>Description</i>	Colorless to light pink solid
<i>Molecular formula</i>	C ₆ H ₅ OH
<i>Molecular weight</i>	94.11 g/mol
<i>Density</i>	1.0576 g/cm ³ @ 20° C
<i>Boiling point</i>	181.75° C
<i>Melting point</i>	40.9° C
<i>Vapor pressure</i>	0.3513 torr @ 25° C
<i>Odor threshold</i>	40 ppb (150 µg/m ³) (Amoore and Hautala, 1983)
<i>Solubility</i>	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
<i>Henry's Law Constant</i>	3.97 x 10 ⁻⁷ ATM-m ³ /mol (25 °C)
<i>Conversion factor</i>	1 ppm = 3.85 mg/m ³

III. Major Uses or Sources (HSDB, 1995)

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

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

IV. Effects of Human Exposures

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

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

Occupational studies make up the majority of subchronic/chronic studies available on human health effects associated with phenol exposure. Merliss (1972) described muscle pain and weakness of unknown etiology, enlarged liver, and elevated serum enzymes (LDH, GOT, and GPT) characteristic of liver damage in an individual with intermittent inhalation and dermal exposures to phenol, cresol and xylene. 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 $\mu\text{g}/\text{m}^3$ of formaldehyde and 800 $\mu\text{g}/\text{m}^3$ 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/m³ (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)

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

PHOSPHINE*(hydrogen phosphide; phosphorus trihydride; Celphos; Phostoxin)***CAS Registry Number: 7803-51-2****I. Chronic Toxicity Summary**

<i>Inhalation reference exposure level</i>	0.8 µg/m³ (0.6 ppb)
<i>Critical effect(s)</i>	Decreased body weight gain in mice
<i>Hazard index target(s)</i>	Respiratory system; alimentary system; nervous system; kidney; hematopoietic system

II. Chemical Property Summary (HSDB, 1995, except as noted)

<i>Description</i>	Colorless gas
<i>Molecular formula</i>	PH ₃
<i>Molecular weight</i>	34 g/mol
<i>Vapor density</i>	1.17 (air = 1)
<i>Boiling point</i>	-87.7°C
<i>Vapor pressure</i>	20 atm @ -3°C (Weast, 1980)
<i>Solubility</i>	0.26 volumes in water @ 20°C; soluble in alcohol, ether (Sax and Lewis, 1989)
<i>Conversion factor</i>	1.39 mg/m ³ per ppm at 25°C

III. Major Uses and Sources

Phosphine is used as an agricultural fumigant against insects and is among the most acutely toxic of the fumigant gases (HSDB, 1995). In its use as a fumigant, application of aluminum, magnesium, or zinc phosphide pellets generates phosphine gas upon exposure to moisture. Because of high volatility, phosphine residue dissipates from treated material upon ventilation. Inadequate sealing of materials during the course of treatment can result in unplanned environmental exposure.

Phosphine is also used by the semiconductor industry as a chemical doping agent for electronic components (n-type semiconductors) (HSDB, 1995). Other minor sources/uses of phosphine are in chemical syntheses: specifically, in preparations of phosphonium halides, for polymerization initiation, and as condensation catalysts. The annual statewide industrial emissions from facilities reporting under the Air Toxics Hot Spots Act in California based on the most recent inventory were estimated to be 3312 pounds of phosphine (CARB, 1999). In 2000, more than 120,000 pounds of phosphide compounds (including 119,519 pounds of aluminum phosphide and 1607 pounds of zinc phosphide) were applied in California agriculture (CDPR, 2001). In the

presence of water these phosphides break down to phosphine. However, the fraction emitted as phosphine is not known.

IV. Effects of Exposures to Humans

Toxicity among 22 workers intermittently exposed to phosphine levels of 0.17-2.11 ppm in air from fumigation activity ranging over 0.5 to 29 years (mean = 11.1 years) was evaluated (Misra *et al.*, 1988). The subjects were interviewed within one day of fumigation activity and reported that symptoms subsided when phosphine was not in use. The most frequently reported symptoms include dyspnea (31.8%), headache (31.8%), chest tightness (27.3%), cough (18.2%), anorexia and epigastric pain (18.2%), finger paresthesia and numbness (13.6%), and giddiness, numbness, and lethargy (13.6%). No change in motor or sensory nerve conduction velocity was found.

A similar spectrum of toxic effects among workers involved in grain storage at a seaboard terminal has been reported (Jones *et al.*, 1964). Among 69 men exposed to breathing zone phosphine levels of 0-35 ppm for as long as 16 hours per day, the authors report symptoms of multiple origins. These include gastrointestinal (diarrhea, nausea, epigastric pain, vomiting), cardio-respiratory (chest tightness, dyspnea, pain in chest, palpitations, retrospinal pain), and central nervous (headache, dizziness, staggering gait) systems. Symptoms were reported to appear only at the time of exposure and apparently were reversible.

In another report of chronic occupational exposure, authors cited the appearance of chronic bronchitis, anemia, and digestive disorders (Eichler, 1934).

Most literature reports of human toxic health effects of phosphine, however, come from case reports of acute exposures. Some are suggestive of potential chronic toxicity endpoints because of the irreversible nature of the effect. In a case report of phosphine poisoning of 29 people exposed by inhalation on a grain freighter, pathological findings included evidence of urinary tract injury (occult blood), liver damage (bilirubinuria and increased SGPT, GGPT, and LDH), and myocardial damage (increased MB fraction of CPK, abnormal ECG) (Wilson *et al.*, 1980). A two-year old child who died as a result of the exposure showed myocardial necrosis with mononuclear infiltrates, pulmonary edema with damaged epithelia, pleural effusion, and an enlarged spleen. In another case report exposure of a 7-months pregnant, 24-year-old woman to aluminum phosphide from a nearby grain storage site was lethal (Garry *et al.*, 1993). There was evidence of severe pulmonary edema, necrosis of individual hepatic cells, and anoxic change in Purkinje cells of the cerebellum. These reported deaths of a small child and a pregnant woman exposed together with individuals who survived exposure to phosphine suggest that there may be sensitive human subpopulations. In another case report of acute phosphine poisoning by inhalation, Schoonbroodt *et al.*, (1992) observed necrosis of the nasal mucosa, delayed onset of pulmonary edema, and myocardial injury. Chopra *et al.* (1986) treated sixteen patients with aluminium phosphide poisoning during 1985. Findings included mucosal necrosis and cardiac abnormalities (due to hypoxemia). Renal failure (1/16), proteinuria (1/16), and increased blood transaminases (2/16) resulted from oral exposure to phosphine. The multi-organ involvement in toxicity suggests that phosphine is a broad-spectrum toxicant.

In a 1994 two deaths and three illnesses were reported due to phosphine fumigation of agricultural products in railroad cars (Perrotta *et al.*, 1994). The annual report for the year 2000 of the American Association of Poison Control Centers' Toxic Exposure Surveillance System does not list fumigants as a separate category of pesticides (Litovitz *et al.*, 2001). However, two of the case reports of fatalities, that were presented in abstract form, were due to aluminum phosphide.

V. Effects of Exposures to Animals

A subchronic inhalation toxicity study of phosphine was conducted in Balb-c mice (Barbosa *et al.*, 1994). Twelve animals/sex/dose group were exposed for 6 hours/day, 5 days/week for 13 weeks to 0, 0.3, 1.0, or 4.5 ppm phosphine. Non-cancer toxicity endpoints included reduction in weight gain and changes in relative organ weights of kidneys, lungs, liver, heart, brain and spleen. In the highest dose group, itching and scratching of the eyes, feet and tail, and decreased overall activity were observed. No diarrhea, loss of equilibrium, convulsions, seizures, or other neurological disturbances were noted. A dose-dependent decrease in total body weight gain was observed at all exposure levels with a greater effect observed in females ($p < 0.0001$). Statistically significant decreases in relative organ weights (kidney, heart, and brain) were observed in males only at the 0.3 ppm exposure level ($p < 0.001$). On the other hand, female mice showed increased relative organ weights (lungs, heart, and spleen) predominantly at higher doses (1.0 and 4.5 ppm; $p < 0.001$). At 4.5 ppm phosphine absolute kidney and spleen weights were significantly increased in females ($p < 0.01$). Increased frequencies of micronuclei in polychromatic erythrocytes from bone marrow and spleen were also seen at 4.5 ppm. This group also conducted a short-term repeated dose experiment. Six mice/sex/group were exposed to 5.5 ppm phosphine for 2 weeks (6 hrs/day, 5 days/wk). No statistically significant changes in weight gain were observed at the end of this exposure period.

In another subchronic inhalation toxicity study, male and female Fischer 344 rats (10/sex/group) were exposed to levels of 0, 0.37, 1.0, and 3.1 ppm phosphine for 6 hours per day, 5 days per week, for 13 weeks (Newton *et al.*, 1993). A higher dose group at 10 ppm was terminated prematurely (at 3 days) because of high mortality. A satellite group exposed to 5.1 ppm for 2 weeks was terminated after 13 days recovery. Observations of overt toxicity and viability were made at the time of each exposure; body weight and food consumption were monitored weekly; ophthalmic examination was done the day before termination; and hematological and clinical chemistry indices were measured after 4 and 13 weeks. Postmortem examination included gross necropsy, with particular attention to orifices, the cranial cavity, surfaces of the brain and spinal cord, nasal cavity and sinuses, the thoracic, abdominal, and pelvic cavities and viscera, and the cervical tissue and organs. Histopathology was performed on 10% buffered formalin-fixed/hematoxylin-eosin-stained tissues. Significant observations after 13 weeks of phosphine exposure included decreased hemoglobin, hematocrit, and erythrocytes in males in the 3.1 ppm dose group. Male rats in the 1 ppm dose group showed decreased weight gain. Increased incidence of small seminal vesicles was noted at 1 and 3.1 ppm, although no histological correlate was observed. Absolute and relative decreases in liver weight were observed in all exposed groups, but there was no evidence that this effect was dose-related. A significant

decrease in serum glutamic pyruvic transaminase (SGPT) was observed at 3.1 ppm, although the authors noted unusually high control levels. None of these effects were observed after the 4 week recovery period. Other effects of a transient nature noted during the exposure include decreased weight gain in female rats at 1 ppm, decreased food consumption at 0.37 ppm in males and females, and increased blood urea nitrogen (BUN) at 3.1 ppm. Observations in the 10 ppm group necropsied after 3 days of exposure included decreased erythrocytes, increased alkaline phosphatase, and increased kidney weight with coagulative necrosis of the tubular epithelium of the outer cortex. In a subchronic study of CD male and female rats under similar conditions (exposure to 0, 0.3, 1, or 3 ppm phosphine 6 h/day, 5 days/week for 13 weeks), no neurotoxicity was observed (Schaefer *et al.*, 1998).

Newton *et al.* (1993) also examined developmental toxicity by exposing 24 pregnant female CD^R rats per group to 0, 0.03, 0.33, 2.8, 4.9, and 7.0 ppm phosphine. The highest dose group was terminated prematurely because of high mortality; all other animals were necropsied after 20 days for evaluation of maternal and fetal toxicity. Maternal toxicity endpoints included weight of ovaries and uteri, number of corpora lutea, pregnancy, and implantation rate. Fetal toxicity was evaluated by weight, number, and location of fetuses and resorptions, visceral malformations and variations, and skeletal changes after alizarin staining. No statistically significant differences from control animals were observed for any parameter at any dose, with the exception of a change in mean number of resorption sites ($p \leq 0.01$), mean resorption/implant ratio ($p \leq 0.05$), and incidence of females with resorption ($p \leq 0.05$), all at 0.03 ppm only. In the absence of this effect at higher dose levels, these observations are not considered useful in establishing a low adverse effect level.

A 35-day phosphine inhalation study was conducted exposing rats continuously to 0, 0.05, 0.2, 1.5, and 8.0 mg/m³ phosphine (0, 0.036, 0.14, 1.1, and 5.8 ppm) in which hematological endpoints and histopathological changes of the lungs and kidneys were examined (Pazynich *et al.*, 1984). Observations include a statistically significant change in erythrocytes (increase followed by a decrease at day 35) and decreased hemoglobin at the 0.05 and 0.2 mg/m³ dose levels, although the 1.5 mg/m³ dose group did not show this change. Other significant changes, noted in the lowest dose group, included decreased peroxidase activity after 35 days exposure, decreased sulfhydryl group content in blood after 27 days, and decreased phagocytotic index after 21 days. Some histological changes were noted in the lungs, kidneys, and to a lesser extent, the liver, particularly in the higher dose groups, although the exact nature of the degenerative change is not well described. Unclear dose-response relationships and temporal aspects of the endpoints also make establishment of a low adverse effect level unreliable.

Two rats were exposed to 20 ppm phosphine for 14 days (4 hours/day) (Waritz and Brown, 1975). Animals were monitored for weight gain, and organs/tissues fixed in Bouin's solution and stained with trichrome were examined histopathologically. There were no reported histopathological effects, although there was slightly reduced weight gain in the exposed animals.

In a chronic study of phosphine (Newton *et al.*, 1999), 60 male and female F344 rats per group were exposed via whole-body inhalation for 6 h/day, 5 days/wk for up to 104 wk to mean concentrations of 0, 0.3, 1, or 3 ppm phosphine. Three ppm (4.17 mg/m³) was the maximum

exposure level because of lethality seen at the high exposure level (7 ppm = 9.73 mg/m³) in previous repeat dose studies (Newton *et al.*, 1993). Ten rats per sex per group were killed after 52 weeks of exposure. Survivors were killed after 104 weeks of exposure. There were no phosphine-related effects seen on clinical observations, body weight, food consumption, hematology, clinical chemistry, urinalysis, or ophthalmology. There were no phosphine-related macroscopic findings or effect on absolute or relative organ weights. No histologic or morphologic alterations attributable to phosphine exposure were seen in the more than 40 organs and tissues examined. Under the conditions of this study, the authors found no treatment-related changes suggestive of a toxic or carcinogenic effect in rats following 52 weeks or 2 years of whole-body inhalation exposure to 0.3, 1, or 3 ppm phosphine. Thus 3 ppm is a chronic NOAEL for rats.

VI. Derivation of Chronic Reference Exposure Level

<i>Study</i>	Barbosa <i>et al.</i> , 1994
<i>Study population</i>	Balb-c mice (12 animals/sex/group)
<i>Exposure method</i>	Discontinuous whole body inhalation exposure (0, 0.3, 1, or 4.5 ppm)
<i>Critical effects</i>	Decrease in body weight gain, increase in relative organ weights; increase in micronuclei
<i>LOAEL</i>	4.5 ppm
<i>NOAEL</i>	1 ppm
<i>Exposure continuity</i>	6 hr/day, 5 days/ week
<i>Average experimental exposure</i>	0.178 ppm for NOAEL group (1 x 6/24 x 5/7)
<i>Human equivalent concentration</i>	0.178 ppm for NOAEL group (gas with systemic effects, based on RGDR = 1.0 using default assumption that lambda (a) = lambda (h))
<i>Exposure duration</i>	13 weeks
<i>LOAEL uncertainty factor</i>	1
<i>Subchronic uncertainty factor</i>	3
<i>Interspecies uncertainty factor</i>	<u>10 (see below)</u>
<i>Intraspecies uncertainty factor</i>	10
<i>Cumulative uncertainty factor</i>	300
<i>Reference exposure level</i>	0.0006 ppm (0.6 ppb; 0.0008 mg/m ³ ; 0.8 µg/m ³)

<i>Supportive study</i>	Newton <i>et al.</i> , 1999
<i>Study population</i>	Rats (60 animals/sex/exposure level)
<i>Exposure method</i>	Discontinuous whole body inhalation exposure (0, 0.3, 1, or 3 ppm)
<i>Critical effects</i>	None
<i>LOAEL</i>	None detected
<i>NOAEL</i>	3 ppm
<i>Exposure continuity</i>	6 hours/day, 5 days/week
<i>Average experimental exposure</i>	0.53 ppm (3 x 6/24 x 5/7)
<i>Human equivalent concentration</i>	0.53 ppm (gas with systemic effects, based on RGDR = 1.0 using default assumption that lambda (a) = lambda (h))
<i>Exposure duration</i>	2 years
<i>LOAEL uncertainty factor</i>	1
<i>Subchronic uncertainty factor</i>	1
<i>Interspecies uncertainty factor</i>	<u>10 (see below)</u>
<i>Intraspecies uncertainty factor</i>	10
<i>Cumulative uncertainty factor</i>	100
<i>Reference exposure level</i>	0.005 ppm (5 ppb, 0.007 mg/m ³ , 7 µg/m ³)

Newton *et al.* (1999) found no treatment-related changes suggestive of a toxic effect in F344 rats following 52 weeks or 2 years of whole-body inhalation exposure to 0.3, 1, or 3 ppm phosphine. Thus in this study 3 ppm is a chronic NOAEL for rats. Three ppm was set as the maximum level because in an earlier subchronic study in rats Newton *et al.* (1993) found lethality at 7 ppm. However, the chronic results of Newton *et al.* (1999) differ from the subchronic results of Newton *et al.* (1993), in which at least transient effects were seen in the hematopoietic system after 13 weeks at 3.1 ppm. In a subchronic study in mice (Barbosa *et al.*, 1994), 4.5 ppm phosphine was a LOAEL and 1 ppm was a NOAEL for decrease in body weight gain. The results of Barbosa *et al.* (1994) indicated that mice may be more sensitive than rats. Thus, it was selected as the key study, and decrease in body weight gain was selected as the critical effect.

OEHHA has applied a subchronic uncertainty factor of 3 to account for the short duration of the Barbosa *et al.* (1994) study and an intraspecies uncertainty factor of 10 to account for human variability. Due to the general inconsistencies among the various studies in the database on phosphine, and in particular with the observation of mortality at 7 ppm in a short-term developmental study in rats (Newton *et al.*, 1993), it was considered prudent to include the full interspecies uncertainty factor of 10 (even though the HEC adjustment procedure could be applied) to acknowledge the severity of effect in at least one comparison study, and the additional uncertainty associated with the apparent wide and unpredictable variability between species and between different studies in the same species (rats). This results in a cumulative uncertainty factor of 100 to be applied to the NOAEL of 1 ppm in the subchronic study by Barbosa *et al.* (1994) and a chronic REL for phosphine of 0.8 µg/m³ (0.6 ppb).

The U.S. EPA based its RfC of 0.3 µg/m³ on the Barbosa *et al.* (1994) study, an adequate subchronic animal study for the derivation of a REL, and included a Modifying Factor (MF) of 3

for database deficiencies (lack of multigenerational reproduction studies). The criteria for use of modifying factors are not well specified by U.S. EPA. USEPA used its default interspecies uncertainty factor of 10 for a 13 week study.

The lack of adequate data on levels of chronic phosphine exposure to humans precludes development of a REL from human studies. The endpoint used in the determination of the REL (total body weight gain) showed a dose-related decrease with phosphine exposure in Balb-c mice. This endpoint is also consistent with that found by Newton *et al.* (1993), who noted dose-dependent decreases in body weight gain in Fischer 344 rats after a 13 week exposure regimen at 1 ppm, and Waritz and Brown (1975), who reported slightly decreased weight gain in rats exposed for 14 days to 20 ppm. Surprisingly Newton *et al.* (1999) did not find differences in body weight gain at either 1 ppm or 3 ppm and they did not comment on the discrepancy between their 2 reports. Although body weight changes or changes in food consumption were not addressed in human studies, the scant human data do relate phosphine exposure to a broad spectrum of toxic effects (gastrointestinal, cardio-respiratory, CNS). The decrease in weight gain found in the animal studies and reported changes in some relative organ weights (Barbosa *et al.*, 1994) suggest systemic toxicity.

VII. Data Strengths and Limitations for Development of the REL

The strengths of the inhalation REL for phosphine include the availability of data on multiple inhalation exposure concentrations and the observation of a NOAEL in a lifetime animal study. Major areas of uncertainty are the lack of adequate human exposure data, the lack of reproductive and developmental toxicity studies, and the inconsistency of the dose-response relationship across rodent studies.

VIII. Potential for Differential Impacts on Children's Health

Based on the lack of a dose-response in developmental toxicity caused by exposing pregnant female rats to 4.9 ppm phosphine (Newton *et al.*, 1993), the proposed REL of $0.8 \mu\text{g}/\text{m}^3$ (0.6 ppb) is likely to be protective of developing humans in utero. However, there is no direct evidence in the literature to quantify a differential effect of phosphine on infants and children.

IX. References

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CHRONIC TOXICITY SUMMARY

PHOSPHORIC ACID*(Orthophosphoric acid)***CAS Registry Number: 7664-38-2****I. Chronic Toxicity Summary**

<i>Inhalation reference exposure level</i>	7 µg/m³
<i>Critical effect(s)</i>	Bronchiolar fibrosis of the respiratory tract in rats
<i>Hazard index target(s)</i>	Respiratory system

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

<i>Description</i>	Clear syrupy liquid or unstable crystals; odorless
<i>Molecular formula</i>	H ₃ PO ₄
<i>Molecular weight</i>	98
<i>Boiling point</i>	213°C
<i>Melting point</i>	42.35°C
<i>Vapor pressure</i>	0.03 torr @ 20°C
<i>Solubility</i>	Very soluble in hot water; 548 g/100 ml cold water; soluble in alcohol
<i>Conversion factor</i>	4.0 µg/m ³ per ppb at 25°C

III. Major Uses and Sources

Phosphoric acid has varied uses (HSDB, 1995). In manufacturing, it is a chemical intermediate or reagent in the production of numerous phosphate fertilizers, agricultural feeds, waxes, polishes, soaps, and detergents. It is added to foods as a preservative, acidifying agent, flavor enhancer, and clarifying agent. Phosphoric acid is also used in processes such as the coagulation of rubber latex, electropolishing, soil stabilization, and as a catalyst in the production of propylene and butene polymers, ethylbenzene, and cumene. By far, largest use of phosphoric acid comes in the production of fertilizers (>75%). The annual statewide industrial emissions from facilities reporting under the Air Toxics Hot Spots Act in California, based on the most recent inventory, were estimated to be 81,103 pounds of phosphoric acid (CARB, 1999).

Airborne phosphoric acid can be produced by the hydrolysis of phosphorus oxides generated from either the spontaneous ignition of white phosphorus in air or the combustion of red phosphorus (Burton *et al.*, 1982; US Department of Defense (US DOD), 1981).

IV. Effects of Human Exposures

The toxic effects to 48 workers exposed (28 unexposed control workers) to oxidation products of phosphorus during the course of phosphorus production were reported (Hughes *et al.*, 1962). Exposure duration ranged from 1 to 17 years. No differences were observed between exposed and control workers with respect to leukocyte count, an effect observed in acute intoxications, or hand bone density, an effect observed in experimentally exposed animals (Inuzuka, 1956).

A prospective study of 131 workers exposed to several compounds including phosphoric acid, phosphorus pentoxide, fluorides and coal tar pitch in the air was conducted at an industrial refinery (Dutton *et al.*, 1993). Mean duration of exposure (employment) was 11.4 years and the maximum exposure level measured was 2.23 mg/m³ (phosphorus pentoxide). Pulmonary function tests were performed annually over a 3 to 7 year period. No significant residual effect was found after adjusting for age and smoking status.

V. Effects of Animal Exposures

Two 13-week inhalation studies of the effects of exposure to the combustion products of 95% red phosphorus and 5% butyl rubber were conducted in male Sprague-Dawley rats, with the first group exposed to 0, 300, 750, or 1200 mg/m³ combustion products, and the second exposed to 0, 50, 180, or 300 mg/m³ combustion products (Aranyi *et al.*, 1988a; Aranyi *et al.*, 1988b). Group numbers in the first study were 176, 84, 176, and 176, respectively. The second study used 40 animals/group. Animals were exposed for 2¼ hours/day on 4 consecutive days/week. Control animals were exposed to filtered air only. Daily particle measurements showed MMADs of 0.49-0.65 µm and σ_gs of 1.56-1.83. Fractional content of phosphoric acid in the aerosol was 71-79%. Nineteen of the 176 animals in the 1200 mg/m³ dose group died of treatment related effects. Post-mortem examination of animals that died during the course of the study showed damage to the laryngeal mucosa, which was probably contributory to mortality. The two highest dose groups in the first study also showed decreased weight gain. Twelve animals from each dose group in the first study were examined histologically and neurobehavioral studies were conducted on other animals. Half the animals in the second study were examined strictly for toxic effects on the respiratory tract, with examination of the trachea, 2 sections of the nasal turbinates, and 5 lobes of the lung. Surviving animals in the high-dose study were observed to have moderate to severe fibrosis of the terminal bronchioles, with minimal severity of this effect in the animals in the low-dose study. The reported incidence of this lesion was 9/20 at 300 mg/m³, 4/20 at 180 mg/m³, and 0/20 at 50 mg/m³. Little to no involvement of pulmonary tissue was observed.

The effects of acid aerosols (particularly sulfuric and phosphoric acid) were studied by U.S. EPA (1989). The respiratory tract was the primary target of toxicity resulting from the irritational effect of the acid on the tissues of the larynx and trachea. The nature of the effect was dependent upon the aerosol particle size, duration of exposure, and the hygroscopic character of the acid.

Sprague-Dawley rats were exposed to the smoke and combustion products of white phosphorus in felt pellets at 192.5 (18 animals/sex), 589 (24 animals/sex), or 1161 mg/m³ (34 males, 43

females) phosphoric acid equivalents for 15 minutes/day, 5 days/week, for 13 weeks (US Department of Defense (US DOD), 1981). Control animals numbering half the size of the treated groups were exposed to air only. Groups of animals were sacrificed at 6 and 13 weeks, and 4 weeks post-exposure. Endpoints examined included: hematology, clinical chemistry, gross- and histo-pathology, ECG, pulmonary function, and behavior. Of the animals in the highest dose group, 56% died as a result of exposure, with the only other death occurring in the control group. Findings were restricted to effects on the respiratory system, with tracheitis and laryngitis incidences of 2/35, 32/47, and 28/31 among surviving animals in the three dose groups. In the post-exposure examination, bronchiolitis occurred with a frequency of 0/12, 5/24, and 6/16 in the three dose groups.

The toxicity of the combustion products of 95% amorphous red phosphorus and 5% polyvinyl butyral BL18 to female Wistar rats, Porton-strain mice, and guinea pigs was reported (Marrs *et al.*, 1989). Rats (50/group), mice (100/group), and guinea pigs (42-48/group) were exposed to concentrations of 0, 16, or 128 mg/m³ for 1 hour/day, 5 days/week for 36 weeks (mice) or 40 weeks (rats and guinea pigs), with an examination conducted at 19 months or when animals appeared unhealthy. All groups, including controls, showed high mortality. Mice showed accumulation of alveolar macrophages with incidences of 2/41, 9/37, and 9/22 in the control, low-, and high-dose groups, respectively. Guinea pigs appeared to be particularly intolerant to the effects of the smoke.

Female rabbits and rats (10/group) were examined for acute toxic effects of smoke generated by the combustion of either 95% red phosphorus / 5% butyl rubber (Smoke I) or 97% red phosphorus / 3% butadiene styrene (Smoke II) (Marrs, 1984). Animals were exposed for 30 minutes and examined one and 14 days later. Smoke I produced inflammation of the larynx and trachea in rats at 1 day with some inflammation still observed at 14 days. Tracheal inflammation was also reported in rabbits exposed to Smoke I. Four of the rats exposed to Smoke II died within the first day, with severe pulmonary congestion observed in the animals.

One hour exposure to the combustion products of 95% red phosphorus / 5% butyl rubber (plus 1% mineral oil) produced epiglottal deformation, laryngeal edema, and laryngeal and tracheal lesions in rats (Burton *et al.*, 1982). A four-hour exposure produced more severe effects of a similar nature plus some hemorrhaging.

Rats (number unspecified) exposed to 150-160 mg/m³ elemental phosphorus for 30 minutes/day for 60 days were examined for toxic effects (Inuzuka, 1956). Limb bone abnormalities were noted and effects included delayed ossification, widening of the epiphysis, and abnormal axial development.

Two studies have addressed the reproductive and developmental toxicity from exposure to the combustion products of white phosphorus and felt for 15 minutes/day during gestational days 6-15 in rats (24/group) (US Department of Defense (US DOD), 1981; US Department of Defense (US DOD), 1982). Fetal effects included increased incidence of some visceral variations and hypoplasia of the xiphoid process although data were incompletely reported. Another study, which exposed dams 3 weeks prior to mating, throughout gestation, and through lactation and males for 10 weeks prior to and during mating, showed decreased pup body weight, 24-hour and

21-day survival, and lactation. An oral study in which elemental phosphorus was administered to male and female rats by gavage in corn oil showed no statistically significant effects (Condray, 1985).

VI. Derivation of the Chronic Reference Exposure Level

<i>Study</i>	Aranyi <i>et al.</i> , 1988a
<i>Study population</i>	Male Sprague-Dawley rats (40-176/group)
<i>Exposure method</i>	Discontinuous whole body inhalation
<i>Critical effects</i>	Bronchiolar fibrosis of the respiratory tract
<i>LOAEL</i>	180 mg/m ³
<i>NOAEL</i>	50 mg/m ³
<i>BMC₀₅</i>	64 mg/m ³
<i>Exposure continuity</i>	2¼ hours/day, 4 days/week
<i>Exposure duration</i>	13 weeks
<i>Average experimental exposure</i>	2.7 mg/m ³ for NOAEL group (estimated as 3.5 mg/m ³ at BMC ₀₅)
<i>Human equivalent concentration</i>	2.2 mg/m ³ at BMC ₀₅ (particle with respiratory effects, RDDR = 0.63) (3.5 x 0.63)
<i>LOAEL uncertainty factor</i>	1 (BMC ₀₅ assumed to be similar to NOAEL)
<i>Subchronic uncertainty factor</i>	10
<i>Interspecies uncertainty factor</i>	3
<i>Intraspecies uncertainty factor</i>	10
<i>Cumulative uncertainty factor</i>	300
<i>Reference exposure level</i>	0.007 mg/m ³ (7 µg/m ³)

OEHHA has used the same study which U.S. EPA used in the development of its Reference Concentration (RfC) of 10 µg/m³. The U.S. EPA has used a benchmark dose methodology for the derivation of the RfC for phosphoric acid from the toxicity data in the Aranyi *et al.* (1988) study (U.S. EPA, 1995). The RfC is restricted to “aerosols of phosphoric acid and phosphorus oxidation products and does not apply to elemental phosphorus or other forms of phosphorus, such as phosphorus salts”.

The U.S. EPA, using the Weibull model, estimated the lower 95% confidence level bound on the maximum likelihood estimate (MLE = 150 mg/m³) resulting in 10% incidence of lesions in the tracheo-bronchiolar region to be 100 mg/m³ (the BMC₁₀). The U.S. EPA considered 10% incidence level to be a correlate to the NOAEL, based on a precedent in the analysis of data with developmental toxicity endpoints (Allen *et al.*, 1994; Faustman *et al.*, 1994). After correction for exposure continuity, a regional deposited dose ratio (RDDR) for the tracheobronchial region of 0.64 was applied due to the availability of data concerning the growth and deposition of phosphoric acid aerosol particles in humans and the similarities in the effects of phosphoric and better-characterized sulfuric acid aerosols. Key assumptions in the generation of this factor include: (1) the lowest σ_g of 1.56 µm cited in the study was used in the calculation; (2) geometric rather than aerodynamic diameter approximations were used; (3) particles of this size reach the deposition / lesion site (bronchioles); 4) these hygroscopic particles become more uniform with

growth; and (5) particle growth is similar in humans and rodents. An uncertainty factor of 10 was applied because of the subchronic duration of the study. A factor of 3 was applied for interspecies extrapolation in light of the fact that some correction for human equivalency was made with the RDDR. Finally, a factor of 10 was applied for protection of potentially sensitive human subpopulations. The resulting RfC for phosphoric acid is 0.01 mg/m^3 .

OEHHA uses a BMC_{05} for development of acute Reference Exposure Levels (OEHHA, 1999; Fowles *et al.*, 1999). OEHHA staff believe that the BMC_{05} is more likely to approximate a NOAEL than a BMC_{10} since 5% is closer than 10% to the lower end of average risk levels associated with a NOAEL (Leisenring and Ryan, 1992). A BMC_{05} is more likely to represent a value close to the limit of most studies to detect an effect, and is therefore more like a NOAEL. In contrast, a BMC_{10} is more likely to represent a LOAEL since it is usually in the detectable range of responses. In the specific case of phosphoric acid the BMC_{10} of 100 mg/m^3 was twice the NOAEL of 50 mg/m^3 . The BMC_{05} was calculated to be 64 mg/m^3 , much closer to the NOAEL. Use of the BMC_{05} results in a chronic REL of $7 \text{ } \mu\text{g/m}^3$.

VII. Data Strengths and Limitations for Development of the REL

The strengths of the inhalation REL for phosphoric acid include the availability of subchronic inhalation exposure data from a well-conducted study with histopathological analysis and the observation of a NOAEL. Major areas of uncertainty are the lack of adequate human exposure data, the lack of chronic inhalation exposure studies, and the discontinuous nature of exposures (only 2 1/4 hours per day).

The Aranyi *et al.* (1988a) study represents the most adequate study for the quantitative evaluation of the toxicity of phosphoric acid. It was conducted with a large number of animals with multiple doses, produced good dose-response data, and examined likely targets of toxicity (respiratory system) of smoke generated from the combustion of phosphorus and butyl rubber. Uncertainties associated with these data, however, include that (1) the study used combustion products of phosphorus rather than phosphoric acid itself, (2) the total exposure time was relatively short and discontinuous over the duration of the experiment, and (3) only one species/strain/sex was studied.

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CHRONIC TOXICITY SUMMARY

PHTHALIC ANHYDRIDE*(1,3-isobenzofurandione; phthalic acid anhydride)***CAS Registry Number: 85-44-9****I. Chronic Toxicity Summary**

<i>Inhalation reference exposure level</i>	20 µg/m³
<i>Critical effect(s)</i>	Eye and respiratory irritation, asthma, and bronchitis in occupationally exposed workers
<i>Hazard index target(s)</i>	Respiratory system

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

<i>Description</i>	White or pale yellow crystals
<i>Molecular formula</i>	C ₈ H ₄ O ₃
<i>Molecular weight</i>	148.11 g/mol
<i>Boiling point</i>	295°C
<i>Melting point</i>	130.8°C
<i>Vapor pressure</i>	5.14 × 10 ⁻⁴ torr @ 25°C; 1 torr @ 96.5°C
<i>Solubility</i>	Soluble in 162 parts water, 125 parts carbon disulfide; soluble in hot benzene
<i>Conversion factor</i>	1 µg/m ³ per ppb at 25°C

III. Major Uses and Sources

The primary use of phthalic anhydride (PA) is as a chemical intermediate in the production of plastics from vinyl chloride. Phthalate esters, which function as plasticizers, are derived from phthalic anhydride. Phthalic anhydride has another major use in the production of polyester resins and other minor uses in the production of alkyd resins used in paints and lacquers, certain dyes (anthraquinone, phthalein, rhodamine, phthalocyanine, fluorescein, and xanthene dyes), insect repellents, and urethane polyester polyols. It has also been used as a rubber scorch inhibitor and retarder (HSDB, 1995; National Cancer Institute (NCI), 1979). The annual statewide industrial emissions from facilities reporting under the Air Toxics Hot Spots Act in California based on the most recent inventory were estimated to be 11,442 pounds of phthalic anhydride (CARB, 2000).

IV. Effects of Human Exposure

Symptoms in workers exposed to phthalic anhydride by inhalation in two plants (A and B) manufacturing alkyd and unsaturated polyester resins were studied (Nielsen *et al.*, 1988). Two groups of exposed workers were identified in each plant. One group worked directly loading the reactors from bags of phthalic anhydride (“heavy” exposure – 35 workers) and the other group was involved with “other work” which led to “low” exposure (25 workers). Mean employment times for the “heavy” and “low” exposure groups were 13.3 and 11.9 years, respectively. Time-weighted average air concentrations for workers from the loading of PA was 6.1 (range: 1.8-14.9) and 6.8 mg PA/m³ (range: 1.5-17.4) in plants A and B, respectively. Similar exposure levels in both plants led to pooling of data. The exposure duration of the “heavy” group was estimated at approximately 30 minutes two times a day, corresponding to the time of loading, and resulted in a full-day time weighted exposure estimate of 0.4 mg PA/m³. For those engaged in “other work” exposure levels were estimated at < 0.1 mg PA/m³ (the limit of detection). Other chemicals in use in smaller amounts included maleic anhydride, isophthalic anhydride, and trimellitic anhydride. Comparison of symptom incidence between the “heavy” and “low” exposure groups included conjunctivitis (46% vs. 20%), rhinitis (40% vs. 20%), rhinoconjunctivitis (17% vs. 12%), asthma (17% vs. 0%), and chronic bronchitis (17% vs. 4%). Serum antibodies were measured in both groups of workers and compared to 22 nonexposed workers (employed at a food processing factory). The only significantly changed level was an increase in specific IgG in the “heavy” exposure group. A correlation was also noted between specific IgG level and exposure level, although not all individuals with elevated specific IgG reported symptoms.

In a study conducted at another plant manufacturing alkyd and/or unsaturated polyester resins, serum immunoglobulins and lung function were examined in 23 workers exposed to phthalic anhydride and 18 control subjects (Nielsen *et al.*, 1991). Estimated exposure levels were 6.6 mg PA/m³ (range: 1.5-17) (Nielsen *et al.*, 1988). Workers were examined for sensitization to PA and other allergens and possible development of small airways disease. Among the exposed workers, there was significantly increased reporting of conjunctivitis and rhinoconjunctivitis. One worker showed an asthmatic response to anhydrides. No significant differences in lung function tests were observed between exposed and unexposed groups.

Symptoms in workers occupationally exposed to PA during the course of producing alkyd and/or polyunsaturated polyester resins were described (Wernfors *et al.*, 1986). Exposure estimates of breathing zone PA levels ranged from 3 to 13 mg/m³ for workers engaged directly with the handling of PA. In other areas the estimated level was <0.3 mg/m³. The study examined 48 workers who were employed at the time of the study and 70 former employees who responded to a survey of symptoms related to exposure. No unexposed control group was included in the study. Workers who were employed for at least two months reported symptoms of rhinitis (28%), chronic bronchitis (11%), and asthma (28%). Among a subset of 11 workers with asthma, 3 had positive skin tests for PA sensitivity. Bronchial provocation tests with 6 or 0.5 mg/m³ PA for 5 or 10 minutes were positive in 2 workers.

V. Effects of Animal Exposure

Male albino rats (6/treatment group) were exposed to phthalic anhydride vapors at 0, 0.02, 0.2, and 1 mg/m³ continuously for 45 days (Protsenko, 1970). After a two week recovery period the testes were examined for spermatozoa motility time as well as for ascorbic acid, dehydroascorbic acid, and nucleic acid content. Motility time was defined as the time it took for spermatozoa to cease motion completely under microscopic examination. Spermatozoa motility time was decreased ~50% in the 1 mg/m³ dose group and ~25% in the 0.2 mg/m³ dose group. Significant decreases in ascorbic acid and dehydroascorbic acid levels were found in animals exposed to 0.2 and 1.0 mg/m³ phthalic anhydride, and dehydroascorbic acid levels were decreased in the 0.02 mg/m³ dose group. At 1 mg/m³, RNA levels and combined RNA and DNA levels were significantly increased over controls. No significant changes were observed in the 0.02 mg/m³ dose group.

Five and six female Hartley guinea pigs were exposed to 0.05-0.2 mg/m³ and 0.6-6 mg/m³ phthalic anhydride dust, respectively, for 3 hours/day for 5 consecutive days (Sarlo and Clark, 1992). Exposures were expressed as ranges due to difficulty in regulating dust levels in the chambers. Sampling of dust showed particles were 65-80% < 10 µm diameter and had a mean mass diameter of 5.8-9.8 µm. Eight control animals were exposed to filtered air only. Two weeks after the last exposure, animals were challenged for 30 minutes with aerosolized PA-guinea pig serum albumin conjugate. All animals in the "high" dose group showed immediate bronchoconstriction and transiently increased respiratory rate. Animals in this dose group also showed elevated IgG antibody titers. No detectable increase in antibody levels was found in the "low" dose group.

Type I hypersensitivity was examined in female Hartley guinea pigs exposed to phthalic anhydride dust (Sarlo *et al.*, 1994). Two groups of 8 animals were exposed to 0.5 or 1.0 mg/m³, and two groups of 16 animals were exposed to 0 (filtered air only) or 5.0 mg/m³ phthalic anhydride dust (respirable size – 5 µm) in stainless steel chambers for 3 hours/day for 5 consecutive days. Groups of 8 animals from the control and 5 mg/m³ groups were challenged after a two week recovery period for 30 minutes with 5.0 mg/m³ phthalic anhydride dust. Respiratory data were collected using a plethysmograph from 30 minutes before the exposure to 60 minutes after the exposure. No significant difference (defined as a change of 3 standard deviations from the same parameter in the control animals) in respiration rate or plethysmograph pressures was found between the exposed and unexposed animals. Eight animals in each of the four exposure groups were also challenged after two weeks of recovery with 2.0 mg/m³ aerosolized PA-guinea pig serum albumin (GPSA) conjugate as described above. Respiratory rate was increased in 4/8 of the high-dose group animals and 1/8 of the low-dose animals. Plethysmograph pressures were increased in 3/8 animals in the high-dose group and one animal each in the low- and mid-dose groups. Serum IgG antibodies to PA-GPSA were elevated in all exposed animal groups and the effect showed a dose-response. Passive cutaneous anaphylaxis testing for anti-phthalic anhydride-GPSA IgG1a immunoglobulins showed positive results for 3/8, 1/8, and 5/8 animals in the 0.5, 1.0, and 5.0 mg/m³ dose groups, respectively. Results in control animals were not described. Three of eight animals in the highest dose group had >189 hemorrhagic foci in their lungs. No control animal had more than 2 such foci. No foci were

observed in animals challenged with albumin conjugate. Serum IgG titer correlated with the presence of these foci.

Slavgorodskiy (1969) studied the toxicity of phthalic anhydride to animals from inhalation exposure. Sixty white male rats (strain not reported; group distribution not stated, but presumed to be 15 animals/treatment group) were exposed in 100 L chambers to 0, 0.18, 0.54, and 1.52 mg PA/m³ aerosol continuously for 70 days. General condition and behavior, body weight, motor chronaxy of flexor and extensor muscles (every 10 days), cholinesterase activity (every two weeks), and hematological parameters were monitored during the course of the study. (Chronaxy is the minimum time for which a current must flow, at a voltage twice the minimal current necessary to produce muscle stimulation, in order to cause a muscle to contract.) No changes in body weight or behavior were observed in the treated animals. In animals in the high-dose group, the chronaxy ratio of flexors and extensors differed from the controls beginning on day 31 of exposure and continued until two weeks after exposure ceased. Significantly decreased whole blood cholinesterase activity occurred in the high- and mid-dose groups, with the change occurring after 42 days of exposure. An increase in thrombocyte count occurred in the high- and mid-dose groups after 70 days of exposure, but returned to normal during the two-week recovery period. Thus, 0.18 mg/m³ PA appears to be a NOAEL in this study.

A chronic feeding study was conducted with phthalic anhydride in rats and mice to evaluate the carcinogenicity of the compound (National Cancer Institute (NCI), 1979). F344 rats (50/sex/dose group plus 20/sex control animals) were treated with diet containing 0, 7500, or 15,000 ppm phthalic anhydride for 105 weeks (which corresponds to approximately 0, 300, and 600 mg/kg-day, assuming that food consumption is 4% body weight/day). Animals were monitored for changes in body weight and for survival, and, upon death or the end of the study, were examined histopathologically. The only group showing significantly lower body weights was male rats in the high-dose group after week 13. No significant change in mortality was observed. Adverse non-cancer effects observed in the dosed groups, but not in the control animals, included “arched back, rough hair coat, ulceration, and corneal opacity”, however, incidences were described as “low”. No significant histopathological effects were found to be associated with exposure to phthalic anhydride. B6C3F₁ mice (50/sex/dose group plus 20/sex control animals) were initially treated with diet containing 0, 25,000, or 50,000 ppm phthalic anhydride (approximately 0, 3000, and 6000 mg/kg-day, assuming that food consumption is 12% body weight/day). Because of excessive weight loss after week 32, exposure levels were reduced during the course of the study such that the time-weighted average exposure for males was 16,346 and 32,692 ppm and for females was 12,019 and 24,038 ppm phthalic anhydride. Evaluation of toxicity was conducted at 104 weeks as with the rats. Mean body weight was reduced in male and female mice in a dose-related manner. No other significant treatment-related adverse effects were observed in the mice.

Pregnant female CD-1 mice (10/dose group) were treated intraperitoneally with phthalic anhydride in 0.5% (w/v) carboxymethyl cellulose solution on gestational days 8-10 (Fabro *et al.*, 1982). Dosing was variable, beginning within the 95% confidence limits of the LD₀₁ and progressing geometrically downward until no effect was observed. Animals were terminated on Day 18 and examined for teratogenic effects including fetal viability and number, resorption, and gross malformations. The 95% lower confidence limit on the dose producing teratogenicity

(grossly observable malformations and fetal internal malformations) in 5% and 50% of animals were 0.40 and 1.37 mmol/kg-day (59 and 203 mg/kg-day), respectively.

VI. Derivation of Chronic Reference Exposure Level (REL)

<i>Study</i>	Neilsen <i>et al.</i> (1988; 1991)
<i>Study population</i>	23 occupationally-exposed workers
<i>Exposure method</i>	Discontinuous occupational inhalation exposures
<i>Critical effects</i>	Increased incidence of conjunctivitis, rhinitis, asthma, and chronic bronchitis
<i>LOAEL</i>	6.5 mg/m ³ (mean of 6.1 and 6.8)
<i>NOAEL</i>	Not observed
<i>Exposure continuity</i>	8 hours/day, 5 days/week
<i>Exposure duration</i>	Mean of 13.3 years
<i>Average experimental exposure</i>	2.3 mg/m ³ for LOAEL group (6.5 mg/m ³ × 10/20 × 5/7)
<i>LOAEL uncertainty factor</i>	10
<i>Subchronic uncertainty factor</i>	1
<i>Interspecies uncertainty factor</i>	1
<i>Intraspecies uncertainty factor</i>	10
<i>Cumulative uncertainty factor</i>	100
<i>Inhalation reference exposure level</i>	0.02 mg/m ³ (20 µg/m ³)

Adverse effects were demonstrated to occur in humans occupationally exposed to phthalic anhydride in the workplace over long periods of time (Nielsen *et al.*, 1988). The symptoms reported primarily affected the respiratory system, with increased incidence of rhinitis, rhinoconjunctivitis, asthma, and chronic bronchitis. Conjunctivitis was also reported in exposed workers. Specific anti-PA IgG was significantly elevated compared to a non-exposed group. Increased incidences of rhinoconjunctivitis, conjunctivitis, or chronic bronchitis have also been reported in workers exposed to similar levels of PA dust (Nielsen *et al.*, 1991; Wernfors *et al.*, 1986). In these reports, adverse effects were clearly observed at the exposure level reported (6.5 mg PA/m³; full-day time weighted exposure of 0.4 mg PA/m³). Although symptoms were reported by Nielsen (1988) in the lower exposure level group, the significance is not clear since a true control group (unexposed workers) was not included in the symptomatology section of the study. The low exposure group's level of exposure was less than the detection limit for phthalic anhydride cited in the study, and this group was considered as a control group.

VII. Data Strengths and Limitations for Development of the REL

The strengths of the inhalation REL for phthalic anhydride include the use of human exposure data from workers exposed over a period of years. Major areas of uncertainty are (1) the uncertainty in estimating exposure, (2) the potential variability in exposure concentration, (3) the

potential low exposures of the group considered as controls, (4) potential confounding by exposures to other chemicals, (5) the limited nature of the study, (6) the lack of reproductive and developmental toxicity studies, and (6) the lack of observation of a NOAEL in the key study. Another area of uncertainty is the apparent 10-fold greater sensitivity to bronchoconstriction from PA exposure in guinea pigs (a model for human asthmatics) in comparison to occupationally exposed workers.

The study in rats by Protsenko (1970) identified a LOAEL of 0.2 mg/m³ and a NOAEL of 0.02 mg/m³ for decreased sperm motility. However, this result from 1970 has not been verified or further explored in more recent toxicological or epidemiological studies. The small sample size of 6/group further weakens confidence in this result. Therefore, the study in workers by Nielson *et al.* (1988, 1991) was chosen as the basis for the REL for PA.

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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**

<i>Inhalation reference exposure level</i>	3,000 µg/m³ (2,000 ppb)
<i>Critical effect(s)</i>	Squamous metaplasia (males and females), epithelial hyperplasia (females only), and inflammation (males only) of the nasal cavity in Fischer 344/N rats
<i>Hazard index target(s)</i>	Respiratory system

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

<i>Description</i>	Colorless gas; practically odorless.
<i>Molecular formula</i>	C ₃ H ₆
<i>Molecular weight</i>	42.08
<i>Boiling point</i>	-47.6 °C
<i>Melting point</i>	-185.2°C
<i>Vapor pressure</i>	8690 torr at 25°C
<i>Solubility</i>	Soluble in alcohol and ether.
<i>Conversion factor</i>	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-hydroxypropyl 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/m³) 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/m³) 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 B6C3F₁ 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)

<i>Study</i>	Quest <i>et al.</i> , 1984
<i>Study population</i>	50 rats/group/sex, 300 total.
<i>Exposure method</i>	Discontinuous whole body inhalation exposure (0 or 4,985 or 9,891 ppm).
<i>Critical effects</i>	Respiratory system; squamous metaplasia (males and females), epithelial hyperplasia (females only), and inflammation (males only) of the nasal cavity
<i>LOAEL</i>	4,985 ppm (8,570 mg/m ³)
<i>NOAEL</i>	Not observed
<i>Exposure continuity</i>	6 hr/day, 5 days/week
<i>Exposure duration</i>	2 years
<i>Average experimental exposure</i>	890 ppm for LOAEL group (4985 x 6/24 x 5/7)
<i>Human equivalent concentration</i>	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)
<i>LOAEL uncertainty factor</i>	3 (low severity)
<i>Subchronic uncertainty factor</i>	1
<i>Interspecies uncertainty factor</i>	3
<i>Intraspecies factor</i>	10
<i>Cumulative uncertainty factor</i>	100
<i>Inhalation reference exposure level</i>	2 ppm (2,000 ppb, 3 mg/m ³ , 3,000 µg/m ³)

VII. Data Strengths and Limitations for Development of the REL

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

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

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

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CHRONIC TOXICITY SUMMARY

PROPYLENE GLYCOL MONOMETHYL ETHER*(1-Methoxy-2-propanol; 1-methoxypropanol; Propapsol solvent M)***CAS Registry Number: 107-98-2****I. Chronic Toxicity Summary**

<i>Inhalation reference exposure level</i>	7,000 µg/m³ (2000 ppb)
<i>Critical effect(s)</i>	Liver effects in rats
<i>Hazard index target(s)</i>	Alimentary system (liver)

II. Physical and Chemical Properties (HSDB, 1995)

<i>Description</i>	Colorless liquid
<i>Molecular formula</i>	C ₄ H ₁₀ O ₂
<i>Molecular weight</i>	90.14
<i>Density</i>	0.962 g/cm ³ @ 20° C
<i>Boiling point</i>	118-118.5°C
<i>Melting point</i>	-96.7°C
<i>Vapor pressure</i>	11.8 torr @ 25°C
<i>Solubility</i>	Soluble in water, methanol, ether, and other organic solvents
<i>Conversion factor</i>	1 ppm = 3.69 mg/m ³ at 25° C

III. Major Uses or Sources

Propylene glycol monomethyl ether (PGME) is used as a solvent for cellulose, acrylics, dyes, inks and stains (HSDB, 1995). Thus, the primary use of PGME is in lacquers and paints. Use of PGME is anticipated to increase due to its low systemic toxicity. The annual specific statewide industrial emissions of PGME from facilities reporting under the Air Toxics Hot Spots Act in California, based on the most recent inventory, were estimated to be 205,769 pounds (CARB, 1999). (Many industries did not estimate emissions of specific glycol ethers so that in 1998 there were also emitted 2,922,744 pounds of the general category glycol ethers, some of which may be PGME.)

IV. Effects of Exposures on Humans

No reports or studies of human toxicity following chronic exposure to PGME were located in the literature. Slight eye irritation was reported by two of six human volunteers exposed to 100 ppm PGME for 2 hours (Stewart *et al.*, 1970). These subjects were exposed for a total of 3 1/2 hours during which no decrement in visual acuity, coordination, neurological responses or reaction time was measured. The same experiment exposed 23 subjects to 250 ppm PGME. After 15 to 30 minutes of exposure, 8/23 reported eye irritation and 3/23 reported throat irritation; lacrimation was observed in 3/23 subjects. Three subjects each reported one of the following symptoms: irritation, headache, and nausea. While the subjects frequently reported the odor to be objectionable upon first entering the chamber, the odor was usually undetectable by the end of the exposure. Clinical chemistry and urinalysis completed following exposure was not altered as compared to pre-exposure measurements.

V. Effects of Exposures on Animals

Male and female rats (10 per sex per concentration) and rabbits (7 per sex per concentration) were exposed by inhalation to 300, 1000, or 3000 ppm PGME 5 hours per day, 5 days per week for 13 weeks (Landry *et al.*, 1983). Relative liver weights were statistically significantly higher than controls in both male and female rats exposed to 3000 ppm PGME. Hepatocellular hypertrophy was observed upon histopathologic examination of high dose females. The authors conclude that these effects are the result of physiologic adaptation rather than a manifestation of toxicity. The key observation in this study was sedation of rats and rabbits exposed to 3000 ppm PGME. The sedative effects were no longer apparent after 1-2 weeks of exposure.

Similar findings of mild CNS depression were observed by Hanley *et al.* (1984). Pregnant rats and rabbits were exposed to 500, 1500, or 3000 ppm PGME 6 hours per day either days 6-15 or days 6-18 of gestation, respectively. During the first 4-5 days of exposure, rats in the 3000 ppm PGME exposure group were lethargic and moderately ataxic. Statistically significant decreases in food consumption and maternal body weight gain were also observed during this period. A statistically significant increase in the incidence of delayed sternal ossification was observed in the 3000 ppm exposure group. Rabbits exposed to 3000 ppm exhibited mild lethargy during the first 1-2 days of exposure with rapid post-exposure recovery. Overall maternal weight gain during the exposure (days 6-18 of gestation) was statistically significantly lower than controls.

No significant effect on fetal birth weight or on pup survival indices (e.g., proportion of pups surviving to day 3 post-delivery) was noted following exposure of pregnant rats to 200 or 600 ppm PGME 6 hours per day on days 6-17 of gestation (Doe *et al.*, 1983). Male rats were exposed to 200 or 600 ppm PGME 6 hours per day for 10 consecutive days. No significant effects on testicular weight or pathology were observed.

Increased liver and kidney weights were observed in male and female rats (10 per sex per concentration) following exposure to 6000 ppm for 7 hours per day, for 81 exposures over a 114-day period (Rowe *et al.*, 1954). No histopathological abnormalities were observed at necropsy.

Ciezlak et al. (1998) evaluated the potential chronic toxicity/oncogenicity and the response of liver and kidney tissue of Fischer 344 rats to propylene glycol monomethyl ether (PGME) at targeted vapor concentrations of 0, 300, 1000 or 3000 ppm. Groups of 50 male and female rats per sex were whole-body exposed under dynamic airflow conditions for 6 hours/day, 5 days/week for up to 2 years. Parameters evaluated included the general appearance and demeanor of animals, in-life body weights, survival, hematology, urinalysis and clinical chemistry determinations, survival, selected organ weights, gross and microscopic pathologic changes and tumor incidence. (The metabolic and morphological bases for PGME-induced sedation, hepatic hypertrophy and renal toxicity were characterized in separate groups of male and female rats exposed to PGME for 6, 12 or 18 months. Hepatic enzyme induction and cellular proliferation, as well as renal cellular proliferation and accumulation of alpha_{2u}-globulin (males only) in the kidneys, were conducted in these separate groups of animals.)

PGME-induced sedation at 3000 ppm resolved in all animals during the second week of exposure in conjunction with the appearance of adaptive changes in the liver (cytochrome P450 induction and hepatocellular proliferation). Cytochrome P450 (pentoxyresorufin O-demethylase) activities dropped to near control concentrations by week 52, coinciding with a return of sedation at 3000 ppm PGME. In male rats, the loss of metabolic adaptation was followed by a dose-related increase in altered hepatocellular foci after two years of exposure to 1000 or 3000 ppm PGME. The kidney toxicity observed in male rats was confirmed immunohistochemically as an alpha_{2u}-globulin nephropathy. No statistically-identified increases in tumors were observed in any tissue. The authors established a NOEL of 300 ppm PGME for the study.

Ethylene glycol methyl ether (EGME), a structurally related compound, exerts considerable toxicity on the blood, thymus, testes, and developing fetus. The toxicity of EGME has been linked to its primary metabolite, methoxyacetic acid. Recent comparative toxicity and metabolism studies (Miller *et al.*, 1983, Miller *et al.*, 1984) indicate that the relatively low systemic toxicity exerted by PGME is due to its different metabolites. Following a single oral dose of PGME, the key urinary metabolites identified in rats were propylene glycol and the sulfate and glucuronide conjugate of PGME (Miller *et al.*, 1983).

VI. Derivation of Reference Exposure Level

<i>Study</i>	Ciezlak <i>et al.</i> , 1998
<i>Study population</i>	Fischer 344 rats (50/sex/concentration)
<i>Exposure method</i>	Discontinuous whole-body inhalation (0, 300, 1000, or 3000 ppm)
<i>Critical effects</i>	Increased eosinophilic foci of altered hepatocytes
<i>LOAEL</i>	1000 ppm
<i>NOAEL</i>	300 ppm
<i>Exposure continuity</i>	6 hours per day, 5 days per week
<i>Average experimental exposure</i>	54 ppm for NOAEL group (300 x 6/24 x 5/7)
<i>Human equivalent concentration</i>	54 ppm for NOAEL group (gas with systemic effects, based on RGDR = 1.0 using default assumption that lambda (a) = lambda (h))
<i>Exposure duration</i>	104 weeks
<i>LOAEL factor</i>	1
<i>Subchronic uncertainty factor</i>	1
<i>Interspecies uncertainty factor</i>	3
<i>Intraspecies uncertainty factor</i>	10
<i>Cumulative uncertainty factor</i>	30
<i>Inhalation reference exposure level</i>	2 ppm (2000 ppb, 7 mg/m ³ , 7000 µg/m ³)

VII. Data Strengths and Limitations for Development of the REL

Strengths of the PGME RfC include the observation of a NOAEL and a LOAEL in the same study, and the availability of chronic exposure studies involving multiple concentrations. A major area of uncertainty is the lack of human data.

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CHRONIC TOXICITY SUMMARY

PROPYLENE OXIDE*(1,2-propylene oxide; methyl ethylene oxide; propene oxide)***CAS Registry Number: 75-56-9****I. Chronic Toxicity Summary**

<i>Inhalation reference exposure level</i>	30 µg/m³ (9 ppb)
<i>Critical effect(s)</i>	Degenerative and hyperplastic changes in the respiratory epithelium of rats
<i>Hazard index target(s)</i>	Respiratory system

II. Physical and Chemical Properties (HSDB, 1994)

<i>Description</i>	Colorless liquid
<i>Molecular formula</i>	C ₃ H ₆ O
<i>Molecular weight</i>	58.08
<i>Density</i>	0.83 g/cm ³ @ 20° C
<i>Boiling point</i>	34.23° C
<i>Melting point</i>	-112.13° C
<i>Vapor pressure</i>	445 torr @ 20° C
<i>Solubility</i>	Soluble in water, miscible in acetone, benzene, carbon tetrachloride, methanol, ether
<i>Conversion factor</i>	2.38 mg/m ³ per ppm at 25° C

III. Major Uses or Sources

Propylene oxide is used as a fumigant such as in the sterilization of packaged foods. It is also used as a chemical intermediate in the production of propylene glycol and glycol ethers and as a solvent. Propylene oxide is used in the preparation of surfactants and oil demulsifiers (HSDB, 1994). The annual statewide emissions from facilities reporting under the Air Toxics Hot Spots Act in California, based on the most recent inventory, were estimated to be 619,494 pounds of propylene oxide.

IV. Effects of Human Exposures

Conclusive data regarding the effects of occupational exposure to propylene oxide were not located.

An epidemiological study examining mortality among workers with exposure to asbestos and several chemicals, including propylene oxide, identified three deaths due to mesothelioma, a rare cancer associated with asbestos exposure, and a statistically significant increase in the number of deaths attributed to forms of heart disease other than ischemia and hypertension (Egedahl *et al.*, 1989). The latter finding was explained by the authors to be the result of differences in diagnostic accuracy between rural and urban, and primary and tertiary medical care settings. A statistically significant decrease in observed deaths was found for all respiratory cancers, cancer of the bronchus and lung, circulatory disease, digestive diseases, cirrhosis and other liver disease, and death due to accidents, poisonings, and violence. These observations may be partially attributed to a "healthy worker effect".

V. Effects of Animal Exposures

Male and female rats were exposed for 124 or 123 weeks (respectively) to 30, 100 or 300 ppm propylene oxide 6 hours per day, 5 days per week (Kuper *et al.*, 1988). Interim sacrifices were performed at 12, 18, and 24 months. Cumulative mortality was statistically significantly different from controls at 115 weeks in rats of both sexes exposed to 300 ppm propylene oxide. Cumulative mortality was also significantly different from controls at 119 weeks in female rats exposed to 100 ppm. However, a contributing factor to the increased mortality in female rats was the presence of mammary tumors. Atrophy of the olfactory epithelium and degenerative changes in the respiratory epithelium were observed in both male and female rats following 28 months of exposure to 30, 100, or 300 ppm propylene oxide. Severe hyperplastic changes in the olfactory epithelium were observed in male and female rats following 28 months exposure to 300 ppm propylene oxide. Mild hyperplastic changes were observed in the olfactory epithelium of female rats exposed to 100 ppm propylene oxide.

Rats and mice were exposed to 200 and 400 ppm propylene oxide 6 hours per day, 5 days per week for 103 weeks (NTP, 1985). Survival in mice was adversely affected in all groups exposed to propylene oxide; a statistically significant decrease in survival was observed in male and female mice exposed to 400 ppm propylene oxide. Survival in rats was not adversely affected by propylene oxide exposure. Rats exhibited exposure-related increases in suppurative inflammation of the nasal cavity, epithelial hyperplasia and squamous metaplasia.

Rats were exposed to 1500 ppm propylene oxide 6 hours per day, 5 days per week for 7 weeks (Ohnishi *et al.*, 1988). After 3-4 weeks of exposure the rats exhibited an awkward gait; the rats were ataxic by the seventh week. Histopathological examination revealed axonal degeneration of myelinated fibers of the hindleg nerve and fasciculus gracilis indicating central-peripheral distal axonopathy.

Eldridge *et al.* (1995) exposed male F344 rats to 0, 10, 20, 50, 150, or 525 ppm propylene oxide vapor for up to 4 weeks (with up to 4 weeks of recovery). Histopathology showed that the incidence and severity of respiratory epithelial hyperplasia increased with exposure time and regressed after termination of exposure, with complete recovery after 4 weeks. Cell proliferation (determined by bromodeoxyuridine incorporation) was elevated following 1 and 4 weeks of exposure, but decreased to control values after 1 week of recovery. Degeneration of the olfactory epithelium was found after 4 weeks of exposure with a decrease in incidence and severity after termination of exposure. Proliferation of olfactory epithelium was elevated during the 4-week exposure period and 1 week post-exposure and returned to control values after 4 weeks of recovery. The authors report a 4-week NOAEL for propylene oxide effects in nasal epithelium of 50 ppm.

Artificially inseminated rabbits were exposed to 500 ppm propylene oxide on days 1-19 or 7-19 of gestation (Hardin *et al.*, 1983). Maternal toxicity as indicated by a significant reduction in food intake and a significant decrease in maternal body weight gain was observed in both exposed groups. An increased number of resorptions per litter, with no change in total resorptions, was observed in rabbits exposed on days 1-19 of gestation. Sternebral and limb anomalies (considered minor by U.S. EPA and the authors) were significantly increased in the offspring of rabbits exposed on days 1-19 of gestation.

The same study also reported similar findings in sperm-positive rats exposed to 500 ppm propylene oxide on either days 1-16 or 7-16 of gestation or daily for 3 weeks prior to mating and then daily on days 1-16 of gestation. Reproductive capacity was impaired in rats exposed prior to breeding; the number of corpora lutea, implantation sites, and live fetuses were reduced. Those dams exposed pregestationally to propylene oxide also exhibited more resorptions. Maternal toxicity as indicated by decreased food intake and decreased body weight gain was observed in all exposed rats. Significant reductions in fetal body weight and fetal crown-rump length were observed in all exposed groups. An increased incidence of wavy ribs and reduced ossification were observed in the offspring of rats exposed from days 1-16 of gestation.

Harris *et al.* (1989) evaluated the developmental toxicity potential of propylene oxide in Fischer 344 rats. Four groups of 25 mated female rats were exposed to 0, 100, 300, and 500 ppm for 6 hours per day on gestation days 6 through 15. Cesarean sections were performed on all females on gestation day 20 and the fetuses were removed for morphological evaluation. Exposure to propylene oxide did not adversely affect survival, appearance, or behavior at any level. Maternal body weight gain and food consumption were reduced significantly at the 500 ppm level during exposure. Only one exposure-related effect was noted with respect to maternal water consumption, organ weights, cesarean section, or fetal morphological observations: increased frequency of seventh cervical ribs in fetuses at the maternally toxic exposure level of 500 ppm. Thus 300 ppm was considered the NOAEL.

VI. Derivation of Chronic REL (U.S. EPA Reference Concentration (IRIS, 1995))

<i>Study</i>	Kuper <i>et al.</i> , 1988
<i>Study population</i>	Rats (male and female)
<i>Exposure method</i>	Inhalation (0, 30, 100 or 300 ppm)
<i>LOAEL</i>	30 ppm
<i>Critical effects</i>	Degenerative and hyperplastic changes in the respiratory epithelium
<i>NOAEL</i>	Not observed
<i>Exposure continuity</i>	6 hr/day for 5 days/week
<i>Exposure duration</i>	124 weeks
<i>Average experimental exposure</i>	5.4 ppm for LOAEL group (30 x 6/24 x 5/7)
<i>Human equivalent concentration</i>	1.2 ppm for LOAEL group (gas with extrathoracic respiratory effects, RGDR = 0.23, based on MV = 0.3 m ³ /day, SA(ET) = 11.6 cm ²)
<i>LOAEL uncertainty factor</i>	3 (mild effects only observed during last 4 months of exposure)
<i>Subchronic uncertainty factor</i>	1
<i>Interspecies uncertainty factor</i>	3
<i>Intraspecies uncertainty factor</i>	10
<i>Cumulative uncertainty factor</i>	100
<i>Inhalation reference exposure level</i>	0.009 ppm (9 ppb, 0.03 mg/m ³ , 30 µg/m ³)

VII. Data Strengths and Limitations for Development of the REL

The chronic REL is equivalent to the US EPA RfC. The major strength of the REL for propylene oxide is the use of a well-conducted, long-term, multi-concentration study with adequate histopathological analyses. Weaknesses include the lack of adequate human data and the lack of a chronic NOAEL observation.

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CHRONIC TOXICITY SUMMARY

SELENIUM AND SELENIUM COMPOUNDS

(other than Hydrogen Selenide)

<i>Molecular Formula</i>	<i>Synonyms</i>	<i>Molecular Weight (g/mol)</i>	<i>CAS Reg. No.</i>
Se	elemental selenium	78.96	7782-49-2
SeO ₂	selenium dioxide; selenium oxide; selenious anhydride	110.96	7446-08-4
H ₂ SeO ₃	selenious acid	128.97	7783-00-8
SeOCl ₂	seleninyl chloride; selenium oxychloride; selenium oxichloric	165.86	7791-23-3
Na ₂ SeO ₃	disodium selenite	263.01	10102-18-8
Na ₂ SeO ₄	disodium selenate	188.94	13410-01-0
SeS	selenium sulfide; sulfur selenide	111.02	7446-34-6

I. Chronic Toxicity Summary

<i>Inhalation reference exposure level</i>	20 µg/m³
<i>Oral reference exposure level</i>	0.005 mg/kg/day (USEPA RfD)
<i>Critical effect(s)</i>	Clinical selenosis
<i>Hazard index target(s)</i>	Alimentary system; cardiovascular system; nervous system

II. Chemical Property Summary (HSDB, 1995; Weast, 1980; Canady and Hodes, 1994; ACGIH, 1992)

<i>Description</i>	Se ⁰ crystal: metallic gray H ₂ SeO ₄ , Na ₂ SeO ₃ : white crystals H ₂ SeO ₃ , Na ₂ SeO ₄ : colorless crystals SeO ₂ : lustrous crystals; yellow vapor SeS: yellow to orange powder
<i>Molecular formula</i>	see above
<i>Molecular weight</i>	see above
<i>Vapor pressure</i>	0.001 torr @ 20°C
<i>Melting point</i>	SeO ₂ : 340°C SeS: decomposes at 118-119°C
<i>Solubility</i>	Se ⁰ : insoluble in water, alcohol; slightly soluble in CS ₂ ; soluble in ether H ₂ SeO ₄ : sol. in water; decomposes in alcohol H ₂ SeO ₃ : sol. in hot water, alcohol Na ₂ SeO ₃ : sol. in water Na ₂ SeO ₄ : 84 g/100 ml water at 35°C SeO ₂ : 38.4 g/100 ml water at 14°C SeS: insoluble in water
<i>Conversion factor</i>	Se ⁰ : not applicable (particulate) SeO ₂ : 4.5 µg/m ³ per ppb at 20°C

III. Major Uses and Sources

Selenium occurs in four valence states: selenates (Se⁶⁺), selenites (Se⁴⁺), selenides (Se²⁻), and elemental selenium (Se⁰) (Goyer, 1991) which include compounds formed with oxygen, sulfur, metals, and/or halogens. Selenium compounds are used in the glass industry as decolorizing agents and in the rubber industry as vulcanizing agents. Selenium compounds are also found in toning baths used in photography and xerography, and in insecticides and photoelectric cells. Selenious acid is a component of gun cleaning chemicals (Quadrani *et al.*, 2000). Selenium sulfide is used in shampoos as an anti-dandruff agent. The most widely used selenium compound in industry is selenium dioxide (SeO₂) which catalyzes reactions of organic compounds and is produced by the oxidation of selenium with nitric acid followed by evaporation or by burning selenium in oxygen (HSDB, 1995). The largest anthropogenic sources of atmospheric selenium are from the combustion of fossil fuels and the production/refining of copper; particulates are the primary expected form of the compound (National Academy of Sciences (NAS), 1976; U.S. EPA, 1984). The annual statewide industrial emissions from facilities reporting under the Air Toxics Hot Spots Act in California based on the most recent inventory were estimated to be 12,417 pounds of selenium and 4846 pounds of selenium sulfide (CARB, 1999).

Selenium is an essential trace element in humans and other species; selenium deficiency leads to cardiomyopathy in humans (Goyer, 1991). For dietary intake, the National Research Council has

set a U.S. Recommended Daily Allowance (RDA) of 0.87 $\mu\text{g}/\text{kg}$ (55-70 $\mu\text{g}/\text{person}/\text{day}$) (Subcommittee on the Tenth Edition of the RDAs, 1989). The average daily oral intake of selenium is 125 $\mu\text{g}/\text{person}$ (U.S. EPA, 1991). Organic selenium compounds (e.g., dimethyl selenide) are known to occur as metabolites and as microbial degradation products in the environment. These compounds appear to have relatively low toxicity.

IV. Effects of Human Exposures

Acute occupational exposure to SeO_2 resulted in bronchospasm, irritation of the upper respiratory passages, violent coughing, and gagging with nausea and vomiting (Wilson, 1962).

The relationship between inhalation exposure to selenium and the presence of selenium in the urine was investigated in a five year study of workers at a selenium rectifying plant (Glover, 1967). Workers were exposed to fumes and dusts of elemental red selenium, which, the author reported, is converted 80% to SeO_2 in the presence of air. Average air concentrations of selenium were reported to be 3.6 mg/m^3 in grinding processes, 0.04 mg/m^3 in annealing processes, and a range of averages of 0.23-0.87 $\text{mg Se}/\text{m}^3$ in various "special" processes, e.g., punching, scraping, sorting, refining, and testing. The same author previously reported symptoms among selenium exposed workers including garlic-like odor of the breath, skin rashes, indigestion, and poorly-defined "socio-psychological" effects including lassitude and irritability (Glover, 1954).

Clinical signs of toxicity were observed among a population exposed to high levels of selenium in soils and food supplies in China (Yang *et al.*, 1983). Approximately half of 248 people in this region showed symptoms including hair and nail loss, discoloration and decay of the teeth, and CNS disturbances including pain and anesthesia of the extremities. Animals in the region were also affected, with hoof damage and horn sloughing reported in water buffalo, cattle, and pigs. Populations in low-, medium-, and high-selenium areas of China were later studied to associate the symptoms with selenium intake. Estimated daily intake for adults in these areas were 70, 195, and 1438 $\mu\text{g Se}$ for males and 62, 198, and 1238 μg for females, respectively (Yang *et al.*, 1989). Selenium intake was highly correlated with whole blood, breast milk, and 24-hour urine selenium levels. The authors also suggested the possibility of liver dysfunction as indicated by a delay in prothrombin time among persons with intake of 750-850 $\mu\text{g Se}/\text{day}$. More clearly recognized and characteristic clinical signs, however, were only observed in a group exposed to greater than 1261 $\mu\text{g Se}/\text{day}$ and not among those exposed to less than 853 $\mu\text{g Se}/\text{day}$. Assuming a 55 kg body weight, these respective daily dose rates were 0.023 and 0.015 $\text{mg}/\text{kg}\text{-day}$.

A population of 142 subjects in seleniferous areas of western South Dakota and eastern Wyoming was examined for signs of selenosis over a two-year period with monitoring of selenium levels in diet, whole blood, serum, urine, and toenails (Longnecker *et al.*, 1991). Subjects completed health questionnaires, underwent physical examinations, provided blood samples for clinical assessment, and provided blood, urine, toenails, and duplicate-plate food collections for selenium analysis. About half of the 142 free-living subjects had selenium intakes greater than 2.54 $\text{m}\mu\text{mol}/\text{day}$ (200 $\mu\text{g}/\text{day}$) (range 0.86-9.20 $\text{m}\mu\text{mol}/\text{day}$, or 68-724 $\mu\text{g}/\text{day}$). Average intake among the population was estimated at 239 $\mu\text{g Se}/\text{day}$. No clinical

signs and no changes in hematological function, clinical chemistry, or liver function were observed in the population, even in subjects whose intake was as high as 9.20 $\mu\text{mol/day}$ (724 $\mu\text{g/day}$).

V. Effects of Animal Exposures

Toxic effects from acute inhalation exposure to selenium dust were examined in rats, guinea pigs, and rabbits (Hall *et al.*, 1951). Twenty female rats were exposed once for 8 hours to $33 \pm 10 \text{ mg Se/m}^3$. Many animals showed signs of pulmonary effects at both one week and 4 weeks after exposure; however, no control group was included in the experiment with which to compare incidence. Similarly, six female rabbits and 10 male guinea pigs were exposed to the same level of selenium dust for four 4-hour periods every 48 hours (8 days total duration). The animals showed signs of interstitial pneumonitis at one week (2 animals of each species) and lung congestion and alveolar infiltration of large macrophages.

Guinea pigs exposed one time to concentrations “less than 0.021 mg $\text{H}_2\text{Se/L}$ ” (22 mg Se/m^3 as hydrogen selenide) for 2, 4, or 8 hours exhibited difficulty breathing and a red-tinged discharge from the nose (Dudley and Miller, 1941). Mortality studies were conducted with guinea pigs (16/group) using the same exposure duration and selenium concentrations ranging from 1 to 43 mg Se/m^3 . Fifty percent mortality was observed at 30 days among animals exposed once for 2 hours to 12 mg Se/m^3 . Mortality after 30 days was 50% among animals exposed once to 1 mg Se/m^3 for 8 hours. Histopathological evaluation of guinea pigs exposed once for 4 hours to 8 mg Se/m^3 showed fatty change to the liver, pneumonia, lymphoid hyperplasia, and increased reticuloendothelial tissue in the spleen. These effects did not begin to resolve until more than 17 days after the exposure.

Several studies have addressed the toxicity of selenium compounds to animals when administered in either food or drinking water. Mice (50/group) treated with 0, 1, 4, or 8 ppm Na_2SeO_3 in drinking water over 50 weeks showed decreased growth rates at 8 ppm (Jacobs and Forst, 1981). The same group reported gross liver pathology in male mice treated by oral gavage for 3 days with 0.5 ml of 64 ppm Na_2SeO_3 . Hamsters (8/sex/group) treated with 0.1 (unsupplemented), 1, 5, 10, or 20 ppm Na_2SeO_3 in the diet for 42 days showed histopathological changes to the liver (Beems and van Beek, 1985). Rats (6-8/group) treated in the diet with SeS_2 , Na_2Se , Na_2SeO_3 , or Na_2SeO_4 showed increased relative liver weights and/or decreased body weight gain at 10 ppm (for each compound) over a 5 week exposure (Dausch and Fullerton, 1993). A 13-week drinking water study of Na_2SeO_3 , and Na_2SeO_4 in rats and mice showed increased mortality, decreased body weights, and histopathological changes to the kidneys in rats and decreased body weight and decreased water consumption in mice (Abdo, 1994). Decreased body weights were observed in rats treated for 6 weeks in drinking water with 2 ppm Na_2SeO_3 or Na_2SeO_4 (Palmer and Olson, 1974).

Decreased percentage of live spermatozoa, altered sperm morphology, and decreased body weight gain were observed in rats (6/group) treated for 5 weeks with 2 ppm Na_2SeO_3 in the diet (Kaur and Parshad, 1994). Rats (7-12/group) exposed to 0, 4, 8, or 16 ppm Na_2SeO_3 in drinking

water for 240 days showed alterations in testicular LDH and β -glucuronidase activity at 4 ppm (Nebbia *et al.*, 1987).

Developmental toxicity endpoints were examined in hamsters (5-10/group) exposed by oral gavage on gestational day 8 to Na_2SeO_3 and Na_2SeO_4 at concentrations ranging from 0 - 110 $\mu\text{mol/kg}$ body weight (Ferm *et al.*, 1990). Effects observed at 100 $\mu\text{mol Na}_2\text{SeO}_3/\text{kg}$ included decreased fetal crown-rump length and increased percentage of abnormal litters. At 90 $\mu\text{mol Na}_2\text{SeO}_4/\text{kg}$, an increased percentage of abnormal litters was observed. Mice (10 or 14/group) treated with 0, 3, or 6 ppm Na_2SeO_3 in drinking water from 30 days pre-gestation through gestation showed altered estrus cycle length, decreased fetal growth, and a decreased number of ossified vertebrae in offspring (Nobunaga *et al.*, 1979).

VI. Derivation of Chronic Reference Exposure Level (REL) (for selenium and selenium compounds other than hydrogen selenide)

<i>Study</i>	Yang <i>et al.</i> , 1989
<i>Study population</i>	400 people in China
<i>Exposure method</i>	Low, medium, & high environmental levels of Se
<i>Critical effects</i>	Clinical selenosis (liver, blood, skin, CNS)
<i>LOAEL</i>	0.023 mg/kg-day* (1.261 mg/day / 55 kg)
<i>NOAEL</i>	0.015 mg/kg-day* (0.853 mg/day / 55 kg)
<i>Exposure continuity</i>	Continuous
<i>Exposure duration</i>	Lifetime
<i>Average experimental exposure</i>	70, 195, and 1438 $\mu\text{g/day}$ for adult males; 62, 198, and 1238 $\mu\text{g/day}$ for adult females
<i>LOAEL uncertainty factor</i>	1
<i>Subchronic uncertainty factor</i>	1
<i>Interspecies factor</i>	1
<i>Intraspecies factor</i>	3
<i>Cumulative uncertainty factor</i>	3
<i>Oral reference exposure level</i>	0.005 mg/kg/day (USEPA RfD)
<i>Inhalation extrapolation factor</i>	3,500 $\mu\text{g/m}^3$ per mg/kg-day
<i>Inhalation reference exposure level</i>	20 $\mu\text{g/m}^3$

*Factors: NOAEL (0.853 mg/day) and LOAEL (1.261 mg/day) calculated from regression analysis ($\log Y = 0.767 \log X - 2.248$, where Y = blood selenium and X = selenium intake) based upon the correlation ($r = 0.962$) between dietary selenium intake and blood selenium level for data showing incidence of clinical selenosis in adults based on an average adult body weight of 55 kg.

The inhalation chronic REL is based on the oral chronic REL, which is the same as the USEPA's oral reference dose (RfD) (U.S. EPA, 1996). In addition to being inhaled, airborne selenium can settle onto crops and soil and enter the body by ingestion. Thus an oral chronic reference exposure level for selenium is also required for Air Toxics Hot Spots health risk assessments. The chronic inhalation REL was derived by route-to-route extrapolation of the RfD. The

principal study used for the REL/RfD was that of Yang *et al.* (1989). Yang *et al.* (1989), in a follow-up to an earlier study (Yang *et al.*, 1983), studied a population of approximately 400 individuals living in an area of China with unusually high environmental concentrations of selenium (Se). The subjects were evaluated for clinical and biochemical signs of Se intoxication. Three geographical areas with low, medium, and high selenium levels in the soil and food supply were chosen for comparison in the studies. The earlier study was conducted in response to endemic selenium intoxication in two separate areas with sample sizes of only 6 and 3. Comparisons were then made to a selenium-adequate area (n=8) and low-selenium area (n=13). The Yang *et al.* (1989) studies provide a much larger sample size and include additional analysis of tissue selenium levels. This allows a more accurate estimation of the dose-response relationship observed for selenium toxicity. Selenium levels in soil and approximately 30 typical food types commonly eaten by the exposed population showed a positive correlation with blood and tissue Se levels. The daily average Se intakes, based on lifetime exposure, were 70, 195, and 1438 μg for adult males and 62, 198, and 1238 μg for adult females in the low-, medium- and high-selenium areas, respectively. Significant correlations, demonstrated between Se concentrations of various tissues, were used to estimate the minimal daily Se intake values that elicited various alterations in biochemical parameters indicative of possible Se-induced liver dysfunction (i.e., prolongation of clotting time and serum glutathione titer) and clinical signs of selenosis (i.e., hair or nail loss, morphological changes of the nails, etc.). In this manner, a marginal safe level of daily Se intake was estimated. Analysis of the results indicated that persistent clinical signs of selenosis were observed only in 5/349 adults, a potentially sensitive subpopulation. The blood selenium concentration in this group ranged from 1.054 to 1.854 mg/L with a mean of 1.346 mg/L. Clinical signs observed included the characteristic "garlic odor" of excess selenium excretion in the breath and urine, thickened and brittle nails, hair and nail loss, lowered hemoglobin levels, mottled teeth, skin lesions, and CNS abnormalities (peripheral anesthesia, acroparesthesia, and pain in the extremities). Alterations in the measured biochemical parameters occurred at dietary intake levels of 750-850 $\mu\text{g}/\text{day}$. These alterations were described as a delay in prothrombin time, i.e., increase in blood coagulation time and reduction in blood glutathione concentration. However, these indicators were poorly characterized and are not typically used as an index for clinical selenosis resulting from chronic exposure to selenium (NAS, 1989). Based upon the blood selenium levels shown to reflect clinical signs of selenium intoxication, a whole blood selenium concentration of 1.35 mg/L corresponding to 1.261 mg of daily selenium intake is indicative of the lowest correlative selenium intake causing overt signs of selenosis. The next lowest whole blood selenium concentration of 1.0 mg/L, corresponding to 0.853 mg selenium/day, produces no clinical signs of selenosis. The NOAEL for this study is 0.85 mg Se/day and the LOAEL is 1.26 mg Se/day.

An intraspecies uncertainty factor of 3 was applied to the NOAEL to account for sensitive individuals. A full factor of 10 was not deemed necessary since similar NOAELs were identified in two moderately-sized human populations exposed to selenium levels in excess of the RDA throughout a lifetime without apparent clinical signs of selenosis. No modifying factor was applied by USEPA. OEHHA accepted the USEPA analysis.

Route-to-route extrapolation assumes by default that a chemical is equally absorbed by the inhalation and the oral routes and that the first pass effect due to metabolism by the liver is not important for the chemical. The latter assumption is applicable to most metals. There are

limited data to evaluate the assumption of equal absorption across the gastrointestinal tract and the lungs. Limited data indicate that 60% (range = 44-100%) of ingested Se is absorbed by the gastrointestinal tract, while in one study 30% (single estimate) of inhaled selenium was deposited in the respiratory tract (Owen, 1990). Deposition is dependent on particle size. The available data are not adequate to depart from the default assumption.

The USEPA stated its confidence in the RfD as: Study - Medium; Data Base - High; and RfD - High. Confidence in the chosen principal study is medium. Although this is a human epidemiological study in which a sizable population with sensitive subpopulations was studied, there are still several possible interactions that were not fully accounted for, e.g., fluoride intake and protein status. Also, except for clinical signs of selenosis there are no other reliable indicators, biochemical or clinical, of selenium toxicity. Confidence in the database is high because many animal studies and epidemiologic studies support the principal study. An additional human study with a freestanding NOAEL (Longnecker et al., 1991) provides support for the NOAEL identified in the principal study. Longnecker *et al.* (1991) found no effects at 238 µg Se per day, which would equate to 0.004 mg/kg-day for a 55 kg person. Therefore, high confidence in the RfD is selected based upon support of the critical study and the high level of confidence in the database.

There are insufficient data relating human inhalation exposure to selenium compounds to adverse health effects to use for the development of a chronic REL although toxicity has been reported from occupational exposure to gases of both H₂Se and SeO₂ (Buchan, 1947; Wilson, 1962). Experiments in animals have shown that H₂Se is toxic following inhalation exposure, with 8-hour exposures to concentrations as low as 1 mg H₂Se/m³ causing “irritation sufficiently damaging to cause pneumonitis” and subsequently increasing 30-day mortality (Dudley, 1937; Dudley and Miller, 1941). Thus the selenium chronic REL is not meant to be applied to H₂Se, which may be considerably more toxic than other selenium compounds. At this time there are inadequate data to develop a REL for H₂Se. It is also not intended to be applied to organic metabolites of selenium.

VII. Data Strengths and Limitations for Development of the REL

The strengths of the REL for selenium include its basis on a study with a large number of human subjects in a non-occupational setting that determined both a NOAEL and a LOAEL. The weaknesses include its basis on a route of exposure other than inhalation and its lack of applicability to hydrogen selenide, the most toxic selenium compound.

VIII. Potential for Differential Impacts on Children's Health

The key study (Yang *et al.*, 1989) included evaluation of children as young as one year old. Thus the chronic REL should be protective of infants and children. No adverse reproductive outcomes were reported, although only 400 people were studied. However, the inhalation REL is based on an oral REL of 0.005 mg/kg-day (0.06 µmol/kg-day). Ferm *et al.* (1990) did not find adverse effects on hamster development with Se doses below 34 µmol/kg. Thus the chronic REL should also be protective of infants and children.

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CHRONIC TOXICITY SUMMARY

SILICA (CRYSTALLINE, RESPIRABLE)*(silicon dioxide, quartz, tridymite, cristobalite)*

CAS Registry Number: 7631-86-9

I. Chronic Toxicity Summary

<i>Inhalation Reference Exposure Level</i>	3 µg/m³ [respirable, as defined occupationally by ACGIH (2004)/ISO (1995)]
<i>Critical effect(s)</i>	Silicosis in miners and other workers
<i>Hazard index target(s)</i>	Respiratory system

II. Physical and Chemical Properties (HSDB, 2001)

<i>Description</i>	Transparent crystals
<i>Molecular formula</i>	SiO ₂
<i>Molecular weight</i>	60.09 g/mol
<i>Density</i>	2.65 g/cm ³ @ 0 °C (quartz)
<i>Melting point</i>	1610 °C
<i>Boiling point</i>	2230 °C (2503.20 °K)
<i>Vapor pressure</i>	10 torr @ 1732 °C
<i>Solubility</i>	Practically insoluble in water or acids, except hydrofluoric acid; very slightly sol. in alkali.
<i>Conversion factor</i>	Not applicable

In crystalline silica, the silicon and oxygen atoms are arranged in a definite regular pattern throughout the crystal. The characteristic crystal faces of a crystalline form of silica are the outward expression of this regular arrangement of the atoms (HSDB, 2001). This REL is meant to be applied only to particles of crystalline silica (quartz, cristobalite, tridymite), of respirable size, as defined by the occupational hygiene methods described by ACGIH (2004)/ISO (1995) which has a 50% cut-point at 4 µm particle aerodynamic diameter. This occupational definition of respirable differs from the environmental definition of respirable, which is PM₁₀. (The occupational particle category “thoracic” has a 50% cut-point at 10 µm particle diameter (ACGIH, 2004) and the category “inhalable” has a 50% cut-point at 100 µm particle diameter (ACGIH, 2004).)

III. Major Uses and Sources

At least 11 chemically identical forms (polymorphs) have been described for crystalline silica. Alpha-quartz is the most abundant polymorph and constitutes 12% of the earth's crust (Elzea, 1997). Silica is also found in the amorphous (non-crystalline) state. The amorphous silica in diatomaceous earth (composed mainly of the cell walls of diatoms) can be converted to the crystalline form cristobalite by heating to 1000-1100 °C (calcining). Silica is often associated

with silicates, which, in addition to silicon and oxygen, contain other metals such as iron, magnesium, aluminum, calcium, potassium, and sodium.

The major uses of silica are in the manufacture of glass, abrasives, ceramics, and enamels, in scouring and grinding compounds, and in molds for castings. Silica is also used in decolorizing and purifying oils and petroleum products; as a clarifying agent; in filtering liquids; and in the manufacture of heat insulators, firebrick, and fire- and acid-proof packing materials. As diatomite (naturally occurring diatomaceous earth), silica is used as a filtration agent, as an abrasive, and as an industrial filler. Sources of ambient respirable crystalline silica in California include mines, quarries, diatomaceous earth calcining plants, sand blasting, and entrained fines (e.g., PM10) from surface soil. The annual statewide industrial emissions from facilities reporting under the Air Toxics Hot Spots Act in California based on the most recent inventory were estimated to be 2,514,981 pounds of crystalline silica (CARB, 2001). The fraction, which is respirable as defined either occupationally or environmentally, is not known.

Measurement of crystalline silica has evolved. Instrumentation has varied by country. In South Africa since the 1930s, dust was collected with a konimeter (Le Roux, 1970; Cherrie and Aitken, 1999). A small volume of air (e.g., 5 cm³ captured in less than a second) was collected (impacted) onto a small area of a glass slide coated with adhesive. Total dust particles were counted and expressed as dust particles per cubic centimeter. Later, slides were heated to 500-550 °C (ignition) to remove carbonaceous materials and immersed in hot 50% hydrochloric acid followed by a second ignition to remove acid-soluble materials. The remainder was mostly silica particles, which could be counted. The konimeter was superseded by the thermal precipitator, which also deposited particles onto glass but could sample larger air volumes at high flow rates (> 1 L/minute) for several hours. With time, particle counting was replaced by estimation of a particle's surface area, initially by examining slides but more recently by an automated method (Kitto, 1960; 1970).

In the United States the impinger method was used from 1922 until 1984 (Lippmann, 2001). Air was drawn into a trap containing fluid, particles in an aliquot of the fluid were counted under magnification, and concentrations were expressed as million particles per cubic foot of air sampled. Later, gravimetric analysis was introduced. Gravimetric analysis is dominated by the larger particles in any given size range.

When it was realized that only a fraction of the dust was responsible for silicosis, respirable dust was collected onto filters using size-specific dust collectors, such as horizontal plate elutriators in South Africa and cyclones in the United States. The sizes of particles collected on the filter were a function of the apparatus used and the rate of airflow through the apparatus. Quartz dust was quantified by examining filters in an electron microscope with a specific X-ray diffraction beam absorbed by crystalline silica. The National Institute of Occupational Sciences and Health (NIOSH, 2003) has approved Method 7500, which uses one of three approved cyclones and a 5 µm PVC membrane filter to sample, and X-ray diffraction to measure crystalline silica. The ARB has used Method 7500 in research projects.

In order to harmonize respirable particulate sampling methodology in workers, an international agreement has been reached to use dust samplers that have a 50% cut point for particles of 4 µm aerodynamic diameter (ISO, 1995; ACGIH, 2004).

Various attempts have been made to estimate the changes in silica levels in workplaces over time (e.g., Seixas et al., 1997 for diatomaceous earth facilities in California; Verma et al., 1989 for Ontario hard rock miners). However, although some conversion factors have been proposed, correlation between dust particle number in earlier studies, when dust concentrations were higher, and dust particle weight in the later studies, when the dust concentrations have been lowered, is imprecise so it is difficult to compare the earlier silica measurements with the more recent ones.

IV. Effects of Human Exposures

Inhalation of crystalline silica initially causes respiratory irritation and an inflammatory reaction in the lungs (e.g., Vallyathan et al., 1995). Acute exposures to high concentrations cause cough, shortness of breath, and pulmonary alveolar lipoproteinosis (acute silicosis). After chronic but lower workplace exposures to silica for six to sixteen years, the small airways become obstructed as measured by pulmonary function tests (e.g., decreased FEV1) in granite quarry workers (no measurement of silica levels reported; Chia et al., 1992). In a report on the hazards of exposure to crystalline silica, the American Thoracic Society (1997) stated: "Studies from many different work environments suggest that exposure to working environments contaminated by silica at dust levels that appear not to cause roentgenographically visible simple silicosis can cause chronic airflow limitation and/or mucus hypersecretion and/or pathologic emphysema." Hnizdo and Vallyathan (2003) also concluded that "chronic levels of silica dust that do not cause disabling silicosis may cause the development of chronic bronchitis, emphysema, and/or small airways disease that can lead to airflow obstruction, even in the absence of radiological silicosis." Fibrotic lesions associated with crystalline silica have also been found at autopsy in the lungs of granite workers who lacked radiological evidence of silicosis (Craighead and Vallyathan, 1980).

Silicosis results from chronic exposure; it is characterized by the presence of histologically unique silicotic nodules and by fibrotic scarring of the lung. The histological progression of silicosis has been described as: (1) granuloma composed of histiocytic cells, collagen, and lymphocytes; (2) cellular fibrotic nodule with irregular collagen at the center and circular collagen at the periphery; (3) more mature nodule with acellular and avascular center; and (4) late mature nodule composed of dust and collagen including a calcified center (Green and Vallyathan, 1996). Lung diseases other than cancer associated with silica exposure include silicosis, tuberculosis/silicotuberculosis, chronic bronchitis, small airways disease, and emphysema (Oxman et al., 1993; Park et al., 2002; Hnizdo and Vallyathan, 2003; Balmes et al., 2003). Silica exposure has been implicated in autoimmune diseases (rheumatoid arthritis, scleroderma, systemic lupus erythematosus) in gold miners and granite workers (Steenland and Goldsmith, 1995; Parks et al., 1999) and in the causation of kidney disease in some occupations (Goldsmith and Goldsmith, 1993; Stratta et al., 2001), possibly by an immune mechanism.

At the cellular level, silica particles are engulfed in the lung by alveolar macrophages (AM). According to the generally assumed pathological model, the AM subsequently release various growth factors and reactive oxygen species (ROS; superoxide anion, hydrogen peroxide, hydroxyl radical) (Lapp and Castranova, 1993; Mossman and Churg, 1998; Ding et al., 2002). ROS and some growth factors (e.g., activator protein-1, platelet activating factor) are inflammatory and attract neutrophils to the site of inflammation, while other factors (fibronectin,

alveolar macrophage-derived growth factor) stimulate fibroblasts to proliferate and to make collagen. Since silica particles cannot be digested by the macrophage, the inflammatory process becomes chronic (frustrated phagocytosis). An increased silica burden leads to more foci of inflammation, nodule formation, and fibrosis. The internal process can continue after external exposure ends. Silica particles also enter into alveolar Type I epithelial cells (Churg, 1996), which can lead to cell death of Type I cells and to hypertrophy and proliferation of Type II epithelial cells to replace the Type I cells. The epithelial repair process is associated with a subsequent increase in collagen formation.

The initial diagnosis of silicosis is often based on chest radiographs. Recent papers have used the 1980 classification by the International Labor Organization (ILO, 1980) to identify and classify silicosis into categories and subcategories of seriousness by comparison of patient radiographs with ILO-supplied reference radiographs taken at various stages of silicosis (Table 1):

Table 1. International Labor Organization categorization of silicosis (ILO, 1980).

<i>ILO Category</i>	<i>Qualitative Description</i>
0/0	No small (up to 1 cm) silicotic opacities (nodules) are present
0/1	Probably no nodules, but some areas of radiograph are suspect [possible silicosis]
1/0	Small silicotic nodules are most likely present, but not certainly [probable silicosis]
1/1	Small silicotic nodules are definitely present
1/2	Small silicotic nodules are definitely present; other areas of the radiograph may indicate more advanced lesions including large opacities (> 1 cm), pleural thickening, etc.
2/1, 2/2, 2/3, 3/2, 3/3	More advanced stages of silicosis/increasing certainty of the presence of lung abnormalities

Some reports (e.g., Kreiss and Zhen, 1996; Hughes et al., 1998) use 1/0 (probable) as the basis of classification of silicosis, since many cases of silicosis are not detected by chest radiographs, yet silicotic nodules and other lesions are found at autopsy (Craighead and Vallayathan, 1980; Hnizdo et al., 1993). Other reports (e.g., Hnizdo and Sluis-Cremer, 1993) use the definite 1/1 as the lowest category indicating silicosis. Some disease is missed by radiography and is determined only by autopsy (Hnizdo et al., 1993). The ILO criteria are intended as an epidemiologic classification and comparison tool, not as a diagnostic classification on an individual basis. In occupational medicine practice, a group of tests is used to clinically diagnose silica-related lung disease including physical examination, X-rays, and high resolution computed tomography (CT) scans of the lung (e.g., Begin et al., 1991; Olivetti et al., 1993).

A. Environmental silicosis

Several studies have reported "environmental silicosis", cases where the silicosis occurs in the absence of an industry usually associated with the disease (reviewed by USEPA, 1996). In one of the stronger examples, Saiyed et al. (1991) investigated non-occupational pneumoconiosis in Ladakh, India, high in the western Himalayas where there are no mines or industries. Among 449 randomly selected inhabitants of three villages, there were many cases of pneumoconiosis

associated with progressive massive fibrosis (nodules > 1 cm) and "egg shell" calcification of hilar glands. The prevalence of pneumoconiosis was 2.0% (3/150) in the village of Saboo, 20.1% (31/149) in Shey, and 45.3% (68/150) in Chushot, and corresponded with the severity of dust storms and the presence or absence of chimneys in the kitchens (i.e., ventilated cooking). Without chimneys (Chushot), dust concentrations in kitchens averaged 7.5 mg/m³ during cooking periods. The free silica content of the dust storms was 60-70%. The authors suggested that exposure to free silica from dust storms and to soot from cooking with domestic fuels caused the pneumoconiosis. Perhaps the interaction of silica and soot led to the disease. Such exposures in this and other studies, such as Bar-Ziv and Goldberg (1974), might be considered to be non-industrial but occupational, since the subjects studied by Saiyed et al. (1991) were involved in the domestic work of cleaning and cooking (USEPA, 1996). In any case, the exposures were very high and thus similar to some occupational exposures.

B. Occupational silicosis

Several relatively recent reports have presented data that allow a quantitative relationship between occupational dust exposure and the development of silicosis in workers to be calculated.

Hard rock miners in Ontario, Canada (Muir *et al.*, 1989)

Muir *et al.* (1989) examined the relationship between cumulative exposure to silica (free crystalline silica, specifically alpha-quartz) and the development of silicosis in 2109 male hard rock (uranium, gold, mixed metals) miners in Ontario, Canada. The miners began work between 1940 and 1959 and were followed either until they ended their dust exposure or until December 31, 1982 (whichever came first). Five X-ray readers examined chest radiographs; one or more readers identified 32 cases of silicosis, defined as ILO category 1/1 or greater with round opacities. All five readers agreed on only six cases, while 12 cases were identified by only one reader (Table 2). A Weibull model of the form

$$R(x) = 1 - \exp[-(\alpha x)^\beta] \quad (x \geq 0, \beta > 0)$$

gave the best fit to the data for cumulative risk R of silicosis as a function of cumulative exposure in units of (mg/m³)-yr. In this model x is the cumulative exposure (lagged five years), α is the Weibull scale parameter, and β is the Weibull shape parameter (Table 2). Estimates of α and β for each reader are given in Table II of Muir *et al.* (1989).

Table 2. Silicosis Risk vs. Cumulative Respirable Silica in (mg/m³)-y (Table IV of Muir *et al.*)

<i>Reader</i>	<i>Cases (n)</i>	<i>1% risk</i> ^a	<i>2% risk</i>	<i>5% risk</i>	<i>10% risk</i>
1	14	3.5 (2.4-5.1)	5.7 (3.9-8.4)	11.2 (6.8-18.2)	18.6 (9.9-35.0)
2	24	2.7 (2.0-3.6)	4.1 (3.2-5.3)	7.1 (5.5-9.1)	10.9 (8.1-14.8)
3	24	3.0 (2.3-3.9)	4.3 (3.4-5.3)	6.9 (5.6-8.5)	9.9 (7.8-12.7)
4	14	3.7 (2.6-5.2)	5.6 (4.1-7.7)	9.8 (6.7-14.3)	15.1 (9.3-24.4)
5	7	5.7 (4.0-8.0)	7.8 (5.5-11.0)	11.9 (7.8-18.3)	16.5 (9.7-28.2)
Any reader	32	2.1 (1.6-2.9)	3.3 (2.6-4.2)	6.0 (4.8-7.5)	9.6 (7.3-12.5)
At least 3	15	3.5 (2.5-4.9)	5.4 (4.0-7.3)	9.5 (6.6-13.6)	14.6 (9.3-23.2)
All readers	6	6.1 (4.1-8.9)	8.5 (5.6-12.8)	13.2 (7.8-22.5)	18.7 (9.7-36.1)

^a In parentheses is the 95% confidence interval (CI) for each risk estimate.

The Ontario cohort gives the shallowest dose-response relationship for silicosis of the several cohorts examined (see Summary Table 15 below) due in part to the lack of follow-up of members who left the mines (either for another type of work or for retirement). Silicosis often develops after leaving employment (Hnizdo and Sluis-Cremer, 1993; Chen *et al.*, 2001). In Hnizdo and Sluis-Cremer (1993), for more than half the cases of silicosis radiographic signs developed at an average of 7.4 years after mining exposure ended. In addition, some of the Ontario miners in the Muir *et al.* study may have changed to a less dusty job if their physician told them that their (annual) radiograph showed abnormalities. The lack of follow-up, leading to under-ascertainment of silicosis, is a serious limitation of this study.

Gray iron foundry workers (Rosenman *et al.*, 1996)

Rosenman *et al.* (1996) evaluated 1,072 (96.8% males) current and retired workers in a Mid-western gray iron foundry, which produces engine blocks for the automotive industry. Medical records and silica exposure data were analyzed for those with at least 5 years of employment as of June 1991. Nearly half had worked at the foundry for 20 years. Sixty had radiographic evidence of pneumoconiosis (ILO categories 1/0 and greater). Twenty-eight workers had radiographs consistent with silicosis; of these 25 had simple silicosis and three had progressive massive fibrosis. The prevalence of radiographic changes consistent with silicosis increased with years at the foundry, work area, quantitative silica exposure, and cigarette smoking. In regard to quantitative silica exposure, the authors stated that 0.3-2.7% of workers at the OSHA standard (90-100 $\mu\text{g}/\text{m}^3$) were silicotic, as were 4.9-9.9% of workers above 100 $\mu\text{g}/\text{m}^3$. After controlling for confounders, Rosenman *et al.* (1996) used a logistic regression analysis based on cumulative silica exposure to determine an odds ratio of 1.45 for developing a radiograph consistent with silicosis after 20 years of work at 100 $\mu\text{g}/\text{m}^3$ and an odds ratio of 2.10 after 40 years of work at 100 $\mu\text{g}/\text{m}^3$ (Tables 3 and 4). This study probably underestimates risk due to lack of follow-up of the current workers. Although silica is not the only toxic chemical in a foundry, the unique nature of the silicotic nodule diminishes the likelihood of confounding by other exposures.

Table 3. Silicosis risk based on Rosenman *et al.* data (Finkelstein, 2000)

<i>Cumulative silica exposure</i>	<i>Prevalence of silicosis</i>
< 2 (mg/m^3)-y	0.4%
2-6 (mg/m^3)-y	2.7%
> 6 (mg/m^3)-y	10%

Table 4. Odds ratios for silicosis (from Table 8 of Rosenman *et al.*)^a

<i>Time-weighted average silica exposure (mg/m³)</i>	<i>20-year cumulative exposure [(mg/m³)-y]</i>	<i>Odds ratio (95% C.I.)</i>	<i>40-year cumulative exposure [(mg/m³)-y]</i>	<i>Odds ratio (95% C.I.)</i>
0.010	0.2	1.04 (1.02-1.15)	0.4	1.08 (1.05-1.11)
0.025	0.5	1.10 (1.06-1.14)	1.0	1.20 (1.12-1.30)
0.050	1.0	1.20 (1.12-1.30)	2.0	1.45 (1.25-1.68)
0.075	1.5	1.32 (1.18-1.47)	3.0	1.74 (1.40-2.17)
0.100	2.0	1.45 (1.25-1.68)	4.0	2.10 (1.15-2.82)
0.150	3.0	1.74 (1.40-2.17)	6.0	3.04 (1.96-4.72)
0.200	4.0	2.10 (1.56-2.82)	8.0	4.40 (2.45-7.93)
0.300	6.0	3.04 (1.96-4.72)	12.0	9.24 (3.83-22.3)

^a Additional mean silica exposures, their calculated odds ratios, and 95% confidence intervals (C.I.) are given in the paper.

Diatomaceous earth workers in California (Hughes *et al.*, 1998; Park *et al.*, 2002)

Hughes *et al.* (1998) investigated 1,809 Caucasian male diatomaceous earth workers in Lompoc, California, who had at least one year of exposure to cristobalite between 1942 and 1987. The crystalline silica isomorph cristobalite is formed when the amorphous silica in diatomaceous earth is calcined at 1000-1100 °C. Quantitative estimates of dust exposure were made and published in the peer-reviewed literature by Seixas *et al.* (1997) based on 6395 air sampling records taken from 1948-1988. The average estimated respirable dust concentrations for 135 jobs were 3.55 ± 1.25 mg/m³ prior to 1949, 1.37 ± 0.48 mg/m³ from 1949-1953, 0.47 ± 0.16 mg/m³ from 1954-1973, and 0.29 ± 0.10 mg/m³ from 1974-1988. The workers had periodic chest radiographs. Based on the median of radiographic readings by three independent readers, 81 workers (4.5%) were judged to have opacities on chest radiographs (small opacities, ILO profusion $\geq 1/0$, and/or large opacities). Age-adjusted relative risk of opacities increased significantly with cumulative exposure to crystalline silica. The concentration of respirable crystalline silica was an important determinant of risk after accounting for cumulative exposure. The workers were split into two categories: those exposed to < 0.50 mg/m³ (or hired after 1950) and those exposed to > 0.50 mg/m³ (or hired before 1950). The risk of opacities for a cumulative exposure to crystalline silica of 2.0 mg/m³-yr is shown in Table 5.

Table 5. Silica exposure and silicosis based on data of Hughes *et al.* (1998)

<i>Average crystalline silica exposure</i>	<i>Cumulative risk of silicotic opacities</i>
< 0.50 mg/m ³ (or hired after 1950)	1.1%
> 0.50 mg/m ³ (or hired before 1950)	3.7%

The findings of Hughes *et al.* (1998) indicate an exposure-response relationship between cumulative exposure to crystalline silica as cristobalite and radiographic opacities. The relationship was substantially steeper among those exposed at the highest average concentrations of crystalline silica. The authors believe that the data do not support the regulatory assumption that cristobalite is more fibrogenic than quartz (i.e., prior to 2000 the occupational limit for cristobalite was half that for quartz), since at average silica levels comparable to other

epidemiologic studies quartz gave a higher incidence of silicosis than did cristobalite in this study. However, since radiography can under-diagnose silicosis, complete accounting for silicosis will require evaluation at autopsy. The ACGIH recently lowered the TLV for alpha-quartz from 100 to 50 $\mu\text{g}/\text{m}^3$, so that it has the same TLV as cristobalite (ACGIH, 2000).

Park *et al.* (2002) carried out a quantitative risk assessment, by Poisson regression methods, of the onset of silicosis among the diatomaceous earth workers in Lompoc. A linear relative risk model gave the best fit to the data. They estimated an excess lifetime risk for radiographic silicosis of 68-75 cases per thousand workers exposed to 50 $\mu\text{g}/\text{m}^3$ silica (cristobalite) for a 45 year work-life, then living to age 85. At 1 $\mu\text{g}/\text{m}^3$ silica the excess lifetime risk was estimated to be 1.6 cases of lung disease other than cancer per thousand workers exposed (Table 6).

Table 6. Excess lifetime risk of silicosis predicted by Park *et al.* (2002)

<i>Silica concentration (mg/m³)</i>	<i>45 year cumulative exposure in mg/m³-y</i>	<i>Radiographic silicosis - all workers</i>	<i>Radiographic silicosis in workers with < 10 mg/m³-y</i>
0.001	0.045	6.2/1000*	1.6/1000
0.005	0.225	17/1000	7.8/1000
0.01	0.45	26/1000	16/1000
0.02	1.8	39/1000	31/1000
0.05	2.25	68/1000	75/1000
0.1	4.5	100/1000	140/1000
0.2	9	150/1000	260/1000

* Excess risk estimates assume that workers were exposed to a constant silica concentration for up to 45 years (ages 20-65). Annual risks are accumulated up to age 85.

White South African gold miners (Hnizdo and Sluis-Cremer, 1993)

Hnizdo and Sluis-Cremer (1993) investigated silicosis risk retrospectively in a cohort of 2,235 white male South African gold miners. Exposure estimates were made for nine separate occupational categories based on a special study of dust levels in these mines done by Beadle in the 1960s (Beadle, 1971). To compensate for the fact that the average hours working in dust ranged among the 9 categories from 4 hours for “other officials” to 8 hours for “shaft sinkers and developers,” exposure was “normalized” to 8-hour shifts. The workers had a minimum of 10 years and an average of 24 years service from 1940 until the early 1970s. Dust levels were fairly constant during this period (see, e.g., Table 2 in Gibbs and DuToit (2002)). The miners had an annual chest radiograph while mining; they were followed until 1991 for radiographic signs of the onset of silicosis. An ILO category 1/1 (definite silicosis) or greater was selected to designate silicosis. Two independent readers initially read the chest films, but only the reader whose interpretations correlated better with autopsy results was used for additional analysis; the use of one reader is a limitation of the study. There were 313 miners (14% of the cohort) who developed radiographic signs of silicosis at an average age of 55.9 years. The latency period was largely independent of the cumulative dust exposure (CDE). In 57% of the silicotics, the radiographic signs developed at an average of 7.4 years after mining exposure ceased. The risk of silicosis determined by chest radiographs increased exponentially with cumulative dust dose. At the highest level of 15-(mg/m³)-years CDE (approximately 37 years of gold mining at an

average respirable dust concentration of 0.4 mg/m³, the cumulative risk for silicosis reached 77% as estimated by the accelerated failure time model using the log-logistic distribution (SAS Proc LIFEREG):

$$CR(t) = 1 - \{1/[1 + \exp (-\mu/\sigma) \times t^{(1/\sigma)}]\}$$

where CR(t) = cumulative risk at time t, and μ (2.439) is the intercept and σ (0.2199) is the scale parameter estimated by SAS’s LIFEREG procedure. The authors concluded that the risk of silicosis was strongly dose-dependent, but that the latency period was largely independent of dose. The life table analysis (SAS Proc LIFETEST) below (Table 7) shows the number of miners who developed silicosis (“cases”), the number of miners considered by the authors to be at risk, and the risk per unit of CDE (also as calculated by the authors). In the table in column 1 (in parentheses) are OEHHA’s determination of the mg/m³-yr respirable silica exposure, based on Hnizdo and Sluis-Cremer’s estimate of 30% silica in the dust, and in column 4 is the total number of miners actually at each midpoint level of CDE or silica. The values in column 4 of Table 7 are the number of workers in the group with the temporally integrated dust exposure in column 1.

Table 7. Life table results - Risk of silicosis per unit Cumulative Dust Exposure (CDE)
(from Table IV of Hnizdo and Sluis-Cremer, 1993)

<i>Midpoint in (mg/m³)-y of CDE (silica)</i>	<i>Cases of silicosis</i>	<i>Number of workers at risk based on life table</i>	<i>Number of workers remaining at this CDE midpoint</i>	<i>“Risk/unit CDE”</i>	<i>Mean years in dust</i>	<i>Mean dust conc. (mg/m³)</i>
1 (0.3)	0	2218	204			
3 (0.9)	9	2014	474	0.002	20.5	0.17
5 (1.5)	48	1540	556	0.016	23.5	0.24
7 (2.1)	85	984	469	0.045	27.2	0.30
9 (2.7)	93	515	318	0.099	28.0	0.33
11 (3.3)	53	197	142	0.156	29.4	0.38
13 (3.9)	20	55	44	0.222	31.5	0.41
15 (4.5)	5	11	11	0.227	37.0	0.42

^a CDE = Σ number of dusty shifts x mean mass respirable dust conc. x average number of hours spent underground / (270 shifts/year x 8 h/shift)

A plot of risk of silicosis per unit of Cumulative Dust Exposure (CDE) versus the mid-point unit CDE, as given in Figure 1 of the Hnizdo and Sluis-Cremer report, and a plot of % silicosis among the workers actually exposed to a given level of silica (Figure 2), as determined by OEHHA staff, respectively, are given below.

Figure 1. Risk of silicosis per unit CDE vs. CDE mid-point

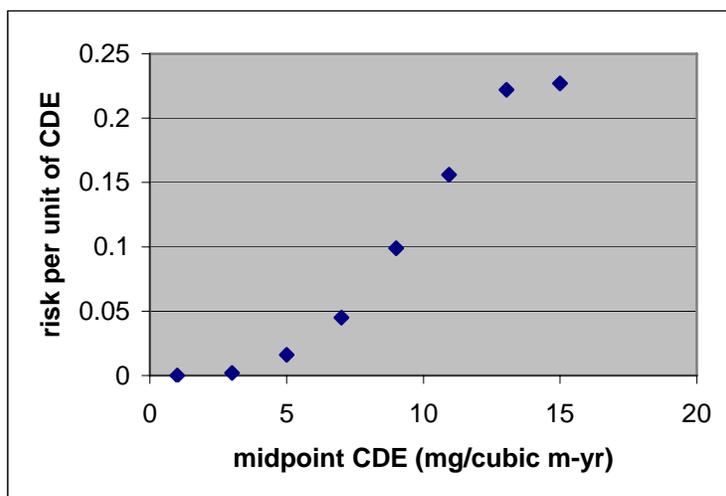
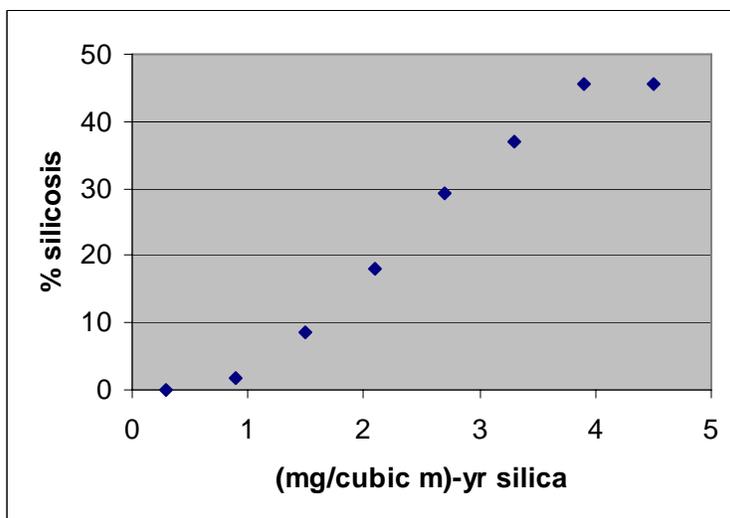


Figure 2. Percent silicosis among workers at each silica level



Black South African gold miners (Churchyard *et al.*, 2004; Murray *et al.*, 1996)

Black migrant contract workers constitute a large majority (85 - 90%) of South African gold miners. In a cross sectional study, Churchyard *et al.* (2004) interviewed and took chest radiographs of 520 black gold miners (mean age = 46.7 years, range = 37.1 – 59.9) who were still mining (average service = 21.8 years, range 6.3-34.5). Two readers examined the radiographs. As in the Hnizdo and Sluis-Cremer study, silicosis was defined as an ILO (1980) profusion of $\geq 1/1$. The mean respirable dust concentration was 0.37 mg/m^3 (0 - 0.70); the mean quartz concentration was 0.053 mg/m^3 (0 - 0.095). The prevalence of silicosis was determined to be 18.3% by one reader and 19.9% by the other (mean 19.1%) (Table 8). This included several workers with more serious silicosis as indicated by ILO profusions $\geq 2/1$ (see Table 1).

Significant trends were found between the prevalence of silicosis and: (1) length of service (OR = 1.69 per 5 years), (2) mean intensity of exposure (OR = 1.18 per 0.01 mg/m³), and (3) cumulative exposure to quartz (OR = 3.2). The study confirms the large burden of silicosis among older black workers in this industry (see next paragraph). The burden is likely to worsen with continuous employment in dusty jobs. For this cohort the prevalence of silicosis will increase even if the miners stop mining immediately. If, as assumed by the authors, the dust levels during the working life of these black miners were constant, silicosis developed while they were exposed to a quartz level below the workplace limit of 0.100 mg/m³.

Table 8. Silicosis in black gold miners (Churchyard *et al.*, 2003; 2004)

<i>Cumulative quartz exposure in mg/m³-yr</i>	<i>Mid-point of cumulative quartz exposure</i>	<i>Number in quintile*</i>	<i>Cases of silicosis</i>	<i>Percent silicosis</i>
0 – 0.80	0.4	103	11	10.7
0.80 – 0.99	0.9	97	8	8.2
0.99 – 1.24	1.12	103	18	17.5
1.24 – 1.48	1.36	104	23	22.1
1.48 – 3.08	2.28	103	33	32.0
(Total)		(510)**	(93)	(18.2)

* Personal communication from Dr. J. teWaterNaude, December 2, 2004.

** Ten of the 520 films were unreadable.

Murray *et al.* (1996) analyzed data from 16,454 black South African gold miners dying from unnatural causes between 1975 and 1991 in order to study change in prevalence in silicosis and pulmonary tuberculosis (TB). TB prevalence increased from 0.9% in 1975 to 3.9% in 1991, while that for silicosis increased from 9.3% to 12.8%. The prevalence of both increased with age and duration of service. Silicosis was the most significant predictor of TB (OR = 1.78, CI = 1.27 - 2.30, p = 0.0001). A highly significant trend for TB, for year of autopsy, remained after adjustment for other variables, such as age and duration of service (OR = 1.04, CI = 1.01 – 1.06, p = 0.0046). (Another 21,202 black gold miners died of natural causes during the study period.)

Hong Kong granite workers (Ng and Chan, 1994)

Ng and Chan (1994) investigated silicosis among 338 male workers, who had worked at least one year between 1967 and 1985 in two granite quarries in Hong Kong. Three readers examined the chest radiographs. Silicosis was defined as an ILO classification of at least 1/1 (for small rounded opacities) or greater, assigned by at least two of the three readers. Exposure was estimated for each worker based on job category and particle counts. Thirty-six workers (10.6%) were designated silicotic. Both a logistic and a linear model fit the data well. The study suffered because only about half of the previously employed granite workers were studied, which probably led to an underestimate of silicosis risk in at least the highest exposure category and maybe in others. The data are summarized in Table 9.

Table 9. Silica exposure and silicosis in Ng and Chan (Finkelstein, 2000)

<i>Mean cumulative exposure (mg/m³)-y</i>	<i>Prevalence of silicosis^a</i>
< 1	0%
3.1	13%
7.1	25%
22	22%

^a rounded opacities determined by at least 2 of 3 readers (Table 3 of Ng and Chan)

Gold miners in South Dakota (Steenland and Brown, 1995)

Steenland and Brown (1995) studied a very large cohort (3330) of white male gold miners in South Dakota, who had worked at least 1 year underground between 1940 and 1965 (average = 9 years underground). The mine dust contained on average 13% silica (range = 1-48%). A job-exposure matrix was created for full-time underground workers grouped into five categories. The authors estimated that most miners were exposed to a median silica level of 0.05 mg/m³, but that those hired before 1930 were exposed to a median level of 0.15 mg/m³. A total of 170 cases of silicosis (5.1% of the cohort) was determined from death certificates only (n = 128 cases), from two cross-sectional radiographic surveys in 1960 and 1976 (n = 29 cases; ILO category 1/1 or greater), or from both (n = 13 cases). Unfortunately, only 25% of living cohort members were surveyed radiographically. The life-time risk of silicosis was less than 1% with a cumulative exposure under 0.5 mg/m³-years and increased to 68% to 84% for the highest cumulative exposure category (more than 4 (mg/m³)-years) (Table 10).

Table 10. Risk of silicosis for cohort by cumulative exposure (Table 3, Steenland and Brown)

<i>Silica exposure in (mg/m³)-yrs: range (midpoint)</i>	<i>Miners with silicosis</i>	<i>Number entering exposure category (from life table)</i>	<i>Number remaining at this exposure level</i>	<i>Cumulative^a Risk</i>	<i>Mean years of exposure</i>	<i>Mean year first exposed</i>
0-0.2 (0.10)	5	3330	1530	0.002	2.9	1953
0.2-0.5 (0.35)	5	1800	740	0.005	9.7	1948
0.5-1.0 (0.75)	15	1060	376	0.017-0.022 ^b	15.4	1942
1.0-2.0 (1.50)	33	684	353	0.060-0.084 ^b	13.2	1931
2.0-3.0 (2.50)	44	331	206	0.167-0.245 ^b	18.8	1926
3.0-4.0 (3.50)	42	125	73	0.403-0.534 ^b	25.5	1921
>4.0	26	52	52	0.678-0.844 ^b	30.6	1914

^a Cumulative risk = 1-exp[-sum of (hazards * interval width)], where the hazards for each category of cumulative exposure are:

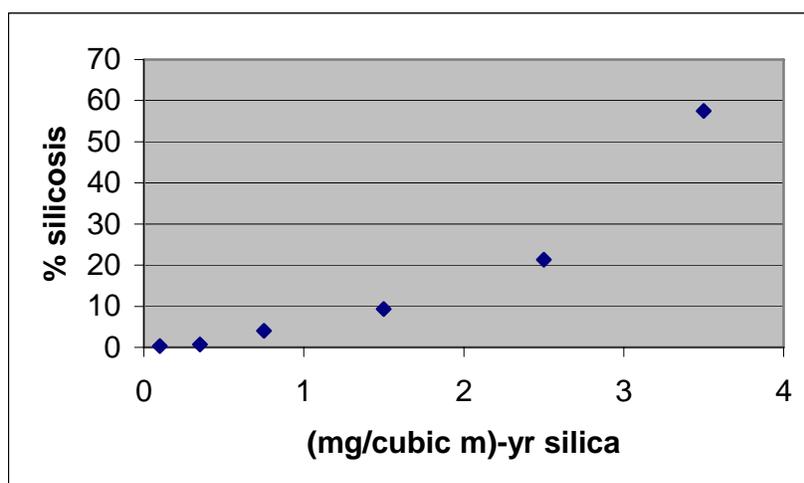
no. cases/(width*(no. entering category - 0.5*no. cases - 0.5*no. withdrawals))

^b Cumulative risk adjusted for age and calendar time (Steenland and Brown, 1995)

The best predictor of disease was cumulative exposure ((mg/m³) - years), followed by duration of exposure (years), and then by average exposure (mg/m³). Figure 1 of Steenland and Brown indicates that a plot of their data for silicosis risk versus cumulative silica exposure was similar

to a plot of the data of Hnizdo and Sluis-Cremer (1993). After adjustment for competing risks of death, Steenland and Brown estimate that a 45-year exposure to 90 - 100 $\mu\text{g}/\text{m}^3$ silica would lead to a lifetime risk of silicosis for gold miners of 35% to 47%. A limitation of this study is the reliance on death certificates rather than on ILO interpretation of radiographs. In addition, no mention was made of validating the data on the death certificates. It was also not clear what, if any, autopsy data were available. A plot of silicosis incidence among the workers (as determined by OEHHA staff) actually exposed to the estimated level of silica is given in Figure 3 below. An accompanying editorial (Wagner, 1995) commended the article for estimating both the risk of silicosis while working and the lifetime risk of silicosis resulting from exposure during work.

Figure 3. % Silicosis vs. silica exposure in Steenland and Brown (see Table 10)



Miners in Leadville, Colorado (Kreiss and Zhen, 1996)

Kreiss and Zhen (1996) investigated the exposure-response relationships for silicosis among 134 male miners over 40 years old in Leadville, Colorado. The men had been studied three years earlier in a random sample of respiratory disease in their community (Kreiss *et al.*, 1989). Of 100 dust-exposed miners, 32 had radiological profusions of small opacities of ILO category 1/0 or greater at a mean of 36.1 years since their first silica exposure. Of miners with cumulative silica, exposures of 2 (mg/m^3)-years or less, 20% had silicosis while 63% of miners accumulating greater than 2 (mg/m^3)-years had silicosis. Average silica exposure was also strongly associated with silicosis prevalence rates (Table 11).

Table 11. Miners studied by Kreiss and Zhen (1996)

<i>Average silica exposure</i>	<i>% silicotics</i>
0.025-0.05 mg/m^3	13% (5/38)
> 0.05-0.1 mg/m^3	34% (15/44)
> 0.1 mg/m^3	75% (9/12)
<i>Cumulative silica exposure</i>	<i>% silicotics</i>
≤ 2 (mg/m^3)-y	20% (14/70)
2 - 4 (mg/m^3)-y	63% (15/24)

Based on logistic regression models of the form $R(x) = [1 + \exp(-\alpha - B'x)]^{-1}$, Kreiss and Zhen concluded that the risk of silicosis was best predicted by elapsed time since last silica exposure together with either (1) cumulative silica exposure or (2) a combination of average silica exposure and duration of exposure. Exposure-response relationships were substantially higher using measured silica exposures (compared to using estimated silica exposures based on measured total dust exposures and assuming a constant silica proportion of dust). The risk of silicosis in this study is higher than in workforce studies having no follow-up of those leaving the mining industry (e.g., Muir *et al.*, 1989) and in studies without job title-specific silica measurements (e.g., Hnizdo and Sluis-Cremer, 1993). However, the risk is comparable to several recent studies of exposure-response relationships for mining dust (e.g., Ng and Chan, 1994; Steenland and Brown, 1995) (see Summary Table 15 below). A limitation relative to other studies is the small number of subjects (100) in the group.

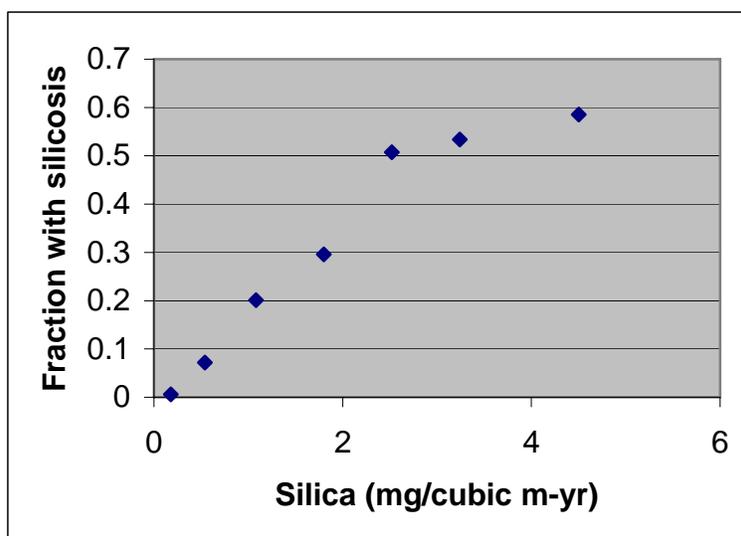
Chinese tin miners (Chen *et al.*, 2001)

Chen *et al.* (2001) found a clear exposure-response relationship between silica dust exposure and silicosis in a cohort of 3010 (2795 male and 215 female) miners employed for at least 1 year during the period 1960-1965 in any of four Chinese tin mines. No other diseases due to silica or tin were mentioned. Each cohort member was followed through 1994. Historical Chinese total dust (CTD) data were used to create a job exposure matrix for each facility, job title, and calendar year. The CTD data were converted to estimates of respirable crystalline silica for comparison with findings from other epidemiological studies of silicosis (including some of those above). Each miner's work history was abstracted from employment records. The diagnosis of silicosis was based on 1986 Chinese Roentgen diagnostic criteria for pneumoconiosis. The criteria classified silicosis as stages I-III, similar to an ILO classification of 1/1 or greater. Of the 3010 miners, 1015 (33.7%) were identified as silicotic (mean age = 48.3 years, with a mean of 21.3 years after first exposure) (Table 12). Among the silicotics, 684 (67.4%) developed silicosis after their tin mine exposure had ended (mean = 3.7 years after). The risk of silicosis was strongly related to cumulative exposure to silica. The Weibull distribution gave a very good fit to the data. The risk of silicosis was less than 0.1% when CTD was less than 10 (mg/m³)-yr (= 0.36 (mg/m³)-yr of respirable crystalline silica). The risk of silicosis increased to 68.7% when CTD exposure was equal to 150 (mg/m³)-yr (= 5.4 (mg/m³)-yr of respirable crystalline silica). Latency period was not correlated to the risk of silicosis or to cumulative dose. From their data, the authors predicted a 55% risk of silicosis for 45 years exposure to 0.1 mg/m³ respirable crystalline silica, the workplace exposure limit (4.5-(mg/m³)-years silica). Figure 4 plots the fraction of the workers in Chen *et al.* with silicosis (column 2 in Table 12 divided by column 4) exposed to a given level of silica (mid-point – in parentheses in column 1 of Table 12), as calculated by OEHHA staff.

**Table 12. Cumulative silicosis risk based on cumulative total dust (CTD)
(Table 5, Chen *et al.*, 2001)**

<i>Range of CTD exposure in (mg/m³)-y/ (silica mid-point)</i>	<i>Cases of silicosis (n)</i>	<i>Workers entering category</i>	<i>Workers at this level of CTD/silica</i>	<i>Cumulative risk based on Weibull model</i>	<i>Mean net exposure (years)</i>	<i>Mean latency (years)</i>
<10 (0.18)	2	3010	333	0.001	2.2	14.7
10-19.99 (0.54)	24	2677	334	0.010	5.3	21.3
20-39.99 (1.08)	126	2343	626	0.070	9.3	22.0
40-59.99 (1.80)	127	1717	429	0.145	11.9	21.5
60-79.99 (2.52)	196	1288	386	0.285	9.9	20.3
80-99.99 (3.24)	141	902	264	0.405	10.8	19.0
100-149.99 (4.50)	244	638	417	0.663	13.1	20.4
≥ 150 (≥ 5.4)	155	221	221	0.917	15.7	25.4

Figure 4. Percent silicosis vs. silica level from Chen *et al.*



Industrial sand workers (McDonald *et al.*, 2001; Hughes *et al.*, 2001; Rando *et al.*, 2001)

McDonald *et al.* (2001) studied a cohort of 2670 men employed before 1980 for 3 years or more and followed through 1994 in one of nine North American sand-producing plants and in a large associated office complex (since most of the office employees had previously worked in the mines). They found 37 deaths due to silicosis and silicotuberculosis. The mean exposure of the cohort was 42 µg/m³ silica (Rando *et al.*, 2001). Odds ratios for silicosis mortality, determined using conditional multiple logistic regression (SAS software), were significantly related to cumulative silica exposure (Hughes *et al.*, 2001) (Table 13). The odds ratios are in general agreement with those in the gray foundry workers of Rosenman *et al.* (1996) (Table 4).

Table 13. Median cumulative silica exposure and odds ratio (Table 3 in Hughes *et al.*, 2001)

<i>No lagging</i>			<i>Lagged 15 yr</i>		
<i>Median exposure in (mg/m³)-y</i>	<i>Silicotics (n)</i>	<i>Odds ratio^a for mortality</i>	<i>Median exposure in (mg/m³)-y</i>	<i>Silicotics (n)</i>	<i>Odds ratio^{a,b} for mortality</i>
0.832	7	1.00	0.142	7	1.00
2.744	7	1.27	1.229	7	2.54
6.916	8	2.62	2.583	7	4.55
12.084	7	2.13	7.990	8	5.16

^a Matched odds ratio relative to lowest cumulative exposure category. Although labeled a cohort study, the data analysis compared cases of silicosis with non-silicotic controls.

^b Significant increasing trend across exposure categories (see Hughes *et al.* for more details)

Ceramic workers (Cavariani *et al.*, 1995; Legrand-Cattan *et al.* (1998)

Cavariani *et al.* (1995) investigated the incidence of silicosis among 2,480 men in the ceramics industry in central Italy. The workers were surveyed during the period 1974-1987 and followed through 1991 with annual chest radiographs. The cumulative risk of silicosis (ILO category 1/1 or greater) was 48% after 30 years of employment. A multivariate Cox's proportional hazards model indicated that silicosis increased linearly up to the period of 25-29 years employment. A hazard risk of 14.6 was found comparing those with ≥ 30 years exposure to those employed 10 years. Smoking significantly contributed to the model, but its role was unclear.

Legrand-Cattan *et al.* (1998) examined the dose-response relationship in two French ceramic plants. A 1992 cross-sectional study included more than 200 silica-exposed workers. Three ILO certified B readers read chest radiographs. Silica was sampled in the airborne dust. The results are tabulated below (Table 14).

Table 14. Silicosis in two French ceramic plants (Legrand-Cattan *et al.*, 1998)

<i>Cumulative exposure to silica in (mg/m³ – years)</i>	<i>Number of workers at this level</i>	<i>Number with small opacities with ILO profusion $\geq 1/0$</i>	<i>Percent</i>
< 0.35	50	2	4
0.35 – 1.08	57	8	14
1.09 – 1.77	55	11	20
> 1.77	55	17	31
Total	217	38	(18)

A dose response relationship is clear; the authors reported a p value of 0.002. However, the study is limited by the lack of follow-up of the workers.

Slate workers (Glover *et al.*, 1980; Saiyed *et al.*, 1985; Saiyed and Bannerjee, 1985)

Slate contains calcium carbonate, iron oxides, silicates, amorphous silica, and crystalline silica. Glover *et al.* (1980) studied slate workers in North Wales. The respirable slate dust contained 13-32% crystalline silica. In the study group were 725 current and former workers exposed only to slate dust, while the controls were 530 men from the same area who had never been exposed to dust. Pneumoconiosis was found in 239 slate workers (33 %), and 10% had degrees of pneumoconiosis (category 2 or higher using the 1971 ILO scheme) that would bring worker's compensation. The prevalence of respiratory symptoms (cough, phlegm, dyspnea) was high. There was evidence of an effect of both simple and complicated pneumoconiosis on lung function (declines in FVC and FEV₁) additional to the effect of age. The high prevalence (40-50%) of radiological lesions suggested the presence of healed tubercular lesions in men over 55. Either pneumoconiosis or old tubercular lesions (or both) could account for the symptomatology and disability of the men.

Saiyed *et al.* (1985) surveyed the slate-pencil industry in India. An industrial hygiene survey revealed very high levels of free silica (2-10 mg/m³), while a medical survey showed that 324 of 593 workers (54.6%) had silicosis. Of these, 105 had "conglomerate" silicosis (progressive massive fibrosis, PMF). Some lung lesions were detectable after less than five years of exposure to slate dust. Saiyed and Bannerjee (1985) conducted a follow-up examination 16 months later. The progression of silicosis was very rapid, and a total of 23 workers had died during this period (mean age = 34.7 years; mean exposure = 11.9 years). The authors attributed the high mortality to high levels of silica leading to early onset of PMF. The progression of silicosis was related to the intensity and duration of dust exposure, and to the severity of silicosis found initially.

Silicosis has been reported in other groups of slate workers in Norway (Bang and Suhr, 1998; Suhr *et al.*, 2003) and in Germany (Mehnert *et al.*, 1990).

Silica particle size

Data on silica particle size in the various workplaces are limited. According to Witschi and Last (2001), silica particles with a diameter of 1 µm (range = 0.5 - 3 µm) appear to be the most fibrotic in humans. NIOSH (1974) reviewed the existing literature and found that in five diatomite plants the mean silica diameter was 1.1 µm (range = 0.5 - 2 µm). For nine potteries, the particle size was 1.2 µm. For 18 foundries, more than 90% of the particles were less than 3 µm. The majority of particles to which shipyard sandblasters were exposed was also less than 3 µm. In the Vermont granite sheds, 10 mppcf (million particles per cubic foot) granite dust were initially estimated to be equal to 0.1 mg/m³ respirable quartz. Steenland and Brown (1995) used this estimate for silica in South Dakota gold mines. Assuming that the density of quartz is 2.65 g/cm³ and that the quartz particles are spherical, the data indicate that the particles have a diameter of 0.59 µm. NIOSH (1974) listed 0.94 µm as the median particle size in metal mines. No indication was given of the dispersion of the particle sizes around the average value. Davis *et al.* (1983) used the value of 10 mppcf in granite sheds as equal to 0.075 mg/m³ silica. For that estimation, OEHHA staff calculated the particle diameter to be 0.53 µm. Thus, existing data indicate that the majority of silica in the workplace is respirable. In most of the occupational studies examined, the exposures were measured using a calibrated cyclone sampler similar to that recommended in the current NIOSH (2003) method. This allows collection of particles

primarily in the 0.5 – 5 μm range, with a collection efficiency profile intended to match the penetration of particles into the alveolar region of the human lung. In the case of the South African gold mine studies (Beadle, 1971; Page-Shipp and Harris, 1972; Hnizdo and Sluis-Cremer, 1993), particle number was determined by an optical method selecting respirable particles (range of 0.5 to 5 μm). Thus, the risk estimates obtained from these studies refer to particles in the size range where penetration occurs into the respiratory region of the lung. This corresponds to the size range of particles thought to be responsible for silicosis. It differs from the definition of “respirable” particles (*i.e.* PM_{10}) commonly used in environmental measurements, which refers to particles capable of penetrating anywhere in the lower respiratory tract (described as “thoracic” particles in occupational studies).

Risk estimation for silicosis from epidemiologic studies

The data from the above studies have been used by a number of investigators (Finkelstein, 2000; Chen *et al.*, 2001; Hughes, 1995) and by OEHHA staff to estimate percent silicosis based on cumulative silica exposure in units of $(\text{mg}/\text{m}^3)\text{-yr}$. The results are summarized in Table 15.

In Table 15, more than 14,000 workers were studied, of whom approximately 12% were classified as silicotic. The 12% is likely an underestimate of the incidence of silicosis due to lack of follow-up by chest radiographs during life in some cohorts and to the lack of an autopsy after death.

Table 15. Summary - Estimates of % silicosis based on cumulative silica exposure in (mg/m³)-y

<i>Study</i>	<i>Population (number with silicosis)</i>	<i>Exposure of 2 (mg/m³)-y</i>	<i>Exposure of 4 (mg/m³)-y</i>	<i>Exposure of 4.5 (mg/m³)-y</i>
Muir <i>et al.</i> , 1989	2109 male Ontario hard rock miners ("15")	0.4 ^{a,c}	1.2 ^{a,c}	2 ^b
Rosenman <i>et al.</i> , 1996	1072 Midwestern foundry workers (28)	2 ^a	10 ^a	3 ^b
Graham <i>et al.</i> , 1991	408 Vermont granite workers (35)	~3 ^c	—	—
Hughes <i>et al.</i> , 1998	1809 white male diatomaceous earth workers (81)	1.1 (low intensity) 3.7 (high intens.) ^a	4 (low) 12 (high) ^a	—
Park <i>et al.</i> , 2002	2342 white male diatomaceous earth workers (80)	~7 ^c	13 ^c	14 ^c
Hnizdo & Sluis-Cremer 1993	2235 white male South African gold miners (313)	5 ^a 10 ^c	52 ^a 60 ^c	77 ^b
Ng & Chan, 1994	338 male Hong Kong granite workers (36)	6 ^a	15 ^a	15-20 ^b
Steenland & Brown, 1995	3330 male S. Dakota gold miners (170)	8 ^a	53 ^a	70 ^b
Kreiss & Zhen, 1996	100 miners in Leadville, CO (32)	11 ^a	53 ^a	92 ^b
Chen <i>et al.</i> , 2001	3010 Chinese tin miners (1015)	14 ^d	47 ^d	55 ^b
Churchyard <i>et al.</i> , 2004	510 black gold miners (93)	~28	-	-

^a From Table II of Finkelstein (2000)

^b From Table 6 of Chen *et al.* (2001)

^c From Tables 3 and 4 of Hughes (1995)

^d Interpolated by OEHHA staff from Fig. 2 of Chen *et al.* (2001).

^e Estimated by OEHHA staff from Table 4 of Park *et al.* (2002)

^f 158 had an ILO reading $\geq 1/0$, while 103 had an ILO reading $\geq 1/1$.

Determination of LOAEL and NOAEL for silicosis (Rice and Stayner, 1995)

In another approach to the data, Rice and Stayner (1995) identified the NOAEL and LOAEL for silicosis in several studies (Table 16). The study of Hnizdo and Sluis-Cremer (1993) yielded both a LOAEL and a NOAEL.

Table 16. Estimates of NOAELs and LOAELs for silicosis (Rice and Stayner, 1995)

<i>Study</i>	<i>Subjects</i>	<i>NOAEL in $\mu\text{g}/\text{m}^3$</i>	<i>LOAEL in $\mu\text{g}/\text{m}^3$</i>
Davis <i>et al.</i> , 1983	969 granite workers	67.5	
Hnizdo and Sluis-Cremer, 1993	2235 gold miners	7	20
McDonald and Oakes, 1984	1321 gold miners	-	8 ^a
	64 gypsum miners	35	49
Muir <i>et al.</i> , 1989	2109 gold miners	Could not determine	Could not determine
Rice <i>et al.</i> , 1986	888 dusty trade workers	80-100	200-252

^a McDonald and Oakes (1984) considered this value to be only an approximation.

Proposals to change the occupational exposure limit

Silicosis is still being diagnosed at death in workers who were supposed to be exposed to occupational levels of 50-100 $\mu\text{g}/\text{m}^3$. Thus, there have been recommendations that the occupational exposure limit for respirable, crystalline silica (specifically alpha-quartz) be lowered from the current level of 100 $\mu\text{g}/\text{m}^3$ to 50 $\mu\text{g}/\text{m}^3$ (NIOSH, 1974; Rosenman *et al.*, 1996; ACGIH, 1999; Finkelstein, 2000). In 2000, the ACGIH lowered its TLV for quartz from 100 to 50 $\mu\text{g}/\text{m}^3$. In 1986, WHO recommended that the occupational level be set at 40 $\mu\text{g}/\text{m}^3$ (WHO, 1986). Greaves (2000) recommended that the TLV be lowered to 10 $\mu\text{g}/\text{m}^3$. Based on existing data Greaves (2000) estimated that at 10 $\mu\text{g}/\text{m}^3$ the incidence rate for ILO grade 1/0 silicosis would be less than 5%, while for grade 1/1 it would be less than 2%. Chen *et al.* (2001) recommended that the TLV be lowered to 5 $\mu\text{g}/\text{m}^3$. "If the lifetime risk of silicosis is to be under 1 in 1000 (a criterion used by OSHA) for a lifetime exposure of 45 years, then the mean Chinese total dust concentration must be lower than 0.14 mg/m^3 (or lower than 0.005 mg/m^3 respirable crystalline silica)" (Chen *et al.*, 2001). Mannetje *et al.* (2002) pooled data from six occupational cohorts. These included four groups discussed above: diatomaceous earth workers, Vermont granite workers, U.S. industrial sand workers, and South Dakota gold miners. Among them 170 deaths from silicosis were reported. The estimated mortality risk from silicosis to age 65 after 45 years of exposure at 100 $\mu\text{g}/\text{m}^3$ silica was 13 per 1000, while the risk of death at 50 $\mu\text{g}/\text{m}^3$ was estimated at 6 per 1000. Both estimates are above the 1 per 1000 risk acceptable to OSHA. Mannetje *et al.* also concluded that the occupational standards for silica should be lowered, but they did not specify a level. They further state that their estimates of silicosis mortality are probably underestimates due to exposure misclassification and to outcome misclassification, since deaths due to silicosis might have been coded to tuberculosis or chronic obstructive pulmonary disease.

C. Silica exposure and lung cancer in workers

In 1997, IARC classified respirable crystalline silica in Class 1, a Known Human Carcinogen, based on occupational epidemiologic studies. However, chronic RELs are not based on cancer endpoints. Further, there is no approved cancer potency factor for silica.

V. Effects of Animal Exposures

Several papers have reported that freshly fractured quartz, which has increased surface activity, causes greater inflammation than "aged" quartz. Vallyathan *et al.* (1991) reported that "fresh" silica was 4.2-fold more potent than silica aged for 1-2 days in decreasing the membrane integrity of male rat macrophages; 50% more potent in activating hydrogen peroxide secretion by macrophages; and 4.6-fold more potent in stimulating cellular chemiluminescence. Vallyathan *et al.* (1995) reported that inhalation of 19.3 mg/m³ aged (for 2 months) quartz for five hours/day for 10 days by male Fischer 344 rats increased the number of cells recoverable by bronchoalveolar lavage (BAL) (Table 17). Aged quartz also gave histopathological evidence of increased pulmonary infiltrates, showed higher levels of biochemical markers of lung injury, increased lipid peroxidation, and increased the ability of pulmonary phagocytes to produce more oxygen radicals than air-exposed controls. These pulmonary responses were significantly more pronounced after inhalation of 22.4 mg/m³ freshly fractured quartz.

Table 17. Cells recovered in bronchoalveolar lavage from rats (Vallyathan *et al.*, 1995)

<i>Cell type</i>	<i>Room air</i>	<i>Aged quartz</i>	<i>Freshly fractured</i>	<i>Fresh/aged</i>
Total cells	7.1±0.78*	9.3±1.2	20.4±2.2	2.2
Macrophages	6.7±0.69	4.7±0.79	5.4±0.78	1.1
Neutrophils	≥ 0.038	5.3±0.66	10.4±1.44	2.0
Lymphocytes	≥ 0.038	1.7±0.25	3.6±0.27	2.1
Red blood cells	≥ 0.038	1.7±0.26	6.0±0.57	3.5

* Cell counts are in millions. Each value is the mean ± standard error of 5 rats.

Burns *et al.* (1980) exposed female Balb/c mice for up to 39 weeks to 4.9 mg/m³ Min-U-Sil brand crystalline silica. By 24 weeks, silica-laden macrophages were present in the lungs. After 39 weeks of exposure, silicotic lesions were seen in the lungs and adjacent lymph nodes (Table 18).

Davis *et al.* (1998) exposed mice to an aerosol of cristobalite silica (mass median aerodynamic diameter (MMAD) = 1.7 µm) for five hours/day in order to examine (1) the effects of exposure dose, (2) the evolution of disease over time, and (3) the variation in responses among strains. In C3H/HeN mice, incremental, cumulative exposure doses of cristobalite (10 mg/m³ for 8 days, 43 mg/m³ for 9 days, and 70 mg/m³ for 12 days) caused (1) increased initial lung dust burden at 12 to 16 weeks post-exposure, (2) progressively intense pathological responses, and (3) increased total lung collagen (as measured by hydroxyproline).

The histopathological changes and total lung collagen increased with time after exposure. Silicosis was compared in four inbred strains of mice (BALB/c, C3H/HeN, MRL/MpJ, New Zealand Black) 16 weeks after aerosol inhalation exposure to cristobalite (70 mg/m³, 5 hours/day, 12 days). C3H/HeN mice had histopathological silicotic lesions, enlarged

intrapulmonary lymphoid tissue, and increased lung wet weight, increased bronchoalveolar lavage (BAL) recoverable macrophages, lymphocytes, and neutrophils, and increased total lung collagen (hydroxyproline analyses). BALB/c mice developed slight pulmonary lesions. MRL/MpJ mice showed prominent pulmonary infiltrates with lymphocytes. New Zealand Black (NZB) mice developed extensive alveolar proteinaceous deposits, inflammation, and fibrosis. The authors found both dose-time-response relationships and a substantial variation of responses among mouse strains to the high level, short duration exposure.

At Brookhaven National Laboratory, groups of Fischer 344 rats were exposed to 0, 2, 10, and 20 mg/m³ Min-U-Sil brand silica (alpha-quartz) for six months (Kutzman, 1984a; as summarized by USEPA, 1996). Other groups of rats had the same exposure, but were allowed to "recover" in air for an additional 6 months (Kutzman, 1984b; as summarized by USEPA, 1996). Significant alterations in total lung weight, total lung collagen, total elastin per unit lung dry weight, and total protein per unit lung dry weight at 2 mg/m³ silica and microscopic evidence of silicotic lesions at the higher silica levels indicated that 2 mg/m³ was a LOAEL for silica effects. After six months in clean air, the silica-induced lesions appeared to worsen.

Muhle *et al.* (1989) exposed groups of 50 male and 50 female rats to 1 mg/m³ DQ12 quartz six hours/day, five days/week for 24 months. DQ12 contains 87% crystalline alpha-quartz, has a mass median aerodynamic diameter (MMAD) of 1.3 μm, and is 74% respirable. Moderate fibrosis was seen in 85 animals, slight fibrosis in 13, and very slight fibrosis in 1 (total rats with fibrosis = 99/100). Varying amounts of peribronchial granulomatous foci were noted in 95 rats.

Muhle *et al.* (1998) reported lung fibrosis in hamsters exposed to 3 mg/m³ DQ12 silica. After 18 months of exposure to DQ12 for 6 h/day, 5 days/week, all hamsters in the group of 15-19 animals necropsied had very slight fibrosis. Approximately 100 silica-exposed animals were exposed for five more months to air only. Afterward 22.2% had very slight fibrosis, 68.7 % had slight fibrosis, and 1% had moderate fibrosis (i.e., more than 90/100 hamsters had lung fibrosis). No collagen measurements were reported. Thus, rats, mice, and hamsters show pulmonary fibrosis after crystalline silica exposure at and above 1 mg/m³.

Wagner *et al.* (1968) exposed dogs up to 2.5 years, guinea pigs up to 18 months, and rats up to 2 years for 6 hours/day, 5 days/week to 61% cristobalite (in calcined diatomaceous earth). Dust exposures were 2 and 5 million particles per cubic foot (mppcf), equivalent to 0.2 and 0.5 mg/m³ cristobalite (USEPA, 1996), with occasional excursions to 50 mppcf. No lung fibrosis was detected at these levels but all levels caused accumulation of inflammatory cells in the lung parenchyma. However, in dogs fibrotic nodules developed in the hilar lymph nodes with more nodules at 5 mppcf than at 2 mppcf.

Scheuchzuber *et al.* (1985) examined immunologic responses in Balb/c mice following inhalation of 1.954 mg/m³ silica for 150, 300, or 570 days. Mice exposed for 570 days were tested immediately post-exposure. Those exposed for 150 or 300 days were tested immediately or were rested for 30 or 150 days to allow for possible recovery from effects of dust inhalation. Silica inhalation suppressed the number of specific plaque-forming cells (PFC) in the spleen produced in response to aerosolized *E. coli*. After 570 days of inhalation, silica also reduced the ability of alveolar macrophages to phagocytize *Staphylococcus aureus in vitro* and impaired the ability to lyse allogeneic tumor cells (from mice other than Balb/c) *in vitro*. Silica inhalation did

not affect antibody-dependent cell-mediated cytotoxic and mitogenic responses by splenic lymphocytes. (Fibrosis was not an endpoint measured, but the effect level is similar to the LOAELs in other animal studies.)

Table 18. Animal studies of silica inhalation analyzed by USEPA (1996)

<i>Study</i>	<i>Species</i>	<i>Duration</i> ^a	<i>LOAEL</i>
Muhle <i>et al.</i> , 1989	Rat	24 mo	1.0 mg/m ³
Scheuchzuber <i>et al.</i> , 1985	Mice	150-570 d	2.0
Burns <i>et al.</i> , 1980	Mice	3-39 wk	4.9
Kutzman, 1984a	Rat	6 mo	2.0
Kutzman, 1984b	Rat	6 mo + 6 mo recovery	2.0
Wagner <i>et al.</i> , 1986	Dog	Up to 2.5 yr	0.2

^a Inhalation exposure was generally for 6 h/day, 5 d/wk.

Quartz has the ability to induce the generation of free radicals and to cause oxidative stress in tissues. Many substances that affect the quartz surface can modify this ability. Some of these modifiers could originate from other minerals, which exist together with quartz in nature. Donaldson and Borm (1998) proposed that the hazard posed by quartz may vary widely depending on the origin of the silica sample or on its contact with other chemicals/minerals. Such mechanistic data could assist in the interpretation of epidemiological studies such as those above. Experimentally their group found that DQ12 quartz, a European quartz standard which is often used in experimental studies of silica effects, is much more inflammatory in rat lung than respirable silica collected from two workplaces (Clouter *et al.*, 2001).

Humans appear to show adverse effects of silica exposure at lower levels than animals (compare LOAELs in Table 18 to LOAELs/NOAELs in Table 16). Rodents tend to be obligate nose-breathers and to have extensive nasal turbinates, which may result in less silica reaching the lower lung. For silica, results in animals may not be a good predictor of human effect levels.

VI. Derivation of Chronic Reference Exposure Level (REL)

<i>Key study</i>	Hnizdo and Sluis-Cremer, 1993
<i>Study population</i>	2235 white South African gold miners
<i>Exposure method</i>	Workplace inhalation
<i>Critical effects</i>	Silicosis (313 miners) (14 %)
<i>LOAEL</i>	3 mg/m ³ -years CDE (9 miners with silicosis)
<i>NOAEL</i>	2 mg/m ³ -years CDE (0 miners with silicosis) or 600 µg/m ³ -years silica (dust = 30% silica)
<i>BMCL₀₁</i>	2.12 (mg/m ³)-yr CDE or 0.636 (mg/m ³)-yr silica
<i>Exposure continuity</i>	8 h/day, 5 d/wk
<i>Exposure duration</i>	Average of 24 years dust exposure (10-39 years)
<i>Average experimental exposure</i>	235 µg/m ³ -yr silica at BMC ₀₁ (636 x 10 m ³ /20 m ³ x 270 shifts/365 days) 235 µg/m ³ -yr/24 yr = 9.8 µg/m ³
<i>Human Equivalent Concentration (HEC)</i>	9.8 µg/m ³
<i>LOAEL uncertainty factor</i>	Not needed in BMC approach
<i>Subchronic uncertainty factor</i>	1
<i>Interspecies uncertainty factor</i>	1
<i>Intraspecies uncertainty factor</i>	3
<i>Cumulative uncertainty factor</i>	3
<i>Inhalation Reference Exposure Level</i>	3 µg/m ³ (based on 30% silica in mine dust) [respirable, as defined occupationally by ACGIH/ISO]
<i>First supportive study</i>	Steenland and Brown, 1995
<i>Study population</i>	3330 S. Dakota gold miners
<i>Exposure method</i>	Workplace inhalation
<i>Critical effects</i>	Silicosis (170 miners) (5.1%)
<i>LOAEL</i>	0-0.2 mg/m ³ -years (5 miners with silicosis)
<i>NOAEL</i>	Not found
<i>BMCL₀₁</i>	0.34 (mg/m ³)-yr (see text below)
<i>Exposure continuity</i>	8 h/day, 5 d/wk
<i>Exposure duration</i>	3-36 years (average 9 years underground)
<i>Average experimental exposure</i>	112 µg/m ³ -y (340 x 10 m ³ /20 m ³ x 5 d/7 d x 48 wk/52 wk) 112 µg/m ³ -y/9 y = 12.4 µg/m ³
<i>Human Equivalent Concentration (HEC)</i>	12.4 µg/m ³
<i>LOAEL uncertainty factor</i>	Not needed in BMC approach
<i>Subchronic uncertainty factor</i>	1
<i>Interspecies uncertainty factor</i>	1
<i>Intraspecies uncertainty factor</i>	3
<i>Cumulative uncertainty factor</i>	3
<i>Inhalation Reference Exposure Level</i>	4 µg/m ³ [respirable, as defined occupationally]

<i>Second supportive study</i>	Hughes <i>et al.</i> , 1998
<i>Study population</i>	1809 California diatomaceous earth workers
<i>Exposure method</i>	Workplace inhalation
<i>Critical effects</i>	Silicosis (81 workers) (4.5%)
<i>LOAEL</i>	> 1, ≤ 3 mg/m ³ -years (17 workers with silicosis)
<i>NOAEL</i>	≤ 1 mg/m ³ -years (6 cases). (Six cases were observed, but Hughes <i>et al.</i> assigned the group a Relative Risk = 1 for silicosis.)
<i>Exposure continuity</i>	8 h/day, 5 d/wk
<i>Exposure duration</i>	1-45 years (mean = 11.5 years)
<i>Average experimental exposure</i>	≤ 330 µg/m ³ -y (1000 x 10/20 x 5/7 x 48/52) ≤ 330 µg/m ³ -y/ 11.5years = ≤ 29 µg/m ³
<i>Human Equivalent Concentration (HEC)</i>	29 µg/m ³
<i>LOAEL uncertainty factor</i>	3 (authors' NOAEL actually is a LOAEL)
<i>Subchronic uncertainty factor</i>	1
<i>Interspecies uncertainty factor</i>	1
<i>Intraspecies uncertainty factor</i>	3
<i>Cumulative uncertainty factor</i>	10
<i>Inhalation Reference Exposure Level</i>	3 µg/m ³ [respirable, as defined occupationally]
<i>Third supportive study</i>	Chen <i>et al.</i> (2001)
<i>Study population</i>	3010 Chinese tin miners
<i>Exposure method</i>	Workplace inhalation
<i>Critical effects</i>	Silicosis (1015 workers) (33.7 %)
<i>LOAEL</i>	10-19.99 mg CTD/m ³ -years (24 cases)
<i>NOAEL</i>	≤ 10 mg CTD/m ³ -years (2 cases) ≤ 360 µg silica/m ³ - years
<i>BMCL₀₁</i>	132 µg silica/m ³ - years
<i>Exposure continuity</i>	8 h/day, 5 d/wk
<i>Exposure duration</i>	2.2 years for NOAEL group
<i>Average experimental exposure</i>	40 µg/m ³ -y (132 x 10/20 x 5/7 x 48/52) 40 µg/m ³ -y/2.2 years = 18 µg/m ³
<i>Human Equivalent Concentration (HEC)</i>	18 µg/m ³
<i>LOAEL uncertainty factor</i>	Not needed in BMC approach
<i>Subchronic uncertainty factor</i>	1
<i>Interspecies uncertainty factor</i>	1
<i>Intraspecies uncertainty factor</i>	3
<i>Cumulative uncertainty factor</i>	3
<i>Inhalation Reference Exposure Level</i>	6 µg/m ³ [respirable, as defined occupationally]

<i>Fourth supportive study</i>	Churchyard <i>et al.</i> , 2004
<i>Study population</i>	510-520 black South African gold miners
<i>Exposure method</i>	Workplace inhalation
<i>Critical effects</i>	Silicosis (93 cases)
<i>LOAEL</i>	0-0.80 mg/m ³ -yr (11 cases)
<i>NOAEL</i>	Not identified
<i>BMCL₀₅</i>	0.673 (mg/m ³)-yr
<i>Exposure continuity</i>	270 shifts/year
<i>Exposure duration</i>	21.8 yr (6.3-34.5)
<i>Average experimental exposure</i>	249 (µg/m ³)-yr (673 x 10/20 x 270shifts/365) 249 (µg/m ³)-yr/21.8 yr= 11.4 µg/m ³
<i>Human equivalent concentration (HEC)</i>	11.4 µg/m ³
<i>LOAEL uncertainty factor</i>	Not needed in BMC approach
<i>Subchronic uncertainty factor</i>	1
<i>Interspecies uncertainty factor</i>	1
<i>Intraspecies uncertainty factor</i>	3
<i>Cumulative uncertainty factor</i>	3
<i>Inhalation Reference Exposure Level</i>	4 µg/m ³ [respirable, as defined occupationally]

The study of 2235 white South African gold miners by Hnizdo and Sluis-Cremer (1993) not only determined a NOAEL of 2 (mg/m³)-yr CDE (600 µg/m³-yr silica), but also had sufficient dose-response data for a BMC derivation. This study was powerful enough to detect a 1.9% incidence of silicosis (9 cases out of 474 exposed) at 0.9 mg/m³-yr silica (0/204 vs. 9/474, $p = 0.064$ by Fisher exact test, two-tailed). Because this incidence represents approximately the sensitivity limit of the data, and silicosis is a severe irreversible endpoint, the BMCL₀₁ (*i.e.*, the lower bound estimate of the concentration at which 1% of the population develops silicosis) was selected as the basis of the chronic REL. In benchmark analysis of chronic animal studies, BMCL₀₅ is typically regarded by OEHHHA as equivalent to a NOAEL. However, the power of this large-scale study is sufficient to demonstrate measurable responses below the 5% incidence level (which cannot then be logically considered a no-effect level). Furthermore, the endpoint measured in this epidemiological study is considered to be severe, since it represents the occurrence of clinically recognizable and irreversible disease, rather than an adverse physiological or biochemical response or a histopathological result seen at autopsy.

Benchmark Concentration (BMC) models, developed by the USEPA (BMDS versions 1.3, 1.3.1, and 1.3.2), were fit to the human data in Hnizdo and Sluis-Cremer (1993) (Table 7 and Figure 2 above). Fitting the probit model to the log dose of the Hnizdo and Sluis-Cremer (1993) data yielded an MLE₀₁ of 2.45 (mg/m³)-yr CDE and a BMCL₀₁ of 2.12 (mg/m³)-yr CDE ($\chi^2 = 0.64$; p value for fit = 0.9957) (Figure 5, Table 19). (For comparison the BMCL₀₅ was 3.73 (mg/m³)-yr CDE.) Fitting the logistic model to the same data yielded a BMCL₀₁ of 1.73 (mg/m³)-yr CDE ($\chi^2 = 2.71$; p value for fit = 0.8446) (Table 19). The BMCL₀₁ from these data is about the same as the apparent NOAEL. In general, a BMC is preferred to a NOAEL because the BMC takes into account all the dose response data in a study. The apparent NOAEL may be either above or below an actual effect level, depending on the study design and distribution of the data.

Figure 5. Probit model fit to the log dose of the Hnizdo and Sluis-Cremer data.

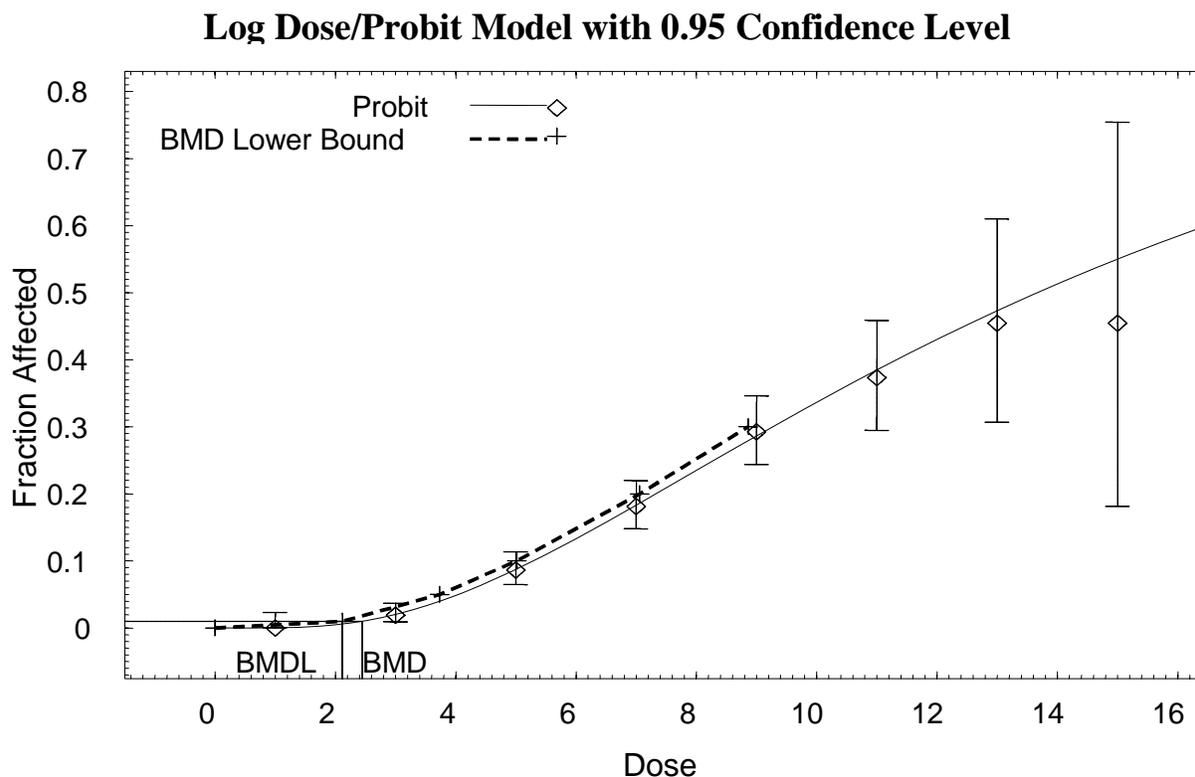


Table 19. Fits of benchmark models to the Hnizdo and Sluis-Cremer (1993) data

<i>BMDS Model</i>	<i>MLE₀₁</i>	<i>BMCL₀₁</i>	<i>p value for fit</i>
Probit-log-dose	2.45 (mg/m ³)-yr CDE	2.12 (mg/m ³)-yr CDE	0.9957
Logistic-log-dose	2.07	1.73	0.8446
Multistage (n=2)	2.47	1.89	0.7213
Quantal-quadratic	1.62	1.54	0.5017
Probit	1.56	1.32	0.0079
Logistic	1.48	1.28	0.0003
Quantal-linear	0.37	0.34	0.0000

For the estimate of 30% silica in the South African gold mine dust, Hnizdo and Sluis-Cremer (1993) relied on estimates for the years 1956-1960 by Beadle (Beadle and Bradley, 1970; Beadle, 1971). The original data, obtained by Corner House Laboratories for the South African Bureau of Mines, are partly presented by Beadle and Bradley (1970), but a more detailed presentation of exposures for various classes of workers is given by Page-Shipp and Harris

(1972). The latter paper also describes in some detail the methodology used to obtain the particle counts, and to convert those data into either respirable surface area or respirable mass values. Gibbs and Du Toit (2002) reviewed the data and methodology used by Hnizdo and Sluis-Cremer (1993) to estimate silica exposures of workers, which apparently depended on an unpublished analysis of the Corner House Laboratories' data done by Du Toit in 1991. Gibbs and Du Toit state that the exact relationship between the observed particle counts and theoretically derived mass concentrations cannot be determined, but that the uncertainties in this conversion do not appear to be severe for the dust characteristics observed in the South African mines. They accept the estimates by Beadle and Bradley (1970) of the quartz percentages in the dust, *i.e.* 54% for incinerated and acid-washed dust and 30% for unmodified dust.

However, Gibbs and Du Toit (2002) assert that Hnizdo and Sluis-Cremer (1993) incorrectly applied the 30% (total dust) silica content to figures for acid-treated dust in calculating the silica exposures of each occupational group. This contention is supported by the footnote to Table II in Hnizdo and Sluis-Cremer (1993) where the respirable dust concentration is described as "After heat and acid treatment". In order to clarify this point, OEHHA reviewed the independent reporting of the underlying data by Page-Shipp and Harris (1972). For most occupational groups, the silica exposures (shown in Table 20) calculated from Appendix I of Page-Shipp and Harris (1972), using the 54% silica content appropriate for acid-washed dust, correspond more closely to those calculated by Hnizdo and Sluis-Cremer (1993) (applying the 30% quartz content to their reported "respirable dust concentrations," *i.e.*, the untreated dust), than to the modified, and higher, quartz exposures proposed by Gibbs and Du Toit (2002). For example, 113 exposure samples were taken for stopers.

Table 20. Estimates of silica exposures in mg/m³ for different occupational groups in South African gold mines.

<i>Occupation</i>	<i>Shaft Sinkers</i>	<i>Developers</i>	<i>Stoppers</i>	<i>Assistant miners/ Trammers</i>	<i>Shift Bosses</i>	<i>Other Officials</i>	<i>Banks/ Skips</i>	<i>Workers Near shafts</i>	<i>Boiler-makers</i>	<i>Other Artisans</i>	<i>Miscellaneous</i>
Page-Shipp and Harris (1972) (Table III and Appendix I)											
Hours/shift (t)	7.70	8.00	7.80	7.70	5.20	4.00	7.50	6.50	6.30	5.70	7.20
Number of samples	10	37	113	157	43	106	33	34	41	61	11
RM x t	4.44	1.96	1.57	1.20	0.87	0.77	1.31	0.56	1.00	0.64	1.01
s.d.	3.94	1.59	1.00	0.93	0.71	0.53	1.38	0.57	0.71	0.51	0.79
Respirable Mass (RM)	0.58	0.25	0.20	0.16	0.17	0.19	0.17	0.09	0.16	0.11	0.14
Silica (54%)	0.31	0.13	0.11	0.08	0.09	0.10	0.09	0.05	0.09	0.06	0.08
(after acid treatment)											
Hnizdo and Sluis-Cremer (1993) (Table II)											
RM		0.48	0.37	0.27	0.30	0.30	0.13	0.10	0.19	0.19	
Silica (30%)		0.14*	0.11*	0.08*	0.09*	0.09*	0.04	0.03	0.06	0.06*	
(before acid treatment)											
Gibbs and Du Toit (2002) (Table 4)											
RM		0.48	0.37	0.27	0.30	0.30	0.13	0.10	0.19	0.19	
Silica (54%)		0.26	0.20	0.15	0.16	0.16	0.07*	0.05*	0.10*	0.10	
(after acid treatment)											

* denotes that value is equal to or closer to the value based on Page-Shipp and Harris

The last line of Appendix I of Page-Shipp and Harris (1972) gives a mean value for stopers of 1.57 (mg/m³)-hours respirable dust mass after acid treatment. Since the average work shift for stopers was 7.8 hours (Page-Shipp and Harris, 1972, Table III, last row), the average exposure level was 0.20 mg/m³. If 54% of this were quartz, the quartz level would be 0.11 mg/m³. Table II of Hnizdo and Sluis-Cremer (1993) lists 0.37 mg/m³ respirable dust for stopers. Thirty % of 0.37 mg/m³ equals 0.11 mg/m³, the same value reported by Page-Shipp and Harris. In Table 4 of Gibbs and Du Toit (2002) stopers are also reported to be exposed to 0.37 mg/m³ respirable dust. If 54% were quartz, as Gibbs and Du Toit contend, the quartz level would be 0.2 mg/m³. For 6 of the 9 categories of workers comprising 83% of the samples taken the silica levels correspond more closely to values used by Hnizdo and Sluis-Cremer than to those suggested by Gibbs and Du Toit.

Several more recent analyses of quartz content of South African mining rock have been reported (Table 21). Kielblock *et al.* (1997) give the overall silica content of the dust as 15% for the late 1980s to early 1990s. Dr. Eva Hnizdo (personal communication, 2003), now with the U.S. National Institute of Occupational Safety and Health (NIOSH), provided a summary of various other estimates that have been made. "Past surveys indicate that the amount of airborne respirable dust in SA gold mines in 1980's and in 1970's was on average around 0.4 mg/m³ with average quartz concentration of 0.08 mg/m³" (about 20%). In a Ph.D. thesis submitted by the late R.E.G. Rendall (1999) on dust in the air of gold mines, the silica percentage averaged 22% during the period from 1964 to 1988. In summary,

- (1) Notwithstanding some apparent contradictions in the various accounts, the silica concentrations in air proposed by Hnizdo and Sluis-Cremer, based on the Corner House Laboratory data, are a reasonable contemporary estimate of the exposures experienced by the workers examined in the study by Hnizdo and Sluis-Cremer (1993).
- (2) Other, more recent estimates of percent silica in the mine dust were lower than the value of 30% used by Hnizdo and Sluis-Cremer (1993). Newer studies, which using more sophisticated methods to measure silica in the dust, indicate lower silica concentrations in the various occupational settings. Since dust levels in the mines were fairly constant for decades and quantification of silica was improving, 30% is more likely to be an overestimate than an underestimate of silica levels.
- (3) Analysis of the data of Page-Shipp and Harris (1972) by OEHHA staff indicated that Hnizdo and Sluis-Cremer (1993) used the correct silica content, despite an erroneous statement in a footnote to Table II of their paper¹.

¹ Dr. Eva Hnizdo reviewed this analysis of the silica content of the dust and agrees with the assessment. ("I am very pleased that you studied carefully all the reports and came to the conclusion that our study was after all reasonably correct. Based on the Churchyard study and the measurements data I have seen in SA during the 1990s, I am also convinced that our results are reasonable estimates of the exposure of the cohort." (Hnizdo, personal communication October 2004))

Table 21. Estimates of respirable silica fraction of South African gold mine dust

<i>Authors</i>	<i>Time frame</i>	<i>% silica</i>		<i>Number of samples</i>	<i>Methods</i>
Beadle and Bradley, 1970	1958-1967	total dust: 25.7%; gravimetric: 28.5%; microscopy. acid-washed: 54%		142 grav; 143 elect ppt	gravimetric; precipitator + microscopy
Hnizdo and Sluis-Cremer (1993)	1956-1960	30%			precipitator + microscopy
Rendall (unpublished thesis)					
Survey 1	1987-8	17%		588	gravimetric
Survey 2	1977	20%		166	gravimetric
Survey 3	1977	17%		90	gravimetric
Survey 4a	1964-7	22%		112	gravimetric
Hnizdo (personal communication)	1970-1989	20%			
Kielblock (1997)	~1990	15.08%			Not stated
Churchyard (2004)	2000-1	14.3%			Gravimetric + X-ray diffraction

In the first supportive study Steenland and Brown (1995) found five cases of silicosis in the lowest dose group of 0 – 0.2 (mg/m³)-yr and considered the group to be a LOAEL (Table 10 above). None of the BMDS models gave an acceptable fit at the $p \geq 0.05$ level using six or seven silica levels. The closest was the quantal quadratic model ($\chi^2 = 9.62$; $p = 0.0473$), which resulted in a BMC₀₁ for silica of 0.43 (mg/m³)-yr using the six lowest levels of silica. In risk assessment, the highest dose or doses are often dropped in order to obtain an acceptable fit of the model to the data. This is reasonable with the benchmark approach since the highest doses should be least informative and the doses in the low dose region near the benchmark should be most informative for the benchmark concentration (USEPA, 1995; Filipsson *et al.*, 2003). Fitting the probit model to the log dose of the five lowest silica levels from Steenland and Brown yielded a BMCL₀₁ of 0.34 (mg/m³)-yr CDE ($\chi^2 = 1.32$; p value for fit = 0.5177). [For comparison, BMCL₀₅ = 0.85 (mg/m³)-yr CDE.] Fitting the quantal quadratic model gave a BMCL₀₁ of 0.45 (mg/m³)-yr ($\chi^2 = 3.36$; $p = 0.3395$). Use of the BMC₀₁ value of 0.34 (mg/m³)-yr CDE from the log dose probit model resulted in a chronic REL estimate for crystalline silica of 4 µg/m³. Steenland and Brown stated that “silicosis has no background rate for non-exposed populations that changes with age or calendar time” and thus they assumed that the five silicotics in the 0 – 0.2 (mg/m³)-yr were exposed to silica in the mines.

In a second supportive study, Hughes *et al.* (1998) found six cases of silicosis in the lowest exposure group of ≤ 1 mg/m³-yr but considered that group to be a NOAEL, not a LOAEL. If the lowest exposure group is used as a NOAEL, a chronic REL of 10 µg/m³ is calculated from the

data. Hughes *et al.* (1998) cite examples of possible non-occupational chest radiograph opacities (due, for example, to age or smoking) to explain the six cases in the lowest exposure group. However, due to the rarity of silicosis the six cases are biologically significant. OEHHA considers that the six cases may be work related, not cases of environmental or background silicosis. When a LOAEL to NOAEL UF of 3 is applied to the data of Hughes *et al.* (1998), the estimated REL is $3 \mu\text{g}/\text{m}^3$.

In a third supportive study, Chen *et al.* (2001) found two cases of silicosis in the lowest exposure group of $\leq 10 \text{ mg CTD}/\text{m}^3\text{-years}$ and considered that exposure level to be a NOAEL. One of the advantages of the benchmark dose analysis is that a NOAEL/LOAEL controversy, such as the one above with the Hughes *et al.* (1998) data, does not impact the procedure. The chart of the Chen *et al.* data above (Figure 4) indicates that the dose response is linear at low doses. Fitting the probit model to the log dose of the four lowest data points yielded a BMC_{01} of $0.132 (\text{mg}/\text{m}^3)\text{-yr CDE}$ ($\chi^2 = 2.19$; p value for fit = 0.335). Use of five, six, or seven data points gave BMC_{01} s of 0.14 to 0.17, but the p values were less than 0.1. For comparison, fitting the logistic model to the log dose of the four lowest data points yielded a BMCL_{01} of $0.093 (\text{mg}/\text{m}^3)\text{-yr CDE}$ ($\chi^2 = 4.86$; p value for fit = 0.0879). An inhalation chronic Reference Exposure Level for crystalline silica of $6 \mu\text{g}/\text{m}^3$ was estimated from the Chen *et al.* data.

The fourth supportive study is that of black South African gold miners by Churchyard *et al.* (2003, 2004). A problem with this data set is the statistical “noise” in the lower exposure groups; e.g., the lowest exposure group has a higher incidence of silicosis (11/103) than the next group (8/97). This noise causes problems in estimating a low benchmark such as the BMCL_{01} used with the Hnizdo and Sluis-Cremer (1993) data and with data from Steenland and Brown (1995) and Chen *et al.* (2001). The calculation therefore uses a 5% BMCL of $0.673 (\text{mg}/\text{m}^3)\text{-yr}$ from the probit log dose model as the benchmark, which is reasonably well within the range of the reliably observed data and which does not differ too widely from the MLE_{05} estimate of $0.955 (\text{mg}/\text{m}^3)\text{-yr}$ for that parameter. This BMCL_{05} is not strictly comparable to the BMCL_{01} calculated from the data of Hnizdo and Sluis-Cremer, but the concerns about the severity of the effect noted in the discussion of that derivation apply with equal or greater force here.

On the other hand, the variability in the low-dose data in this study implies that an unobserved factor is affecting the data. All the models predict that the “background” is substantially (as much as 5 – 10%) above zero, which is intrinsically implausible for silicosis unless there is an unrecorded additional source of silica exposure. Possibly, there were occasional excursions in the exposure of the workers in less exposed jobs, which were not captured by the systematic assessments for these job classifications. Alternatively, perhaps the assignment of an assumed zero exposure value to “non-dusty” job classifications noted in the paper was in fact inaccurate for some individuals. There may also be some distortion of the curve resulting from the “binning” of the exposure categories; certainly, the bin widths hinder the attempt to calculate a BMDL_{01} in this case. In relation to the model fit, other models (including the quantal linear model, which emphasizes the likely more reliable incidences at higher dose levels) are consistent with the BMCL_{05} results from the log probit model used here. Finally, the total number of cases and controls examined is fewer than in Hnizdo and Sluis-Cremer’s study, which reduces the precision.

None of these issues can be resolved without recourse to the individual data, which were not available for this analysis, and may not be resolved even then. However, the derivation of an HEC of $11.2 \mu\text{g}/\text{m}^3$ (and thus a comparison REL of $4 \mu\text{g}/\text{m}^3$) indicates at least that the results of the earlier analysis are unlikely to have underestimated the proper value of the REL. Although the uncertainties in the Churchyard *et al.* data prevent us from being more precise, we cannot eliminate the possibility that the REL should be set lower. However, the California ambient monitoring data, although subject to considerable uncertainty as to the relevant particle size distributions, suggest a plausible lower bound on the REL, which is consistent with our analysis of Hnizdo and Sluis-Cremer's data.

Other investigators have approached the possibility that some opacities on radiographs may be due to background influences such as age and smoking. In regard to smoking, Blanc and Gamsu (1988) reviewed the literature and concluded that smoking would not interfere with the determination of silicosis by the ILO system. Based on reading 1422 films of unexposed blue-collar workers, Castellan *et al.* (1985) stated that the use of the median result of 3 readers (the same number used by Hughes *et al.*) rarely results in interpreting a chest radiograph as ILO category $\geq 1/0$ in workers who were not exposed to dust (and regardless of smoking status).

The USEPA (1996) did a benchmark analysis with the Hnizdo and Sluis-Cremer (1993) data. They estimated that the lower bound for a 1% risk for silicosis (BMCL_{01}) was $1.31 (\text{mg}/\text{m}^3)\text{-yr}$, which by their methods is equivalent to a continuous, 70-year exposure to $6.7 \mu\text{g}/\text{m}^3$ silica. However, USEPA did not do a formal Reference Concentration (RfC) derivation for silica by either the BMC/UF or NOAEL/UF approach.

The key (Hnizdo and Sluis-Cremer, 1993) and supporting (Steenland and Brown, 1995; Hughes *et al.*, 1998; Chen *et al.*, 2001) studies were of human adults, nearly all males, who were presumably healthy, at least initially, since they were able to work. Thus there is need to protect the sensitive members of the population, especially children, in whose airways penetration of silica particles will be greater (Phalen *et al.*, 1985; Schiller-Scotland *et al.*, 1994; Oldham *et al.*, 1997; Bennett and Zeman, 1998). In addition, women may be more sensitive than men to the development of silicosis (Gerhardsson and Ahlmark, 1985; Katsnelson *et al.*, 1986). The selection of three as the intraspecies uncertainty factor (UF_H) was based on several considerations.

- (1) The workers who developed silicosis at low silica concentrations are by definition the most sensitive workers to silica-induced silicosis. Because of the large population of workers examined in these studies (more than 14,000), the sensitive individuals represent at least part of the range of sensitivity to be expected in the general population. This may justify reducing the UF_H from the default value of 10. Since these workers did not include children, the elderly, or females (except for the 215 females in Chen *et al.*), some uncertainty related to inter-individual variability remains. Therefore, a UF_H of 3 rather than 1 is chosen.
- (2) Mukherji *et al.* (1993) reported mean ambient silica levels (in PM_{10}) at three locations in the northern part of Santa Barbara County, California (see the Appendix to this report). At Santa Maria (an urban site) the level was $2.3 \mu\text{g}/\text{m}^3$; in Santa Ynez (a rural site) $0.6 \mu\text{g}/\text{m}^3$; and in Buellton (a remote background site) $0.2 \mu\text{g}/\text{m}^3$ crystalline silica. Thus, use

of a human intraspecies uncertainty factor (UF_H) of 10 with the data from the key study would result in an estimated chronic REL of $0.9 \mu\text{g}/\text{m}^3$ (ACGIH method), a level in the range of ambient levels in California. Although the reported levels at the urban site may (according to the authors) have reflected some anthropogenic contributions such as disturbance and tracking of siliceous road dust, the rural and remote site values are apparently (perhaps conservatively) reflective of the natural background to which all California residents are exposed. (U.S. EPA (1996) found slightly higher average ambient levels of silica in PM_{10} ; this average may include some sites affected by disturbance and emissions.) There is no evidence that these background levels of silica are causing silicosis. On the other hand, silicosis in the general population is not a target for medical attention, and autopsy rates are very low, so the possibility of a low frequency of response at these levels cannot be entirely dismissed. On balance, it appears plausible that a REL of $3 \mu\text{g}/\text{m}^3$ (benchmark + $UF_H = 3$) would be protective of the general population.

- (3) The dose-response curve for silicosis due to inhalation of crystalline silica is steep, and an upward curvature of this dose response was seen in some studies (Figure 7-1 in USEPA, 1996). It is notable that, whereas exposures in the $1\text{-}3 \mu\text{g}/\text{m}^3$ range are apparently without effect (based on the benchmark calculations and the California ambient background data), Rice and Stayner (1995) described a LOAEL for silicosis of $8 \mu\text{g}/\text{m}^3$ in gold miners (Table 16; based on data from McDonald and Oakes [1984]). This finding may partly reflect differences in physical state of the silica, and co-exposures, but it might indicate that, although the chronic REL should be protective of public health, chronic exposures only moderately exceeding the REL may lead to clinically observable disease.

The animal studies gave LOAELs for silica of $0.2 \text{ mg}/\text{m}^3$ in dogs and from 1 to $4.9 \text{ mg}/\text{m}^3$ in rodents. After extrapolation to equivalent continuous time and application of LOAEL to NOAEL, interspecies, and intraspecies UFs, the estimated chronic RELs from animal data are all less than $1 \mu\text{g}/\text{m}^3$. This reflects in part the greater uncertainty in extrapolating from animal studies to predicted human health effects.

The silica particles of concern in the causation of silicosis are those of respirable size. California EPA defines 'respirable' as particles $10 \mu\text{m}$ or less MMAD. This reflects one usual type of sampler (for " PM_{10} ") used for ambient air sampling in the general environment. The other usual type of environmental sampler, $\text{PM}_{2.5}$, collects even smaller particles. There are differences in the size range distribution between a typical PM_{10} measuring device and the NIOSH type personal samplers, or other devices with similar size selection properties, used by the investigators in the epidemiological studies. The NIOSH-type samplers capture 50% of particles with a MMAD of $4 \mu\text{m}$, and higher percentages of smaller particles. A smaller proportion of larger particles between 4 and $10 \mu\text{m}$ in aerodynamic diameter will also be collected. Figure 5, from Volume I of U.S. EPA's Third External Review Draft of Air Quality Criteria for Particulate Matter (April 2002), includes particle penetration curves for PM_{10} , $\text{PM}_{2.5}$, and occupational samplers.

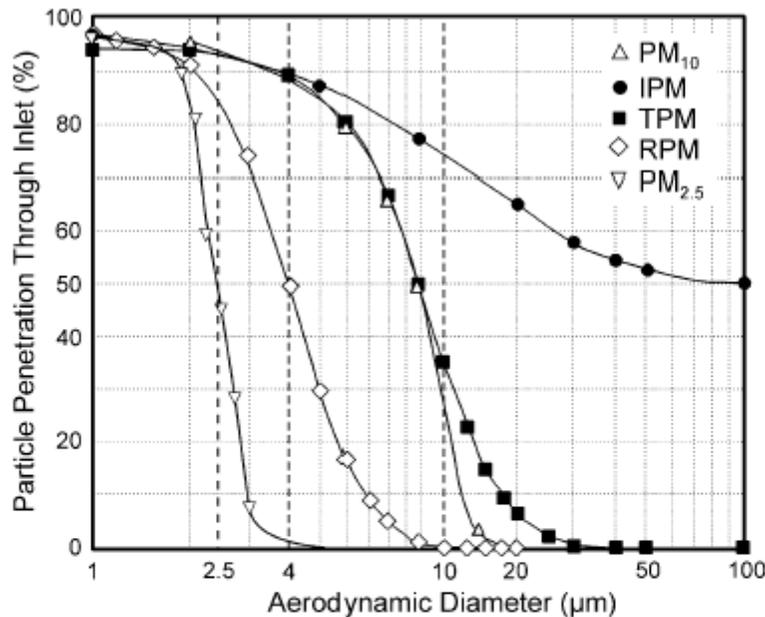
Figure 5. Size cut curves of particle penetration

Figure 2-6. Specified particle penetration (size-cut curves) through an ideal (no-particle-loss) inlet for five different size-selective sampling criteria. Regulatory size cuts are defined in the Code of Federal Regulations; PM_{2.5} (2001c), PM₁₀ (2001a). PM_{2.5} is also defined in the Federal Register (1997). Size-cut curves for inhalable particulate matter (IPM), thoracic particulate matter (TPM) and respirable particulate matter (RPM) size cuts are computed from definitions given by American Conference of Governmental and Industrial Hygienists (1994).

The NIOSH samplers are designed to mimic the size range of particles that reach into the bronchiolar and alveolar spaces (what the occupational community calls respirable). PM₁₀ samplers are meant to capture particles that penetrate the entire length of the lower respiratory tree, including those that penetrate to the tracheobronchial and alveolar regions. Penetration (and therefore presumably deposition) by particle size is complex, and is dependent on the aerodynamic diameter, hygroscopicity, and electrostatic charge of the particles, and on a number of host factors including airway structure and geometry, as well as depth, rate, and mode of breathing (nasal vs. oronasal). The fractional penetration in the various regions of the respiratory tract is not linear with respect to size. Generally, though, larger particles impact higher in the respiratory tree (the extrathoracic and tracheobronchial regions), while smaller particles show greater penetration to the lower tracheobronchial and alveolar regions. There are a number of models of regional penetration and deposition in the respiratory tract, as well as some measurements. Chan and Lippmann (1980) showed peak alveolar deposition for particles about 3 μm MMAD with deposition dropping above and below that. Their data and model indicate that tracheobronchial deposition rises rapidly above about 3 μm MMAD. Available data also

indicate significant inter-individual variability in fractional deposition. The ICRP (1994) model used in evaluating risk from radioactive particles indicates that total deposition in the respiratory tract for particles 3 μm in activity median thermodynamic diameter (AMTD) is about 0.78 with a regional deposition fraction of 0.077 for the alveolar region for a reference male worker during nasal breathing. The same model predicts a total deposition in the respiratory tract of 0.77 for 10 μm AMTD particles and a deposition fraction of 0.024 in the alveolar region. Thus, many particles with a 10 μm MMAD get into the alveolar space. A smaller difference in regional penetration and deposition is predicted for mouth breathers. Therefore, if only the size range measured by the samplers used in the studies were considered, the measurement might underestimate the amount of silica that reaches the gas exchange regions of the lung, depending on the actual particle size distributions in the occupational studies and in the environments in which the REL is to be applied. Unfortunately, neither the occupational nor the environmental silica particle size distributions are known in detail; measurements have been reported only in terms of NIOSH sampler results or PM_{10} cutoff values.

It is generally assumed that the silicosis is induced by that fraction of the silica that reaches the alveoli. Nevertheless, no actual data exonerate the coarser particles in the 4 - 10 μm range. A fraction of these particles can enter the bronchioles and alveoli. However, some data from South African gold mines indicate that more than 99% of the crystalline silica dust can be in the $\text{PM}_{2.5}$ fraction (Sichel, 1957). Thus, the samplers used in the key study appear to be collecting the biologically relevant range of particles in that situation.

In the absence of comprehensive data on the silicosis-inducing activity of different particle sizes, it is not possible to adjust the REL for different particle size distributions, which might be found in the general environment, or for different measurement methods. The REL is therefore specified as applicable to concentrations of particles having a size range (and reactivity) similar to those measured in the occupational studies [respirable as defined occupationally (ISO, 1995; NIOSH, 2003; ACGIH, 2004)]. Results obtained by other sampling methods would need to be corrected for any difference in size selectivity of the method used. Such a correction factor would be specific to the particle size distribution present at the site studied, so no general correction factors can be proposed. A more inclusive sampling procedure, such as that used for PM_{10} , would overestimate the relevant exposure in any situation, and so would be inappropriate for precise risk quantification. However, PM_{10} would be useful as a screening method to establish that a particular situation is unlikely to present a hazard. For example, if the silica concentration in PM_{10} modeled at a receptor is less than the REL ($3 \mu\text{g}/\text{m}^3$), occupationally respirable silica will also be less than $3 \mu\text{g}/\text{m}^3$, so a facility would not pose a risk due to silica at that receptor. If the silica concentration in $\text{PM}_{2.5}$ modeled at a receptor is less than $3 \mu\text{g}/\text{m}^3$ but PM_{10} is greater than $3 \mu\text{g}/\text{m}^3$, further testing would be needed. If both $\text{PM}_{2.5}$ and PM_{10} exceeded the REL, the chronic Hazard Index would exceed 1 to an undetermined extent, suggesting a need for risk management. More precise determination of the amount of material in the respirable size fraction for environmental samples may require further work on measurement methodology, since ISO (1995) and similar occupational methods have not been validated for the lower levels encountered in environmental samples.

VII. Data Strengths and Limitations for Development of the REL

The strengths of the inhalation REL for silica include:

- (1) The availability of several long-term studies of inhalation in workers at varying exposure concentrations (see Summary Table 15 above), with adequate histopathological and radiological analysis, and with adequate follow-up.
- (2) The finding of a dose-response effect for silicosis in several of the studies (e.g., Hnizdo and Sluis-Cremer, 1993; Steenland and Brown, 1995; Chen *et al.*, 2001).
- (3) The observation of a NOAEL in some studies including the key study (summarized by Rice and Stayner, 1995).
- (4) The power of the Hnizdo and Sluis-Cremer (1993) data to detect a small effect.

Major areas of uncertainty are:

- (5) The limited follow-up of the cohort members in some studies (e.g., Muir *et al.*, 1989; Rosenman *et al.*, 1996) with consequent under-ascertainment of silicosis (even to the extent that such studies are useless for determining exposure-response).
- (6) The general underestimation of silicosis by radiography alone (Hnizdo *et al.*, 1993), which results in higher, less health-protective chronic REL estimates.
- (7) The possible underreporting of silicosis where complete radiographic data and autopsy data are not available (Steenland and Brown, 1995).
- (8) The uncertainties in exposure estimation, especially when reconstructing historical levels of silica exposure (Seixas *et al.*, 1997; Gibbs and Du Toit, 2002) including the variability in the estimates of percent quartz in the South African mine dust (Beadle, 1971; Hnizdo and Sluis-Cremer, 1993; Kielblock *et al.*, 1997; Gibbs and Du Toit, 2002; Hnizdo, personal communication) and when converting particle counts to mass.
- (9) The differences in percent silicosis in different studies at what were considered similar silica levels and similar exposure duration (see Summary Table 15 above).
- (10) The variability in toxicity of various forms of silica (e.g., freshly fractured vs. aged quartz; cristobalite vs. quartz) although all forms have toxicity (Table 17).
- (11) The limited information on silica particle size (including its variability) in the epidemiological studies, other than that the silica was respirable, and the variability in particle penetration and deposition as a function of particle size in the respiratory tract in the human population (e.g., Heyder *et al.*, 1982; ICRP, 1994; Hattis *et al.*, 2001).
- (12) The use of area samplers rather than personal samplers to estimate exposure, which usually results in an underestimation of silica exposure (Cherrie, 1999).

VIII. Potential for Differential Impacts on Children's Health

Silica is a respiratory irritant and a modifier of immune function. Since the key study involved over 2000 men, some were likely to be more sensitive to silica than others. In addition, we used a benchmark of 1% adverse effect, rather than the usual 5%. Thus, use of the human intraspecies uncertainty factor (UF_H) of 3 should result in a REL that adequately protects most members of the general population. Exacerbation of asthma, which has a more severe impact on children than on adults, is a known response to some respiratory irritants. However, there is no data on such a response to silica in infants or children. The epidemiological studies used in the derivation of the REL did not include children. If children's susceptibility were much greater than that of adults, it would be expected that clinical disease would be evident in children following exposures in the upper range of the respirable silica levels measured in ambient air in California. No such reports have been identified in the literature. There are no data on silica's effects on the immune system of children.

OEHHA is currently evaluating its risk assessment methodology, in particular the UF_H , for its adequacy in protecting infants and children. Since children have smaller airways than adults and breathe more air on a body weight basis, penetration and deposition of particles in the airways and alveoli in children is likely greater than that in adults exposed to the same concentration (Phalen *et al.*, 1985; Schiller-Scotland *et al.*, 1994; Oldham *et al.*, 1997; Bennett and Zeman, 1998).

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X. Appendix*Particulate Levels of Interest for Exposure to Respirable Crystalline Silica Isomorphs*

150 $\mu\text{g}/\text{m}^3$	Federal 24 hour PM_{10} standard
65 $\mu\text{g}/\text{m}^3$	Federal 24 hour $\text{PM}_{2.5}$ standard
50 $\mu\text{g}/\text{m}^3$	California 24 hour PM_{10} standard
50 $\mu\text{g}/\text{m}^3$	Federal PM_{10} annual standard (chronic exposure)
50 $\mu\text{g}/\text{m}^3$	8 hour TLV for quartz, cristobalite, and tridymite for workers (ACGIH Method)
50 $\mu\text{g}/\text{m}^3$	estimated workplace LOAEL for silicosis from studies by Theriault <i>et al.</i> (“)
20 $\mu\text{g}/\text{m}^3$	CA annual PM_{10} standard (chronic exposure) (arithmetic mean)
15 $\mu\text{g}/\text{m}^3$	Federal annual $\text{PM}_{2.5}$ standard (chronic exposure)
12 $\mu\text{g}/\text{m}^3$	CA annual $\text{PM}_{2.5}$ standard (chronic exposure) (arithmetic mean)
12 $\mu\text{g}/\text{m}^3$	current silica TLV adjusted to equivalent continuous exposure (50 $\mu\text{g}/\text{m}^3 \times 8 \text{ h}/24 \text{ h} \times 5 \text{ d}/7\text{d}$) (ACGIH)
10 $\mu\text{g}/\text{m}^3$	TLV for silica proposed by Greaves (2000) (ACGIH)
8 $\mu\text{g}/\text{m}^3$	current silica TLV further adjusted by 46/70 years occupational exposure (ACGIH)
8 $\mu\text{g}/\text{m}^3$	estimated high-end ambient crystalline silica level in US (USEPA, 1996) (PM_{10})
6.7 $\mu\text{g}/\text{m}^3$	lower bound on 1% risk of silicosis estimated by USEPA (1996) (PM_{10})
5 $\mu\text{g}/\text{m}^3$	TLV for silica proposed by Chen <i>et al.</i> (2001) (ACGIH)
5 $\mu\text{g}/\text{m}^3$	“acceptable” ambient level for silica (10% of PM_{10}) (USEPA, 1996)
5 $\mu\text{g}/\text{m}^3$	RfC for diesel exhaust particulate, a respirable PM ($\text{PM}_{2.5}$)
3 $\mu\text{g}/\text{m}^3$	estimated average ambient exposure to crystalline silica (USEPA, 1996) (PM_{10})
3 $\mu\text{g}/\text{m}^3$	draft silica chronic REL proposed by OEHHA (ACGIH)
2.3 $\mu\text{g}/\text{m}^3$ (1.17-3.46; n=12)*	silica level during 1989 in Santa Maria, CA (urban site) (PM_{10})
0.6 $\mu\text{g}/\text{m}^3$ (0-1.44; n=16)*	silica level during 1989 in Santa Ynez, CA (rural site) (“)
0.2 $\mu\text{g}/\text{m}^3$ (0-1.15; n=18)*	silica level during 1989 in Buellton, CA (remote background)(“)

* mean, range, and number of crystalline silica measurements (Mukherji *et al.*, 1993)

CHRONIC TOXICITY SUMMARY

STYRENE*(ethenylbenzene, phenylethylene, vinylbenzene)***CAS Registry Number: 100-42-5****I. Chronic Toxicity Summary**

<i>Inhalation reference exposure level</i>	900 µg/m³ (200 ppb)
<i>Critical effects(s)</i>	Neuropsychological deficits in humans as measured by memory and sensory/motor function tests
<i>Hazard index target(s)</i>	Nervous system

II. Chemical Property Summary

<i>Description</i>	Colorless to slightly yellow liquid with sweet, floral odor (HSDB, 1999)
<i>Molecular formula</i>	C ₈ H ₈
<i>Molecular weight</i>	104.16
<i>Boiling point</i>	145.2 °C
<i>Melting point</i>	-31°C (HSDB, 1999)
<i>Vapor pressure</i>	10 torr at 31°C, polymerizes at 82°C and above (Weast, 1979)
<i>Solubility</i>	310 µg/ml (Dean, 1985)
<i>Conversion factor</i>	4.26 µg/m ³ per ppb at 25°C

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 (Fourereman, 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 styrene 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/m³). 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 OEHA 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	$\geq 3^c$
Controls	50	4/50	2/50	0/50
< 150 (mean = 75 \pm 33) ^d	14	6/14	3/14	2/14
150-299 (mean = 216 \pm 45)	9	6/9	5/9	3/9
300 - 450 (mean = 367 \pm 49)	14	10/14	7/14	5/14
> 450 (mean = 571 \pm 108)	13	11/13	8/13	6/13

^a Data from Table IV in Mutti *et al.* (1984b).

^b "Next-morning" styrene urinary metabolites.

^c The quantal grouping of the number of subjects that performed abnormally in ≥ 3 tests based on their styrene urinary metabolite concentrations, both shown in bold, were used in a benchmark concentration (BMC) analysis for the derivation of the REL (see Section VI below).

^d Based on Guillemin *et al.* (1982), a linear relationship exists for converting the urinary metabolite concentrations to ppm air styrene levels (4.97 mmoles MA+PGA/mole creatinine is equivalent to 1 ppm styrene). Thus, the mean styrene concentrations per group are 0, 15, 44, 74,

and 115 ppm. In addition to dyschromatopsia observed by Chia *et al.* (1994), Gobba and Cavalleri (1993) and Campagna *et al.* (1995) also reported this visual dysfunction among styrene workers in the reinforced plastics industry. Workers (n=36) exposed to an average of 16 ppm styrene exhibited significantly greater dyschromatopsia than controls, matched for age, ethanol consumption and tobacco smoking (Gobba and Cavalleri, 1993). Among the study population, only 1/36 styrene-exposed workers (compared to 16/36 controls) performed the test with 100 percent accuracy. When a different group of styrene-exposed workers was tested, those exposed to > 50 ppm styrene exhibited greater dyschromatopsia than those exposed to ≤ 50 ppm, and within this group, a subset exhibited a similar decrement after returning from a one month vacation. In the Campagna *et al.* (1995) study, the test for dyschromatopsia was given to 81 reinforced plastics industry workers (79 male and 2 female) exposed to 4.6, 10.1, and 88.8 ppm styrene (first quartile, median, and third quartile, respectively). No control group was used in this study. Statistical analysis revealed a correlation of color vision loss with exposure to styrene (defined as next-morning urinary mandelic acid), age, and ethanol consumption.

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

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

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

Immune system alterations were reported in a study conducted by Bergamaschi *et al.* (1995). Reinforced plastics industry workers (n=32 female/39 male, average age = 32 years, average exposure duration = 7 years) were compared with non-styrene exposed factory workers and matched for age, sex, tobacco use and ethanol consumption. Air styrene levels, among the different factories, varied between 10 - 50 ppm, and individual worker exposure was measured

by urinary metabolites the morning after the last shift (15 hours post-exposure). Among all workers in the study (median exposure = 16 ppm - according to the data of Guillemin *et al.* (1982)), the proportion of 12/18 lymphocyte subsets and the prevalence of abnormal values of immunologic phenotypes for 11/18 subsets were statistically different from the controls ($p < 0.001$ to < 0.05). When the workers were placed into three exposure groups (0, < 25 ppm, and > 25 ppm styrene), dose-response relationships were observed for prevalences of abnormal responses for four lymphocyte subsets and, in the case of two subsets, abnormal responses were observed in the group exposed to < 25 ppm styrene. Natural killer cell activity (a lymphocyte function), measured in a different group of workers in the same study, was decreased compared to unexposed worker controls. The median exposure, given in terms of urinary metabolites, was calculated as 21 ppm based on the data of Guillemin *et al.* (1982). The data show that exposure of these workers to air styrene levels below 50 ppm, and probably at levels near 25 ppm, resulted in alterations of the immune system.

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

V. Effects of Animal Exposure

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

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

Kishi *et al.* (1995) carried out a developmental study on rat pups born to dams exposed by inhalation to styrene (0, 50, 300 ppm; 6-hr/d; gestation days 7-21). Although the small number of litters (n=2) at the 50 ppm dose prevented detailed statistical analysis, the data suggest that exposure of the dams to 50 ppm styrene resulted in deficits and delays in some motor and coordination abilities among the pups. Pups born to dams exposed to 300 ppm exhibited statistically significant increases in spontaneous activity and in the delay of some neurobehavioral functions. Many of the effects became diminished as the pups aged. Measurements of reproductive toxicity (maternal weight gain, length of gestation, number of live

births) did not change. Postnatal body weights were lower among the styrene-exposed pups, but the differences became less as the pups aged to 125-days.

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

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

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

<i>Study</i>	Mutti <i>et al.</i> (1984b)
<i>Study populations</i>	Human (occupational)
<i>Exposure method</i>	Inhalation
<i>Critical effects</i>	Central nervous system
<i>LOAEL</i>	15 ppm
<i>NOAEL</i>	Not established
<i>BMC₀₅</i>	1.7 ppm
<i>Exposure continuity</i>	8 hr/d (10 m ³ per 20 m ³ day), 5 d/wk
<i>Exposure duration</i>	8.6 years (average years at work)
<i>Average occupational exposure</i>	0.61 ppm (1.7 x 10/20 x 5/7)
<i>Human equivalent concentration</i>	0.61 ppm
<i>LOAEL uncertainty factor</i>	Not needed in the BMC approach
<i>Subchronic uncertainty factor</i>	1 (average exposure 12.3% of lifetime)
<i>Interspecies uncertainty factor</i>	1
<i>Intraspecies uncertainty factor</i>	3
<i>Cumulative uncertainty factor</i>	3
<i>Inhalation reference exposure level</i>	0.2 ppm (200 ppb; 0.9 mg/m ³ ; 900 µg/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 ≥ 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 m³ per 20 m³ 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/m³) 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. Collapsing 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 m³ 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/m³).

The U.S. EPA (1996) calculated a reference concentration (RfC) of 0.3 ppm (1 mg/m³), which is slightly higher than the OEHHA-derived chronic REL of 0.2 ppm (0.9 mg/m³). 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 BMC₀₅ 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 ≥ 1 and ≥ 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: $25 \times (8/24 \times 5/7) / 10 \times 10$ 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 $(6/24 \times 5/7) = 0.18$. 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 $(20 \text{ ppm} \times 0.18) / 300$ which equals 0.01 ppm or 10 ppb ($40 \mu\text{g}/\text{m}^3$). 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 ($1300 \mu\text{g}/\text{m}^3$) 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 BMC₀₅ 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***SULFURIC ACID***(dithionic acid; pyrosulphuric acid)***CAS Registry Number: 7664-93-9****II. I. Chronic Toxicity Summary**

<i>Inhalation reference exposure level</i>	1 µg/m³
<i>Critical effect(s)</i>	Bronchiolar epithelial hyperplasia, and thickening of the bronchial walls in monkeys
<i>Hazard index target(s)</i>	Respiratory system

III. Physical and Chemical Properties (HSDB, 1995; CRC, 1994; CARB, 1997)

<i>Description</i>	Colorless liquid
<i>Molecular formula</i>	H ₂ SO ₄
Molecular weight	98.1 g/mol
<i>Density</i>	1.84 g/cm ³ @ 15° C
<i>Boiling point</i>	330±0.5°C (100%)
<i>Melting point</i>	10.36°C (100%)
<i>Vapor pressure</i>	<0.001 torr @ 25° C; 1 torr @ 145.8° C
<i>Solubility</i>	Soluble in water
<i>Conversion factor</i>	Not applicable

III. Major Uses or Sources

Sulfuric acid is a strong acid used as an intermediate in the synthesis of linear alkylbenzene sulfonation surfactants used in dyes, in petroleum refining, for the nitration of explosives, in the manufacture of nitrocellulose, in caprolactam manufacturing, as the electrolyte in lead-acid batteries, and as a drying agent for chlorine and nitric acid. Sulfuric acid is formed in the atmosphere from sulfur dioxide, from sulfur trioxide, and from oleum (a combination of sulfur trioxide and sulfuric acid used industrially). The annual statewide industrial emissions from facilities reporting under the Air Toxics Hot Spots Act in California based on the most recent inventory were estimated to be 4460 pounds of sulfuric acid (CARB, 1999).

IV. Effects of Human Exposures

Workers in the lead battery industry showed etching and erosion of the teeth after 4 months exposure to an average concentration of 0.23 mg/m³ H₂SO₄ (Gamble *et al.*, 1984). Dental erosion increased in a dose-dependent manner with longer duration of exposure.

A study of 33 storage battery plant workers exposed to H₂SO₄ concentrations as high as 35 mg/m³ showed a greater group mean decrease in FEV₁ across the time of their work shift compared to workers who were not exposed to sulfuric acid (El-Saddik *et al.*, 1972). The salivary pH of the sulfuric acid exposed workers, a qualitative measure of acid exposure, was lower than the controls during the course of the work shift.

OEHHA recently reviewed the California Ambient Air Quality Standard (CAAQS) for sulfates (25 µg/m³ for 24 hours) to see if it adequately protects children (OEHHA, 2000). The report was peer-reviewed by the Air Quality Advisory Committee. The report indicates that H⁺ itself may play a role in the effects seen in epidemiological studies of sulfate air pollution. Controlled acute inhalation studies in humans and laboratory animals of pH neutral or nearly neutral sulfate salts (e.g., ammonium sulfate) (Utell *et al.*, 1983; Lippman *et al.*, 1987; Schlesinger *et al.*, 1990), even at relatively high concentrations, do not produce the effects reported from epidemiologic studies of sulfates (asthma exacerbation, bronchoconstriction, decrements in lung function) that might be expected from short-term excursions. The controlled exposure studies show that sulfate aerosols containing strong acids, such as sulfuric acid and, to a lesser extent, ammonium bisulfate, produce functional and structural changes in healthy subjects consistent with those observed in epidemiological studies. A working hypothesis is that H⁺ is a causal factor for adverse human health effects (e.g., see Lippmann and Thurston, 1996) and that, among the commonly measured particulate matter (PM) indices, SO₄⁻ is the best surrogate metric for H⁺.

A large number of epidemiologic studies have been conducted showing that elevated levels of several air pollutants, including acid aerosols, sulfur and nitrogen oxides, and particulate sulfates are correlated with an increased prevalence of pulmonary disease (U.S. EPA, 1989; OEHHA 2000). Elevated sulfate levels (1.6 ppb or 6.6 µg/m³) have been associated with statistically significant decrements in FVC and FEV₁ in a cohort of Canadian children (Stern *et al.*, 1989). Further analysis of these data led Bates and Sitzo (1989) to conclude that H₂SO₄ was the most likely cause for the pulmonary changes observed. Similarly, Ostro *et al.* (1989) reported a statistical association between asthma-related symptoms reported by 209 asthmatics and sulfate and acidity levels in ambient air in Denver. Delfino *et al.* (1997) found that ambient H⁺ was associated with emergency room visits by children for respiratory symptoms in a study in Montreal. Additionally, Damokosh *et al.* (1993) in a follow-up analysis of the 6-City study suggested associations between average H⁺ concentration and chronic bronchitic symptoms. The relative odds of bronchitic symptoms with the highest acid concentration (58 nmoles/m³ H⁺) versus the lowest concentration (16 nmoles/m³) was 2.4 (95% CI: 1.9 to 3.2). Furthermore in a study of children in 24 U.S. and Canadian communities (Dockery *et al.*, 1996) in which the analysis was adjusted for the effects of gender, age, parental asthma, parental education, and parental allergies, bronchitic symptoms were confirmed to be significantly associated with strongly acidic PM (OR= 1.66; 95% CI 1.11-2.48). It was also found that FVC and FEV₁ were lower in locales with high particle acidity (Raizenne *et al.*, 1996). Gwynn *et al.* (2000) reported an association between both H⁺ and sulfate particles and respiratory hospital admissions and mortality in Buffalo, NY. Acidic sulfates may act to increase the toxicity of particles by enhancing the availability of metals present in the particles to generate reactive oxygen species in the respiratory epithelium. This may account for some of the effects seen in these epidemiological studies and makes it difficult to use these studies as a basis for a Reference Exposure Level for sulfuric acid. The relationship between the effect levels observed in these

studies and the proposed REL is discussed in the section below on the potential for differential impacts on children's health.

The occupational standard for sulfuric acid is based on a study in human subjects by Amdur *et al.* (1952). In their study, 22 healthy male subjects were exposed to 0, 0.35, 0.4, 0.5, 1, 2, or 5 mg/m³ for 5-15 minutes. The odor, taste, and irritation threshold was 1 mg/m³. Since the basis for this standard is an acute exposure, it is not useful in determining a chronic non-cancer REL for sulfuric acid. A review of chronic human exposures to sulfuric acid and resulting carcinogenicity outcomes can be found in IARC (1992). However, none of the studies in that review examined non-cancer endpoints.

Sulfuric acid and oleum (supersaturated anhydrous sulfuric acid with varying concentrations of free sulfur trioxide) are absorbed as salts of sulfate anion (SO₄²⁻), and are excreted as organic sulfates, neutral sulfur, or neutral sulfur compounds such as sulfur-containing amino acids. The low systemic toxicity of these metabolites is likely of secondary importance to the irritation caused by the inhaled acid.

V. Effects of Animal Exposures

An exposure of 9 cynomolgus monkeys per group to H₂SO₄ concentrations of 0, 0.38, 0.48, 2.43, and 4.79 mg/m³ continuously for 78 weeks resulted in dose-dependent adverse histological changes in lung and bronchiolar epithelial and parenchymal tissue in addition to a dose-dependent decrease in blood oxygenation (Alarie *et al.*, 1973). In the animals exposed to 0.38 mg/m³, significant bronchiolar epithelial hyperplasia was observed in 5 of 9 animals; thickening of the bronchiolar walls was observed in 3 of 9 animals. A slight focal bronchial epithelial hyperplasia was present in 4 of the 9 animals. One animal died after 4 weeks exposure to 0.38 mg/m³. Although signs of pulmonary edema and cardiac hypertrophy were found, the cause of death was not determined.

Respiratory system effects of H₂SO₄ exposure in monkeys (Alarie *et al.*, 1973)

H ₂ SO ₄ (g/m ³)	Particle size MMD	Bronchiolar epithelial hyperplasia Incidence – severity	Thickening of walls of respiratory bronchioles Incidence – severity	Increase in thickness of alveolar walls Incidence – severity
0		0/9	0/9	0/9
0.38	2.15	5/8 – slight	3/8 - slight	0/8
0.48	0.54	0/8	0/8	0/8
2.43	3.60	8/8 – moderate	8/8 – moderate	8/8 – moderate
4.79	0.73	8/8 – moderate to severe	8/8 – moderate to severe	0/8

Alarie *et al.* (1973) also exposed groups of 50 guinea pigs of each sex to 0, 0.08, or 0.1 mg/m³ H₂SO₄ continuously for 52 weeks. The group exposed to 0.1 mg/m³ also received larger sized particulates than the 0.08 mg/m³ group (2.78 μm vs. 0.84 μm, respectively). No exposure related effects were observed in the animals exposed to 0.08 mg/m³, whereas exposure of 0.1 mg/m³ resulted in decreased body weights in the female guinea pigs. No other histological changes in any organs were observed at the end of the 52-week study.

Rabbits (4 per group) were exposed to $250 \mu\text{g}/\text{m}^3$ H_2SO_4 1 hour/day, 5 days/week for 4, 8, or 12 months. They showed significantly increased bronchoconstriction upon acetylcholine challenge after 8 and 12 months exposure, compared with a control group of 4 animals that received no H_2SO_4 (Gearhart and Schlesinger, 1986, 1988). Mucociliary clearance was also impaired by H_2SO_4 exposure and did not improve 3 months after cessation of exposure. A decline in dynamic lung compliance was observed after 12 months exposure. There was no evidence of inflammatory cell infiltration in the lungs of the exposed animals.

In guinea pigs, significantly slower and irregular breathing patterns were noted when the animals had inhaled albumin followed by 30 minute exposures to H_2SO_4 at $1.91 \text{ mg}/\text{m}^3$ twice per week for 5 weeks (Kitabatake *et al.*, 1979). Similarly, when guinea pigs were exposed to $2.49 \text{ mg H}_2\text{SO}_4/\text{m}^3$ for 4 hours/day, 6 days/week for 4 weeks, *in vitro* lung histamine release was significantly enhanced following heterogeneous albumin inhalation, compared to control animals unexposed to albumin (Fujisawa *et al.*, 1986; Iguchi *et al.*, 1986). In guinea pigs, sulfuric acid caused significantly greater lung function changes when adsorbed on the surface of zinc oxide particles as compared with pure sulfuric acid (Amdur and Chen, 1989). An exposure to $24 \mu\text{g}/\text{m}^3$ sulfuric acid, layered on zinc oxide, produced significant reductions in lung function when followed by a brief exposure to 0.15 ppm ozone (Chen *et al.*, 1991).

A chronic exposure of beagle dogs to an average concentration of $889 \mu\text{g}/\text{m}^3$ H_2SO_4 for 21 hours/day over a 620 day period resulted in increased expiratory resistance, reduced carbon monoxide diffusing capacity, reduced total and residual lung volume, and decreased lung and heart weights (Lewis *et al.*, 1973).

In apparent contrast to the above studies, rats and guinea pigs exposed to H_2SO_4 at $10 \text{ mg}/\text{m}^3$ for 6 hours/day, 5 days/week for 6 months exhibited no adverse histologic changes in lung tissue. Lung function measurements were not reported in this study (Cavender *et al.*, 1978).

Mice inhaled sulfuric acid mist at a concentration of $1.4 \text{ mg}/\text{m}^3$ in combination with a carbon particle mixture ($1.5 \text{ mg}/\text{m}^3$) for 3 hours/day, 5 days/week for up to 20 weeks. The exposure resulted in significant alterations in specific antibody titer (decreased IgG, Ig_{2a}, IgM; increased IgG_{2b}), depression of primary splenic antibody response, and decreased resistance to respiratory infection as measured by mortality and survival time compared to controls (Fenters *et al.*, 1979).

There are no reliable studies indicating that sulfuric acid is a developmental or reproductive toxicant. In the absence of massive overexposure leading to maternal acidemia, H_2SO_4 will be neutralized in the maternal circulation and is unlikely to reach the fetus.

VI. Derivation of Chronic Reference Exposure Level

<i>Study</i>	Alarie <i>et al.</i> , 1973
<i>Study population</i>	Cynomolgus monkeys (5 males and 4 females per group or vice versa)
<i>Exposure method</i>	Continuous inhalation exposures (0, 380, 480, 2400, or 4800 $\mu\text{g}/\text{m}^3$) for 78 weeks
<i>Critical effects</i>	Significantly increased bronchial epithelial hyperplasia and bronchial thickening
<i>LOAEL</i>	380 $\mu\text{g}/\text{m}^3$
<i>NOAEL</i>	Not observed
<i>Exposure continuity</i>	The exposure was continuous during the experiment.
<i>Exposure duration</i>	78 weeks
<i>Average experimental exposure</i>	380 $\mu\text{g}/\text{m}^3$ for the LOAEL group
<i>Human equivalent concentration</i>	380 $\mu\text{g}/\text{m}^3$
<i>LOAEL uncertainty factor</i>	3 (slight effects)
<i>Subchronic uncertainty factor</i>	3
<i>Interspecies uncertainty factor</i>	3 (non-human primate)
<i>Intraspecies uncertainty factor</i>	10
<i>Cumulative uncertainty factor</i>	300
<i>Reference exposure level</i>	1 $\mu\text{g}/\text{m}^3$

The study by Alarie *et al.* (1973) identified a LOAEL for chronic exposure to sulfuric acid of 380 $\mu\text{g}/\text{m}^3$. The principal uncertainties of this study are the small sample size of the test groups and the absence of an observed NOAEL. A lower chronic LOAEL for bronchial reactivity is presented by Gearhart and Schlesinger (1986, 1988) for rabbits (250 $\mu\text{g}/\text{m}^3$). This study was not selected as the basis of the REL because Gearhart and Schlesinger used only a single concentration of sulfuric acid, exposed the animals only for 1 hour per day for 5 days/week, used only 4 animals per group, and measured effects over the course of up to 12 months. The predominant weakness in the rabbit study, however, was the extreme discontinuity of the exposures (1 hour/day, 5 days/week), which would have necessitated use of a very large continuity adjustment. For these reasons, in addition to obvious physiological and genetic similarity arguments, the study in monkeys by Alarie *et al.* (1973) was felt to be more appropriate as the basis for the chronic REL for sulfuric acid. Alarie *et al.* (1975) determined a NOAEL for sulfuric acid in monkeys of 0.1 mg/m^3 . However, other particulate matter (fly ash) was also present during the exposure. The Alarie *et al.* (1973) report provides data from exposure to sulfuric acid alone.

A free-standing NOAEL for histological changes in 100 guinea pigs exposed continuously for 1 year to 0.08 mg/m^3 was reported by Alarie *et al.* (1973). Guinea pigs respond to high concentrations of sulfuric acid by occasional laryngeal spasms that appear similar to a human asthmatic attack (Silbaugh *et al.*, 1981; Amdur and Chen, 1989). As a result, guinea pigs are thought to be sensitive models for the acute effects of sulfuric acid. For chronic effects of sulfuric acid on the lung, monkeys are likely a suitable model due to their physiological and structural similarities to humans.

For comparison, a chronic REL based on the guinea pig free-standing NOAEL of 0.08 mg/m³ in animals exposed continuously for one year (Alarie *et al.*, 1973) would be 0.8 µg/m³.

VII. Data Strengths and Limitations for Development of the REL

The major strength of the study on sulfuric acid is the use of health effects observations from continuous long-term exposures to a primate. The major weaknesses are the lack of adequate human health effects data and the lack of a NOAEL observation.

VIII. Potential for Differential Impacts on Children's Health

There are no reliable studies indicating that sulfuric acid is a developmental or reproductive toxicant. Children are likely to be at greater risk from long-term exposures because their bodies are growing, and their developmental processes, especially in the lung, may well be impacted by air pollution exposures. Elevated sulfate levels (1.6 ppb or 6.6 µg/m³) have been associated with statistically significant decrements in FVC and FEV1 in a cohort of Canadian children (Stern *et al.*, 1989). The chronic REL for sulfuric acid of 1 µg/m³ is below the level associated with those decrements in pulmonary function. However, in a study of moderately to severe asthmatic children (ages 7-13) (Thurston *et al.*, 1997), a sensitive subpopulation for sulfate effects, approximately 1 µg/m³ was the lowest level of ambient sulfate measured. The mean daily morning to afternoon peak airflow change, the use of beta-agonist medication, and the number of chest symptoms versus sulfate concentration in these children extrapolated linearly down to 1 µg/m³. Thurston *et al.* (1997) also examined earlier data from Ontario (Burnett *et al.*, 1994) on respiratory admissions to hospitals, and concluded that the sulfate threshold of effects, if it exists, lies below 5 µg/m³, perhaps at about 2 µg/m³. It should be noted that the sulfate and hydrogen ion effects are difficult to disentangle from each other and from the effects of other PM constituents. The chronic REL of 1 µg/m³ appears to have a relatively low margin of safety with respect to the epidemiological studies, but these observations are consistent with the proposed REL of 1 µg/m³ since asthmatic children appear to be the critically sensitive human population for exposure to sulfuric acid (or sulfate).

IX. References

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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; \approx 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/m³ of toluene, respectively; however, before 1980 the exposure levels had exceeded 300 mg/m³ 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/m³) toluene while the

control workers inhaled 13 ppm (49 mg/m³). 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/m³) 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/m³ (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 D₂ 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-³H(N)]-propylnorapomorphine ([³H]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 [³H]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 D₂ 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 [³H]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 [³H]raclopride-binding in the caudate-putamen and the subcortical limbic area, or the effect of dopamine on IC_{50} values at [³H]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 D₂ 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 D₂ 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/m³) 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/m³) 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/m³) 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/m³) 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 F₁ pups were exposed 80 times to the appropriate exposure level and then randomly mated to members of the same exposure group. The F₁ generation showed significantly decreased body weight which persisted throughout lactation. No effects were observed on histopathology. No data were presented for the F₂ generation.

Da Silva *et al.* (1990) exposed rats and hamsters to 0 or 800 mg/m³ 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 <i>et al.</i> (1995); supported by Orbaek and Nise (1989), Foo <i>et al.</i> (1990)
<i>Study population</i>	Male Sprague-Dawley rats
<i>Exposure method</i>	Inhalation
<i>Critical effects</i>	Decreased brain (subcortical limbic area) weight, altered dopamine receptor (caudate-putamen) binding
<i>LOAEL</i>	80 ppm
<i>NOAEL</i>	40 ppm
<i>Exposure continuity</i>	6 hours/day, 5 days/week
<i>Exposure duration</i>	4 weeks, followed by 29-40 days recovery
<i>Average experimental exposure</i>	7 ppm (40 × 6/24 hours × 5/7 days)
<i>Human equivalent concentration</i>	7 ppm (gas with systemic effects, based on RGDR = 1.0 using default assumption that $\lambda_a = \lambda_h$)
<i>Subchronic uncertainty factor</i>	10
<i>Interspecies uncertainty factor</i>	1 (see below)
<i>Intraspecies uncertainty factor</i>	10
<i>Cumulative uncertainty factor</i>	100
<i>Inhalation reference exposure level</i>	0.07 ppm (70 ppb; 0.3 mg/m ³ ; 300 µg/m ³)
<i>Supportive human study</i>	Foo <i>et al.</i> , 1990
<i>Study population</i>	30 female workers in an electronic assembly plant
<i>Exposure method</i>	Occupational inhalation
<i>Critical effects</i>	Neurobehavioral deficits in 6 out of 8 tests
<i>LOAEL</i>	88 ppm
<i>NOAEL</i>	Not observed
<i>Exposure continuity</i>	10 m ³ /day occupational inhalation rate, 5 days/week
<i>Average occupational exposure</i>	31.4 ppm (88 ppm × 10/20 × 5/7)
<i>Exposure duration</i>	5.7 ± 3.2 years (exposed group); 2.5 ± 2.7 years (controls)
<i>LOAEL uncertainty factor</i>	10
<i>Subchronic uncertainty factor</i>	3
<i>Interspecies uncertainty factor</i>	1
<i>Intraspecies uncertainty factor</i>	10
<i>Cumulative uncertainty factor</i>	300
<i>Inhalation reference exposure level</i>	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 <i>et al.</i> (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 $\mu\text{g}/\text{m}^3$ (rounded to 300 $\mu\text{g}/\text{m}^3$ 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

2,4- and 2,6-TOLUENE DIISOCYANATE

(2,4- and 2,6-TDI; 2,4- and 2,6-diisocyanato-1-methylbenzene; 2,4- and 2,6-diisocyanatoluene)

CAS Registry Number: 584-84-9 or 26471-62-5 (mixture)

I. Chronic Toxicity Summary

<i>Inhalation reference exposure level</i>	0.07 $\mu\text{g}/\text{m}^3$ (0.01 ppb)
<i>Critical effect(s)</i>	Decreased lung function in occupationally exposed workers
<i>Hazard index target(s)</i>	Respiratory system

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

<i>Description</i>	Colorless to pale yellow liquid
<i>Molecular formula</i>	$\text{C}_9\text{H}_6\text{N}_2\text{O}_2$
<i>Molecular weight</i>	174.15 g/mol
<i>Boiling point</i>	2,4-TDI: 251°C
<i>Melting point</i>	2,4-TDI: 20.5°C 2,6-TDI: 18.3°C
<i>Vapor pressure</i>	2,4-TDI: 0.008 torr @ 20°C
<i>Solubility</i>	Miscible with ether, acetone, benzene, carbon tetrachloride, chlorobenzene, diglycol monomethyl ether, kerosene, olive oil, alcohol; soluble in ethyl acetate
<i>Conversion factor</i>	7.1 $\mu\text{g}/\text{m}^3$ per ppb at 25°C

III. Major Uses and Sources

Commercial toluene diisocyanate is comprised of approximately 80% 2,4-TDI and 20% 2,6-TDI. TDI is used in the manufacture of polyurethane foams, elastomers, and coatings (HSDB, 1995; Howard, 1989). It is also used in the manufacture of floor and wood finishes, lacquers, foam plastics, polyurethane foam coated fabrics, and insulation materials (HSDB, 1995; Howard, 1989; Duncan *et al.*, 1962). Emissions of TDI to the atmosphere can occur during production, handling, and processing of polyurethane foam (Howard, 1989) and coatings. The annual statewide industrial emissions from facilities reporting under the Air Toxics Hot Spots Act in California based on the most recent inventory were estimated to be 13,223 pounds of toluene diisocyanates, 35,663 pounds of toluene-2,4-diisocyanate, and 754 pounds of toluene-2,6-diisocyanate (CARB, 1999).

IV. Effects of Human Exposures

Diem *et al.* (1982) conducted a prospective study beginning in 1973 of 277 male workers involved in the production of TDI. The study examined pulmonary function, with nine examinations conducted over a five year period. A large group of workers (168) with no previously reported TDI exposure was examined 6 months prior to TDI production in the plant to provide baseline pulmonary function measurements. Personal sampling by continuous tape monitors provided exposure levels, but was not used until 2 years after the study was initiated. Sampling information resulted in a division of the workers into two groups: those exposed to levels below 68.2 ppb-months (which reflects the level of exposure of a worker for the entire 5 year duration in the low-exposure area (geometric mean = 1.1 ppb)) and those above this level. The arithmetic mean exposure level for the non-smokers was 1.9 ppb TDI in the high-exposure group and 0.9 ppb TDI in the low-exposure group (calculated by Hughes, 1993). The higher exposure group was further limited to those individuals who showed a normal FEV₁ to height ratio. Data were analyzed by the maximum likelihood weighted regression approach (Diem and Liukkonen, 1988). Both FEV₁ and forced expiratory flow (25-75%) [FEF (25-75%)] among workers who never smoked were found to be significantly reduced in the high-exposure group (n = 21) compared to the low-exposure group (n = 35). Categorizing workers based on time spent at exposure levels above 20 ppb demonstrated a significant difference in FEV₁ and FEF(25-75%) and this effect was also observed among current smokers. Among low-exposure workers, a smoking effect was observed, with smokers showing a significant decline in FEV₁.

A similar longitudinal study of lung function was conducted among workers exposed to TDI during the course of polyurethane foam production (Jones *et al.*, 1992). Participants (181 males and 46 females) were required to have 3 or more spirometric examinations over the 5 year study period. Exposure of males was evaluated by personal monitors and resulted in arithmetic mean low exposure levels of 0.3, 0.4, and 0.4 ppb TDI for never-smokers, ex-smokers, and current smokers, respectively. Among workers with high-level exposure, mean TDI levels were reported to be 1.3, 1.2, and 1.2 ppb for never-, ex-, and current smokers, respectively. Stepwise multiple linear regression methods (excluding asthmatics) were used in evaluating the data (Diem and Liukkonen, 1988). No relationship between TDI exposure and change in lung function was observed, although the prevalence of chronic bronchitis was significantly associated with exposure.

A longitudinal study of 780 workers exposed to TDI in the production of polyurethane foam was also conducted (Bugler *et al.*, 1991; unpublished). Exposure levels were established using continuous-tape personal monitoring devices. The mean exposure level was 1.2 ± 1.1 (SD) ppb TDI among 521 workers and 0.3 ± 0.18 ppb TDI in the control group. Another control group who handled cold urethane products had an 8 hour time-weighted average exposure of 0.6 ppb TDI. No significant longitudinal changes in FEV₁ were found after regression analysis, although FEV₁ decline was high among the control group. Exposure levels among the different groups were close, limiting the power of the study to detect changes. Approximately 3% of the 780 workers showed signs of TDI sensitization and, of these, over 80% were in the group exposed to 1.2 ppb.

Meta-analysis of the three data sets (Jones *et al.*, 1992; Bugler *et al.*, 1991; Diem *et al.*, 1982) showed that the difference in significance among the findings of each of the studies could have been due to chance. The change in the probability density for the decline in FEV₁ shifted in the same direction for all data sets and the smoker/non-smoker slope difference became less meaningful with the data set combination (Hasselblad, 1993).

Another toxicological area of concern with exposure to TDI is the development of sensitization, resulting in a well-documented condition known as “isocyanate asthma” of either immediate or delayed-type onset (Moscato *et al.*, 1991). The level of exposure required to either develop or trigger a sensitization reaction is not well documented, however. Weaknesses of studies showing pulmonary effects of TDI exposure include use of area sampling vs. breathing-zone measurement of exposure, poor statement of criteria for evaluating hypersensitivity, and the presence of other compounds in the environment which may influence lung function.

V. Effects of Animal Exposures

Mice were exposed to TDI concentrations ranging from 0.007 to 1.18 ppm for 3 hours/day for 5 days consecutively (Sangha and Alarie, 1979); decreased respiratory rate was observed in groups exposed to levels higher than 0.023 ppm TDI. Groups of four mice were also exposed to 0.031 and 0.250 ppm TDI for 3 hours/day for 3 days. Lesions of the external nares and respiratory epithelium were observed in the high dose group.

Female guinea pigs were exposed to 0.12, 0.36, 0.61, 0.96, and 10.00 ppm TDI (head-only) for 3 hours/day for 5 consecutive days (short protocol) or to 0.02 ppm TDI (whole body) plus controls for 6 hours/day, 5 days/week for 70 days (long protocol). The animals showed decreased respiration rate two hours into exposure at levels above 0.12 ppm TDI and had a cytophilic antibody response at 0.96 ppm and above (Karol, 1983). All animals exposed to 10 ppm died. Dermal sensitivity was evident among animals in the short protocol down to 0.12 ppm TDI. No antibody response or dermal sensitivity developed in the animals exposed to 0.02 ppm TDI in the long protocol.

Similarly, guinea pigs (8 females) were exposed head only to 1.40 ppm TDI for 3 hours/day for 4 days (no control group). In a second exposure regimen, animals (n = 24) were exposed to 0.02 ppm TDI for 6 hours/day, 4 days/week for 70 days (whole body) including a control group (n = 8) exposed to room air in a similar manner (Wong *et al.*, 1985). Half the animals (4/8) exposed to 1.40 ppm TDI showed pulmonary hypersensitivity (measured on days 37 and 38) and all developed TDI-specific IgE antibodies, whereas none of the animals in the 0.02 ppm TDI group showed either of these effects. Histopathological effects in the 1.40 ppm TDI group included interstitial inflammation, pleural thickening, and peripheral lymphoid hyperplasia. Interstitial inflammation was noted in 2/24 animals exposed to 0.02 ppm TDI.

SD rats and CD-1 mice were exposed to 0.05 or 0.15 ppm TDI for 6 hours/day, 5 days/week for 2 years (Loeser, 1983; nasal histopathology reported by Owen, 1984). Among female rats at both dose levels and male rats at the high dose level, histopathological effects observed included necrotic rhinitis, metaplasia, and inflammation of the respiratory epithelium. Female animals

showed dose-dependent increases in incidence and severity of this effect. Similar lesions were reported in mice, although they were not well characterized.

Reproductive toxicity of TDI was evaluated in a two-generation study conducted in rats (Tyl and Neepser-Bradley, 1989). Weanling rats (28/sex/dose) were exposed to 0, 0.020, 0.079, and 0.290 ppm TDI for 6 hours/day, 5 days/week, for 10 weeks, at which time the animals were randomly mated. Exposure of the females continued through gestation (excepting gestational day 20 through the fourth day postpartum), and exposure of the males continued only until the delivery of the F₁ generation. Weanlings in the F₁ generation were exposed in a manner similar to the parental (P₀) generation and bred after weaning to produce the F₂ generation. Body weights were significantly reduced among animals of both sexes in the highest dose group and weight gain was reduced among males in the highest dose group. Effects on the respiratory system in the P₀ generation animals included rhinitis of the epithelium in the two highest dose groups of both male and female animals. Hyperplasia of the respiratory epithelium was also increased in the high dose groups of both sexes among P₀ animals. Among males in the F₁ generation, the incidence of rhinitis was significantly increased at all exposure levels and the incidence of submucosal lymphoid infiltrates of the larynx and trachea was increased in the highest dose group. F₂ generation animals showed reduced pup weight and weight gain during the lactation period in the two highest dose groups.

Developmental toxicity of TDI was evaluated by exposing pregnant Sprague-Dawley rats (25/group) for 6 hours/day on gestational days 6-15 to 0, 0.021, 0.120, or 0.48 ppm TDI (Tyl, 1988). Reduced maternal body weight, decreased food consumption, and resorptions occurred among the dams in the 0.48 ppm TDI dose group. A significant fetal effect, a statistically significant increase in a specific skeletal malformation, was reported in the highest dose group.

VI. Derivation of the Chronic Reference Exposure Level

<i>Study</i>	Diem <i>et al.</i> , 1982
<i>Study population</i>	Human TDI production workers (n = 168)
<i>Exposure method</i>	Occupational inhalation exposure
<i>Critical effects</i>	Decreased lung function
<i>LOAEL</i>	0.014 mg/m ³ (1.9 ppb)
<i>NOAEL</i>	0.006 mg/m ³ (0.9 ppb) (non-smokers)
<i>Exposure continuity</i>	8 h/day (10 m ³ /day occupational exposure), 5 d/wk
<i>Exposure duration</i>	5 years
<i>Average occupational exposure</i>	0.002 mg/m ³ for NOAEL group (0.006 x 10/20 x 5/7)
<i>Human equivalent concentration</i>	0.002 mg/m ³ for NOAEL group
<i>LOAEL uncertainty factor</i>	1
<i>Subchronic uncertainty factor</i>	3
<i>Interspecies uncertainty factor</i>	1
<i>Intraspecies uncertainty factor</i>	10
<i>Cumulative uncertainty factor</i>	30
<i>Inhalation reference exposure level</i>	0.00007 mg/m ³ (0.07 µg/m ³ ; 0.01 ppb)

The chronic REL is equivalent to the U.S. EPA RfC. OEHHA agreed with the U.S. EPA analysis and the selection of Diem *et al.* (1982) as the most appropriate study to use for the REL. The rationale for selection of this study is as follows. This study presented evidence of a decline in lung function, as indicated by decrements in FEV₁, among workers involved in TDI production. Other factors supporting its quality include:

- (1) the absence of other confounding compounds in the work environment,
- (2) the establishment of baseline lung function prior to exposure to TDI,
- (3) a “parallel internal comparison” of study groups for lung function,
- (4) an appropriate statistical analysis which took into account interindividual variability,
- (5) breathing zone measurement of TDI (although commenced 2 years into the study), and
- (6) a smoking effect on lung function.

VII. Data Strengths and Limitations for Development of the REL

The major strengths of the chronic REL for TDI are the use of human exposure data from workers exposed over a period of years and the observation of a NOAEL. The major weaknesses are the uncertainty in estimating exposure, the potential variability in exposure concentration, and the limited nature of the study that focused on lung effects.

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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

<i>Inhalation reference exposure level</i>	600 µg/m³ (100 ppb)
<i>Critical effect(s)</i>	Neurotoxicological effects (drowsiness, fatigue, headache) and eye irritation in workers.
<i>Hazard index target(s)</i>	Nervous system; eyes

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

<i>Description</i>	Colorless liquid/vapor; sweetish, chloroform-like odor
<i>Molecular formula</i>	C ₂ HCl ₃
<i>Molecular weight</i>	131.4
<i>Density</i>	1.47 g/cm ³ @ 20°C
<i>Boiling point</i>	87.2 °C
<i>Melting point</i>	-84.7°C
<i>Vapor pressure</i>	77 torr @ 25°C
<i>Vapor density</i>	4.5 (air = 1)
<i>Solubility</i>	Soluble in alcohol, ethers, petroleum distillates and other halogenated solvents
<i>Conversion factor</i>	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/m³ (32-78 ppm). Nineteen workers (including the 11 workers whose work areas were sampled) completed a questionnaire and reported the following symptoms: 73% eye irritation, 70% drowsiness, 58% heart palpitations, 58% cough, 53% weakness and 52% dizziness. About half of the 19 exposed workers reported that consumption of small amounts of alcohol outside of work resulted in changes of skin color and severe intoxication. One worker of the 19 reported no adverse effects from the occupational exposure. Nine control workers experienced none of the above symptoms. Urine samples from the 19 exposed and 9 unexposed workers were collected before and after the work shift and examined for the TCE metabolites trichloroacetic acid (TCA) and trichloroethanol (TRI). TRI levels ranged from 4-260 mg/l and TCA levels ranged from 4-197 mg/l. Results of the urine assays showed a range of TCE metabolite concentrations and, therefore, confirmed that the workers were exposed to a variety of concentrations in their environments.

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

Kimmerle and Eben (1973) exposed 4 human subjects (3 males and 1 female) to a subacute regimen of 48 ± 3 ppm trichloroethylene (TCE) for 4 hours a day over a period of 5 days. Levels of TCE and the metabolites trichloroethanol (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/m³ TCE (40-170 ppm) as did case #5 after two weeks of exposure to 370 mg/m³ TCE (69 ppm). Case #4 involved a 39 year old man exposed to <50 mg/m³ 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 ³H-aminobutyric acid (GABA) and ¹⁴C-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 microtubules, 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 ± 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 P450 activity in 9 rats exposed to 50 ppm trichloroethylene for 28 days, compared with 9 control rats.

VI. Derivation of Chronic Reference Exposure Level

<i>Study</i>	Vandervort and Polnkoff (1973)
<i>Study population</i>	19 workers and 9 controls
<i>Exposure method</i>	Discontinuous occupational inhalation exposure
<i>Critical effects</i>	Drowsiness, fatigue, headache, and eye irritation
<i>LOAEL</i>	32 ppm (170 mg/m ³) in the heavy assembly area
<i>NOAEL</i>	Not observed
<i>Exposure continuity</i>	8 hours a day (10 m ³ /day occupational inhalation rate), 5 days a week
<i>Exposure duration</i>	8 years
<i>Average occupational exposure</i>	11.4 ppm for LOAEL group (32 x 10/20 x 5/7)
<i>Human equivalent concentration</i>	11.4 ppm for LOAEL group
<i>LOAEL uncertainty factor</i>	10
<i>Subchronic uncertainty factor</i>	1
<i>Interspecies uncertainty factor</i>	1
<i>Intraspecies uncertainty factor</i>	10
<i>Cumulative uncertainty factor</i>	100
<i>Inhalation reference exposure level</i>	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/m³) 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. Nomiya *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

TRIETHYLAMINE*(diethylaminoethane; ethanamine; N,N-diethylethanamine)***CAS Registry Number: 121-44-8****I. Chronic Toxicity Summary**

<i>Inhalation reference exposure level</i>	200 $\mu\text{g}/\text{m}^3$ (40 ppb)
<i>Critical effect(s)</i>	Eye effects in rats and humans
<i>Hazard index target(s)</i>	Eyes

II. Physical and Chemical Properties (Nelson and Bull, 1990, except as noted)

<i>Description</i>	Colorless, volatile liquid
<i>Molecular formula</i>	$\text{C}_6\text{H}_{15}\text{N}$
<i>Molecular weight</i>	101.9 g/mol
<i>Density</i>	0.726 g/cm ³ @ 25°C
<i>Boiling point</i>	89.3°C
<i>Melting point</i>	-114.7°C (CRC, 1994)
<i>Vapor pressure</i>	400 torr @ 31.5°C
<i>Odor threshold</i>	480 ppb (Amoore and Hautala, 1983)
<i>Solubility</i>	soluble in acetone, benzene and chloroform
<i>Conversion factor</i>	1 ppm = 4.14 mg/m ³ 25°C

III. Major Uses or Sources

Triethylamine (TEA) is primarily used as a cross-linking catalyst in the production of polyurethane foam used in the manufacture of cores for metal castings (Albrecht and Stephenson, 1988). Triethylamine is also used as a catalyst for epoxy resins, and as a corrosion inhibitor for polymers (Nelson and Bull, 1990). TEA is one of the amines emitted from cattle feedlots (Mosier *et al.*, 1973). In the gas phase TEA can react with nitric acid to form amine nitrates that become part of atmospheric particulates. The annual statewide industrial emissions from facilities reporting under the Air Toxics Hot Spots Act in California based on the most recent inventory were estimated to be 4152 pounds of triethylamine (CARB, 2000).

IV. Effects of Human Exposures

Acute, high level triethylamine exposures (20 mg/m³ (4.8 ppm) for 8 hours) resulted in reversible ocular effects that included corneal swelling and halo vision in 4 out of 5 volunteer subjects (Akersson *et al.*, 1988). Jarvinen *et al.* (1999) reported exposure chamber studies of ocular

responses to TEA in volunteer subjects, who were industrial workers exposed to TEA during the course of their normal jobs (but had good general and ocular health). Four people were exposed for 4 hours to 3.0, 6.5, or 40.6 mg/m³ triethylamine. Corneal thickness was measured by ultrasonography, clinical observations were recorded using ocular microscopy, and statistical analysis of the size, density and distribution of corneal endothelial cells was performed by automated analysis of photographs. Visual acuity and contrast sensitivity were evaluated using test charts. After exposure to 40.6 mg/m³ there was a marked edema in the corneal epithelium, and subepithelial microcysts. However, corneal thickness increased only minimally. Vision was blurred in all subjects and visual acuity and contrast sensitivity decreased in three of the four. After exposure to 6.5 mg/m³ two subjects experienced symptoms (e.g., blurred vision), and contrast sensitivity decreased in three of the four. There were no symptoms or decreases in contrast sensitivity after exposure to 3.0 mg/m³ triethylamine for 4 hours.

A medical examination of 19 workers exposed at a polyurethane foam production plant to a time-weighted average concentration of 13 mg/m³ (3.1 ppm) TEA showed reversible corneal edema in 5 workers (Akesson *et al.*, 1986). Peak concentrations were up to twice the time-weighted average level. A questionnaire on self-reported symptoms of visual disturbances revealed repeated occurrences of temporary eye irritation and “foggy vision.” Small quantities of dimethylethanolamine, toluene diisocyanate, and methylene diphenyl isocyanate were also present in the workplace atmosphere.

Jarvinen and Hyvärinen (1997) reported loss of visual acuity and contrast sensitivity in 41 foundry workers (core makers) exposed to TEA. Concentrations of TEA were reported to have a mean of 46 mg/m³ and a maximum of 486 mg/m³, but were highly variable with numerous large excursions above a background of about 20 mg/m³ during a two-hour period of continuous monitoring. It is therefore difficult to determine an effect level for the observed symptoms. Jarvinen (1998) also reported that cold box core makers exposed to TEA had a somewhat increased incidence of mild headaches.

V. Effects of Animal Exposures

Lynch *et al.* (1990) exposed male and female Fischer 344 rats to triethylamine at concentrations of 0, 25, or 247 ppm (0, 103.4, or 1022.2 mg/m³) for 6 hours/day, 5 days/week. Groups of rats were necropsied at approximately 30, 60, and 120 days of exposure. The last corresponds to an elapsed time of 28 weeks. Endpoints examined included gross and histopathological examination of all major organs, including the lungs, nasal passages, and eyes. Clinical enzyme and nitrogen levels (BUN, ALT, AST, CPK, and creatinine), and hematological values (hemoglobin, RBC count) were also measured. No gross or histological effects in any organ were observed in any group. Clinical and hematological parameters were unchanged with exposure. However, all rats exposed to 247 ppm TEA manifested irritation. “At 247 ppm TEA the rats kept their eyes closed and noses buried in their fur during the entire exposure period.” Thus 247 ppm is a LOAEL and 25 ppm is a NOAEL for eye and nose irritation in the rat.

In a short-term study by the same authors for Virginia Chemicals (1987), necrotizing inflammation of the nasal cavity, metaplasia of the trachea, and thymic atrophy were observed

after exposure to 1000 ppm (4140 mg/m³) triethylamine 6 hours per day for 10 days. Two of five males and one of five females died from pulmonary edema after the seventh day. Thymic atrophy was noted in 7 out of 10 animals, and all animals exhibited necrotizing inflammation in the nasal epithelium.

Rabbits (6-12 per group), exposed to 48 or 100 ppm (199 or 414 mg/m³) triethylamine for 7 hours/day, 5 days/week, for 6 weeks, showed concentration-dependent pathology in the eyes, lungs, liver, kidney, and heart (Brieger and Hodes, 1951). The eyes showed multiple punctate erosions of the corneal epithelium, and corneal edema at 48 ppm. Lung lesions included thickening of vascular walls; liver lesions included parenchymal degeneration. Overall the lesions in the 48 ppm group were less severe than those seen in the 100 ppm group. No control animals were included in this study, nor were the incidences of histologic effects among the exposed animals reported. All animals did survive the exposures. The lesser effects at 48 ppm in the rabbit (compared to those at 100 ppm) are consistent with the findings of Lynch *et al.* (1990) where 25 ppm was a NOAEL in the rat for eye and nose irritation.

A chronic 3-generation reproductive study in rats (10/sex/group) was inconclusive due to excessive mortality in controls (Davison *et al.*, 1965). In this study, rats were exposed to 0, 2, or 200 ppm triethylamine. The third generation of the 200 ppm group was changed to 500 ppm since no effects were noted in the 200 ppm group. Exposure of this group to 500 ppm resulted in decreased body weight and decreased water consumption.

VI. Derivation of Reference Exposure Level

<i>Study</i>	Lynch <i>et al.</i> , 1990; Brieger and Hodes, 1951
<i>Study population</i>	Rats; rabbits
<i>Exposure method</i>	Discontinuous whole-body inhalation
<i>Critical effects</i>	Eye irritation; lung and liver toxicity
<i>LOAEL</i>	48 ppm (Brieger and Hodes, 1951)
<i>NOAEL</i>	25 ppm (Lynch <i>et al.</i> , 1990)
<i>Exposure continuity</i>	6 or 7 hours/day, 5 days/week
<i>Exposure duration</i>	28 weeks; 6 weeks
<i>Average experimental exposure</i>	4.46 ppm for NOAEL group (25 x 6/24 x 5/7)
<i>Human equivalent concentration</i>	4.46 ppm (18.5 mg/m ³) for NOAEL group
<i>LOAEL uncertainty factor</i>	1
<i>Subchronic uncertainty factor</i>	1 (NOAEL is based on a 28 wk study in rats)
<i>Interspecies uncertainty factor</i>	10
<i>Intraspecies uncertainty factor</i>	10
<i>Cumulative uncertainty factor</i>	100
<i>Reference exposure level</i>	0.04 ppm (40 ppb; 200 µg/m ³)

The U.S. EPA (1995) based its Reference Concentration (RfC) of 7 µg/m³ (2 ppb) for triethylamine on Lynch *et al.* (1990) but included a Modifying Factor (MF) of 10 for “database deficiencies” - “lack of developmental and reproductive effects, and of appropriate data in a second species.” The criteria for use of modifying factors are not well specified by U.S. EPA.

Such modifying factors were not used by OEHHA. In addition OEHHA applied a subchronic UF of 1 since 24 male and 24 female rats in the NOAEL group were exposed to 25 ppm TEA for 28 weeks, while USEPA used a subchronic UF of 10. U.S. EPA considered 247 ppm to be a NOAEL. However, the Lynch *et al.* (1990) study indicates that the animals closed their eyes and buried their noses in their fur, likely to prevent the irritant effects of TEA on their eyes and respiratory tract. Thus adverse effects occurred at 247 ppm, although they could be considered repeated acute effects. Brieger and Hodes (1951) observed adverse effects in the eyes, lungs, and livers of rabbits after six weeks of discontinuous exposure to TEA. Thus 48 ppm is a LOAEL in this study.

For comparison, the five affected workers studied by Akesson *et al.* (1986) showed symptoms at 12-13 mg/m³ TEA, which is equivalent to 4.5 mg/m³ continuous exposure. (Other tasks were at 4-5 mg/m³ TEA or 1.6 mg/m³ continuous exposure.) Selection of a LOAEL UF of 3 (26% incidence of a reversible effect), a subchronic UF of 1 since the workers had been employed for 9.7 years (range = 4-11), and an intraspecies UF of 10 results in an estimated REL of 200 µg/m³ based on human data. These workers experienced some short-term peak exposures to TEA and were also exposed to dimethylethanolamine (<0.1 mg/m³), toluene diisocyanate, and methylene diphenyl isocyanate.

VII. Data Strengths and Limitations for Development of the REL

The major strengths of the triethylamine REL are the observation of a NOAEL in a controlled exposure experiment and finding of the same adverse effect, eye irritation, in humans and animals. The major weaknesses are the minimal amount of adequate human health effects information, the lack of dose-response data in a single experiment, and the lack of long-term exposure data.

VIII. Potential for Differential Impacts on Children's Health

There is no direct evidence in the literature to quantify a differential effect of TEA in infants and children. However, it is a respiratory irritant and thus has the potential to exacerbate asthma. In addition, other alkylamines are known to be associated with occupational asthma (Bernstein *et al.*, 1999). There is some concern that TEA could have a similar effect.

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CHRONIC TOXICITY SUMMARY

VINYL ACETATE

(1-acetoxyethylene; acetic acid, vinyl ester; acetic acid, ethenyl ester; VAC; vinyl A monomer; ethenyl ethanoate)

CAS Registry Number: 108-05-4

I. Chronic Toxicity Summary

<i>Inhalation reference exposure level</i>	200 µg/m³ (50 ppb)
<i>Critical effect(s)</i>	Nasal epithelial lesions in rats and mice
<i>Hazard index target(s)</i>	Respiratory system

II. Physical and Chemical Properties (HSDB, 1994)

<i>Description</i>	Colorless liquid
<i>Molecular formula</i>	C ₄ H ₆ O ₂
<i>Molecular weight</i>	86.09 g/mol
<i>Density</i>	0.932 g/cm ³ @ 20°C
<i>Boiling point</i>	72.7° C
<i>Melting point</i>	-93.2°C
<i>Vapor pressure</i>	115 torr @ 25°C
<i>Solubility</i>	Slightly soluble in water, soluble in ethane, acetone, chloroform; >10% soluble in ethanol and benzene
<i>Conversion factor</i>	1 ppm = 3.52 mg/m ³ @ 25°C

III. Major Uses and Sources

The major use of vinyl acetate monomer is in the manufacture of polyvinyl and vinyl acetate copolymers, which are used in water-based paints, adhesives, paper coatings, and applications not requiring service at extreme temperatures (HSDB, 1994). It is also used in safety glass interlayers and in hair sprays (HSDB, 1994). In the atmosphere vinyl acetate breakdown can result in formation of acetaldehyde. The annual statewide industrial emissions from facilities reporting under the Air Toxics Hot Spots Act in California based on the most recent inventory were estimated to be 3855 pounds of vinyl acetate (CARB, 2000).

IV. Effects of Human Exposures

Deese and Joyner (1969) conducted an occupational study of 21 chemical workers with a mean length of employment of 15.2 years and exposed to a time-weighted average of 8.6 ppm (30.3 mg/m³) VA. No adverse effects were noted following chest x-ray, electrocardiogram, blood

chemistry, and urinalysis. The control group (sample size unspecified) consisted of workers in units not exposed to VA. Deese and Joyner (1969) also showed intolerable eye irritation in 3 out of 3 subjects exposed for an unspecified extended period of time to 21.6 ppm (76 mg/m³) VA. Upper respiratory irritation was also experienced by a majority of 5 subjects. Odor was detected at 0.4 ppm (1.4 mg/m³) in 3 out of 3 subjects.

V. Effects of Animal Exposures

A 104-week inhalation study in rats and mice (90/sex/group) was conducted using concentrations of 0, 50, 200, or 600 ppm (0, 176, 704, or 2113 mg/m³) vinyl acetate (VA) (Owen, 1988). The study was later published by Bogdanffy *et al.* (1994). Exposures were for 6 hours/day, 5 days/week. Histology was performed on all major organs. There was no mortality resulting from these exposures. A close examination of the effects of VA on the lung and nasal passages showed significant lesions in the nasal cavity, bronchi, and lungs of rats exposed to 600 ppm VA. Lesions included olfactory epithelial metaplasia/atrophy (see table below) and nest-like epithelial folds in the nasal cavity, exfoliation of bronchial epithelium, fibrous intraluminal projections in the bronchi, and pigmented histiocyte accumulation in the lungs. Body weight gain of rats was significantly decreased in the 600 ppm VA group. Rats treated with 200 ppm VA showed some evidence of epithelial atrophy and metaplasia in the nasal cavity. No effects were observed in the rats exposed to 50 ppm VA.

Number of male rats with olfactory epithelial atrophy (Bogdanffy *et al.* 1994)

VA (ppm)	N in group	Very slight	Slight	Moderate	Severe
0	58	0	0	0	0
50	59	1	2	0	0
200	60	4	47***	2	0
600	60	0	7*	33***	10***

* p<0.05; ***p<0.001 by Fisher's pair-wise test compared to control group

Mice also exhibited significant histological lesions in the respiratory tract following exposure to 200 ppm VA or greater. The lesions included atrophy of the olfactory epithelium and submucosal gland. At 600 ppm, hyperplasia of the trachea was observed, in addition to exfoliation/flattening of the bronchial epithelium and decreased body weight gain. Relative brain and kidney weights were increased in the 600 ppm group at the end of the study, and absolute liver, heart and kidney weights were also significantly elevated. No adverse effects were observed in the 50 ppm group.

A 13-week study on the effects of VA in mice was conducted by Owen (1980a). Mice (10/sex/concentration) were exposed to 0, 50, 200, or 1000 ppm (0, 176, 704, or 3520 mg/m³) VA for 6 hours/day, 5 days/week for 13 weeks. A concentration-dependent increase in the incidence of diffuse rhinitis, beginning at the 200 ppm concentration, was detected using histopathological examination. Focal pneumonitis was observed in the 1000 ppm treatment group. No adverse effects were seen in the 50 ppm treatment group. An identical study in rats was also conducted by Owen (1980b). In this study, body weight gain was significantly reduced

in male and female rats exposed to 1000 ppm VA. An increase in the incidence of mild histiocytic alveolitis was observed in the 1000 ppm group.

Irvine (1980) conducted a study on the developmental toxicity of VA in rats. Groups of 24 pregnant female rats were exposed to 0, 52, 198, or 1004 ppm (0, 182, 696, or 3533 mg/m³) VA for 6 hours/day on days 6-15 of gestation. Significant maternal toxicity, as measured by reduced weight gain from day 10 through day 15, was observed in animals exposed to 1004 ppm. Fetotoxicity, as measured by reduced crown-rump length, reduced body weight, and increased incidence of ossification defects in the sternbrae and occipital regions, was observed in the 1004 ppm group. No maternal or fetal effects were seen at the lower two VA treatments.

In another developmental toxicity study, groups of 23-24 CrI:CD(SD)BR rats were given 0, 200, 1000, or 5000 ppm VA in drinking water or exposed 6 hr/day to 0, 50, 200, or 1000 ppm VA on gestation days 6-15 of gestation. The authors (Hurtt *et al.*, 1995) estimated that the doses by both routes were approximately 0, 25, 100, or 500 mg/kg/day. VA in the drinking water produced no evidence of maternal or developmental toxicity at any dose. In the inhalation study, maternal toxicity was indicated by a reduction in weight gain of dams exposed to 1000 ppm. Fetal toxicity was evident by a significant decrease in mean fetal weight and mean crown-rump length in fetuses from the 1000-ppm group and by a significant increase in the incidence of minor skeletal alterations (especially delayed ossification) in fetuses from dams exposed to 1000 ppm VA. These results indicated to the authors that VA is not uniquely toxic to the conceptus. The NOAEL was greater than 5000 ppm via the drinking water and 200 ppm by the inhalation route.

VI. Derivation of Chronic Reference Exposure Level

<i>Study</i>	Bogdanffy <i>et al.</i> , 1994
<i>Study population</i>	Male and female Sprague-Dawley rats and CD-1 mice (90/sex/group)
<i>Exposure method</i>	Discontinuous inhalation exposures (0, 50, 200, or 600 ppm) over 104 weeks
<i>Critical effects</i>	Histological lesions of the nasal epithelium
<i>LOAEL</i>	200 ppm
<i>NOAEL</i>	50 ppm
<i>Exposure continuity</i>	6 hours/day, 5 days/week
<i>Exposure duration</i>	104 weeks
<i>Average experimental exposure</i>	8.9 ppm for NOAEL group (50 x 6/24 x 5/7)
<i>Human Equivalent Concentration (HEC)</i>	1.4 ppm for NOAEL group (RGDR = 0.15 based on a gas with respiratory effects in both rats and mice)
<i>LOAEL uncertainty factor</i>	1
<i>Subchronic uncertainty factor</i>	1
<i>Interspecies uncertainty factor</i>	3
<i>Intraspecies uncertainty factor</i>	10
<i>Cumulative uncertainty factor</i>	30
<i>Inhalation reference exposure level</i>	0.05 ppm (50 ppb, 0.2 mg/m ³ , 200 µg/m ³)

The chronic REL is the U.S. EPA RfC (U.S. EPA, 1995) for vinyl acetate. Acetaldehyde, a hydrolysis product of vinyl acetate, was present in the Owen (1988) study at a concentration of 49 ppm (89 mg/m³). The duration-adjusted concentration for acetaldehyde was 16 mg/m³, whereas the NOAEL for histological lesions in rats by Appleman *et al.* (1982) was 48.75 mg/m³ acetaldehyde. Therefore, the concentration of acetaldehyde was not considered to account for significant irritation in the Owen (1988) study. OEHHA accepted the U.S. EPA analysis.

For comparison, Irvine (1980) obtained a NOAEL of 198 ppm for fetotoxicity in rats exposed 6 hours/day on days 6-15 of gestation. This is equivalent to 50 ppm continuous exposure during development. Multiplying by an RGDR of 1 and dividing by a total UF of 30 (3 for interspecies and 10 for intraspecies) results in a REL estimate based on fetotoxicity of 1.7 ppm. The results of Hurtt *et al.* (1995) also yield an estimate of 1.7 ppm.

VII. Data Strengths and Limitations for Development of the REL

The strengths of the inhalation REL for vinyl acetate include the availability of controlled exposure lifetime inhalation studies in multiple species at multiple exposure concentrations and with adequate histopathological analysis, and the observation of a NOAEL. The major area of uncertainty is the lack of adequate human exposure data.

VIII. Potential for Differential Impacts on Children's Health

Since the chronic REL (0.05 ppm) is lower than the comparison estimate based on developmental effects (1.7 ppm), the REL is likely to be protective of children. However, there is no direct evidence in the literature to quantify a differential effect of vinyl acetate in infants and children relative to adults.

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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

<i>Inhalation reference exposure level</i>	700 µg/m³ (200 ppb) (for technical or mixed xylenes or sum of individual isomers of xylene)
<i>Critical effect(s)</i>	CNS effects in humans; irritation of the eyes, nose, and throat
<i>Hazard index target(s)</i>	Nervous system; respiratory system

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

<i>Description</i>	Colorless liquid
<i>Molecular formula</i>	C ₈ H ₁₀
<i>Molecular weight</i>	106.16 g/mol
<i>Density</i>	0.864 g/cm ³ @ 20°C(technical mixture); 0.881 (o-); 0.860 (m-); 0.861 (p-)
<i>Boiling point</i>	137-140°C @ 760 TorrHg (technical mixture); 144.5 °C (o-); 139.1°C (m-); 138.3 °C (p-)
<i>Melting point</i>	-25.2 °C (o-); -47.8°C (m-); +13.2 °C (p-)
<i>Vapor pressure</i>	6.6 torr (o-); 8.39 torr (m-); 8.87 torr (p-) all @ 25°C.
<i>Solubility</i>	Practically insoluble in water; miscible with absolute alcohol, ether and many other organic solvents
<i>Conversion factor</i>	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 $\mu\text{g}/\text{cm}^2/\text{min}$ (Engstrom *et al.*, 1977) to 75-160 $\mu\text{g}/\text{cm}^2/\text{min}$ (Dutkiewicz and Tyras, 1968).

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

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

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

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

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

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

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

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

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

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

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

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

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

V. Effects of Animal Exposure

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

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

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

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

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

of endoplasmic reticulum, changes in distribution of hepatocellular nuclei, and liver degeneration (Bowers *et al.*, 1982; Condie *et al.*, 1988; Elovaara, 1982; Elovaara *et al.*, 1980; Muralidhara and Krishnakumari 1980; Patel *et al.*, 1979; Pyykko 1980; Tatrai and Ungvary, 1980; Tatrai *et al.*, 1981; Toftgard and Nilsen, 1981; 1982; Toftgard *et al.*, 1981; Ungvary *et al.*, 1980).

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

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

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

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

With respect to rats, Mirkova *et al.* (1983) exposed groups of pregnant rats (unspecified strain of white rats) to clean air or 2.3, 12, or 120 ppm of xylene (unspecified composition) for 6 h/day on days 1-21 of gestation. They reported increased postimplantation losses and fetotoxicity (reduced fetal weights) as well as a statistically increased incidence of visceral abnormalities (including ossification defects in bones of the skull) at xylene air concentrations of 12 ppm and above. The ATSDR has suggested that the Mirkova *et al.* (1983) study results may have been influenced by poor animal husbandry as indicated by the low conception rates and the high incidence of fetal hemorrhages seen in the controls. Hass and Jakobsen (1993) attempted to replicate the findings of Mirkova *et al.* (1983). Hass and Jakobsen (1993) exposed groups of 36 pregnant Wistar rats to clean air or 200 ppm of xylene for 6 h/day on days 4-20 of gestation.

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

Ungvary *et al.* (1985) exposed CFY rats by inhalation to air concentrations of xylene (60 ppm, 440 ppm, 800 ppm) for 24 h/day on days 7-15 of gestation. Maternal toxicity was described as moderate and dose-dependent. They observed weight retarded fetuses at all air concentrations. However, there was no increase in malformations, and an increase in minor anomalies and resorbed fetuses occurred only at the highest concentration. In a separate study investigating the interactions between solvents and other agents, Ungvary (1985) exposed CFY rats to either 140 ppm or 440 ppm of xylene on days 10-13 of gestation and also reported increases for either condition in weight retarded and skeletal retarded fetuses without any increase in malformations. Hudak and Ungvary (1978) had earlier examined the effect of 230 ppm xylene (24 h/day, days 9-14 of pregnancy) in the CFY rat and reported effects on skeletal development (e.g., fused sternebrae). In contrast to the other Ungvary findings, no effect on fetal weight was observed. Bio/dynamics (1983) conducted an inhalation exposure study in the rat (CrL-CD (SD) BR strain). Rats were exposed 6 h/day during pre-mating, 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 bromosulphophthalein 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)

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

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

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

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

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

VII. Data Strengths and Limitations for Development of the REL

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

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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 µg/m³ (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 B6C3F₁ 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 ($9 \mu\text{g}/\text{m}^3$) 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 not 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 not 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 µg/m³) 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 $\mu\text{g}/\text{m}^3$ 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 $\mu\text{g}/\text{m}^3$.

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 electrowinning 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 $\mu\text{g}/\text{m}^3$, 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 only 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. OEHHHA staff developed a chronic REL of 400 $\mu\text{g}/\text{m}^3$ 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 $\mu\text{g}/\text{m}^3$. 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 $\mu\text{g}/\text{m}^3$, 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 $\mu\text{g}/\text{m}^3$. 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 $\mu\text{g}/\text{m}^3$.

$$\text{mg}/\text{m}^3 \text{ at } 25^\circ\text{C and } 760 \text{ mm Hg} = \frac{\text{ppm} \times \text{molecular weight (EG} = 62.07)}{24.45}$$
$$1 \text{ ppm} = 2.53865 \text{ mg}/\text{m}^3$$
$$0.2 \text{ ppm} = 0.508 \text{ mg}/\text{m}^3 \text{ or } 508$$

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 $\mu\text{g}/\text{m}^3$. Unfortunately both values were subsequently rounded separately to 0.2 ppm and 400 $\mu\text{g}/\text{m}^3$. When the 0.167 ppm is rounded first to 0.2 ppm, the equivalent value is 508 $\mu\text{g}/\text{m}^3$, which is rounded to 500 $\mu\text{g}/\text{m}^3$. Based on this comment OEHHHA staff will revise the chronic REL to 500 $\mu\text{g}/\text{m}^3$.

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/m³ in the derivation equation and the appropriate conversion factor between "mg/m³ 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/m³ (20 ppm) as the No-Observed-Adverse-Effect-Level (NOAEL), not the highest high value of 67 mg/m³ (rounded from 66.8). (See Table 1 from Wills et al. below.) OEHHA staff believe that 49 mg/m³ is the appropriate NOAEL.

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

Days	Concentration of ethylene glycol in air, mg/m ³		
	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/m³ (the LOAEL), ACGIH selected 100 mg/m³ 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/m³.

With the corrected human equivalent concentration and the appropriate uncertainty factors applied, the chronic inhalation REL for ethylene glycol should be at least 730 $\mu\text{g}/\text{m}^3$ (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/m³, 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/m³ 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/m³ and there were no absolute or relative weight differences in three other inhalation studies at concentrations as high as 2500 mg/m³. 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/m³ (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 $\mu\text{g}/\text{m}^3$ 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

<u>Test Submission</u>	<u>Of Final Report</u>
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/m³, 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 <i>et al</i> 1997)
Critical Toxic Effect:	Chronic progressive nephropathy
Study NOAEL (ppm):	500 ppm
Study NOAEL (mg/m ³):	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.

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

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 differences 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 $1,000 \mu\text{g}/\text{m}^3$ (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 $3.3 \text{ mg}/\text{m}^3$ (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 $3.3 \text{ mg}/\text{in}^3$ (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 CalEPA 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 $\mu\text{g}/\text{m}^3$) 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 $\mu\text{g}/\text{m}^3$) was derived (table below).

OEHHA revised chronic REL

Study	Cavender <i>et al.</i> , 1983
<i>Study population</i>	Rats
<i>Exposure method</i>	Inhalation for 90 days
<i>Critical effects</i>	Increased liver weight and relative kidney weight in males and females
<i>LOAEL</i>	5,041 ppm
<i>NOAEL</i>	2,518 ppm
<i>Exposure continuity</i>	6 hours/day; 5 days/week
<i>Average experimental exposure</i>	449.6 ppm for NOAEL group
<i>Human equivalent concentration</i>	449.6 ppm for NOAEL group (gas with systemic effects, based on RGDR = 1.0 using default assumption that $\lambda(a) = \lambda(h)$)
<i>Exposure duration</i>	90 days
<i>LOAEL UF</i>	1
<i>Subchronic UF</i>	3
<i>Interspecies UF</i>	3
<i>Intraspecies UF</i>	10
<i>Cumulative UF</i>	100
<i>Chronic inhalation reference exposure level (REL)</i>	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 $0.05 \mu\text{g}/\text{m}^3$ 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 $\mu\text{g}/\text{m}^3$ 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 reversibility 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:

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

The values used by EPA for minute volumes of rats and humans are $2.3996 \times 10^{-4} \text{ m}^3/\text{min}$ and $0.0138 \text{ m}^3/\text{min}$, respectively (EPA, 1994). Using the values cited in the OEHHA Technical Support Document, the total respiratory tract surface areas are $3,440 \text{ cm}^2$ and $543,400 \text{ cm}^2$, 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 <i>et al.</i> , 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. OEHHHA proposed a chronic REL of 600 $\mu\text{g}/\text{m}^3$ for phenol based on reports of systemic effects in mice, rats and monkeys inhaling phenol.

The Task Group urges OEHHHA to withdraw its draft chronic toxicity summary and proposed reference exposure level (REL) for phenol. The studies on which OEHHHA 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 OEHHHA 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 OEHHHA's use of the Guidelines to assess or regulate any chemical, including phenol. OEHHHA staff provide detailed responses to the appended comments below.

Comment 1. *The studies on which OEHHHA relies are not adequate to derive a REL.* OEHHHA has based its proposed REL of 0.2 parts per million (ppm) (600 micrograms per cubic meter ($\mu\text{g}/\text{m}^3$)) 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 OEHHHA relies are not adequate to derive a REL for phenol, and the proposed REL should be withdrawn.

Even if the studies on which OEHHHA relies were an adequate basis for deriving a REL, the approach OEHHHA 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 (NIOSH) 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 methodology 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 ($20,002 \mu\text{g}/\text{m}^3$) 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 ($4 \times 10/20 \times 5/7$). Applying an uncertainty factor of 10 to account for variability in sensitivity in the human population results in a REL of 143 ppb or $550 \mu\text{g}/\text{m}^3$, in good agreement with the $600 \mu\text{g}/\text{m}^3$ REL derived from animal studies. The comment time-adjusted the TLV of 5 ppm to 1 ppm ($3,850 \mu\text{g}/\text{m}^3$) but did not make any allowance for variability in sensitivity in the human population. Thus OEHHA is still proposing $600 \mu\text{g}/\text{m}^3$ 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. The 1997 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 exposed 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, OEHHHA 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 exposed 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 $\mu\text{g}/\text{m}^3$ 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. *Diethylhexylphthalate.* 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 ($\mu\text{g}/\text{m}^3$) 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 $\mu\text{g}/\text{m}^3$ based on neurotoxicity.

Comment 1. The TSD proposes an REL for PGME of 2,000 $\mu\text{g}/\text{m}^3$ (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 $700 \mu\text{g}/\text{m}^3$ 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 ($200 \mu\text{g}/\text{m}^3$) 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 overall 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. (1997) 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 only the prior day's exposure contributed to the observed symptoms, then the dose response analysis below would suggest the daily 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.

	<u>Daily Exposure</u>	<u>Annual Exposure</u>
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 all 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 sternbrae) 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 sternbrae should not be considered biologically significant in the absence of other conventional signs of embryotoxicity (e.g., increased malformations, increased embryoletality 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:

<u>Study</u>	<u>Strain/Species</u>	<u>Exposure Duration</u>	<u>NOAEL</u>	<u>LOAEL</u>
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 ppm	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 µg/m³ 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 ($0.9 \mu\text{g}/\text{m}^3$) 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 H₂S 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 $9 \mu\text{g}/\text{m}^3$ (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 H₂S 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 H₂S is 0.03 ppm. (2) The proposed REL of 0.7 ppb is one thousandth of the upper concentration for H₂S naturally occurring in human breath. (3) Low levels of H₂S 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 H₂S REL is based, should be decreased by at least one order of magnitude. (5) New studies on the toxicity of H₂S 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 µg/m³ (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 H₂S 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 H₂S, since 1990. This means that the H₂S 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 H₂S 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 H₂S. The chronic REL is for much longer exposures and should be less than the acute REL. Although odor is one consideration with H₂S, 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 H₂S as a federal Hazardous Air Pollutant as well as the known toxicity of H₂S, 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 $\mu\text{g}/\text{m}^3$) 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 H₂S 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 $9 \mu\text{g}/\text{m}^3$ (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 $0.01 \mu\text{g}/\text{m}^3$ is 350-fold lower than the earlier CAPCOA value of $3.5 \mu\text{g}/\text{m}^3$. 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 $4.2 \text{ E-}3 (\mu\text{g}/\text{m}^3)^{-1}$. (At the proposed chronic REL the estimated lifetime cancer risk would be forty in a million.)

<u>Chemical of Interest to MFASC</u>	<u>OEHHA Proposed REL</u> $\mu\text{g}/\text{m}^3$	<u>1992 CAPCOA Guidance Value</u> $\mu\text{g}/\text{m}^3$	<u>Basis of 1992 CAPCOA Value</u>	<u>Ratio of CAPCOA to OEHHA</u>
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 (PM₁₀, 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 µg/m³ and a NOAEL of 8 µg/m³ for copper fumes. The only other available inhalation toxicity study was a subchronic mouse study that established a LOAEL of 130 µg/m³. 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.

Uncertainty Factor	Geometric Mean of Uncertainty Factors	
	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
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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 H₂S.

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 incorporates 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 NO₂, 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 µg/m³) to “prevent health risk and improve visibility.” California has a 1-hour standard for nitrogen dioxide of 0.25 ppm (470 µg/m³). 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 µg/m³ (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 NO₂ standard of 100 µg/m³ or the state daily NO₂ standard of 470 µg/m³ was exceeded. But ambient concentrations exceeded the proposed NO₂ REL of 20 µg/m³ (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/emisin/95inven195inv.htm>). Those fuel combustion sources

regulated under the “hot spots” program typically emit much more NO than NO₂, and it would be difficult for NO_x dominated “hot spot” sources to accurately estimate what percentage of their NO emissions might convert to NO₂ 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 NO₂, conversion in the center of an NO dominated plume can be incomplete 60 km downwind (page 8, Air & Waste Management Association paper 95-RA113A.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 NO₂ 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 NO₂, 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 NO₂ 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 NO₂ data. While OEHHA has prepared an excellent 8 page summary on its proposed NO₂ 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 NO₂ 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 µg/m³) is to "prevent health risk and improve visibility." The proposed chronic REL of 20 µg/m³ (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 $42 \mu\text{g}/\text{m}^3$, 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 $2 \mu\text{g}/\text{m}^3$, 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 $9 \mu\text{g}/\text{m}^3$ (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 $94 \mu\text{g}/\text{m}^3$.

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 $2 \mu\text{g}/\text{m}^3$ 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 $3 \mu\text{g}/\text{m}^3$ 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 -----	= $2.54 \mu\text{g}/\text{m}^3$
and		which cancer
6 in a million/ $(\mu\text{g}/\text{m}^3)$ [unit risk]	46 years [working]	hazards
equate		

But most exposures vary from year to year. In our opinion $9 \mu\text{g}/\text{m}^3$ 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 $\mu\text{g}/\text{m}^3$ 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 ($0.9 \mu\text{g}/\text{m}^3$) as the chronic REL.

Comment 1. After thorough review of the H₂S 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 H₂S 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 H₂S 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 H₂S 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 H₂S REL is based, should be decreased by at least an order of magnitude. (5) New studies on the toxicity of H₂S 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 H₂S 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 H₂S is around 10-20 ppb, while the characteristic "rotten egg" odor associated with H₂S 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 H₂S 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 H₂S 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 µg/m³), with a 30-minute averaging time (WHO, 1987; National Research Council, 1979; Lindvall, 1970). The RfC of 0.9 µg/m³ (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 H₂S at levels around the odor threshold causes adverse health effects. One study of a community in Rotorua, 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 H₂S.

Response. If chronic low-level exposure to H₂S 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. H₂S 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. H₂S 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 H₂S 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) H₂S. 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) H₂S, 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 H₂S through a mouthpiece.

Response. Many toxic chemicals are produced by metabolism: CO, acetaldehyde, formate, NO, and H₂S. 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 H₂S is based on the USEPA reference concentration (RfC) promulgated in 1994. A number of studies on the toxicity of H₂S 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 Geysers' Geothermal using the same study as U.S. EPA, but different uncertainty factors. The new proposed REL is 9 µg/m³ (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 H₂S. 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 H₂S, these subchronic rodent studies performed at high H₂S concentrations (much higher than the OEHHA acute level II REL for H₂S) 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 H₂S 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 H₂S. 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 H₂S 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 H₂S 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 $\mu\text{g}/\text{m}^3$.

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 hypersusceptibility 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 $\mu\text{g}/\text{m}^3$ 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 Cyp2E1 (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-2E1, 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 study¹ 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 (NQO1) 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.

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 Rinsky³ (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 Rinsky⁴ (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

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 B6C3F₁ 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, C57Bl 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.

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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 $\mu\text{g}/\text{m}^3$) 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 H₂S. Additional studies on H₂S 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 H₂S should be 0.7 parts-per-billion (0.9 $\mu\text{g}/\text{m}^3$). The calculation and documentation of this value have numerous flaws. WSPA recommends that OEHHA consider a value between 9 and 30 ppb H₂S as the reference exposure level in California. The California ambient air standard for H₂S is 30 ppb to prevent nuisance odor situations. There is no evidence or expectation of toxicity at this concentration. The odor detection level for H₂S 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 H₂S (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 $1 \mu\text{g}/\text{m}^3$.

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 H₂S and its ubiquitous presence in the environment. The estimated ambient air concentrations of H₂S 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 H₂S. 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 H₂S 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 H₂S. A larger and more carefully controlled study by Bhambhani et al. (1994) found no effects in human subjects exposed to 5 ppm H₂S. Other studies by Bhambhani et al. (1996 and 1997) in human subjects did observe small but statistically significant changes after exposure to 10 ppm H₂S. OEHHA also cites Bhambhani and Singh, 1985, to suggest, "... either that humans are more sensitive to H₂S, 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 H₂S 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 H₂S without experiencing a significant reduction in their maximum physical work capacity during short-term incremental exercise." Bhambhani et al. (1996) found that exposure to H₂S 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 H₂S.

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 µg/m³ for hydrogen sulfide.

References cited by commentator 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 $0.05 \mu\text{g}/\text{m}^3$ 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

^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 Toxicology 11(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 A1 - 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/m³) and soluble nickel compounds on the other (TLV = 0.1 mg Ni/m³).^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

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.

^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(1):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/m³) was roughly one-half of the LOAEL for nickel subsulfide (0.11 mg Ni/m³) and an order of magnitude lower than the LOAEL for nickel oxide (0.5 mg Ni/m³).

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 document⁵, 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,

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.” Notwithstanding 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 $\mu\text{g}/\text{m}^3$ 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 $\mu\text{g}/\text{m}^3$ 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 B6C3F₁ 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/m³). 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/m³ 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/m³) 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/m³) 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/m³) 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}. OEHHA⁹ 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

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 speculate.

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 ($0.33 \mu\text{g}/\text{m}^3$) and (soluble) nickel sulfate ($0.29 \mu\text{g}/\text{m}^3$) 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 ($0.3 \mu\text{g}/\text{m}^3$). 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 $0.05 \mu\text{g}/\text{m}^3$ is more appropriate. The difference between the value for nickel oxide of $0.33 \mu\text{g}/\text{m}^3$ and the value for nickel sulfate of $0.05 \mu\text{g}/\text{m}^3$ 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 lung¹¹:

Study duration:	<u>13 weeks</u>		<u>2 years</u>	
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 7/5th 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 Sub sulfide, 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/m³, 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/m³ 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/m³ was equivalent to 0.5 mg Ni/m³. See id. at 40.

$$\text{REL} = \frac{500 \mu\text{g Ni/m}^3 \times 0.179 \times 0.29}{3 \times 3 \times 10} = \frac{30 \mu\text{g Ni/m}^3}{90} = 0.33 \mu\text{g Ni/m}^3$$

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/m³, 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.⁹ 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

$$\text{REL} = \frac{110 \mu\text{g Ni/m}^3 \times 0.179 \times 0.29}{3 \times 3 \times 10} = \frac{5.7 \mu\text{g Ni/m}^3}{90} = 0.06 \mu\text{g Ni/m}^3$$

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.

⁹ See NTP, Toxicology and Carcinogenesis Studies of Nickel Subsulfide in F344/N Rats and B6C3F₁ 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/m³ was equivalent to 0.11 mgNi/m³. 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 $0.1 \mu\text{g Ni/m}^3$. This value is twice the value ($0.05 \mu\text{g Ni/m}^3$) developed by OEHHA.

The comment stated that its nickel oxide alternative REL of $0.33 \mu\text{g Ni/m}^3$ 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 $0.33 \mu\text{g/m}^3$ for nickel oxide is also substantially different from the comment's REL of $0.06 \mu\text{g/m}^3$ 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 $0.06 \mu\text{g/m}^3$ would be preferred for all other forms of insoluble nickel compounds¹⁴.

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 ($0.33 \mu\text{g/m}^3$). However, for these reasons, OEHHA also believes that the proposed REL value of $0.05 \mu\text{g/m}^3$ 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/m³.

In developing its response to these comments, OEHHA has identified the following possible REL values:

<u>Form of Nickel</u>	<u>REL Value</u>	<u>Based Upon</u>	<u>Cumulative Safety Factor</u>
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/m³ 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/m³ 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

^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 $0.33 \mu\text{g Ni}/\text{m}^3$. 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.

Responses to Comments on the October 1997 Draft on Noncancer Chronic RELs
Do not cite or quote. SRP Draft – 2nd set of chemicals

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 Second Set of 40 Chemicals

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Chemical Industry Institute of Toxicology (CIIT)

Comments on the chronic REL for **ethylene oxide** were received from Drs. Preston, Fennell and Janszen of the Chemical Industry Institute of Toxicology (CIIT). OEHHA developed a chronic REL of 5 µg/m³ from a 1995 study of hospital workers by Schulte and coworkers.

Comment 1. In regard to exposure, major uncertainties exist in estimating ethylene oxide exposure to the workers and in interpreting the variability in exposure in the human study used to develop the cREL. The ethylene oxide analyses and calculations are not clearly explained. There may not be a significant association between individual exposure and hemoglobin adducts.

Source data for ethylene oxide assessment: The exposure response data used as the source for chronic exposure limits for ethylene oxide are those published by Schulte et al. (*Molecular, Cytogenetic, and Hematologic Effects of Ethylene Oxide on Female Hospital Workers*, Journal of Occupational and Environmental Medicine 37, 313-320, 1995). In order to adequately assess the data and conclusions drawn, it is necessary to also refer to a previous paper that presents much of the original exposure response data (Schulte, P.A. et al., *Biologic markers in hospital workers exposed to low levels of ethylene oxide*, Mutation Research 278, 237-251, 1992). The more significant differences between the two publications is that only female workers were considered in the analysis presented in the 1995 paper (see discussion below), and hematologic effects were analyzed in the 1995 paper. The relevance of the latter markers to risk assessment remains unclear, and for this and other reasons they are not considered further in this commentary.

Response: Staff have again reviewed both the papers by Schulte and coworkers (1992, 1995) to evaluate exposure issues. Based on these comments, those of the CMA, and OEHHA staff's re-evaluation, we decided not to use the study of Schulte et al. as the basis of the REL. Instead we have developed a revised chronic REL for ethylene oxide of 30 µg/m³ based on the neurotoxicity study of Klees et al. (1990).

Comment 2. There are three broad areas of concern with the data as presented and these will be considered sequentially as exposure, statistical analyses and biological data. (a) Exposure: As noted in the draft Chronic Toxicity Summary on Ethylene Oxide, major uncertainties exist in estimating exposure, and in interpreting the variability in exposure concentration. In addition, Schulte et al. (1995) did not give adequate information on ethylene oxide analyses and calculations. More details of the exposure assessment and biomarker measurements were provided for this study population in Schulte et al. (1992). The data obtained on hemoglobin adducts may have the power to substantiate the assessment of exposure, since hemoglobin adducts represent a dose integrated over the lifespan of the erythrocyte. However, the uncorrected data were not presented in sufficient detail to enable this comparison to be made, and many of the important features of the data may not be readily apparent as a result of the particular nature of the presentation.

Response. The draft Chronic Toxicity Summary on Ethylene Oxide discussed the major uncertainties that exist in estimating exposure in the 1995 study by Schulte et al. Major areas of uncertainty are the usual uncertainty in estimating human exposure, the potential variability in exposure concentration, and the small number of subjects studied at each location. Schulte et al. also did not give adequate information on their EtO analyses and calculations in their report.

Comment 3. A critical question is whether there is a significant association between the calculated exposures for each individual and the hemoglobin adducts measured. The range observed for the adjusted hemoglobin adduct levels in the U.S. study participants in Figure 2 of Schulte et al. (1992) is extremely broad, and, as noted in the comments on the statistical analysis presented below, a horizontal line indicating a lack of correlation between hemoglobin adducts and the estimated exposure could equally well be valid. A hemoglobin adduct is a measure of the actual internal dose of ethylene oxide achieved in each individual, and is a more reliable estimate of exposure than those generated in Schulte et al. (1992). Unexpected variability of the data is demonstrated by the fact that for 7 individuals with the same log cumulative exposure of 3.4 (30 ppm.hr), the range of hemoglobin adducts was approximately 10 fold, from approximately 0.036 to 0.36 pmol/mg hemoglobin (calculated from the graph). Four of the participants from the >0-32 ppm.hr group had the same exposure assigned as individuals in the 0 ppm.hr category. These values were all plotted together with an exposure value corresponding to approximately 0.5 ppm.hr, and not 0 ppm.hr. No justification was provided for the choice of this value.

Response. Staff assume that the commentators believe that a 10-fold range is unexpectedly high variability of the data. Ten-fold is the common uncertainty factor used for intraspecies (human) variability by both OEHHA and USEPA. OEHHA staff are aware of only one instance in which USEPA has used a UF_H less than 10 when using the NOAEL/UF approach for an RfC. Recent studies by Hattis and coworkers indicate that for many chemicals the variability is more than 10-fold (e.g., Hattis D. 1996. Variability in susceptibility – how big, how often, for what responses to what agents? *Environmental Toxicology and Pharmacology*. 2:133-145; Hattis D et al. Distributions of individual susceptibility among humans for toxic effects – For what fraction of which kinds of chemicals and effects does the traditional 10-fold factor provide how much protection? *Annals NY Academy of Sciences*, submitted). As one example, in a study of DNA adducts from PAHs the interindividual variability was about 24-fold (Dickey C, Santella RM, Hattis D, Tang D, Hsu Y, Cooper T, Young TL, Perera FP. Variability in PAH-DNA adduct measurements in peripheral mononuclear cells: implications for quantitative cancer risk assessment. *Risk Anal* 1997;17(5):649-656).

The choice of 0.5 ppm-h as a cut-off is not an unreasonable choice based on the available data.

Comment 4. The shortcomings of the exposure measurements are discussed by Schulte et al. (1992). The estimates of exposure were based on 2-4 days of ethylene oxide measurements to model cumulative exposure. Exposure that occurred prior to the four-month period of the

exposure assessment may be more relevant for the generation of effects in lymphocytes. Given the uncertainty of the exposure assessment, and the potential utility of the hemoglobin adduct data as a dose measure, it is very surprising that an analysis of this data set has not been reported using hemoglobin adducts as the dose measure against the various measures of effect. Before using these studies (Schulte et al., 1995) as the basis of a risk assessment, it is important that the data stand up to reasonable scrutiny. Using hemoglobin adducts in place of an uncertain exposure measure would provide a means of reducing the uncertainty of a risk assessment.

Response. Exposure assessment is often a problem in epidemiologic studies and we can only use the data presented. If the pattern of exposure is fairly consistent, 2 to 4 days may be a representative sample. Sterilization is a routine procedure in hospitals and the study is published in a reputable journal. On the other hand, if the exposure is sporadic and variable, 2 to 4 days may be a poor sample. These uncertainties, coupled with the availability of Klees *et al.* (1990), were some of the reasons OEHHA is no longer using the studies of Schulte and coworkers.

Comment 5. The hemoglobin adduct measurements were made with an immunoassay method that can have considerable variability in specificity and in background levels of adduct between batches of antibody used (Tornqvist et al., *Ring test for low levels of (2-hydroxyethyl)valine in human hemoglobin*, *Anal Biochem* 203, 357-360). It is not clear whether a single batch of antibody was used in the Schulte et al. (1992) study. Failure to do so could affect the results and their interpretation.

Response. OEHHA staff appreciate the identification of this shortcoming. However, we use the data that are available in this peer-reviewed article, while aware of limitations.

Comment 6. (b) Statistical Analyses: The following issues raise questions of whether the statistical analyses for the Schulte et al. dataset were appropriate, and whether the results from a statistical viewpoint are soundly based or valid. (i) Use of same data set for model building and hypothesis testing: In epidemiological studies, one is frequently interested in two basic issues: 1) which factors are important for explaining the observed data; and 2) are the observed differences between groups, as defined by one or more categorical variables, statistically different with regard to a particular response variable. Frequently, as in the study performed by Schulte et al., the same data set is used to answer both questions, although it is not valid to do so. The reason is that this practice involves a type of circular reasoning.

Whenever any kind of stepwise regression is performed, one is interested in building a model of those factors that are deemed to be important for explaining the observed results. This process is designed to choose those factors out of many which significantly contribute to the response of interest. To use this data set to create a model is valid. But to create a model and then test to see if there are differences between groups which were determined by the data (via analysis of covariance) is not a valid exercise. Furthermore, the investigators are implying that the regression coefficients obtained from this small investigational study are

representative of the entire population. Unfortunately, a comparable second study group was not available to test this assumption. The investigators did decide to force certain variables into the model, which were occasionally significant.

A further example is given in the Schulte et al. (1992) article. The investigators arbitrarily decided where the breakpoint should be for creating a grouping variable for cumulative exposure to ethylene oxide. Then they tested to see if there was a difference between the two groups.

Response. OEHHA staff agree that the authors have attempted to make their study both exploratory and confirmatory. In addition to the theoretical undesirability of that approach, the authors' data are very variable. If the data had been more distinctively bimodal, the data might be more credible from a biological standpoint, if not from a statistical one. . These limitations constitute another reason for not using the studies of Schulte and coworkers.

Comment 7. Statistical analyses: (ii) Univariate versus multivariate analyses: Since three outcomes (hemoglobin adducts, SCE, and micronuclei) were measured on each subject, a multivariate analysis should have been performed, which would have taken into consideration the correlation between the responses. This is especially true and necessary for the hematologic effects analyses. A separate regression model for each biomarker response was created from the same data set. Because of the multiplicity of models being created from one data set, some sort of protection against over-significance should have been included, e.g., a p-value might need to be <0.005 for a particular variable to be declared significant. This is analogous to the multiple t-test problem.

Response. $p < 0.005$ is a very stringent decision criterion. Another approach might be to modify $p < 0.05$ by the Bonferroni correction for multiple analyses, especially if one is hunting for differences. It might not be necessary in this instance. Hemoglobin adducts will have a biologically separate mechanism from that for micronuclei and SCEs. However hemoglobin adducts are a surrogate for DNA adducts. DNA adducts can lead to mispairing of DNA, and both SCE and micronuclei result from alterations in the DNA.

Comment 8. Statistical analyses: (iii) Significance of regression coefficients: For each biomarker or hematological response a multiple regression model was created. P-values for each variable in the model are given, and the implication is made that variables with small p-values are important for explaining the observed outcome. However, what is not stated and is true, is that the "significance" of a variable is totally *dependent* upon the presence in the model of the other variables. In other words, if there is a high degree of correlation between one independent variable and another (multicollinearity), this would explain the observed significance. Unfortunately, there is no statistical method to separate the dependence of one variable from another and still assess the importance of a given variable. However, in the Schulte et al. (1992) article, this assessment has been done graphically. In Figure 2, for example, the adjusted log hemoglobin adducts are plotted against log cumulative ethylene oxide exposure. The slope (from the multiple regression model) is 0.18, and the p-value is

given as 0.0006. This p-value is dependent on the model given in Table 4. This same argument applies to Figure 4, in which the adjusted SCE are plotted values against log cumulative ethylene oxide exposure. The true degree of significance can be determined as follows: if the regression line can be rotated about the point that represents the average value for each axis so that it is horizontal and between the 95% confidence intervals, then the relationship between the independent and dependent variable is not significant. Hence, in truth there appears to be no significant relationship between log cumulative ethylene oxide exposure and the adjusted log transformed biomarker responses. One might consider these p-values to be statistical "oddities" with no real interpretation. A similar argument can be presented for analysis of the hematological data. Although the data were not presented in detail in Schulte et al. (1995), it seems highly plausible that the reported statistically significant regression analysis for hematocrit, lymphocytes and neutrophils fall equally into the category of statistically uncertain.

Response. OEHHA staff do not agree that it is true that the "significance" of a variable is *totally* dependent upon the presence in the model of the other variables. The significance may be dependent on, and influenced by, the other variables but it is not totally dependent on them.

Staff also do not agree that the "if the regression line can be rotated about the point that represents the average value for each axis so that it is horizontal and between the 95% confidence intervals, then the relationship between the independent and dependent variable is not significant." If the regression line as calculated is horizontal ($b = 0$), then one can say that there is no association. If the line has a slope, then the slope can be calculated and its significance assessed. The slope can be shallow and statistically significant. The rotation test is interesting but not the accepted method to test significant correlation.

The comment also implies that there is serious confounding. The study controlled for age, smoking and liquor. Smoking is a definite confounder; age and liquor probably less so. Unless identified, it is just a guess that there is another confounder.

The reference to "statistical "oddities" with no real interpretation" is confusing. Something is either statistically significant using the decision criterion specified, or it is not. Whether a statistically significant difference is biologically meaningful is a separate question.

Comment 9. Biological Data: (i) Controls: Population monitoring studies are basically small epidemiological studies that require that confounders of response be accounted for. As noted above, some attempt was made to do this through a statistical approach that has its own inherent problems, but this has to be considered as only a partial attempt to account for confounders. The selection of an appropriate and adequately sized control population can help diminish the influence of confounders. In the study of Schulte et al., the controls are woefully inadequate, being eight in number for the US hospital group and one for the Mexican hospital. Comparing responses from "high" and "low" exposure groups is not a substitute for a comparison between control and exposed, because this will be further complicated by the adequacy of the exposure assessment.

The inadequacy of the control selection is quite possibly the reason for the low mean SCE values presented for the US group (4.61 per cell). In other large control population studies, the mean SCE values are considerably higher, even though the methods used were the same or very similar. Bender et al. (*Chromosomal aberration and sister chromatid exchange frequencies in peripheral blood lymphocytes of a large human population sample*, Mutation Research 204, 425-433, 1988) reported a mean control SCE frequency of 8.29 ± 0.08 for 353 individuals, and Tucker et al. (*Variation in the human lymphocyte sister-chromatid exchange frequency: Results of a long-term longitudinal study*, Mutation Research 204, 435-444, 1988) one of 9.32 for 22 non-smoking individuals. Also of note, smoking was a considerable confounder, accounting for a mean of 1.85 extra SCE per cell. It *appears* to be less so in Schulte et al., but it is not possible to extract the actual data nor to establish the distribution of smokers among the different groups. Suffice it to say that the control data alone are sufficient to provide a very real concern about the validity of the conclusions.

Response. OEHHA staff agree that the number of controls in the Mexican hospital is problematic; as to the U.S. hospitals the adequacy of 8 controls depends on the tightness of the data. OEHHA is not aware of a widely accepted value for SCE in controls. All means in the Schulte study, both unexposed and exposed to ethylene oxide, are <7 which is less than the means of the 2 control groups cited by the commentator. The Bender et al. data seem surprisingly homogeneous while the commentators do not indicate the variability of the Tucker et al. data. Review of the Tucker paper indicated differences in SCE between smokers and non-smokers. Only the eight non-smokers studied by Tucker et al. can be considered controls. The commentators appear to have added the 8 nonsmokers, the 4 smoke-enders and the 10 variable smokers in Table 3 together to arrive at their sum of 22 non-smoking individuals, a questionable summation since the paper shows that smokers have higher levels of SCE and that it takes at least 12 months for SCE to return to normal levels. The Schulte et al. study also had 8 U.S. controls; their smoking status is not obvious.

A 1984 report (Laurent C, Frederic J, Leonard AY. Sister chromatid exchange frequency in workers exposed to high levels of ethylene oxide, in a hospital sterilization service. *Int Arch Occup Environ Health* 1984;54(1):33-43) found 7.52 ± 0.82 SCEs per cell in 15 non-smoking controls, a value lower than that quoted by the commentator. In the absence of an accepted standard for SCEs in controls, we judge the consistency and believability of the data itself as presented in the study.

Comment 10. Biological data: (ii) Micronuclei: Micronuclei can be formed from acentric chromosome fragments or whole chromosomes that failed to segregate at mitosis, and as such represent a mutagenic endpoint in contrast to SCE that are a genotoxic endpoint since they have not been associated directly with any cellular phenotype. In Schulte et al. (1992) the frequencies of micronuclei were not significantly different among the three sample groups (control, "high" exposure, "low" exposure) in the US sample. In Schulte et al. (1995), a significant difference between the high and low exposure group was reported. This was basically the same data set as that in Schulte et al. (1992) except that the analysis was only for female workers. However, there was no significant effect of gender on micronucleus frequency ($p = 0.57$) and so it is difficult to establish the reasons for the different conclusions

from the two analyses, absent a statistical quirk. There was no increase in micronucleus frequency in the Mexican hospital sample, but the single control individual makes this an unusable conclusion.

Response: OEHHA staff agree that the lack of SCE data for the one control is problematic.

Comment 11. Biological data: (iii) Relationship to risk assessment: As noted in Schulte et al. (1992) with regard to the interpretation of the analysis of responses (micronuclei, SCE and hemoglobin adducts) in peripheral lymphocytes, "It is not known whether these changes may be indicative of increased risk of disease; however, they do appear to reflect exposure to relatively low levels of ethylene oxide. The exact meaning of these changes is unknown." There has been a persistent concern on the utility of cytogenetic data, for example, collected in population monitoring studies. It is generally agreed that they can be used to demonstrate an exposure, but not absence of exposure. However, even in this mode, it can be argued that confounders could be of concern. The reason being that peripheral lymphocytes are terminally differentiated, non cycling cells. Chromosome alterations (micronuclei and SCE) produced by the great majority of chemicals, including ethylene oxide, require DNA replications for their formation. Thus, any cytogenetic alterations observed from the way the assays are conducted are produced as errors of DNA replication *in vitro* (i.e. in culture) from DNA damage that remains at the time of this *in vitro* replication. Given that DNA repair processes are operational in peripheral lymphocytes, most alterations will have been derived from recent exposure. This makes it very difficult to establish a relationship between exposure and response except in the case of rather high, accidental exposures. Thus, even as a measure of exposure, the assessment of cytogenetic alterations in peripheral lymphocytes has serious limitations.

Given that no risk can be assigned to genetic alterations that arise *in vitro*, following an *in vivo* exposure, it seems highly inappropriate to use such data in the development of chronic reference exposure levels for ethylene oxide, or indeed for a very broad range of chemicals that produce their biological responses by a similar mechanism.

Response: OEHHA appreciates the thoroughness of the comments. OEHHA staff used the Schulte studies because they were the best human data we could find. Indeed the results may be more applicable as an indicator of genotoxic damage and carcinogenic potential than of other types of toxicity. The commentators do not suggest an alternative, superior, human or animal study to use. OEHHA staff has recalculated a chronic REL for ethylene oxide using human and animal data on neurotoxicity, an endpoint reported in both. OEHHA is now proposing a chronic REL of 30 $\mu\text{g}/\text{m}^3$ based on human data reported by Klees et al. (1990).

Klees *et al.* (1990) observed cognitive impairment and personality dysfunction more frequently in hospital workers chronically exposed to EtO, compared to a control group. A group of 22 hospital workers who had been exposed to an 8-hour TWA of 4.7 ppm EtO for a mean of 6.13 years (range 1-11 years) were matched with 24 control subjects. Worker neuropsychological function was classified as normal or impaired on the basis of the questionnaires and neuropsychological tests by 2 clinical psychologists, who were unaware of

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exposure status. (If the classification of the two clinicians did not agree, the subject was classified as “disagreement” which occurred in 7/23 (30%) of the controls and 10/22 (45%) of the exposed.) Exposed subjects were significantly more frequently classified as impaired (5/12) compared to controls (1/16) ($\chi^2 = 6.0861$; $p < 0.05$).

Derivation of the revised chronic REL for ethylene oxide

<i>Study population</i>	22 hospital workers (and 24 controls)
<i>Exposure method</i>	Workplace exposure
<i>Critical effects</i>	Impaired neurological function
<i>LOAEL</i>	4.7 ppm
<i>NOAEL</i>	Not observed
<i>Exposure continuity</i>	8-hours/day (10 m ³ occupational inhalation rate), 5 days/week
<i>Exposure duration</i>	6.13 years (range 1-11 years)
<i>Average experimental exposure</i>	1.68 ppm (4.7 x 10/20 x 5/7)
<i>Human equivalent concentration</i>	1.68 ppm
<i>LOAEL uncertainty factor</i>	3
<i>Subchronic uncertainty factor</i>	3
<i>Interspecies uncertainty factor</i>	1
<i>Intraspecies uncertainty factor</i>	10
<i>Cumulative uncertainty factor</i>	100
<i>Inhalation reference exposure level</i>	16.8 ppb (30 µg/m ³)

Chemical Manufacturers Association - Ethylene Oxide Industry Council

Comments on the chronic REL for **ethylene oxide** were received from the Ethylene Oxide Industry Council (EOIC) of the Chemical Manufacturers Association (CMA) in a letter signed by Courtney M. Price dated January 29, 1998. OEHHA developed a chronic REL of 5 µg/m³ from a 1995 study of hospital workers by Schulte and coworkers.

Comment 1. The OEHHA guidelines establish criteria for the determination of RELs. The 1995 Schulte study cited in the TSD, and the equally relevant 1992 study that is not cited, must be evaluated subject to Cal EPA guidelines on interpretation of human studies. Cal EPA OEHHA guidelines recognize that "[e]xposure measures frequently represent the greatest weakness of available epidemiological studies." Short-term exposure monitoring must frequently be used where long-term data are not available. "The degree to which air concentrations can be adequately measured is critical in determining the usefulness of an epidemiological study." "Covariables and confounding variables should be controlled or removed from the study." A limitation of controlled human exposure studies, in addition to their short duration, is that they usually involve small sample sizes. In evaluating evidence, OEHHA considers "strengths and uncertainties of each REL.... Issues such as observation of dose-response relationship, reproducibility of findings, and mechanism of action" are given weight in evaluating RELs. "Consistency of an association between chemical exposure and adverse effect is also evaluated. Relevant observations include similarity of effects noted in different studies and among different populations and/or species" When these guidelines are applied to the 1992 and 1995 Schulte studies, significant questions are raised concerning the validity of the findings.

Response. OEHHA acknowledges that human studies (including the 1992 and 1995 Schulte studies) often suffer from deficiencies in the assessment of exposure. The deficiencies were detailed by OEHHA in the TSD. We have since re-evaluated the utility of the Schulte et al. (1995) study in deriving the chronic REL and, as a result, are proposing to use a study by Klees et al. (1990) on neurotoxicity.

Comment 2. Cal EPA should not utilize the Schulte studies to establish a REL for EO since the control population is too small. A valid epidemiologic study must have an adequate number of controls to yield reliable estimates of risk and permit adjustment for potential confounders that can bias results. The number of controls was much too small in the 1995 Schulte study - eight U.S. workers and one in the Mexican worker group. There is indication that the insufficient number of controls is not merely a formal deficiency, but undermines reliance on the TSD's conclusion that Schulte found a significant excess in SCE values and hematology values. Taking SCE values, Schulte's U.S. control group shows SCE mean values of 4.61 per cell. Other larger studies report SCE values of 8.29 ± 0.08 for 353 individuals, and 9.32 for 22 non-smoking individuals. It is recognized that smoking is a considerable confounder and thus an adequate number of controls is especially important to a valid study. As a result of the small size of Schulte's control group and the anomalous level of SCEs reported in these controls, Schulte's findings lack the indicia of validity to be selected as the basis for the EO REL.

Response. The smaller the control group is, the more obvious the effect must be in the exposed group. The possibility that these controls have unusually low SCE values is important and may be a reason to doubt the small, purported increase in SCE in the EO exposed workers. As noted above, OEHHA is now proposing the use of a study on neurotoxicity as the basis for the chronic REL.

Comment 3. Cal EPA should not utilize the Schulte studies to establish a REL for EO since the relevance of SCE data to risk assessment has not been demonstrated. Schulte et al. recognized in the 1992 paper a significant limitation not quoted by Cal EPA: micronuclei, SCE, and hemoglobin adducts appear to reflect exposure to relatively low levels of EO but it is not known whether they are indicative of increased risk of disease. Thus SCE data are biomarkers of EO exposure but it is not known whether they have any clinical significance or indicate any disease endpoint. Schulte himself recognizes that "the predictive value of SCEs and micronuclei to cancer is undetermined." Mutation Research, Vol. 278 at 239. Schulte states that "the significance of our findings [increased numbers of hemoglobin adducts and SCEs] for the long term health of workers is unknown." Id. at 248. Other investigators in addition to Schulte acknowledge that these cytogenetic changes have no known clinical significance. E.g., Stolley et al., "Sister-chromatid exchanges in association with occupational exposure to ethylene oxide," Mutation Research 129:89-102 (1984). It is unwarranted to treat these biomarkers of exposure as indices of health risk.

Response. Although the relevance of SCE data to risk assessment of ethylene oxide has not been demonstrated, the finding of increased SCE in Bloom's syndrome, in which the risk of cancer is increased several fold, indicates that SCE, a rearrangement of the genetic material, may be linked to cancer. However, OEHHA agrees that for noncancer, chronic risk assessment, the use of this endpoint is questionable. As such, OEHHA is proposing to use the Klees et al. (1990) study of neurotoxicity.

Comment 4. Cal EPA should not utilize the Schulte studies to establish a REL for EO since the 1992 Schulte study does not indicate dose response for micronuclei. In the 1992 Schulte Study, frequencies of micronuclei were not significantly different in the U.S. population between controls, low and high exposure. Although the 1995 study overlapped the 1992 data set, an unexplained difference in results was observed which is not rationalized by the fact that the 1995 study was limited to female workers. When the 1992 data are considered, there is not an adequate dose-response to suggest causal association under OEHHA guidelines.

Response. In the U.S. data there is a statistically significant difference between the 0 exposure and the >32 category. The SCE are higher in the high exposure group in the 1995 report (Table 3). The p value is 0.02.

Comment 5. Cal EPA should not utilize the Schulte studies to establish a REL for EO since the exposure assessment in the Schulte assessment was recognized by the author as a

weakness of the study. In the 1992 Schulte study, the estimate of four months of cumulative exposure was based on only two to four days of EO measurements. Schulte acknowledges as study "weaknesses" the fact that "the estimate of exposure was based on 2-4 days of EO measurements to model the cumulative exposure. The impact of peak exposures or other variations from the mean of those measurements could not be assessed." U.S. OSHA adopts a short term excursion limit of 5 ppm for EO given relevance of peaks of exposure. The Schulte data are flawed in their inability to adequately characterize exposure and to take intensity of exposure into account. The 1992 Schulte study simply does not account for the short term exposures (STEs) in conclusions or reporting. There is no indication of the magnitude or frequency of the exposures, even with multiple statements that the STEs are the primary source of exposure. Schulte simply takes all exposure measurements and calculates the ppm hour or cumulative time weighted exposure. Schulte then concludes from this number that effects are observed at exposure levels below the OSHA standard. This is a flawed conclusion because it ignores the implications of the OSHA excursion limit. OSHA has recognized the significance of STEs relative to health effects in the establishment of the EL. If an employer exceeds either the 8-hour limit or the 15 minute limit, the employer has violated the OSHA limits. It is unjustified to assume that health effects caused by exposures above an OSHA standard would apply below the standard. In addition, improper sampling techniques used by Schulte may have lead to inappropriate conclusions. In the study, results from different sampling techniques (personal monitoring, breathing zone, area samples) were used for the same study population and considered together, which would not be considered an appropriate method.

Response. OEHHA acknowledges the limitations of the exposure data in these 2 studies (and in many other human studies), the problems with measuring exposure to humans in such situations, and the problems associated with short-term excursions, especially with ethylene oxide in health care settings. OEHHA prefers to use human studies in developing RELs. We have revisited the use of this study as the basis of the chronic REL and have decided to use the study of Klees at al. (1990) instead.

Comment 6. Cal EPA should not utilize the Schulte studies to establish a REL for EO since the complete blood count data are not significant given the small number of controls and the frequency of iron deficiency in a population of young women. Data on minor hematologic changes do not provide a sound basis for the REL, especially given inadequate sample size. The level of reduction in hemoglobin is well within the expected range for a population of female workers who may be iron-deficient for a variety of reasons.

Small differences were noted in hemoglobin and hematocrit between mid-dose and high-dose exposed workers but not between unexposed workers and either low-dose or high-dose groups. The differences between mid-dose and high-dose groups were not clinically significant. See attached report by Dr. Mark Udden, Baylor College of Medicine. None of the subjects' hemoglobin levels were below the range of normal women as reported in the authoritative reference, Williams' Hematology. Moreover, Schulte does not appear to have addressed some other potential causes for their hematologic status such as folate or other nutritional deficiency.

Schulte's claim that EO causes changes in the CBC data of a population are primarily based on granulocyte and lymphocyte changes. However, as Dr. Udden observes, it is not clear that these changes have biological significance given that there was no statistically significant effect on the total white cell count of EO-exposed women versus unexposed women. The shifts in granulocyte levels (10%) did not decrease to the low level associated with neutropenia, nor was there evidence of lymphocytosis. The study also lacks internal consistency. Although Mexican workers had higher average cumulative exposures than U.S. women, the Mexican workers did not show statistically significant percentage changes in lymphocytes or neutrophils as might be expected if there were a real biological effect. The findings, based on multiple linear regression (Table VII), do not indicate a statistically significant relationship with increasing cumulative exposure.

In addition, there is lack of external consistency or consistency across studies. Schulte identified in his 1995 paper three other studies that did not find the effects he reported. Thus there is not found a consistency of association between EO exposure and hematologic effects across studies. See TSD Guideline § 2.2.2,

A much larger number of women would need to be studied before any conclusion can be drawn that CBC data represent meaningful biological effects of EO exposure.

Response. The inconsistency of the data in the 2 Schulte reports with data in other reports in the literature is important. For this and other reasons OEHHA staff have reconsidered the basis of the chronic REL and are now proposing to use neurotoxicity data from Klees et al. (1990).

Comment 7. Cal EPA should not utilize the Schulte studies to establish a REL for EO since the relevance of EO blood count data to worker health has been questioned. In June 1997 hearings at U.S. OSHA reviewing the current occupational standard on EO, Dr. Anthony LaMontagne appeared as the principal witness for the unions. In discussing recommended revisions to various ancillary requirements, Dr. LaMontagne stated that he questioned the usefulness of the complete blood count and differential in EO medical surveillance. See June 30, 1997 OSHA hearing transcript at 70-73 and exhibit to Dr. LaMontagne's testimony, "The Massachusetts Hospital Eto Health and Safety Study: A Summary Report for Study Participants and Supporters" (1996) at 37. Dr. LaMontagne recommended that the CBC count be eliminated from surveillance requirements, citing his publication, LaMontagne et al., "The utility of the complete blood count in routine medical surveillance for ethylene oxide exposure," *Am. J. Ind. Med.* 24:191-206 (1993). In this article, LaMontagne concludes that "a cross-sectional comparison of the CBC data from the EtO exposed workers to data from non-EtO exposed hospital workers showed no significant differences, ruling out an association of relative lymphocytosis with EtO exposure." The authors conclude that the CBC with lymphocyte differential is not useful in EO medical surveillance.

Response. Staff appreciate being apprised of Dr. LaMontagne's testimony. However, blood count was only one of the endpoints OEHHA considered. Also, as noted above, we have decided not to use the study by Schulte and coworkers as the basis of the chronic REL.

Comment 8. CONCLUSION: Individual epidemiologic studies addressing potential carcinogenicity of EO include hundreds or thousands of workers. It is inappropriate for Cal EPA to use the 1995 Schulte study with its small handful of workers in setting a REL for chronic effects given the significant limitations of the Schulte data.

Response. OEHHA appreciates the thoroughness of the comments. OEHHA staff used the Schulte studies because they were the best human data we could find. Indeed the results may be more applicable as an indicator of genotoxic damage and carcinogenic potential than of other types of toxicity. The commentators do not suggest an alternative, superior, human or animal study which OEHHA should use. OEHHA staff has recalculated the REL using human and animal data on neurotoxicity, an endpoint reported in both. OEHHA is now proposing a chronic REL of 30 $\mu\text{g}/\text{m}^3$ based on human data reported by Klees et al. (1990).

Klees *et al.* (1990) observed cognitive impairment and personality dysfunction more frequently in hospital workers chronically exposed to EtO, compared to a control group. A group of 22 hospital workers who had been exposed to an 8-hour TWA of 4.7 ppm EtO for a mean of 6.13 years (range 1-11 years) were matched with 24 control subjects. Worker neuropsychological function was classified as normal or impaired on the basis of the questionnaires and neuropsychological tests by 2 clinical psychologists unaware of exposure status. (If the classification of the two clinicians did not agree, the subject was classified as “disagreement” which occurred in 7/23 (30%) of the controls and 10/22 (45%) of the exposed.) Exposed subjects were significantly more frequently classified as impaired (5/12) compared to controls (1/16) ($\chi^2 = 6.0861$; $p < 0.05$).

Derivation of the revised chronic REL for ethylene oxide

<i>Study population</i>	22 hospital workers (and 24 controls)
<i>Exposure method</i>	Workplace exposure
<i>Critical effects</i>	Impaired neurological function
<i>LOAEL</i>	4.7 ppm
<i>NOAEL</i>	Not observed
<i>Exposure continuity</i>	8-hours/day (10 m ³ occupational inhalation rate), 5 days/week
<i>Exposure duration</i>	6.13 years (range 1-11 years)
<i>Average experimental exposure</i>	1.68 ppm (4.7 x 10/20 x 5/7)
<i>Human equivalent concentration</i>	1.68 ppm
<i>LOAEL uncertainty factor</i>	3
<i>Subchronic uncertainty factor</i>	3
<i>Interspecies uncertainty factor</i>	1
<i>Intraspecies uncertainty factor</i>	10
<i>Cumulative uncertainty factor</i>	100
<i>Inhalation reference exposure level</i>	16.8 ppb (30 µg/m ³)

Chemical Manufacturers Association - Alkanolamines Panel

Comments on the chronic REL for diethanolamine were made by the Alkanolamines Panel (Panel) of the Chemical Manufacturers Association in a letter from Courtney M. Price dated January 29, 1998. The Panel is comprised of the major domestic producers of diethanolamine (The Dow Chemical Company, Huntsman Corporation, Union Carbide Corporation, and Occidental Chemical Corporation). The Panel urges OEHHA to withdraw its draft toxicity summary and proposed reference exposure level (REL) for diethanolamine. The Panel states that the study on which OEHHA has relied is inadequate to derive a REL, and the draft toxicity summary does not reflect accurately diethanolamine's toxicity database, particularly for reproductive and developmental effects. Also, the summary should be revised to characterize diethanolamine's vapor pressure accurately. In restricting its comments to the toxicity summary and related REL, however, the Panel does not endorse the risk assessment practices, policies, and methods set forth in those Guidelines in whole or in part. Moreover, the Panel reserves the right to challenge OEHHA's use of the Guidelines to assess or regulate any chemical, including DEA. OEHHA developed a chronic REL for diethanolamine of 20 $\mu\text{g}/\text{m}^3$ based on hematologic changes in female rats exposed to the chemical in drinking water.

Comment 1. OEHHA should derive its REL for DEA from inhalation studies, not from a drinking water study. The California Toxic Air Contaminants 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 has stated that, because it is required to develop chronic inhalation RELs, “[s]trong weight is given to inhalation exposure-based health effects data. Oral exposure data are used only if adequate inhalation data are unavailable.

In deriving its REL for DEA, OEHHA stated that no inhalation studies with diethanolamine were located. For this reason, OEHHA derived its REL for DEA from a subchronic drinking water study conducted in rats. As shown below, however, a substantial database exists on DEA's potential inhalation toxicity. None of these studies is referenced in the toxicity summary. These studies provide data that is far more relevant to DEA's potential inhalation effects than the drinking water study on which OEHHA has relied. OEHHA must review these studies to fulfill its obligations under the Toxic Air Contaminants Program, comply with its own Guidelines, and derive an up-to-date and scientifically defensible REL.

A substantial database exists on DEA's potential inhalation toxicity. According to OEHHA's chronic toxicity summary, the direct effects of DEA on the respiratory system are unknown since no subchronic or chronic inhalation studies have been conducted. A number of inhalation studies have been conducted with DEA, however. These studies include:

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BG Chemie (1993): In this 14-day inhalation study, DEA was administered to rats in an aerosol. No effects were observed in response to the 0.2 mg/l dose. For the 0.4 mg/l dose, rats exhibited slightly decreased body weight and retarded body weight gain in the males, slightly decreased serum cholesterol in both sexes, and increased relative and absolute liver weight in the females. The study concludes that “[u]nder the conditions of the test the degree of toxic effects as reported in the literature after inhalation of 6 ppm, 25 ppm, and 200 ppm DEA-vapor could not be confirmed.” It further finds that “[n]eurotoxic effects as reported after 13 week application in the drinking water were not found after 2-weeks inhalation.” [BG Chemie (1993). Study on the inhalation Toxicity Including Neurotoxicological Examinations of Diethanolamine as a Liquid Aerosol in Rats (14 Day Test). Project No. 3610233/90008. A copy of this study is appended as Attachment 1.]

Gamer et al. (1996): In this 90-day liquid aerosol inhalation study, thirteen male and thirteen female Wistar rats were exposed head-nose to liquid aerosols of DEA for six hours per working day for 90 days. The target concentrations were 15, 150, and 400 mg/m³. The study found no functional or morphological evidence of neurotoxicity. Retardation of body weight was observed in animals that received high concentrations. No systemic effects occurred at the low dose, but liver, kidney, male reproductive system and red blood systemic effects occurred in the high concentration dose group. In the mid dose group, mild liver and kidney effects were present. Local irritation of the larynx and trachea was found in the high and mid dose groups, with irritating laryngeal effects also detected in the low dose group. [Gamer, et al. (1996). Diethanolamine – 90-Day Liquid Aerosol - Inhalation Study in Wistar Rats. BASF Project No. 5010075/93011. A copy of this study is appended as Attachment 2.]

BASF (1966): In this study, rats were administered a saturated vapor of DEA for eight hours. No mortality was reported.

Foster (1972): In this study, rats administered 1,471 ppm of DEA via inhalation experienced lung edema and died less than two hours after exposure. [Foster, G. (1972) . “Studies of the Acute and Subacute Toxicologic Responses to Diethanolamine in the Rat.” Dissert. Abst. B32:6549.]

Union Carbide Corp. (1950): Rats were administered a saturated vapor of DEA at 25°C for six hours. No deaths resulted. Rats were also administered DEA in a saturated mist for eight hours with no deaths resulting. [Union Carbide Corp. (1950). Bushy Run Research Center Report 13-67.]

Schaper and Detwiler-Okabayashi (1996): This three-hour inhalation study in mice compared the sensory and pulmonary irritating effects of amines found in metalworking fluids containing DEA. The RD50 (sensory irritation) for ethanolamines ranged from 500 to 1,500 mg/m³. [Schaper, M. and Detwiler-Okabayashi, K. (1996). "Comparison of Sensory and Pulmonary Irritating Effects of Amines Found in Metal Working Fluids (MWF) . " Toxicologist 301:18 (abstract)] .

Knaak et al. (1997): The authors reported a study in which rats were administered 25 ppm. of DEA vapor for a period of nine days by continuous inhalation (23.5 hours/day). Increased

liver and kidney weights, elevated blood urea nitrogen, and serum glutamate oxaloacetate transferase reported. [Knaak J, et al. (1997) "Toxicology of Mono-, Di-, and Triethanolamine" in Ware, G (ed.). Reviews Environ. Contam. Toxicol.

Eastman Kodak Co. (1967): In this 90-day subchronic inhalation study, dogs, weanling rats, adult rats, and guinea pigs were administered saturated vapor concentrations of about 0.26 ppm DEA. Exposure did not produce any identifiable gross or microscopic alterations in organs that could be attributed to DEA in any species. [Eastman Kodak Company (1967). Health and Safety Studies for Diethanolamine, Laboratory Tests to Determine Effect of Inhalation of Two Ethanolamines - Diethanolamine (DEA), Methylaminoethanol (MAE), Formulation 485K - Histological Addendum to Final Report. TSCA 8d Submission 86-890000205, Microfiche Number OTS0516742. Washington, D.C.: OPPT, U.S. EPA.]

Eastman Kodak Co. (1967): As an extension of the study summarized above, rats, guinea pigs, and dogs were, for 45 days, administered atmospheric concentrations of approximately 0.5 ppm DEA. All animals survived the study, and their behavior and appearance appeared normal. No systematic toxic effects or irritation were observed. The clinical examination also revealed no abnormal response, except that a "slight retardation in growth rate in rats may have occurred." [Subacute - Inhalation Toxicity of Diethanolamine and Bimat Imbibant (485 K)]

Hartung et al. (1970): The authors report a subchronic study in which inhalation of 6 ppm vapor by male rats on a workday schedule for 13 weeks caused depressed growth rates, increased lung and kidney weights, and some mortality. [Hartung, R., et al. (1970). "Acute and Chronic Toxicity of Diethanolamine." Toxicol. Appl. Pharmacol. 17:308]

The significance of the more recent studies conducted with DEA in predicting DEA's potential health effects was acknowledged recently during the deliberations of the Organization for Economic Cooperation and Development (OECD) Programme for the Investigation of High Production Volume Chemicals. This program, initiated in 1990, was established to gather data on chemicals produced in large quantities by member nations, provide for an initial screening of the potential risks to human health or the environment presented by these chemicals, and develop recommendations for further testing. The sponsor country for DEA, the United Kingdom, completed its Screening Information Data Set (SIDS) Dossier in 1993 [OECD, Screening - Information Data Set (SIDS) Dossier, OECD Am Chemicals Programme (June 1993) (prepared by the United Kingdom, Department of the Environment) (OECD SIDS Dossier)], and in 1995 submitted a SIDS Initial Assessment Report (SIAR). The SIAR, based on a comprehensive review of data, concluded that no further testing was necessary.

Some additional testing was nevertheless proposed at the SIDS Initial Assessment Meeting (SIAM) where the SIAR was discussed, although initially it was agreed at the SIAM that no further testing was necessary. In the OECD SIAR prepared to address the concerns raised at the SIAM, the National Centre for Ecotoxicity and Hazardous Substances of the United Kingdom's Environment Agency reiterated:

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“Since SIAM 4 the results of good quality 2- and 13-week inhalation toxicity studies have been incorporated into the SIAR. These studies [OECD (1997). SIDS - Initial Assessment Report: Diethanolamine] included specific evaluations of subgroups for neurotoxicity. Also good quality developmental toxicity data has been incorporated. It is therefore concluded that further animal testing of diethanolamine is unnecessary.”

The Panel believes that OEHHA must review and evaluate all available inhalation data including recent unpublished studies that OEHHA has characterized as being "of good quality," in order to reach sound conclusions about DEA's potential inhalation effects.

Response. OEHHA appreciates the suggestion of additional inhalation studies and the furnishing to OEHHA of some of the studies. However, many of the studies are acute or subacute studies:

- Foster (1972) - 2 hours;
- Schaper and Detwiler-Okabayashi (1996) – 3 hours;
- Union Carbide Corp. (1950) – 6 hours;
- BASF (1966) - 8 hours;
- Knaak et al. (1997) – 9 days;
- BG Chemie (1993) 14 days.

These studies are of little use for developing a chronic REL.

Of more relevance to the development of a chronic REL may be:

- the Gamer et al. (1996) 90-day liquid aerosol inhalation study in rats,
- the Hartung et al. (1970) 13 week (90 day) inhalation study of 6 ppm DEA in rats, and
- the Eastman Kodak Co. (1967) 90-day subchronic inhalation study in dogs, weanling rats, adult rats, and guinea pigs administered saturated DEA vapor concentrations of about 0.26 ppm.

The Gamer et al. study has not appeared in the peer-reviewed medical and toxicological literature as of March 1999. The Eastman Kodak study also has not appeared in the peer-reviewed literature; it provides a free-standing NOAEL for inhalation of 0.26 ppm. The Hartung et al. (1970) report on the effects of 6 ppm DEA is likely an abstract since it could not be located on Medline. Hartung and Cornish reported on the acute and short-term oral toxicity of 2-N-ethylaminoethanol in rats in 1969 (Food and Cosmetic Toxicology 7(6):595-602).

The Gamer et al. (1996) aerosol inhalation study can be used as a check against the chronic REL of 20 µg/m³ proposed by OEHHA. The NOAEL from the Gamer et al. (1996) study was 15 mg/m³ diethanolamine based on a 6 hour/day, 5 day/week exposure. The equivalent continuous exposure would be 2.7 mg/m³. After dividing by 1,000 (10 each for subchronic to chronic, animal to human, and intraspecies uncertainty/variability), the REL would be 2.7 µg/m³, one-seventh of the REL proposed. If this study is published in the peer-reviewed literature, OEHHA will consider lowering the REL to 2.7 µg/m³.

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As another check, the Hartung et al. (1970) free-standing LOAEL of 6 ppm (25.8 mg/m³) for a 13 week exposure of male rats would require time adjustment for continuous exposure to 4.6 mg/m³ and the maximum UF of 3,000 which results in a REL of 1.5 µg/m³ (also below the proposed chronic REL).

Comment 2. OEHHA should derive its REL for DEA from the Gamer et al. (1996) inhalation study. The Panel believes that the recent Gamer et al. (1996) study provides adequate data on which to base a REL and should be used for that purpose instead of the Melnick et al. (1994) study. As OEHHA has acknowledged in its Guidelines, oral data are considered only where inhalation data are unavailable. Moreover by using a cumulative uncertainty factor of 3,000 to derive a REL for DEA from this study - the highest uncertainty factor used by OEHHA for any chemical - OEHHA also has acknowledged the relative weakness of this study for predicting DEA's potential toxicity.

OECD (1997) (Section Addressing Recommendations for Further Work) Recent studies reviewed in connection with the OECD SIAR include the BG Chemie (1993) and Gamer et al. (1996) studies.

In the Gamer et al. study, conducted in the Republic of Germany, male and female Wistar rats were exposed by head-nose to liquid aerosols of DEA for 6 hours per working day for 90 days. The target concentrations of treatment groups were 15, 150, and 400 mg/m³. A complete necropsy and gross pathological examination was conducted on animals in the experimental and control groups.

The only clinically detectable effect was a reduction of body weight development among high dose (400 mg/m³) males. No systemic effects occurred at the low dose, but liver, kidney, male reproductive system, and red blood systemic effects occurred in the high dose group. In the mid dose group, mild liver and kidney effects were observed. Local irritation of the larynx and trachea was found in the high and mid dose groups, with irritating laryngeal effects also detected in the low dose group.

The authors concluded that the liver, kidney, male reproductive system, and red blood were target organs for systemic effects at the high concentration tested, but that no systemic effects occurred in the low concentration. They concluded further that the no observed effect level (NOEL) for its systemic effects lies between 15 and 150 mg/m³. The Panel believes that OEHHA should use the no observed adverse effect levels (NOAELs) from this study, together with the standard uncertainty factors set forth in the Guidelines, to compute a REL for DEA.

Response. OEHHA calculated a REL based on the Gamer et al. study in the response to the first comment. Systemic effects on the blood were seen in both the Melnick and Gamer studies, which indicates that DEA causes the same effects by both routes. The laryngeal irritation effects, detected in the low dose group, is of interest because it is an effect specific to the route of exposure.

Comment 3. OEHHA should revise its draft toxicity summary to describe accurately DEA's potential health effects and vapor pressure. The Panel also urges OEHHA to revise its draft chronic toxicity summary for DEA to characterize more accurately the chemical's potential chronic health effects. Although OEHHA states, for example, that there is a lack of reproductive and developmental toxicity studies on DEA, the database on these endpoints is robust. Among studies that provide data relevant to DEA's potential developmental toxicity are:

Bushy Run Research Center (1991): In this study, pregnant rats were dosed cutaneously with 150, 500, and 1,500 mg/kg/day of DEA in distilled water on gestation days 6-15. No mortality was observed during the study, and the pregnancy rate was equivalent for all groups. No evidence of embryotoxicity or malformations was observed; there were no decreases in the mean fetal body weight; and no treatment related differences in the incidence of external or visceral variations were seen. There was an increase in the incidence of fetal skeletal variations at 1,500 mg/kg/day. Maternal toxicity was observed primarily at 1,500 mg/kg/day. [Bushy Run Research Center. Definitive Developmental Toxicity Evaluation of Diethanolamine (DEA) Administered Cutaneously to Sprague Dawley Rats (Final Draft Report) (Unpublished) (Sept. 9, 1991)] .

BASF (1993): In this study, pregnant Wistar rats were dosed with DEA in an aerosol (nose-only on gestation days 6-15. The concentrations tested were 0.01, 0.05, and 0.2 mg/l (10, 50 and 200 mg/m³). Maternal toxicity (vaginal hemorrhage) and embryo fetotoxicity (increased incidence of skeletal variations) were observed at the highest dose level. No teratogenic effects were seen at any dose level. NOAELs were computed as follows: maternal toxicity (50 mg/m³); embryofetal effects (50 mg/m³); and teratogenicity (greater than 200 mg/m³). [BASF (1993). Study of the Prenatal Toxicity of Diethanolamin in Rats after Inhalation. Project No. 31RO233/90010].

Neeper-Bradley and Kubena (1993): Pregnant rabbits were treated by occluded cutaneous application to three concentrations of DEA for 6 hours a day on gestation days 6-18. Maternal toxicity (severe skin irritation) was seen at the highest dose. No teratogenic or embryofetal toxic effects were seen at any dose tested. NOELs were computed as follows: maternal toxicity (100 mg/kg) ; embryofetal effects (350 mg/kg) ; and teratogenicity (greater than 350 mg/kg). [Neeper-Bradley, T. L. and Kubena, M. F. (1993) . Diethanolamine: Developmental Toxicity Study of Cutaneous Administration to New Zealand White Rabbits. Union Carbide Corp. Bushy Run Research Center Project Report 91NO136.]

Environmental Health Research and Testing, Inc. (1990): In a range-finding developmental toxicity study, Sprague-Dawley rats were administered aqueous solutions of DEA by gavage at levels of 0, 50, 200, 500, 800, or 1,200 mg/kg from gestation days 6-15. The dosing volume was held constant at 5 ml/kg. Fetuses were delivered by Cesarean section on day 20 of gestation. The number of implantation sites, resorptions, dead or live fetuses, and the gravid uterine weight were recorded. All animals at the 500 mg/kg or higher level died or were moribund and sacrificed. No maternal mortality was observed in the 50 or 200 mg/kg groups. Maternal body weight gain was significantly reduced in the 200 mg/kg group. At scheduled sacrifice, a litter was found to be completely resorbed in one dam in the 200 mg/kg

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group. None of the recorded gestational parameters were significantly different between the treatment groups and controls, however. [Environmental Health Research and Testing, Inc. (1990) . Report: Range Finding Studies: Developmental Toxicity Diethanolamine When Administered Via Gavage in CD SpragueDawley Rats. NTP-89-RF/DT-002].

Burnett et al. (1976): No embryotoxic or teratogenic effects were produced by topical administration of 2 ml/kg semipermanent hair dye preparations containing 2 percent DEA (equivalent to about 40 mg/kg DEA) to the shaved backs of pregnant Charles River CD rats on gestation days, 1, 4, 7, 10, 13, 16, and 19. [Burnett, C. et al. (1976) "Teratology and percutaneous toxicity studies in hair dyes." J. Toxicol. Environ. Health 1:1027-1040.]

The Panel notes in this regard that the OECD SIAR reviewed these studies, particularly the BASF (1993) study, which it characterizes as "good quality developmental toxicity data," in repeating its recommendation that "further animal testing of diethanolamine is unnecessary. OEHHA should, therefore, assess and incorporate these studies into its chronic toxicity summary and also revise the text of its summary to reflect accurately the robust nature of DEA's toxicological database.

OEHHA similarly has failed to discuss or even reference reproductive studies conducted with DEA. These include:

Battelle Columbus (1989): Reproductive effects were reported in male rats administered DEA concentrations of 2.5 and 5 mg/ml in drinking water (233 mg/kg and 487 mg/kg body weights, respectively). " -Effects included atrophy of the seminal vesicle, hypospermia, and a decrease in sperm motility and sperm count. The doses at which adverse effects were seen, however, approximate toxic levels - evidenced by the fact that the rats exhibited a large depression in their group mean body weight. Body weight gains relative to controls were significantly depressed in all male and female treatment groups. Weight depressions ranged from 66 percent in male rats administered 5 mg/ml DEA, to 11 percent in females administered the lowest dose (0.16 mg/ml). The authors acknowledged that "these doses are much too high for a chronic study," and recommended that doses for a chronic study should not exceed 0.16 mg/ml (the lowest dose used in the Battelle Columbus study). As noted in a toxicology review recently conducted in connection with the OECD's Programme on the Cooperative Investigation of High Production Volume Chemicals, the results observed in this study for DEA are "unlikely to be indicative of specific reproductive toxicity," and "further reproductive effects toxicity studies in animals cannot be justified. [Battelle Columbus (1989). Diethanolamine: Subchronic Dosed Water and Dermal Studies in F344 Rats and B6C3F1 Mice - Final Report for Prechronic Dosed Water Study for Diethanolamine in Fischer 344 Rats. TSCA FYI Submission FYI-OTS-1189-0721, Microfiche Number OTS0000721. Washington, D.C.: OPPT, U.S. EPA.]

Battelle Columbus (1989): In a 14-day oral dosed water study, for example, DEA was administered to mice at concentrations ranging from 0.63 to 10 mg/ml of water. Exposure to the test solutions resulted in a calculated intake of 110 to 1,362 mg/kg/day for male mice and 197 to 2,169 mg/kg/day for female mice. No effects on the reproductive system were

detected in either gross necropsy or during histopathologic examination of high dose mice of both sexes. Battelle Columbus (1989) at 4 and Tables 5 and 6.

Hejtmancik et al. (1988): In a follow-up 13-week subchronic oral dosed water study, B6C3F1 mice were administered DEA concentrations of up to 10 mg/ml. As in the 14-day screening study, reproductive effects were found following gross necropsy or histologic examination. [Hejtmancik, M, et al. (1988a) . Prechronic Dosed Water Study of Diethanolamine (CAS 111-42-2) in B6C3F1 Mice (Report prepared by Battelle, Columbus, Ohio)].

Response. OEHHA appreciates the additional information on the effects of DEA on development. As much as possible OEHHA based its chronic RELs on articles appearing in the peer-reviewed toxicologic and medical literature. Published reports on the reproductive/developmental effects of DEA are lacking. The studies cited by the commentator are nearly all unpublished, in-house reports. They also are either by the cutaneous (skin) or oral (gavage, drinking water) routes. An exception to these routes is the unpublished BASF (1993) study of inhalation of aerosolized DEA, which resulted in NOAELs of 50 mg/m³ both for embryofetal effects and for maternal toxicity. However, OEHHA would prefer to use data other than a 10 day study for developing a chronic REL.

Comment 4. OEHHA should also ensure that the draft toxicity summary adequately characterizes DEA's physical characteristics. OEHHA's draft summary states, for example, that DEA's vapor pressure is less than 0.01 mm Hg at 20 degrees Celsius. DEA's vapor pressure is, however, much lower - less than 0.00015 mm Hg at that temperature. OEHHA should revise the summary to correct this error. The public must be provided with accurate information regarding DEA's vapor pressure because it ensures that ambient air concentrations of DEA are extremely low.

Response. The commentator requests that we be more accurate in reporting the vapor pressure of DEA. Indeed HSDB (1997) reports the value of 0.00014 mm Hg at 25°C, which is found in Dow Chemical's Alkanolamine Handbook (1980). OEHHA will revise the value.

Chemical Manufacturers Association – Arsenic

Comments on the chronic REL for **arsenic** were made by the Arsenic Acid Task Force of the Chemical Manufacturers Association Biocides Panel in a letter from Courtney M. Price dated January 28, 1998. The members of the Chemical Manufacturers Association Biocides Panel Arsenic Acid Task Force are: American Chrome & Chemicals; Chemical Specialties, Inc.; Hickson Corporation; J.H. Baxter & Company; Osmose Wood Preserving, Inc.; Occidental Chemical Corporation; Peninsula Copper Company; and Phibro-Tech, Inc. OEHHA proposed a chronic REL of 0.03 µg/m³ based on reduced fetal body weight in mice exposed to arsenic during days 9-12 of gestation.

Comment 1. The Task Force has reviewed the OEHHA draft chronic inhalation and oral Reference Exposure Level (REL) for arsenic. With regard to the chronic inhalation REL, the Task Force is concerned that OEHHA's analysis relies primarily on one study and fails to account for the well-known differences in toxicity among arsenic compounds based on the chemical oxidation state and the differences in animal metabolism of arsenic. Similarly, the Task Force is concerned about the development of an REL under the "Hot Spots" program that is dependent on oral exposure, as well as the primary reliance in the chronic oral REL on the Taiwanese drinking water studies, especially in light of questions raised about those studies. The Task Force's concerns about each of these points is discussed in more detail below. The Task Force asks that OEHHA carefully consider these comments and make the appropriate revisions to the chronic REL for arsenic.

Response. OEHHA staff recognize that there are differences in toxicity among arsenic compounds based on the chemical oxidation state. However, in the Hot Spots program industries do not speciate their arsenic emissions. Also there are differences in animal metabolism of arsenic. A PBPK model is needed to address this but only one has appeared in the peer-reviewed literature. OEHHA staff address the more detailed comments below.

Comment 2. OEHHA's chronic inhalation REL for arsenic is based primarily on a single publication by Nagymajtenyi et al. that describes the results of an inhalation developmental toxicity study in mice exposed to arsenic trioxide (As₂O₃). In this study, pregnant mice were exposed to trivalent inorganic arsenic (As₂O₃) at concentrations of 28.5, 2.9 or 0.26 mg/m³, which equates to total arsenic concentrations of 21.6, 2.2 or 0.2 mg/m³ as arsenic. Even if one discounts maternal toxicity and considers delayed ossification as a fetal malformation, adverse effects were seen at the highest dose level only:

<u>As₂O₃, mg/m³ Exposure</u>	<u>As mg/m³ Exposure</u>	<u>Fetal effect reported</u>
28.5	21.6	29% (fetal body weight; delayed ossification)
2.9	2.2	9% (fetal body weight)
0.6	0.2	3% (fetal body weight)

Only the effects observed at the highest dose have biological significance and of those, only reduced fetal body weight can be viewed as meaningful because the recoverability of the delay in bone maturation was not assessed in the study. Weight decrements of 9% and certainly 3% are not biologically meaningful.

OEHHA interpreted the Nagymajtenyi data as demonstrative of an adverse effect at each dose level; accordingly, a No-Observed-Adverse-Effect-Level (NOAEL) was not considered in the interpretation of the study data. Also, well-known differences in toxicity among arsenic compounds based on the chemical oxidation state and differences in animal metabolism of arsenic were not taken into account by OEHHA in the proposed arsenic chronic inhalation REL.

Response. The weight decrements of 9.9% and 3% were both statistically significant. A weight difference of 9.9% may be biologically meaningful in a very small, developing animal. The weight decrement of 3% might not be biologically significant if the loss is generally distributed. If it were specific, it could be. 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 since it increases 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 the animal fetuses may be biologically significant.

In order to investigate the effects of environmental arsenic on human reproduction, Ihrig *et al.* (1998) conducted a hospital-based case-control study of stillbirths in a central Texas community. (Ihrig MM, Shalat SL, Baynes C. A hospital-based case-control study of stillbirths and environmental exposure to arsenic using an atmospheric dispersion model linked to a geographical information system. *Epidemiology* 1998 May;9(3):290-294). The community included a facility with a more than 60 year history of producing arsenic-based agricultural products. Data were collected on 119 stillbirth cases and 267 controls (randomly selected from healthy live births at the hospital, matched for year of birth). Arsenic exposure levels were estimated from airborne emission estimates and an atmospheric dispersion model; the results were linked to a geographical information system (GIS) database. Exposure was linked to residence address at time of delivery. A conditional logistic regression model was fit to the data including maternal age, race/ethnicity, parity, income group, exposure as a categorical variable, and exposure-race/ethnicity interaction. The prevalence odds ratio (OR) for stillbirths observed for Hispanics in the high-exposure group ($>0.1 \mu\text{g}/\text{m}^3$ As) was 8.4 (95% confidence interval = 1.4-50.1). Based on these statistically significant results in people, the proposed REL of $0.03 \mu\text{g}/\text{m}^3$ for arsenic does not appear to be too conservative

since LOAEL/NOAEL and intraspecies UFs would need to be applied to the human data to develop a chronic REL.

Comment 3. According to Garcia-Vargas and Cebrian (in Toxicology of Metals, 1996) and the US EPA (EPA, 1984), inorganic trivalent arsenic is generally regarded as being more acutely toxic than inorganic pentavalent arsenic. According to Marie Vahter (in Arsenic Exposure and Health, 1994), a prominent and perhaps leading authority on arsenic metabolism: The methylation of inorganic arsenic in mammals functions as a detoxification mechanism. The methylated metabolites are less acutely toxic than inorganic arsenic. They are also less reactive with tissue components and faster excreted in urine than is inorganic arsenic.

The inorganic arsenic, especially As(III), is the main form of arsenic interacting with tissue constituents. This means that factors influencing the methylation (of arsenic) may influence arsenic toxicity.

Vahter presents comparative metabolism data that show mice methylate inorganic arsenite (trivalent arsenic) about 3.6 times more efficiently than humans for a given dose of arsenic (Vahter, 1994).

Response. Comment noted. OEHHA acknowledges that there are differences in metabolism and in toxicity between trivalent and pentavalent arsenic. However, arsenic emissions are not speciated in the Hot Spots program. Thus we prefer to use data on the more toxic species.

Comment 4. OEHHA should have considered these facts in proposing a chronic REL for arsenic. Using these facts, the derivation of a chronic inhalation REL for arsenic would be:

LOAEL	2.2 mg/m ³ as arsenic (Nagymajtenyi, 1985)
NOAEL	0.2 mg/m ³ as arsenic (Nagymaitenyi, 1985)
LOAEL Uncertainty Factor	1
Interspecies Uncertainty Factor	3.6
Intraspecies Uncertainty Factor	10
Cumulative Uncertainty Factor	36

$$\text{Inhalation Reference Exposure Level } 0.2 \text{ mg/m}^3 \times 36 = 7.2 \text{ mg/m}^3$$

OEHHA should revise the chronic inhalation REL for arsenic to take into account the points presented above and repropose a chronic inhalation REL of 7.2 mg/m³ total arsenic. An REL of 7.2 mg/m³ takes into account relevant inhalation toxicity data for arsenic compounds and contains a safety factor in addition to those listed by California. In OEHHA's calculations, the REL is based on trivalent inorganic arsenic toxicity data and assumes that all exposure to arsenic is to the trivalent form - the most toxic form of inorganic arsenic. Real-world exposures are not limited exclusively to trivalent arsenic, but include exposure to the less toxic forms as well. Thus, calculating the chronic inhalation REL using the

above-referenced conservative assumptions will protect against adverse effects from trivalent arsenic, which also overprotects against exposure to all other forms of arsenic.

Response. The commentator appears to have confused calculation of a REL with calculation of a Margin of Exposure. The chronic REL of 7.2 mg/m³ proposed in the comment is 720 times the ACGIH TLV for arsenic of 0.01 mg/m³. The 50 minute LC₅₀ for arsenic in mice (acute lethality) is 99 mg/m³, only 12x the chronic REL proposed by the commentator. If the suggested NOAEL of 0.22 mg/m³ is divided by the suggested cumulative UF of 36, a tentative REL of 5.5 µg/m³ is estimated. However, OEHHA staff do not agree with the choice of the NOAEL for the study. In addition the suggested interspecies UF would require special consideration.

In the absence of a superior study in the peer-reviewed literature on which to base a REL, the chronic inhalation REL proposed for arsenic is still 0.03 µg/m³

Comment 5. OEHHA has reestablished, in addition to an inhalation REL, an oral REL for arsenic. As a threshold matter, the Task Force objects to the inclusion of a chronic oral REL in the guidelines at all, since the purpose of the guidelines is to derive risk levels for airborne toxic contaminants. These risk levels, in turn, will be used to characterize the hazards associated with routine industrial releases of chemicals to the atmosphere. Nothing in the "Hot Spots" program requires or authorizes OEHHA to develop oral RELs. Even if OEHHA was otherwise authorized to develop oral RELs, the chronic oral REL for arsenic is based exclusively on the US EPA Oral Reference Dose (RfD) for arsenic in drinking water. The US EPA RfD for arsenic is based on the Taiwanese drinking water studies published by Tseng (1968,1977). These studies have been the subject of much scientific review and are not without criticism as to methodology (analytical chemistry and epidemiology) and applicability to US populations. This criticism is presented by Brown (1994, 1996) and suggests that reliance on the Taiwanese studies to establish US regulatory limits for arsenic exposure is not appropriate because of the necessity to extrapolate from high-dose exposures to low-dose exposures and across cultural lines.

Specifically, Brown has stated that a more detailed exposure classification than previously used is needed for reliable descriptions of cancer mortality in Taiwanese villagers and arsenic concentrations in drinking water. Brown also states that the cancer mortality dose-response curve for the Taiwanese cohorts is nonlinear at the low-dose end (arsenic drinking water concentrations of <0.05mg/L) suggesting that there may be a low-dose threshold for the observation of human cancer. US EPA surveys of US drinking water have shown that 95% of the samples collected and analyzed have arsenic levels of less than 0.005mg/L; the highest value recorded was 0.082mg/L (Borum and Abernathy, 1994). Finally, Brown has pointed out evidence that arsenic may be adequately methylated and detoxified at drinking water concentrations below 0.05mg/L. The adverse health risks, particularly cancer, ascribed to ingestion of arsenic in drinking water may be inaccurately stated for US populations when based on the Taiwanese studies. Accordingly, OEHHA's reliance on the Tseng studies (via US EPA) is inappropriate for establishing a chronic oral REL, even if oral RELs were authorized under the "Hot Spots" program.

Response. The Air Toxics Hot Spots risk assessments of facilities include an analysis of all potential pathways of exposure. Oral RELs are needed in the Hot Spots program to do multipathway analysis of chemicals that are emitted as particulates. Not only are these materials inhaled but they also are deposited on and ingested from home-grown crops and from soil, and can be absorbed following dermal contact with contaminated surfaces. Multipathway analyses have been part of the Hot Spots program since its inception. Proper parameters to use are discussed in the 1993 CAPCOA Guidelines and in the draft Exposure Assessment and Stochastic Analysis Technical Support Document. USEPA RfDs are being used as oral RELs. 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 RfDs as oral RELs was one action that OEHHA took to address the RAAC recommendations and to implement the Executive Order.

Comments on the deficiencies in the RfD for arsenic should be directed to USEPA.

Chemical Manufacturers Association (CMA) - Carbon Disulfide Panel

Comments on the chronic REL for **carbon disulfide** were made by the CMA Carbon Disulfide Panel. OEHHA proposes use of the US EPA Reference Concentration of 700 $\mu\text{g}/\text{m}^3$, based on effects on the nervous system

Comment 1. These comments address the chronic toxicity summary and proposed reference exposure level (REL) for carbon disulfide presented in the "Air Toxics Hot Spots Program Risk Assessment Guidelines Part III: Technical Support Document for the Determination of Noncancer Chronic Reference Exposure Levels" (Guidelines). In restricting its comments to the toxicity summary and related REL, however, the Panel does not endorse the risk assessment practices, policies, and methods set forth in those Guidelines in whole or in part. Moreover, the Panel reserves the right to challenge OEHHA's use of the Guidelines to assess or regulate any chemical, including carbon disulfide.

OEHHA should not characterize the database supporting the REL as "limited." The carbon disulfide database is robust, as other agencies reviewing it have found. In its toxicity summary, OEHHA states that one major uncertainty in the REL is the "limited nature of health effects studies conducted. The database supporting the REL cannot fairly be characterized as "limited," however, given the numerous epidemiological and animal studies of carbon disulfide's inhalation effects. The findings of other agencies that have reviewed this substantial body of data support this conclusion. For example, in proposing a test rule under Section 4 of the Toxic Substances Control Act (TSCA) for chemicals listed as hazardous air pollutants (HAPs) under the federal Clean Air Act (CAA), EPA decided not to pursue toxicity testing for carbon disulfide. EPA stated unequivocally that carbon disulfide has "a large inhalation toxicology database." As another example, the Toxicological Profile prepared by the Agency for Toxic Substances and Disease Registry (ATSDR) reviewed the numerous animal and human studies conducted with carbon disulfide. With respect to neurological effects, for example, the Toxicological Profile discussed occupational epidemiological studies in a variety of settings and summarized a number of animal studies. With respect to other endpoints, the Toxicological Profile stated that human data provide information on acute and chronic effects from inhalation exposure to carbon disulfide, as well as immunologic, neurologic, developmental, and reproductive effects. Animal inhalation data are available on intermediate systemic, neurologic, developmental, and reproductive effects.

Moreover, the key epidemiological study underlying the proposed REL, conducted by Johnson et al. (1983), has been subject to both external and internal peer review, and EPA concluded in its Integrated Risk Information System (IRIS) that it is "well designed and conducted, uses adequate numbers of subjects, and is well supported by other occupational studies examining the same effect. Because of its greater confidence in human data, ATSDR relied on this study to establish a minimum risk level (MRL) for carbon disulfide. In light of the large body of human and animal data on carbon disulfide's inhalation effects, and given the review of and reliance on by other agencies of the key study on which the REL is based, OEHHA should delete the reference to the "limited nature" of health effects studies conducted.

Response. OEHHA has reexamined the description of the quality of the health effects database and agrees with the commentator that the term “limited” is not warranted. The text has been changed to reflect this. However, the database for this chemical also can not be viewed as exhaustive. As noted by US EPA, significant areas of uncertainty include the exposure histories of workers examined in the key study and the possibility of developmental effects in humans.

Comment 2. OEHHA should eliminate the use of the modifying factor of 3. This factor is not needed, given carbon disulfide's extensive database. The Panel also believes that no uncertainty or modifying factor should be applied to address any purported deficiencies in the toxicological database for carbon disulfide. OEHHA does not discuss why it has accepted EPA's 3-fold modifying factor for database deficiencies, or why any modifying factor at all is appropriate. Indeed, OEHHA itself has expressed skepticism about the propriety of any modifying factor to address purported database deficiencies. When deriving chronic RELs using its own Guidelines, OEHHA does not employ a modifying factor to address database weaknesses. Given the extensive toxicological database on carbon disulfide's inhalation effects, such an uncertainty factor is particularly inappropriate here.

Response. As a result of both scientific judgement and legislative mandate, OEHHA considers US EPA an authoritative scientific body whose prior scientific assessments carry great weight. Furthermore, OEHHA has been directed to harmonize with US EPA as regards guidance levels for exposure of the general public to chemicals by both the Risk Assessment Advisory Committee (RAAC) and Governor's Executive Order W-137-96. Minor differences in scientific conclusions between two agencies such as OEHHA and US EPA are likely to arise, but such differences add a burden to those attempting to address two differing sets of recommendations. Thus, unless the difference of opinion is substantial, OEHHA will incorporate US EPA guidance into its programs. This does lead to the result that risk assessment recommendations for two different chemicals may be based on slightly different assumptions, as noted by the commentator.

Comment 3. OEHHA should revise the chronic toxicity summary for carbon disulfide to provide a more balanced and accurate summary of the scientific database on carbon disulfide's chronic health effects. The Panel believes that the toxicity summary provided in EPA's recent Sector Notebook for the Plastic, Resin and Manmade Fiber Industry provides such a summary and urges OEHHA to adopt that discussion.

Response. The health effects reviews presented in the chronic reference exposure level document are not intended to be exhaustive but rather to highlight the most important scientific data. Information on health effects of and risk assessment guidelines for more than 100 chemicals are presented in the document, which totals more than 700 pages in length. In addition, for chemicals such as carbon disulfide which have previously been addressed by USEPA in its Reference Concentration (RfC) program, OEHHA gives considerable weight to

US EPA's position as a recognized authoritative body and in most cases has proposed adopting the USEPA RfC.

Comment 4. OEHHA's summary of "effects of human exposure from carbon disulfide" is inaccurate and misleading. OEHHA's chronic toxicity summary for carbon disulfide fails to provide a balanced or accurate summary of the scientific database on carbon disulfide's chronic health effects. For example, the summary of the section entitled "Effects of Human Exposure" states:

"[A] primary target of carbon disulfide (CS₂) toxicity is the nervous system. The major neurotoxic action of carbon disulfide is the development of mental disturbances, such as change of personality, irritability, and forgetfulness, often with accompanying neurophysiological and neuropathology changes after prolonged exposure. Alterations in behavioral indices have been historically associated with high levels of CS₂, often in the excess of 20 ppm."

OEHHA's summary is misleading in not stating clearly that it is only high levels of exposure, well in excess of current regulatory levels, that may result in such effects. EPA's recent Sector Notebook for the Plastic, Resin and Manmade Fiber Industry (Sector Notebook) more accurately states that long-term (chronic) exposure to high levels [of carbon disulfide] in excess of regulatory standards may result in peripheral nerve damage (involving the nerves that control feet, legs, hands, and arms) and cardiovascular effects. The Panel thus urges OEHHA to revise the summary, and in this regard, the Panel urges OEHHA to consider adopting the Sector Notebook summary.

Response. The examples cited do not indicate the OEHHA summary was inaccurate. The current TLV is 10 mg/m³. However, the sections have been reviewed in light of the comment and changes made in the presentation to better clarify the type of exposures that have been associated with observable adverse health effects.

Comment 5. OEHHA's summary of carbon disulfide's reproductive toxicity is similarly misleading and inaccurate. With respect to this end-point, OEHHA says simply that "carbon disulfide causes reproductive toxicity in both males and females. This statement fails, however, to reflect accurately the robust database on carbon disulfide's potential reproductive toxicity and the scientific uncertainty regarding the no effect level that should be used based on these studies. Although there are substantial data bearing on carbon disulfide's potential reproductive effects, as discussed above, there remains substantial uncertainty about the significance of these effects and the no effect level that can be derived from these studies. This uncertainty should be reflected in any statements regarding carbon disulfide's potential reproductive toxicity.

Similarly, the EPA Sector Notebook notes that "[A] few studies contend that chronic exposure may also result in potential reproductive effects. The Panel urges OEHHA to revise its summary and in this regard to consider adopting the Sector Notebook summary,

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Do not cite or quote. SRP Draft – 2nd set of chemicals

which accurately reflects the scientific uncertainty underlying carbon disulfide's potential reproduction effects.

Response. Again, the examples cited do not demonstrate that the OEHHA summary was inaccurate. Similarly, however, the sections have been reviewed in response to the comment and changes made in the chapter to better clarify the evidence for reproductive toxicity from carbon disulfide exposures.

Comment 6. The Panel additionally urges OEHHA to review and rely on the following two recent publications on carbon disulfide's potential toxicity, which are appended as Attachments 1 and 2:

Price, B., *et al.* (1996). A Benchmark Concentration for Carbon Disulfide: Analysis of the NIOSH Carbon Disulfide Exposure Database. *Regulatory Toxicol. Pharmacol.* 24:171-176.

Price, B., *et al.* (1997). A Review of Carbon Disulfide Exposure Data and the Association between Carbon Disulfide Exposure and Ischemic Heart Disease Mortality. *Regulatory Toxicol. Pharmacol.* 26:119-128.

Response. The two papers cited have been reviewed and their findings have been summarized in the revised toxicity summary for carbon disulfide.

Chemical Manufacturers Association – Cresols Panel

Comments on the chronic REL for **cresols** were made by the Cresols Panel of the CMA in a letter from Courtney M. Price dated January 29, 1998. The members of the Cresols Panel are Concord Chemical Company, CRI Fine Chemicals, Dakota Gasification Company, General Electric Company, Merichem Company, Mitsui Petrochemicals (America) Ltd., PMC Specialties Group, Inc., and Sumitomo Chemical Americas, Inc. In the draft TSD OEHHA proposed a chronic REL of 4 $\mu\text{g}/\text{m}^3$ based on the Uzhdavini et al. (1972) discontinuous, 4 month inhalation study in rats which resulted in alterations in bone marrow cellularity.

Comment 1. As discussed in the appended comments, the Panel urges OEHHA to withdraw its draft toxicity summary and proposed reference exposure level (REL for cresol mixtures (cresols)). The studies on which OEHHA has relied cannot support a REL, and cresols do not merit priority attention for evaluation or regulation. These comments address the chronic toxicity summary and proposed inhalation reference exposure level (REL) of 4 $\mu\text{g}/\text{m}^3$ for cresol mixtures (cresols) presented in the Guidelines. In restricting its comments to the toxicity summary and related REL, however, the Panel does not endorse the risk assessment practices, policies, and methods set forth in those Guidelines in whole or in part. Moreover, the Panel reserves the right to challenge OEHHA's use of the Guidelines to assess or regulate any chemical, including cresols.

The Panel urges OEHHA to withdraw its draft toxicity summary and proposed REL for cresols for the following reasons:

- The proposed REL for cresols is based on a single, poorly reported study that does not comply with Good Laboratory Practices, and other data do not support the findings of that study or the proposed REL.
- In any event, cresols do not merit priority attention for assessment or regulation because they are present in the ambient air only in very small concentrations. Available data show very low workplace and general population exposure concentrations - well below those that implicate health concerns.

Response. The detailed comments are individually addressed below.

Comment 2. The Uzhdavini et al. and Kurliandskii et al. studies are of insufficient quality to derive or support a REL. OEHHA derived its REL for cresols from a Russian inhalation study conducted with rats in 1972. OEHHA refers to a second Russian study of the same era as providing support for the REL. Neither study, however, is of sufficient quality to derive or support a REL and OEHHA should, therefore, withdraw the proposed REL.

The Uzhdavini et. al (1972) study is of insufficient quality to support a REL. OEHHA's proposed chronic toxicity REL for cresols is based on the Uzhdavini et al. (1972) observations regarding o-cresol exposure in rats. Uzhdavini et al. reported that rats exposed to 9 mg/m^3 o-cresol by inhalation showed an increase in white blood cells, and a statistically

significant change in the leuko-to-erythmo ratio in the bone marrow. The authors also reported an extension of hexobarbital narcosis duration in treated animals. The Uzhdavini et al. study - which was performed more than 25 years ago in the then Soviet Union under conditions that do not approximate current scientific methods and standards - cannot be used to support a REL. The study findings are difficult to interpret for a variety of reasons. The study parameters reported are vague; the specific data are not included (only summary statements) and the conclusions relate only to imprecisely measured concentrations of "vapor/aerosol." Additionally, the published study report does not describe chamber generation methods, precise analytical methods, exposure details, animal characteristics (weight, age, sex, strain), observational information (times, frequency, duration, specific conditions examined), or specific experimental conditions. From the summary nature of the information presented and the absence of information about the study design, a dose-response relationship cannot be determined. This study would be judged inadequate under GLP requirements for use in determining potential risk to humans. Relying on the study clearly contravenes OEHHA's own Guidelines, which state unequivocally that any animal data supporting a REL "should have a clear rationale and protocol, use [GLP] Standards, and use appropriate analysis methods.

With respect to the specific findings at issue, the results - even if credited - do not indicate adverse effects from exposure to cresols. For example, while white blood cell counts reportedly were elevated in some treated animals, these effects were observed in male animals only, and blood counts returned to normal after cessation of exposure. The reversibility of the effects, and the fact that effects were seen in male animals only, suggests that they were neither serious nor clearly associated with exposure to cresols. Moreover, the authors report with respect to this study that no changes were found in the leuko-erythmo ratio in the second species tested - guinea pigs. Additionally, Uzhdavini reported that:

- the vapor pressure of cresols was so low that acute inhalation toxicity could not be induced with vapor alone, only with a mixed vapor aerosol of cresols could adverse effects be produced;
- nonspecific irritation was produced in the respiratory tract by high concentrations of cresols aerosols;
- in repeated exposure experiments, cresols did not exhibit cumulative toxicity; and
- in rats, where recovery studies were made, recovery from cresols effects (blood parameters) was seen.

Thus, the Uzhdavini et al. findings simply cannot support OEHHA's proposed REL. Indeed, other agencies have discounted the Uzhdavini et al. (1972) study observations regarding o-cresol exposure in rats, as well as the additional limited information reported in the Uzhdavini study regarding effects from inhalation exposure to o-cresol in several species, including humans. [For example, the study reported a human threshold for respiratory irritation (dryness, constriction in the nose, irritation of the throat, a taste in the mouth) of 6 mg/m³ (1.4 ppm) Uzhdavini et al. (1972).] The American Conference of Governmental Industrial Hygienists (ACGIH) considered the Uzhdavini et al. study, but elected not to rely on it to establish its 8-hour threshold limit value (TLV) for exposure to cresols of 22 mg/m³ (5 ppm). Similarly, the National Institute for Occupational Safety and Health (NIOSH) rejected

the Uzhdavini data when it recommended an "immediately dangerous to life or health" (IDLH) population exposure limit of 1,123 mg/m³ (250 ppm), and a number of countries, in addition to the United States, have established inhalation exposure levels for cresols at 22 mg/m³.

ACGIH (1991) (Documentation of the Threshold Limit Values and Biological Exposure Indices (1991) at 341) noted that eight of ten human subjects exposed to 1.4 ppm of o-cresol in the Uzhdavini et al. study complained of upper respiratory tract irritation, but criticized the study because the minimal exposure levels and duration associated with the irritation had not been reliably documented. The U.S. Occupational Safety and Health Administration (OSHA) also has established a time-weighted average (TWA) for all cresol isomers of 5 ppm (29 C.F.R. Part 1910).

Moreover, the U.S. Environmental Protection Agency's (EPA) Health Effects Assessment for Cresols, on which OEHHA also relied in drafting the toxicity summary, has criticized the two Russian studies. Because of the absence of detail regarding the severity or type of changes reported, EPA concluded that "it would be imprudent to use either of these studies to derive a value for an AIS [Acceptable Intake Subchronic] without further information. EPA also noted that NIOSH had concluded that the two Russian studies "were considered inadequate as a result of the incomplete presentation of experimental design and the confusing presentation of results.

Given the many defects and omissions in the Uzhdavini et al. study discussed above, the results cannot be deemed reliable for predicting the chronic health effects potentially associated with exposure to cresols. It is not surprising that the study has been accorded little weight in decision-making by regulatory agencies in the United States and elsewhere. OEHHA likewise should not rely on the results obtained in the Uzhdavini et al. study to reach conclusions about cresols, potential chronic effects, or to derive a REL.

Response. OEHHA originally selected the Uzhdavini et al. (1972) study in order to base as many chronic RELs as possible on inhalation data. The study reports unusual endpoints by today's standards. However, the study had been reported on by both NIOSH and ATSDR in their documents. Therefore the study was selected as the basis for the REL despite its shortcomings. On reconsideration we have decided to base the chronic REL on the USEPA RfD. The U.S. EPA RfD was based on 90 day animal toxicity studies done by USEPA and reported in 1986.

The commentator states: "The reversibility of the effects, and the fact that effects were seen in male animals only, suggests that they were neither serious nor clearly associated with exposure to cresols. Moreover, the authors report with respect to this study that no changes were found in the leuko-erythmo ratio in the second species tested - guinea pigs." OEHHA staff do not agree that these are useful criteria for addressing toxicity results. Elevated carboxyhemoglobin levels are both potentially adverse and reversible. Certain chemicals have the propensity to be more toxic to, or only toxic to, one sex versus the other. Limonene and dichlorobenzene cause kidney tumors only in rats and only in male rats. Other agents may cause adverse effects in only one species or strain or not cause adverse effects in

only one species. Benzo(a)pyrene is highly carcinogenic in all species and strains except DBA2 mice. Thalidomide is teratogenic except in rabbits. Even the lethal level of a chemical can vary among species. The LD₅₀ of TCDD varies widely (guinea pig = 0.001-0.002 mg/kg; male rat = 0.022 mg/kg; female rat = 0.045 mg/kg; mouse = 0.114 mg/kg; hamster = 1.157 mg/kg).

Comment 3. The Kurliandskii et al. (1975) study is of insufficient quality to support a REL. Although OEHHA did not rely on the Kurliandskii et al. (1975) study to derive the REL, it cited the study as further support for the REL and as evidence that chronic adverse health effects may occur in animals exposed to cresols at levels lower than those reported by Uzhdavini et al.

The Kurliandskii et al. study, however, suffers from the same inadequacies that plague the Uzhdavini et al. study. Among other methodological defects, the study lacks information necessary to interpret the findings; fails to report how many hours per day animals were exposed; and fails to report whether the exposure was daily. NIOSH found the findings difficult to assess "because of unexplained differences in the experimental results" and "unanswered questions concerning the procedures used to measure central nervous system function." Like the Uzhdavini et al. study discussed above, the Kurliandskii study also fails to comply with fundamental GLPs and, pursuant to the OEHHA Guidelines, is thus inadequate to derive or support a REL.

Response. OEHHA agrees that the Kurliandskii et al. study also has shortcomings. In addition it indicates that 0.05 mg/m³ is a LOAEL and 0.0052 mg/m³ is a NOAEL for some endpoints, whereas 9 mg/m³ was considered a LOAEL in the Uzhdavini et al. study.

Comment 4. Other data show no adverse effects from exposure to cresols. Not only do the Uzhdavini et al. and Kurliandskii et al. studies not support a REL, but other data show no adverse effects following inhalation exposure to cresols. These include:

- Mellon Institute of Industrial Research (1949): In this acute toxicity study conducted with rats, animals were exposed to a saturated vapor of m-cresol on a single day for eight hours (saturated vapor concentration of m-cresol at room temperature is estimated to be 0.3 mg/L (300 mg/m³ or 68.2 ppm)). No effects were observed, except that one rat failed to gain weight.
- CONOCO (1975): Rats exposed to a single 6-hour exposure of o-cresol vapor by inhalation at doses up to 4,500 ppm (19,800 mg/m³ or 19,800,000 mg/L) did not experience mortality or clinical signs of toxicity other than eye irritation, which cleared up within 24 hours after exposure.

Similarly, a number of oral studies conducted with cresols show none of the blood chemistry changes reported in the Uzhdavini et al. (1972) study. These studies include:

- Hornshaw et al. (1986) Spleen weight and white blood cell (WBC) count were unaffected when o-cresol was administered in feed at doses up to 400-720 mg/kg/day in ferrets and 320-480 mg/kg/day in mink. Similarly, no effect was seen on spleen weight or WBC count in a reproduction study where mink were administered 105-190 mg/kg/day of o-cresol in feed for six months.
- Microbiological Associates, Inc. (1988a, b, c): No mortality or illness due to infections were seen in mice or rats receiving either o-cresol or m/p-cresol mixture in feed for 90 days at concentrations up to 20,000 ppm or 30,000 ppm (for mice and rats, respectively). Hematology parameters including WBCs, lymphocytes, monocytes, and eosinophils were unremarkable at all dose concentrations. In mice, changes in spleen or thymus were observed at 15,000 and 30,000 ppm, but there were no changes observed following gross or microscopic examination.
- Bushy Run Research Center (BRRC) (1989a, b, c). Two-generation reproduction studies were conducted by oral gavage in rats with each cresol isomer. The dose levels used achieved systemic toxicity in adult animals (lethality). First and second generation parents were necropsied, and selected organs, tissues, and all gross lesions were examined. The adrenal gland, spleen, mandibular and mesenteric lymph nodes, pituitary, thyroid, and thymus region were among the tissues examined. The study pathologist reported no compound-related effects in any of these tissues for any of the cresol isomers. [BRRC (1989a). Two-generation reproduction study of o-cresol (CAS No. 95-48-7) administered by gavage to Sprague-Dawley (CD) rats. Project Report 51-614. Unpublished data submitted to the Chemical Manufacturers Association Cresols Panel. Washington, D.C.; BRRC (1989b). Two-generation reproduction study of p-cresol (CAS No. 106-44-5) administered by gavage to Sprague-Dawley (CD) rats. Project Report 52-512. Unpublished data submitted to the Chemical Manufacturers Association Cresols Panel. Washington, D.C.; BRRC (1989c). Two-generation reproduction study of m-cresol (CAS No. 108-39-4) administered by gavage to Sprague-Dawley (CD) rats. Project Report 51-634. Unpublished data submitted to the Chemical Manufacturers Association Cresols Panel. Washington, D. C. These data were submitted to EPA by Union Carbide. See Union Carbide Corporation, "Two-generation reproduction studies on ortho-, meta-, and para-cresols administered by gavage to Sprague-Dawley (CD) rats (final reports) with attachments and cover letter dated 12-06-89." TSCA 4 submission 40-8960311, microfiche number OTS0529224. Washington, D.C. OPPT, U.S. EPA (Nov. 9, 1989).]
- U.S. National Toxicology Program (NTP) (1992): In these studies, groups of mice and rats were administered oral doses of cresol isomers and mixture for 13 weeks. A full battery of hematology parameters were evaluated. No blood alterations were seen in rats exposed to o-cresol or a m/p-cresol mixture up to 30,000 ppm. Mice exposed to 20,000 ppm of o-cresol also displayed no hematological changes. Mice exposed to up to 10,000 ppm m/p-cresol showed a mild decrease in hemoglobin at study termination, but no blood changes similar to those reported by Uzhdavini. The author of the NTP study report concluded that the hematology changes observed in mice following exposure to m/p cresol were "largely unremarkable."

Response. The commentator presents 2 acute inhalation studies of 8 and 6 hours (Mellon and CONOCO, respectively), which showed no adverse effects, and several oral studies that indicate that cresols do not affect hematology parameters, which the Uzhdavini et al. study (1972) claimed cresols affect. As stated above, due to the shortcomings in the Russian studies OEHHA has decided to base the chronic REL on the U.S. EPA RfD.

Comment 5. Cresols are present in the ambient air at very low concentrations, and do not merit priority consideration for evaluation or regulation. The California Toxic Air Contaminants Program (Program) provides that, in evaluating the health effects of toxic air contaminants, OEHHA "shall give priority to the evaluation and regulation of substances based on factors related to the risk of harm to public health, amount or potential amount of emissions, manner of, and exposure to, usage of the substance in California, persistence in the atmosphere, and ambient concentrations in the community." Because cresols concentrations in the ambient air are very low - well below those that implicate health concerns -cresols merit neither evaluation nor regulatory action under the Program.

Response. ARB estimates that at least 12,000 pounds of cresols are released annually into California air. While statewide ambient concentrations are probably low overall, the Hot Spots program addresses ambient concentrations around facilities that are potential "Hot Spots" for emissions of cresols.

Comment 6. Available monitoring data show very low cresols ambient air concentrations Available data show very low cresols concentrations in the atmosphere, even near manufacturing facilities. Monitoring data include:

- EPA 1982 Survey: In a survey of volatile organic compounds (VOCS) found in the atmosphere commissioned by EPA, cresols were found near source-dominated sites (adjacent to chemical plants) at levels ranging from 0.1 to 30 parts per billion (ppb), with a median of 1.6 ppb. [Brodzinsky, R. and Singh H. (1982). Volatile organic Compounds in the Atmosphere: An Assessment of Available Data, EPA Office of Research and Development. Research Triangle Park, North Carolina]
- EPA's Hazardous Substances Databank Entries: Entries for cresols note that o-cresol was detected near a phenolic resin factory in Japan at a maximum concentration of 40 ppb^a and that m-cresol and p-cresol were not detected at all in air samples taken in both urban and rural areas of western Colorado and Utah.
- Gordon (1976): On behalf of EPA, Gordon (1976) estimated cresols air concentrations at a hypothetical facility producing 80 million pounds of cresols annually and emitting 160,000 pounds of cresols per year - an amount greater than actual emissions reported by any one facility for 1994. The estimated air concentration 500 meters downwind of the hypothetical plant was 0.163 mg/m³ or 37 ppb - an amount well below the OSHA, ACGIH, and NIOSH limits for full day occupational exposures. Thus, the population

living near a major source is at a very low risk of exposure from industrial cresols emissions.

- Merichem Data: Merichem Company modeled cresols concentrations at its Houston facility in 1991. At 2,000 feet from the facility, at the fence line, the concentration of cresol isomers was 38 pg/m³. This is far below the OSHA, ACGIH, and NIOSH worker exposure limits (10,000 - 22,000 pg/m³).
- ATSDR Toxicological Profile: In its 1992 Toxicological Profile discussion of general population exposure to cresols, the Agency for Toxic Substances and Disease Registry (ATSDR) concluded that “[m]onitoring data have not shown cresols to be widely occurring. The median air concentration of o-cresol at source-dominated sites is 0.359 ppb for 32 samples.”

The Program requires consideration of exposure data (including emissions data and data on estimated actual exposure) when selecting chemical substances for priority for evaluation and regulation. In light of their low documented emissions and exposure potential, cresols do not merit priority consideration for evaluation or regulation.

Response. It is encouraging that cresols are not wide-spread toxic air contaminants like benzene or butadiene. However, as stated above, the Hot Spots program addresses ambient concentrations around facilities that may be Hot Spots for emissions of cresols. Also cresols are not being given priority consideration.

Comment 7. Modeling data show that even under extreme conditions, highly unlikely to occur, exposure levels are very low. Accidental release modeling shows that even under extreme conditions, cresol vapors would quickly disperse to levels below regulatory levels of concern. Dakota Gasification Company modeled two accident scenarios using the ARCHIE computer program and assuming EPA's worst-case weather conditions of 68° F and 3.4 miles per hour wind speed. The first scenario modeled was a 100,000 gallon tank rupturing and 879,452 pounds of cresols spilling out within one minute. The cresols product temperature was modeled at 68°F. The model predicted that peak cresols concentrations at 1,536 feet (468 meters) from the tank would be 4 ppm (18 mg/m³), which is below the previously established OSHA and ACGIH 8-hour average limit of 22 mg/m³. At 2,333 feet (711 meters), the concentration would be only 2.1 ppm (9.3 mg/m³), less than the NIOSH recommended 10-hour average limit of 10 mg/m³.

The second scenario assumed that a distillation column failed instantaneously, releasing 75,762 pounds of cresols at 365°F. Modeled concentrations from this extreme scenario were 9.6 ppm (42 mg/m³) at 2,194 feet (669 meters), which is well below the NIOSH IDLH of 250 ppm. By 3,450 feet (1,052 meters), the concentration is less than 5 ppm, and by 5,962 feet (1,817 meters), the modeled concentration is 2 ppm (8.8 mg/m³), which is below the NIOSH recommended limit.

These modeled accidental release scenarios represent conditions under which the highest air concentrations of cresols could reasonably be expected (that is, large amounts released within a short interval of time during worst-case weather conditions). Even under these extreme conditions, the modeled air concentrations of cresols in the near vicinity of the facility - concentrations which would persist for only a brief period of time - are on the order of concentrations which are considered acceptable for occupational exposure, i.e., acceptable for 40 hours per week.

The amount of cresols modeled to be released in the second scenario - 75,762 pounds - is more than the amount reported by most facilities as their annual emission quantity. The amount modeled as released in the first scenario - 879,452 pounds far exceeds the total reported cresols air emissions for all cresols manufacturing facilities for 1994. Clearly, then, cresols air concentrations in the vicinity of emitting facilities due to normal operations - that is, concentrations due to emissions of much smaller quantities over an extended period of time - are low, demonstrating that cresols should not be given priority consideration for evaluation or regulation under the Act.

Response. Comment noted. Again cresols are listed as listed Hot Spots chemicals. OEHHA staff attempted to develop as many health guidance values for Hot Spots chemicals as it could find data for. Since the industrial emissions of cresols are low, the ground level concentrations should be well below the chronic REL.

Comment 8. Cresols' physical characteristics ensure low concentrations in the ambient air. The physical characteristics of cresols help explain the very low concentrations found in the ambient air. Cresols air concentrations are limited by the short lifetime of cresols in the atmosphere. During the day, cresols are removed by reaction with hydroxyl radicals. At night, nitrate radical reactions predominate. ATSDR reports cresols half-lives (calculated from kinetic data) as being less than ten minutes at night and less than ten hours during the day. As ATSDR summarized, "cresols have a short residence time in both day- and night-time air; despite continual releases of cresols to the atmosphere, levels are probably low."

Because cresols air concentrations are so low and cresols so rapidly degrade when emitted, the Panel does not believe that cresols in the air present general population exposure concerns. Indeed, EPA stated that it "has also determined that cresols released to the atmosphere are not expected to create an exposure problem." EPA further stated: "Cresols are not expected to persist in the atmosphere because (1) cresols have low estimated half-lives of less than 1 day; (2) they are sensitive to photolysis; and (3) the water solubility of cresols may be expected to cause transport from the atmosphere to the soil or aqueous environment."

Accordingly, the available data show that exposure to cresols is uniformly low, that cresols have a low potential for "persistence in the atmosphere" within the meaning of the Act, and that cresols should not, therefore, be considered a priority for evaluation and regulation.

Response. OEHHA staff attempted to develop as many health guidance values for listed Hot Spots chemicals as it could find data for. Some chemicals will be of more concern than others. Cresols may well be of lesser concern than most listed compounds. Otherwise the commentator encourages OEHHA not to develop a chronic inhalation REL for cresols because there is not a problem. But OEHHA's way of addressing the situation is to develop a chronic inhalation REL and then compare it with ambient levels and with modeled levels around Hot Spots facilities to determine if the levels are above or below the REL.

Comment 9. A majority of the general population exposure is not the result of manufacturing operations, but naturally occurring and other sources. Cresols are ubiquitous in the environment. The vast majority of cresols found in the environment are derived from natural sources. Cresols are formed as metabolites of microbial activity and are excreted in the urine of mammals, including humans. They are present in the lipids of a number of different plant species and are found in foods such as tomatoes, cooked asparagus, cheese, butter, oil, red wine, coffee, and black tea. The Panel has estimated that releases from naturally-occurring sources of cresols are at least 15 million pounds a year - nearly an order of magnitude greater than the 1.7 million pounds reported on EPA's Toxics Release Inventory (TRI) in 1995.

Cresols also are products of combustion both from natural and anthropogenic sources. Cresols are released to the air from fires associated with lightning and volcanic activity.

According to a study performed on behalf of EPA, the "major ambient source [of cresols] is automotive emissions. Cresols also have been detected in stack emissions from municipal waste incinerators, in emissions from vegetable material incineration, in fly ash from coal combustion, in emissions from wood combustion, and in cigarette smoke.

Thus, there are numerous and diverse sources of cresols air emissions. A significant portion of cresols air emissions is due to natural sources -- which are not a concern of California's laws governing toxic air contaminants. Indeed, releases from natural sources dwarf those from manufacturing operations and further confirm that cresols emissions from industrial facilities should not be given priority for evaluation and regulation under the Program.

Response. Since the chronic REL will be compared to off-site, annual ground level concentrations based on modeled facility emissions and not on monitoring data, the background concentrations should not interfere with use of the REL. On the other hand, if cresols are monitored, facility contributions to the outdoor concentration could be detected based on comparison of upwind and downwind concentrations of cresols. Many other compounds emitted by facilities also have measurable natural emissions.

Comment 10. New NESHAPS will further reduce the potential for exposure to cresols. Concentrations of cresols would be expected to be greatest in the vicinity of a facility that emits relatively large quantities of cresols to the air. A review of the TRI database for cresols indicates that, of the facilities which individually have relatively high emissions of cresols,

nearly all are or will be subject to NESHAPs pursuant to the 1990 Clean Air Act (CAA) Amendments. Implementation of these NESHAPs will reduce even further potential exposure to cresols. (These attachments are based on data received from the EPA TRI User Support Library and the National Library of Medicine's ToxNet database.)

Attachments 2, 3, and 4 summarize the top twenty emitters of cresol isomers and mixed cresols in 1993, 1994, and 1995, as reported to the TRI. Only one facility in California was among the top twenty emitters in 1993 and 1994 and no California facility is among the top twenty emitters in 1995 - the latest year for which TRI data is available. In each year, the top twenty emitters represented nearly 100 percent of all reported cresol isomers emissions and between 60 to 94 percent of mixed cresols emissions. Review of the Standard Industrial Classification (SIC) codes associated with each of these facilities indicates that they already are, or soon will be, subject to maximum achievable control technology (MACT) standards under various NESHAPs.

Attachment 5 lists the twenty SIC codes with the highest reported TRI emissions of cresol isomers and mixed cresols in 1995. These SIC codes include primarily pulp and paper operations, chemical manufacturers, surface coating operations, and other sources that are or will be subject to MACT standards established by forthcoming NESHAPs. These include the following:

- The HON: Many manufacturers of cresols themselves are subject to the hazardous organic NESHAP (HON) for the Synthetic organic Chemical Manufacturing Industry. For individual isomers, between 33 percent and 81 percent of emissions are associated with facilities in SIC Code 2865 for Cyclic Crudes and Intermediates or SIC Code 2869 for Industrial Organic Chemicals, both of which are subject to the HON. Cresols emissions will be reduced even further because many of the principal uses of cresols are as chemical intermediates in the manufacture of other chemicals that also are subject to the HON.
- Metal Coil (Surface Coating) Source Category: In 1995, approximately 60 percent of emissions of m-cresol and 28 percent of p-cresol and mixed cresols air releases are reported by facilities in SIC Code 3357 - Nonferrous Wire Drawing and Insulating. Cresols are used at these facilities as a solvent for wire enamel. MACT standards for the Metal Coil (Surface Coating) source category are scheduled for promulgation by the year 2000.L3-1 This category will address hazardous air pollutants (HAP) emissions from facilities that engage in the surface coating of continuous metal strips that are packaged in a roll or coil, such as wire.
- Amino and phenolic resin production: O-cresol is used in the production of epoxy-o-cresol resins and other resins. A presumptive MACT standard has been issued for amino and phenolic resin production that will require controls for cresols emissions.
- Pulp and paper mills: Over 55 percent of releases of mixed cresols isomers in 1995 were produced as a byproduct of pulp, paper, paperboard, and related manufacturing operations. Air emissions from pulp mills, paper mills, and paperboard mills are now subject to a NESHAP. This NESHAP is expected to reduce VOC emissions, including cresols, by

716,000 megagrams (Mg) annually. Existing mills became subject to the NESHAP in December 1996 and reductions in emissions will be reflected in future TRI reports.

- Petroleum refining: Cresols are produced as a byproduct of petroleum distillation. A NESHAP for petroleum operations has been promulgated and is expected to reduce total air emissions by 59 percent.
- Agricultural chemical production: Cresols are used in the production of agricultural chemicals such as 4,6-dinitro-o-cresol, which are subject to MACT controls.
- 4-chlor-2-methyl phenoxyacetic acid production: Cresols are used in the production of 4-chlor-2-methyl phenoxyacetic acid, for which MACT standards will be issued.
- Anthropogenic sources of cresols: Cresols also are produced as a byproduct of various combination operations. These sources of cresols will be reduced by MACT standards for hazardous waste boilers and incinerators; and off-site waste recovery operations.

In summary, most of the individual sources of cresols emissions will be regulated under a NESHAP within the next few years. The Panel believes that cresols emissions also will be reduced significantly in the near future as a result of voluntary efforts undertaken by industry. Panel members who are CMA member companies, for example, are committed to CMA's Responsible Care program, pursuant to which they have agreed to reduce emissions continually. All of these efforts will reduce concentrations of cresols in the ambient air and the need for evaluation or regulatory action under either the Program or the Act.

Response. OEHHA acknowledges that many individual emission sources of cresols will be regulated under various NESHAPs. A chronic REL will still be useful to gauge how far below the health benchmark the ambient concentration of cresols actually falls. In addition there are other environmental sources of cresol, such as cigarette smoke, for which a chronic REL will be useful as a health benchmark.

Comment 11. Measures implemented to reduce ozone levels will reduce emissions that include cresols from mobile and stationary sources. During the past several years, EPA has implemented an aggressive set of programs for achieving the National Ambient Air Quality Standard (NAAQS) for ozone, driven in large part by the new nonattainment provisions enacted by the 1990 CAA Amendments. These programs address ozone precursor emissions from both stationary and mobile sources, and thus are directed toward the reduction of smog levels in affected areas. The very low environmental concentrations of cresols will fall even lower as EPA makes continuing progress toward attaining the NAAQS for ozone, thus further reducing smog exposure levels in current "nonattainment" areas. EPA recently issued a final rule that has tightened the NAAQS for ozone, for example.

These programs will reduce cresols emissions, to which the general population is exposed, from automobile and diesel exhaust, coal-fired power plants, and other operations. These measures will reduce the levels of VOCs, such as cresols, and their contribution to

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ozone formation and to urban smog. Since actual emissions inventory data (and TRI data) must be considered in selecting chemical substances for priority evaluation and regulation, these current and future reductions in cresols emissions further demonstrate the inappropriateness of according priority treatment to cresols.

Response. Cresols are not being given priority treatment. OEHHA staff developed health guidance values for as many chemicals as possible listed under the Hot Spots program, which includes cresols. OEHHA has decided to base the chronic REL, not on the Uzhdavini et al. (1972) study, but on the USEPA RfD. The U.S. EPA RfD was based on 90 day toxicity studies done by USEPA and reported in 1986. The RfD is 0.05 mg/kg/day and the equivalent chronic REL is 180 $\mu\text{g}/\text{m}^3$. The critical effects are decreased body weight and neurotoxicity and the target organ is the nervous system.

Chemical Manufacturers Association – Diisocyanates Panel

Comments on the Proposed Toxicity Summaries and Reference Exposure Levels for **methylene diphenyl isocyanate (MDI) polymer** and **2,4- and 2,6-toluene diisocyanate (TDI)** were made by the Chemical Manufacturers Association Diisocyanates Panel. The Diisocyanates Panel represents the major domestic producers of methylene diphenyl isocyanate ("MDI") and toluene diisocyanate ("TDI"). Members of the Panel are: ARCO Chemical Company; BASF Corporation; Bayer Corporation; The Dow Chemical Company; and ICI Americas, Inc. OEHHA used the original USEPA RfC of 0.02 $\mu\text{g}/\text{m}^3$ based on hyperplasia of the olfactory epithelium in rats as the chronic REL for MDI polymer. For TDI OEHHA used the original USEPA RfC of 0.07 $\mu\text{g}/\text{m}^3$ based on decreased lung function in occupationally exposed workers as the chronic REL.

Comment 1: CALCULATION OF THE REL FOR POLYMERIC MDI. OEHHA's proposed Chronic Toxicity Summary and REL for polymeric MDI are based on the U.S. EPA's IRIS Summary and inhalation RfC. In April 1996, U.S. EPA announced a Pilot Program to update the IRIS database entries for eleven chemicals, including MDI. Pursuant to this program, U.S. EPA currently is reviewing and revising the IRIS summary and RfC for MDI. U.S. EPA expects to finalize the IRIS entry for MDI in February 1998. The Diisocyanates Panel urges OEHHA to defer its recommendation of an REL for MDI pending completion of the updated IRIS assessment and revised RfC.

In connection with the IRIS Pilot Program, U.S. EPA has circulated, for peer review, a draft Toxicological Review for MDI. The Draft Toxicological Review provides an updated summary of the available data for 4,4'-MDI ("monomeric MDI") and polymeric MDI. Based on this review, U.S. EPA proposed a revised RfC of $2 \times 10^{-4} \text{ mg}/\text{m}^3$ for MDI. The proposed RfC was based on the adjusted NOAEL of 0.036 mg/m^3 for nasal effects reported by Reuzel *et al.* (1994). EPA recalculated the human equivalent concentration for the NOAEL group ("NOAEL HEC") in the Reuzel Study based on a revised Regional Deposited Dose Ratio (RDDR) for MDI of 0.453. U.S. EPA's revised NOAEL HEC for MDI is 0.016 mg/m^3 . In calculating its revised RfC, EPA applied three uncertainty factors to the NOAEL HEC: (1) a factor of 10 was applied for intraindividual variation; (2) a factor of 3 was applied for database deficiencies; and (3) a factor of 3 was applied for intraspecies variation. Thus, U.S. EPA proposed an RfC for polymeric and monomeric MDI of $2 \times 10^{-4} \text{ mg}/\text{m}^3$, rather than the value of $2 \times 10^{-5} \text{ mg}/\text{m}^3$ that was previously calculated by EPA and on which OEHHA has relied for its proposed REL.

The CMA Diisocyanates Panel met with U.S. EPA and submitted comments on the IRIS assessment for MDI. The Panel presented a benchmark analysis of the Reuzel data developed by Drs. Bruce Allen and Melvin Andersen of ICF Kaiser. Based on this analysis, the Panel calculated an RfC for MDI of $9.64 \times 10^{-4} \text{ mg}/\text{m}^3$. The Panel urged U.S. EPA to adopt the benchmark methodology in calculating the RfC for MDI. The benchmark approach has received broad scientific support and U.S. EPA and others have recognized the advantages of the benchmark analysis as an alternative to relying on the NOAEL for non-cancer risk assessment. Advantages of the benchmark approach include reduced dependency on dose selection and spacing, more appropriate reflection of sample size, and better inclusion

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of dose-response information. The Panel's comments to EPA presenting its proposed RfC calculation based on the benchmark approach are included in Attachment I (Allen and Andersen (1997) is appended thereto).

EPA has not yet finalized the Toxicological Review and RfC for MDI. However, EPA staff have informed Panel representatives that the Agency intends to use the benchmark analysis in deriving the RfC. The Panel recommends that the State of California similarly adopt the benchmark approach in establishing its REL for MDI. For the reasons presented in the Panel's comments to EPA, the Panel believes that the REL for MDI should be 9.6×10^{-4} mg/m³.

Response: All USEPA Reference Concentrations (RfCs), available when the draft Technical Support Document (TSD) on chronic Reference Exposure Levels was released 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'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. Staff plan to review the scientific basis of each revised RfC when it becomes available and determine whether the scientific literature cited in the RfC is current. Appropriate RfCs will be submitted to the SRP for review and possible endorsement. OEHHA has reviewed the updated IRIS value for this chemical but it was released after October 1997 and OEHHA has not automatically accepted new RfCs. The new RfC for MDI is based on a benchmark dose approach, specifically a BMC10. OEHHA staff believe that consensus has not been reached on benchmark dose methodology. Both BMC10 and BMC05 approaches have their advantages and their proponents. The BMC10 is usually in the linear range of most models while the BMC05 more closely resembles a NOAEL than the BMC10 does. We will continue to review the updated RfC and present it to the SRP in our first update of chronic RELs. In the interim we have revised our proposed chronic REL from 0.02 µg/m³ to 0.5 µg/m³. (See next response.)

Comment 2: APPLICATION OF THE REL TO 4,4'-MDI MONOMER. OEHHA has stated that the "major limitation" of the proposed REL for MDI "is that it is based on data on exposures to MDI polymers." OEHHA states that, because "monomers frequently are much more toxic than polymers, ... OEHHA considers the value is only predictive of adverse effects of polymeric MDI. Effects of monomeric MDI may occur at concentrations several orders of magnitude lower than in the reported study on MDI polymer." This conclusion is not supported by the available data. The study by Reuzel *et al.* (1994) was conducted using the substance described commercially as polymeric MDI. This substance is not, however, a true

MDI polymer. Rather, it is more accurately characterized as MDI oligomer and is comprised of approximately 40 to 60% monomeric MDI and diminishing proportions of MDI dimer and other low-order MDI oligomers. Polymeric MDI also is the more commercially relevant *NMI* product and accounts for greater than 90% of MDI sold domestically.

In addition, the pulmonary effects reported by Reuzel *et al.* (1994) are generally consistent with those reported in the whole body inhalation study of monomeric MDI in rats by Hoymann *et al.* (1995) (abstract only) which reported effects, which were related primarily to the impairment of *MDI* clearance, only in the highest dose group. The International Isocyanate Institute (“III”) currently is sponsoring a comparison of the Hoymann data with the Reuzel (1994) data. Pathologists are reviewing the salient slides from the respiratory tract and the lung to assess the toxicology and also to understand the likely origin of the lesions observed in the two studies. Thus, it appears that monomeric *MDI* has a toxicity that is approximately the same as that of the polymeric MDI evaluated by Reuzel, and monomeric MDI does not have an effect level that is several orders of magnitude lower.

Further evidence of a lack of significant difference between polymeric and monomeric *MDI* is in parallel teratology studies performed by Garner *et al.* (1995) and Buschmann *et al.* (1996), respectively. In similar exposure scenarios, the no embryotoxic effect level was observed at 3 mg/m³ for monomeric MD, and 4 mg/m³ polymeric *MDI*. Maternal effects also were comparable between the polymeric MDI used in the Gainer study and the monomeric MDI in the Buschmann study. This further supports the conclusion that the toxicity of monomeric and polymeric MDI is similar.

Response: OEHHA has revised the text to account for the fact that nearly half the airborne material was monomer. OEHHA has also removed the database modifying factor of 10 since new studies on teratology have been published by Buschmann and others. The HEC calculation has also been revised. OEHHA has recalculated the chronic REL to be 0.5 µg/m³.

Comment 3: 2,6- AND 2,4-TOLUENE DIISOCYANATE: ON-GOING EPIDEMIOLOGY STUDIES OF TDI-EXPOSED WORKERS. OEHHA also relied on the IRIS RfC in proposing an REL for 2,4- and 2,6-TDI. The RfC for TDI is based on a 1982 epidemiology study by Diem *et al.* showing lung function decrement in workers occupationally exposed to TDI. ARCO Chemical Company is looking into the feasibility of updating the Diem study. In addition, efforts currently are underway to complete several other epidemiology studies of TDI-exposed workers. Studies of workers in TDI production facilities are being conducted by Dow Chemical Company and BASF. These studies are expected to be completed in 1998. These additional studies will expand and improve the available epidemiology database related to the human health effects of TDI exposure. Thus, the Panel urges OEHHA to await the results of these studies before finalizing its REL for TDI.

Response: The adoption of USEPA RfCs by OEHHA was described above. OEHHA is pleased that better data may become available and will review the studies when they are finished. We assume that USEPA will do the same. As of April 1999 OEHHA had not

received the updated studies. The current chronic REL is based on the data currently available.

Comment 4: CALCULATION OF THE RfC FOR TDI. U.S. EPA based the RfC for TDI on the epidemiology study of occupationally exposed workers by Diem *et al.* (1982). In calculating the RfC, U.S. EPA relied on the analysis of the data from the Diem study by Hasselblad (1993) to derive a NOAEL of 0.006 mg/m (0.9 ppb) for TDI.

The Diisocyanates Panel does not agree with Hasselblad's conclusion that the Diem study supports a NOAEL of 0.006 mg/m³. As explained in the attached letter by Dr. Gerald Ott of BASF Corporation (copy enclosed as Attachment 1), the statistical analysis of the epidemiology data conducted by Hasselblad is flawed in several respects. First, it selectively applies the data from Diem *et al.* and the other available epidemiology studies (in particular, by failing to consider the findings within the "former smoker" subpopulation). It also employs questionable procedures to estimate TDI concentrations consistent with the reported decline in forced expiratory volume (FEV1) and overlooks important biological parameters in deriving the NOAEL.

Moreover, the Diem Study was not designed to support the derivation of an overall NOAEL for TDI. The study evaluates cumulative exposure categories, which limits examination of exposure intensities. According to Garabrant and Levine (1994), the lung function decrement observed in the study was more likely related to episodic exposure to TDI at 6 levels above 20 ppb than to exposures in the 5 to 10 ppb range. Although the Diem *et al.* study does not permit the examination of exposure intensities, we believe that it is consistent with an overall NOAEL for TDI of 5 ppb.

The Panel further believes that U.S. EPA's use of the Diem Study to derive a NOAEL of 0.9 ppb for TDI is inconsistent with the TDI epidemiological database as a whole. The results reported by Diem *et al.* have not been replicated in larger and more recent studies of TDI-exposed workers, which rely on more precise methods for estimating exposures below 5 ppb. *see* Bulger *et al.* (1991); Jones *et al.* (1992); *see also* Allport *et al.* (1993). Overall, eight studies have failed to demonstrate lung function decrement from exposure to TDI at concentrations below 5 ppb. Thus, the overwhelming epidemiological evidence supports the conclusion that 5 ppb (0.036 mg/m³) is the no-effect-level for exposure to TDI with decreased living function being the most sensitive endpoint.

Response: These concerns should be addressed to the USEPA for possible reevaluation of the RfC.

Comment 5: The Diisocyanates Panel believes that the additional studies currently being conducted will strengthen the TDI database and provide a better data set from which to derive a NOAEL for TDI. For this additional reason, the Panel suggests that OEHHA await the results of these studies before finalizing its REL for TDI.

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Response: USEPA last updated the RfC for TDI on IRIS in September 1995. OEHHA is proceeding with the finalizing of the chronic REL based on information currently available but will review the new data when made available.

Chemical Manufacturers Association (CMA) - Ethylene Glycol Ethers Panel

Comments on the chronic REL for **ethylene glycol butyl ether** (EGBE) were received from the Ethylene Glycol Ethers Panel of the Chemical Manufacturers Association (CMA). The Chemical Manufacturers Association (CMA) Ethylene Glycol Ethers Panel is made up of the Dow Chemical Company, Eastman Chemical Company, Occidental Chemical Corporation, Shell Chemical Company, and Union Carbide Corporation. In the original TSD OEHHA derived a chronic REL of 200 $\mu\text{g}/\text{m}^3$ for EGBE based on a 1983 study by Dodd *et al.* showing decreased red blood cells in female rats. (The chronic REL has been revised to 700 $\mu\text{g}/\text{m}^3$ as described below.)

Comment 1: Significant new information should be employed in the calculation for EGBE. The TSD (pp. A-274 to A-278) proposes an REL for EGBE of 0.04 ppm (200 $\mu\text{g}/\text{m}^3$). This REL is derived by applying a cumulative uncertainty factor of 100 to an average experimental exposure No Observed Adverse Effect Level (NOAEL) of 4.5 ppm, which is equated to a Human Equivalent Concentration (HEC) based on default assumptions. The NOAEL is obtained from the Dodd (1983) 90-day inhalation study in rats that found a NOAEL of 25 ppm with 30 hour/week exposures (converted to continuous exposure by multiplying by 6/24 x 5/7). The cumulative uncertainty factor represents uncertainty factors of 10 each for (a) absence of a chronic study (the subchronic uncertainty factor) and (b) potential intraspecies differences.

A more appropriate REL for EGBE can be established by taking into account significant data on EGBE developed in recent years. These data, described below, should be employed to determine the HEC more accurately and to diminish the need for ten-fold uncertainty factors for intraspecies differences and for the absence of chronic data.

First, a validated physiologically-based pharmacokinetic (PBPK) model has been developed for EGBE. This PBPK model makes EGBE a compound for which "[c]omparison of human and animal pharmacokinetics and metabolism may be useful in selecting the relevant animal model for predicting human health effects" (TSD, at p. 17). As the enclosed publication describing the model (Corley et al.) shows, a more accurate determination of the HEC can be achieved by use of the PBPK model than is obtained by the standard default calculations employed in the TSD to convert discontinuous to continuous exposures (TSD, at p. 23).

Response: According to the Summary, Corley et al developed a PBPK model to describe the disposition of EGBE and its major metabolite, EGBEA (2-butoxyacetic acid), in rats and humans (Corley RA, Bormett GA, Ghanayem BI. Physiologically based pharmacokinetics of 2-butoxyethanol and its major metabolite, 2-butoxyacetic acid, in rats and humans. Toxicol Appl Pharmacol 1994;129(1):61-79). The model predicts that rats metabolize EGBE and eliminate the EGBEA faster per kg body weight than humans do. The balance of these two processes plus physiological differences between species result in higher predicted peak blood concentrations as well as total areas under the blood concentration time curves for EGBEA for rats versus humans. These species differences (and the fact that human blood is significantly less susceptible than rat blood to the hemolytic effects of EGBEA) indicate that

there is considerably less risk for hemolysis in humans from exposure to EGBE than predicted solely from standard toxicity studies with rats. In the original REL, instead of the interspecies UF default value of 10, OEHHA used an interspecies UF of 1, which indicates no likely interspecies differences. There is presently no guidance for using a factor of less than 1. To use a factor of less than 1, there would need to be reproducible data showing that the AUC of BAA in animals was a specific multiple of the AUC of BAA in humans.

Comment 2: Second, research conducted by Dr. Mark M. Udden at Baylor College of Medicine has demonstrated that blood from the elderly and from patients with hemolytic disorders does not show an increased sensitivity to the hemolytic effects of EGBE (which, as the TSD finds, are the critical toxicologic effects for establishment of an REL for EGBE). Enclosed are Dr. Udden's 1994 publications, which demonstrate that an uncertainty factor of ten for intraspecies differences is unwarranted.

Response: The demonstration that blood from the elderly and from patients with hemolytic disorders does not show an increased sensitivity to the hemolytic effects of EGBE is reason to depart from the intraspecies UF default value of 10. Since there may still be other sources of intraspecies uncertainty or variability, OEHHA staff have changed the intraspecies UF to 3. The cumulative UF is then 30 and the revised chronic REL is 0.15 ppm ($724.5 \mu\text{g}/\text{m}^3$, which rounds to $700 \mu\text{g}/\text{m}^3$).

Comment 3: The PBPK and Udden work are both described in more detail and employed in the enclosed Draft IRIS Support Document developed jointly by U.S. EPA scientists (Drs. Jeff Gift, Annie Jarabek, and Vicki Dellarco) and scientists from Panel member companies. Although the Support Document is not yet final and EPA's scientists have not yet reviewed all sections of it, the Panel believes its recommendations for an IRIS Reference Concentration (RfC) for EGBE are consistent with the views of all the scientists working on the IRIS Document. We anticipate working with EPA in 1998 to complete the Document and establish an RfC for EGBE.

We call to your attention, particularly, the derivation of an RfC in Chapter 6 of the IRIS Draft Document. The Draft calculates RfC's by several methods: (1) the standard IRIS RfC method, which is quite similar to California's REL methodology; (2) a methodology that incorporates information from the PBPK model; (3) a benchmark dose methodology; and (4) a methodology incorporating both the PBPK model and the benchmark dose methodology.

The Draft IRIS Support Document recommends adoption of the fourth method because it most fully employs the complete database. That methodology yields an RfC (or REL) of 15 ppm ($73 \text{ mg}/\text{m}^3$). We urge California to do the same. At a minimum, the State should take advantage of the PBPK model to adopt an REL of 6 ppm ($27 \text{ mg}/\text{m}^3$); such an REL would represent a more refined determination of the HEC based on the PBPK model and an acknowledgment that the Udden data shows an intraspecies uncertainty factor of 3 is fully sufficient.

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The Panel urges CalEPA to make use of the significant information we enclose in adopting an REL for EGBE. Alternatively, the State may wish to await EPA's adoption of an IRIS RfC. EPA announced this month that it intends to complete its IRIS review of EGBE in 1998 (63 Fed. Reg. 74, 75, Jan. 2, 1998). By waiting for a short period, CalEPA could also take advantage of the results of chronic bioassays with EGBE in mice and rats to be announced soon by the National Toxicology Program.

Response: The draft TSD was released in October 1997. As of June 1999 IRIS has no listing for EGBE or butoxyethanol. If and when it is finalized, OEHHA will review it, consider whether or not OEHHA should adopt the USEPA RfC, and forward its findings to the Scientific Review Panel for its consideration. We are not willing to wait for the USEPA RfC since there is no date certain for its completion. For now we are proposing a revised chronic REL of 0.15 ppm (700 µg/m³).

Chemical Manufacturers Association - Hydrazine Panel

Comments on the chronic REL for hydrazine were made by the Hydrazine Panel of the Chemical Manufacturers Association in a letter dated January 29, 1998. OEHHA developed a chronic REL of 0.2 µg/m³ based on the critical effects of amyloidosis of the liver and thyroid in hamsters (Vernot et al., 1985). OEHHA considered the lowest dose used (0.25 ppm) in hamsters to be a LOAEL since at this level the authors noted weight depression, mineralization of the kidney, and amyloidosis of the thyroid.

Comment 1: The Panel agrees that Vernot et al. is the appropriate study for derivation of the hydrazine chronic REL, but disagrees with OEHHA's interpretation of that study. The Panel believes that the 0.25 ppm dose level should be considered a NOAEL, not a LOAEL. Although the frequency of amyloidosis in hamsters exposed at this level was increased compared to controls, the frequency levels nonetheless were within the range reported in the literature for control animals.

Response: Controls reported in the same study are more relevant than historical controls for several reasons. Same study controls were exposed to the same environmental and dietary conditions and potential pathogen exposures as the exposed group. Also, the study control and exposed groups use the same strain of animal, whereas historical controls may have significant genetic differences from the test group. In addition to amyloidosis of the liver, thyroid and adrenal glands, male hamsters exposed to 0.25 ppm hydrazine showed other statistically significant increases over controls in liver hemosiderosis, bile duct hyperplasia, lymphadenitis of the lymph nodes, and mineralization of the kidney.

Comment 2: Even if the 0.25 ppm dose level is considered a LOAEL, an uncertainty factor often is overly conservative to extrapolate from a LOAEL to a NOAEL for these effects. The reported amyloidosis was at most an acceleration of a natural aging process, the incidence of the effect was within the levels normally seen in control populations, and hamster amyloidosis is an effect that may have questionable relevance for human health hazard assessment. For these reasons, an uncertainty factor of no more than three is appropriate to extrapolate from a LOAEL to a NOAEL.

Response: The relevance of historical controls was discussed in response to Comment 1. The basis for the statement that amyloidosis is not relevant to human health hazard assessment is unclear. A diverse array of human medical disorders, both neurologic and systemic, are associated with extensive amyloidosis. Human amyloidosis can be severe, with some forms associated with a median survival duration after diagnosis of as low as 25 months (Raikumar SV, Gertz MA, Kyle RA. Prognosis of patients with primary systemic amyloidosis who present with dominant neuropathy. *Am J Med* 1998 Mar;104(3):232-7). Amyloid deposits may cause direct harm or may be markers for an underlying metabolic disorder. Thus amyloidosis does not fit the mild effect category described in the OEHHA chronic REL document.

Comment 3. Alternatively, it may be advantageous to calculate the hydrazine REL using a benchmark concentration approach. Such an approach uses all of the available study data and avoids the difficulties associated with determining whether a NOAEL has been identified in a given study.

Response. The potential use of benchmark concentration (BMC) modeling was extensively evaluated. Dose-response modeling of the data of Vernot and associates (1985) illustrates some of the complexities of using BMCs. Several mathematical models (probit, Weibull, quantal quadratic, quantal linear, and gamma models using USEPA BMDS software) were fit to the data. None of the models fit well the unusual dose-response relationship where all three concentrations, covering a 20-fold range, were associated with a significantly increased incidence of liver amyloidosis relative to controls, but where the dose-response slope appears very shallow over this range. The models able to converge on a solution tended to project a BMC₁₀ of 1 to 3 ppm. However, all the fits are questionable since they are based on assuming (1) that the true control and low dose incidence are both 30-35%, when the observed incidences were 23% and 42% respectively, and (2) that the dose-response slopes are modeled to be much steeper than actually observed. This represents one possible explanation: that the true dose-response relationship is steeper than observed due to sampling error. However, alternative explanations, more consistent with the observed data, can not be ruled out. One explanation would be that the dose-response relationship is not unimodal; there may be a susceptible subgroup at increased risk of amyloidosis at relatively low concentrations and a second more resistant subgroup. Secondly, caution against using poorly modeled BMCs or those exceeding a LOAEL has been emphasized (Gaylor et al., 1998, Procedures for calculating benchmark doses for health risk assessment, Regul. Toxicol. Pharmacol. 28, 150-164). For the above reasons a BMC can not substitute for the experimental observations in this case.

Comment 4: OEHHA should remove the reference to endocrine effects from its chronic toxicity summary for hydrazine. Although amyloidosis was seen in the thyroid of hamsters, no effects on the endocrine system were noted even at the highest doses studied. Nor have any other studies reported adverse effects on the endocrine system from exposure to hydrazine.

Response: The categorization of adverse health effects is intended to denote only the general category of organ system affected. Thus, as thyroid amyloidosis was observed and the thyroid is an endocrine gland, the effect is noted as “endocrine,” and is only meant to imply an endocrine gland was affected, and not to imply that abnormalities in hormone production are anticipated.

Comment 5: The chronic toxicity summary gives undue weight to the poorly-reported findings in the Sotaniemi case report. Other epidemiological studies that are not discussed by OEHHA do not corroborate the findings of Sotaniemi. The Panel therefore requests that OEHHA revise its discussion on the effects of human exposure to hydrazine to provide a more balanced presentation of the available data.

Response: The Sotaniemi paper is not an epidemiological study but rather a case report. Both this paper and the description of this case report as presented in the draft chronic REL document were reviewed. The case was well presented in the original report and the chronic REL review was found to be accurate. Some additional text is being added to clarify some aspects of the case: (1) a cause and effect relationship between the hydrazine exposures and the sudden death of the worker is strongly suggested but not proven; (2) the worker was 59 years old and healthy prior to hydrazine exposure; and (3) the worker's once per week exposure was reported to be routinely followed by 1-2 days of conjunctivitis and tremor.

Only a single epidemiological study of human hydrazine exposures was found and a description is being added to the OEHHA document. This study (Wald, 1984, *IARC Scientific Publication* 65:75-80; Wald et al., 1984, *British Journal of Industrial Medicine* 41:31-34) was based on a review of medical records of 406 of 427 male workers at a single chemical factory. Only 78 of these workers were believed to have had more than incidental exposure to hydrazine. Only cumulative mortality was reviewed. Health effects reported during or after hydrazine exposure were not examined. No increase in mortality was noted for lung cancer, other cancers, or causes other than cancer. However, this small study has little power to detect increased mortality, and age of death was not examined.

Chemical Manufacturers Association (CMA) - Hydrogen Fluoride Panel

The Chemical Manufacturers Association Hydrogen Fluoride Panel (Panel) on January 29, 1998 submitted comments on the October 1997 draft OEHHA chronic inhalation reference exposure level (REL) for fluorides, including hydrogen fluoride (HF). The Hydrogen Fluoride Panel includes 3M Company, Allied Signal Inc., Aluminum Company of America, Chemtech Products, Inc., Daikin America Inc., DuPont, Elf Atochem, NA, Inc., General Chemical, Industrial Quimica de Mexico, S.A. de C.V., LaRoche Industries Inc., LCI/Norfluor, Occidental Chemical Corp., OSRAM Sylvania Inc., and Quimica Fluor S.A.

Comment 1. In general, the Panel believes the chronic toxic summary for fluorides prepared by OEHHA is well-written. However, for reasons set forth below, the Panel believes the REL should be higher by a factor of three. In the case of hydrogen fluoride and other fluorides, an uncertainty factor of three should be sufficient to protect sensitive individuals.

The Technical Support Document discusses the application of an uncertainty factor to account for "the potential for greater susceptibility in subpopulations, including infants and children (p. 29-30). OEHHA indicates it generally will use an uncertainty factor of ten to protect sensitive individuals (p. 30). In the presentation at the OEHHA Workshop held in Long Beach, California on December 4, 1997, however, OEHHA staff presented a slide showing the possibility of using uncertainty factors of one, three or ten for "sensitive subgroups" when justified. The Panel believes a factor of three is scientifically appropriate in the case of fluorides.

As noted in the Technical Support Document, the steepness of the dose-response relationship affects the adequacy of the uncertainty factor for sensitive individuals. The Panel believes that the abundant information available on fluorides, with studies of large and varied human populations, documents a dose-response which would justify an uncertainty factor of three, rather than ten. Much of this information is summarized in a recent National Research Council (NRC) publication ("Health Effects of Ingested Fluoride," National Research Council, National Academy Press, 1993). The NRC publication addresses oral data, and the Panel recognizes that OEHHA typically would prefer to base an inhalation REL on inhalation studies. Nevertheless, it is generally recognized that oral exposure data can provide valuable information (Technical Support Document, p. 30-31) and, specifically in the case of fluorides, it is known that 75 to 90 percent of ingested fluoride is absorbed (Ekstrand, J., Boreus, A.L.O. and Norlin A., 1977, Pharmacokinetics of Fluoride in Man after Single and Multiple Oral Doses, Eur. J. Clin. Pharmacol. 12:311-317). The level of absorption is certainly equivalent to the amount absorbed via inhalation (approximately 99%) (Morris, J.B. and Smith, F.A., 1982, Regional Deposition and Absorption of Inhaled Hydrogen Fluoride in the Rat, Toxicol. Appl. Pharmacol. 62:91-99). Thus, the Panel believes the extensive oral data provide a scientifically sound basis for evaluating the appropriate uncertainty factor for protecting sensitive individuals. Further, since fluoride elimination is primarily via renal clearance, people with impaired renal function or nutritional deficiencies, e.g., Vitamin C or calcium, may be expected to have a greater susceptibility to fluoride toxicity. However, data from Spencer et al. (Spencer, H., Kramer, L., Gatzka, C.A., 1980, Fluoride Metabolism in Patients with Chronic Renal Failure. Arch. Intern. Med. 140:1331-35) indicate that retention is not

more than about three-fold between those with normal renal clearance and those with impaired clearance. Therefore, these data would support the use of a less conservative uncertainty factor.

As a scientific "reality check," one can compare OEHHA's proposed REL of 0.03 mg/m³ with the oral reference dose (RfD) of 0.06 mg/kg/day published by U.S. EPA in its Integrated Risk Information System (IRIS) database. Assuming a person breathes 20 cubic meters of air per day and the air contains HF at a concentration equal to the proposed REL, that person would inhale (but not absorb) 0.6 mg fluoride per day. By comparison, ingesting fluoride at the level of the oral RfD, a 50 kg adult would ingest 3.0 mg per day. One could also use for comparison California's Drinking Water Standard of 1400-2400 µg/L fluoride ion (compared to USEPA's 4000-8000 µg/L, under which an adult could safely ingest at least 2.8 mg (2 liters x 1400 µg) fluoride ion per day. These comparisons show that the proposed chronic inhalation REL for fluorides is approximately five-fold more conservative than USEPA's RfD or the State of California's existing drinking water standard. Using an uncertainty factor of three to account for potential human variability would produce an REL that is consistent with these other regulatory standards.

Response. The intent of the OEHHA reference exposure levels is to provide health-based guidance. Thus regulatory standards, which consider other issues in addition to health effects, were not considered in the development of the RELs. OEHHA RELs are intended to protect the general public, including potentially sensitive groups such as children, the elderly, and those with chronic illness. Chronic RELs, similar to USEPA RfC values, are meant to be protective to the general public rather than predictive of risk. Thus, exposure to a REL concentration may or may not be associated with adverse effects. But because of uncertainties in available data, RELs are calculated at some lower concentration than that at which adverse effects have been observed. The cumulative uncertainty factor of 10 for HF is one of the lowest used among more than 100 OEHHA chronic RELs and USEPA RfCs.

Comment 2. In summarizing the article by Derryberry et al. (1963), the chronic toxicity summary overstates the extent to which bone density increases were observed in workers. The chronic toxicity summary characterizes bone density for several workers as "high" (Table 1). However, the actual Derryberry et al. article simply notes with an asterisk those individuals who had "bone density changes." The study originally planned to include three categories of osseous changes: 1) normal skeletal density; 2) minimal or questionable bone changes indicative of increased bone density; and 3) positive characteristics of increased bone density. Derryberry reported no individuals in the latter category. According to the radiologist, none of the x-rays showed sufficient increase in bone density to be recognized as such in routine radiological practice. Thus, the authors did not express the opinion that "[t]he increased bone density observed was considered as indicating adverse effects had occurred" (Chronic Toxicity Summary, p. A-315). The study by Riggs et al. (1990), which also is cited in the chronic toxicity summary, employed pharmacologic doses of fluorides at levels almost four times those known to result in crippling fluorosis (USEPA, National Primary Drinking Water Regulations; Fluoride; 50 Fed. Reg. 47,142, Nov. 14, 1985). While it may be reasonable to use such questionable radiologic changes as the endpoint for determining a

lowest observed effect level (LOEL) or no observed effect level (NOEL), OEHHA has provided insufficient justification to show that the levels chosen represent a lowest observed adverse effect level or a no observed adverse effect level.

Response. Changes are being made in response to this comment. Text modifications will better clarify the minimal changes in bone density reported by Derryberry and associates. However, the minimal extent of the findings do not mean they are not relevant to developing RELs to protect the general public. In general, it is necessary to consider studies where statistically significant changes of questionable biological significance are consistent with frank adverse effects at higher exposures in other studies. A similar situation would be where high exposures to a chemical were established as causing clear liver toxicity and lower exposures in another study caused minimal effects such as increased liver weight without other observable effects. In these cases it is a reasonable public health goal to avoid exposures which begin going down the path from minimal to frank adverse effects, especially as subgroups in the populations may have preexisting conditions that render them especially susceptible to changes in a particular organ.

Comment 3. Section IV of the chronic toxicity summary ("Effects of Human Exposure") summarizes the few, and mostly older, reports of the effects of human inhalation exposure to generally very high levels of hydrofluoric acid. There is no mention of the abundant literature on human exposure to fluorides by the oral route, nor is there any indication that a certain level of fluoride intake is recommended by public health authorities for the prevention of dental caries (NRC publication, *supra*, n.6). This information should be included so that readers will be aware that fluoride is among those substances, which have beneficial effects at certain levels, with harmful effects only at higher levels.

The study on which OEHHA is relying to set the REL (Derryberry et al.) reports airborne exposures to fluorides. However, the authors note, "The principal routes through which these compounds are introduced into the human system are by ingestion or swallowing of dust containing fluorides and by inhalation of fluoride compounds." Thus, ingestion of fluorides was a major source of fluoride exposure even for those workers studied by Derryberry et al.

Response. *Text is being added to review normal dietary exposures to fluorides and the use of fluoride supplements and to augment generally the health effects of fluorides other than hydrogen fluoride.*

Comment 4. The document should be revised to state more clearly that the REL applies to all fluorides, not just to hydrogen fluoride. The title of the chronic toxicity summary is "FLUORIDES including HYDROGEN FLUORIDE," with subtitles referencing hydrofluoric acid (aqueous solution) and hydrogen fluoride (as a gas). The only substance discussed under "Major Sources and Uses" is hydrogen fluoride, and the literature review also mostly addresses hydrogen fluoride. The article by Derryberry et al., however, is based on exposures to fluorides, not exposure to HF. Fluoride from HF and fluoride from other sources are

essentially indistinguishable by the human body (as well as by air sampling methods). There are many sources of fluoride other than HF. The Panel agrees that it is appropriate to recommend an REL for all fluorides, not just hydrogen fluoride. The Panel recommends that an additional statement be added to the introduction to the chronic toxicity summary to make clear that the REL applies to all fluorides, not just to hydrogen fluoride, even though much of the underlying data is derived from HF studies.

Response. Changes have been made in response to this comment. As noted in the comment, OEHHA relied primarily on health effects data on hydrogen fluoride because most of the available fluoride inhalation data are for this chemical.

Chemical Manufacturers Association (CMA) - Maleic Anhydride Panel

The Chemical Manufacturers Association (CMA) Maleic Anhydride Panel (Amoco Chemical Company, Ashland Chemical Company, Bayer Corporation, Huntsman Corporation) submitted comments on the OEHHA proposed chronic Reference Exposure Level for **maleic anhydride** on January 29, 1998. In the draft TSD OEHHA developed a chronic REL of 0.2 $\mu\text{g}/\text{m}^3$ based on respiratory tract effects in rats, hamsters, and monkeys. (The chronic REL has been revised as described below in the Responses to Comments 4 and 5.)

Comment 1. As we detail below, the strongest basis for a REL is the monkey data by Short et al., which leads to an inhalation REL of 0.06 mg/m^3 (60 $\mu\text{g}/\text{m}^3$). This is the preferred approach for maleic anhydride, which is highly reactive in nasal tissues, because of the strength of the Short monkey data and because the monkey respiratory system is more like that of humans than are rats or hamsters.

Response. In light of these comments, OEHHA has undertaken a reevaluation of the proposed maleic anhydride chronic REL, as presented below. However, as noted below in the response to Comment 2, OEHHA staff do not believe that the monkey data should be used to develop the REL.

Comment 2. California proposes an REL for maleic anhydride of 0.0002 mg/m^3 (0.2 $\mu\text{g}/\text{m}^3$, 0.05 ppb) based on the 1.1 mg/m^3 Lowest Observed Adverse Effect Level (LOAEL) it found for rats, hamsters and monkeys in the Short, et al., six-month inhalation studies (R.D. Short, et al., 1988, A six-month multispecies inhalation study with maleic anhydride, *Fundamen. Appl. Toxicol.* 10:517-524). The State says the study did not find a No Observed Adverse Effect Level (NOAEL) and cites as the critical effects hyperplastic change and neutrophilic infiltration of the nasal epithelium and respiratory irritation. It proposes converting the 6-hour/day, 5 days/week LOAEL exposures of 1.1 mg/m^3 to an average experimental exposure of 0.20 mg/m^3 and converting that value to a human equivalent concentration (HEC), using standard default values for gases, of 0.019 mg/m^3 . To calculate the REL, the HEC is divided by 100 to account for uncertainty factors of 3 for use of a LOAEL rather than a NOAEL, 3 for interspecies variability, and 10 for intraspecies variability.

In developing its REL, California relied on highly conservative assumptions that do not present an accurate and balanced assessment of the human health risks from exposure to maleic anhydride. As we explain below, the REL should be based on the Short monkey data that are more relevant to humans. Maleic anhydride is highly irritating to nasal tissue. The Short studies of inhalation exposure for six months resulted in histological changes to nasal tissue that were indicative of such irritation.

In rats and hamsters, the histological changes observed by Short consisted of nasal epithelial hyperplasia (trace to mild) and/or metaplasia and inflammation (neutrophilic infiltration). Such lesions occurring as a result of inhalation exposure to a known strong irritant such as maleic anhydride are considered a reversible and adaptive response rather than

an adverse effect. (Monticello, T.M., K.T. Morgan, L. Uriah, 1990, Nonneoplastic lesions in rats and mice, *Environ. Health Perspect.*, 85:249-274; Reuben, Z. and C.G. Rousseaux, 1991, The limitations of toxicologic pathology, In *Handbook of Toxicologic Pathology*, pp. 131-142, San Diego, Academic Press). Considerations of the adversity of hyperplastic and metaplastic lesions in rodent nasal cavities have been evaluated in the context of determining a critical effect for setting an EPA RfC (Foureman, G.L., M.M. Greenberg, G.K. Sangha, B.P. Stuart, R.N. Shiotsuka and J.H. Thyssen, 1994, Evaluation of nasal tract lesions in derivation of the inhalation reference concentration for hexamethylene diisocyanate, *Inhalation Toxicology*, 6(suppl): 341-355) and have been adopted by EPA for an RfC (Greenberg, M.M. and G.L. Foureman, 1995, Derivation of the inhalation reference concentration for hexamethylene diisocyanate, *Toxic Substances Mechanisms*, 14: 151-167).

By contrast, only slight inflammation, consisting of an infiltration of neutrophils, was observed by Short in the nasal tissues of monkeys. Pulmonary function tests in monkeys revealed no compound-related effects.

California chose the hamster data from the Short study as the basis for the REL, but the hamster data provides an inappropriate model for human health risk assessment and significantly overstates potential risks. The Panel recommends use of the monkey data because these results would provide a better estimate of the possible effect of maleic anhydride on the human nasal airway. Both monkeys and humans are nose and mouth breathers, whereas rodents are obligate nose breathers (Proctor, D.E., and Chang, J.C.F., 1983, Comparative anatomy and physiology of the nasal cavity, In: *Nasal Tumors in Animals and Man*, Vol. III, pp. 1-33 (G. Reznik and S.F. Stinson, Eds.), CRC Press, Boca Raton, FL; Bridger, M.W., and van Nostrand, A.W., 1978, The nose and paranasal sinuses - applied surgical anatomy, *J Otolaryngol.* 7 (suppl. 6): 1-33; Morgan, K.T., and Monticello, T.M., 1990, Airflow, gas deposition, and lesion distribution in the nasal passages. *Environ. Health Persp.* 88: 209-218; Harkema, JR., 1991, Comparative aspects of nasal airway.) Further, the anatomical structure of the nasal cavity of the monkey is more like the human nasal cavity compared to rodents (Harkema, JR., 1990, Comparative pathology of the nasal mucosa in laboratory animals exposed to inhaled irritants. *Environ. Health Perspect.* 85: 231-238). Thus, for a highly reactive chemical such as maleic anhydride, which produces nasal irritation with no systemic toxicity, human risk assessment should use the monkey data.

Response. The observation by Short and colleagues that monkeys, unlike rats and hamsters, did not develop hyperplastic changes of the nasal epithelium was discussed in the presentation of the proposed chronic REL. The difficulties in adopting the primate data as the sole basis for deriving a REL are: (1) neutrophilic infiltration of the nasal epithelium and irritation were observed in primates at all dose levels, and (2) only 3 monkeys per sex per dose were studied by Short et al. (1988) thus giving little evidence whether such changes might occur in a significant minority of monkeys. With only 3 animals per group there are only 16 possible outcomes of the experiment and, on chance alone, each one would occur with a probability of 0.0625. Thus no outcome has a $p < 0.05$. In addition, as noted in the document, challenge with particulate maleic anhydride at an average concentration of 0.83 mg/m^3 has resulted in acute asthmatic response in a sensitized worker.

Comment 3. The Short study finds a NOAEL for monkeys of 9.8 mg/m³. Monkeys exhibited mucosal and/or submucosal infiltration of neutrophils into the nasal tissues at all exposure levels, but no morphological changes such as hyperplasia were observed. Since maleic anhydride is known to be very irritating to nasal tissue, this slight inflammatory response in monkeys is considered to result from the acute irritating properties of maleic anhydride.

Response. The common situation where the primary adverse effect observed for a chemical is an *acute* irritation response presents a special difficulty in developing an appropriate *chronic* REL. The chronic REL must still be protective against such effects that can be repeatedly or chronically induced as a result of long-term exposures to acutely irritating substances. The scenario in which a subset of sensitized individuals develop an atopic response to lower levels than might be a concern for non-sensitized individuals is an additional complication. Both of these issues apply to maleic anhydride.

Comment 4. The Panel thus proposes that the REL be based on the monkey data as follows:

NOAEL	9.8 mg/m ³
Average experimental exposure:	1.75 mg/m ³ for NOAEL group
Human equivalent concentration:	1.75 mg/m ³ for NOAEL group (monkeys considered equal to humans based on similar anatomy of the nasal cavity and similar surface area to volume ratio)
Subchronic uncertainty factor:	1
Interspecies uncertainty factor:	3
Intraspecies uncertainty factor:	10
Cumulative uncertainty factor:	30
Inhalation REL:	0.06 mg/m ³ (60 µg/m ³)

This monkey data-derived REL is both based on the best animal model for human risk assessment of maleic anhydride and within an order of magnitude of the REL values that would apply if the Short rat or hamster data were used, as shown below. Because the only systemic effects found in rodents in the Short studies are the weight losses at the highest doses in male and female rats, a REL derived from that data would be:

NOAEL	3.3 mg/m ³
Exposure continuity:	6 h/day, 5 days/week
Average experimental exposure:	0.6 mg/m ³ for NOAEL group
RGDR:	$(0.395 \text{ m}^3 / 15 \text{ cm}^2) / (20 \text{ m}^3 / 200 \text{ cm}^2) = 0.263$
Human equivalent concentration:	$0.6 \text{ mg/m}^3 \times 0.263 = 0.16 \text{ mg/m}^3$
Exposure duration:	6 months
Subchronic uncertainty factor:	1
Interspecies uncertainty factor:	3
Intraspecies uncertainty factor:	10

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Cumulative uncertainty factor: 30
 Inhalation reference exposure level: 0.005 mg/m³ or 5 µg/m³

Similarly, a hamster-based REL would be based on a NOAEL as described below. The mild to trace hyperplasia and metaplasia observed in hamsters are not considered to be adverse effects for the reasons described above at page 2. The incidence of these lesions appears to be slightly lower in hamsters than in rats. As there were no compound-related effects observed for body weight in hamsters, the concentration of 9.8 mg/m³ is a NOAEL for hamsters. Thus, the REL would be calculated as follows:

NOAEL: 9.8 mg/m³
 Exposure Continuity: 6 hr/day, 5 days/week
 Average experimental exposure: 1.75 mg/m³
 RGDR 0.096 (hamster)
 Human equivalent concentration: 1.75 mg/m³ x 0.096 = 0.168 mg/m³
 Exposure duration: 6 months
 Subchronic uncertainty factor 1
 Interspecies uncertainty factor 3
 Intraspecies uncertainty factor 10
 Cumulative uncertainty factor 30
 Inhalation reference exposure level 0.006 mg/m³ or 6 µg/m³

Response. The derivation of the chronic REL for maleic anhydride was reexamined in light of these comments. The results of three alternative analyses are presented in the following tables.

A. Alternative analysis for the repeated acute effects of irritation and inflammatory responses among the larger experimental group size rodent study.

<i>Study</i>	Short <i>et al.</i> , 1988
<i>Study population</i>	Rats (15/sex/group), hamsters (15/sex/group)
<i>Exposure method</i>	Discontinuous inhalation exposure (0, 1.1, 3.3, or 9.8 mg/m ³)
<i>Critical effects</i>	Neutrophilic infiltration of the nasal epithelium, respiratory irritation in all species
<i>LOAEL</i>	1.1 mg/m ³
<i>NOAEL</i>	Not observed
<i>Exposure continuity</i>	6 hr/day, 5 days/week
<i>Average experimental exposure</i>	Relevant exposure assumed to be 1.1 mg/m ³ for repetitive acute exposures
<i>Human equivalent concentration</i>	0.100 mg/m ³ for LOAEL group (gas with extrathoracic respiratory effects, RGDR = 0.096, based on hamster data)
<i>Exposure duration</i>	6 months
<i>LOAEL uncertainty factor</i>	3
<i>Subchronic uncertainty factor</i>	1

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<i>Interspecies uncertainty factor</i>	3
<i>Intraspecies uncertainty factor</i>	10
<i>Cumulative uncertainty factor</i>	100
<i>Inhalation reference exposure level</i>	0.001 mg/m ³ (1 µg/m ³ , 0.0002 ppm, 0.2 ppb)

B. Alternative analysis for repeated acute irritation and inflammatory responses in the smaller experimental group size monkey study

<i>Study</i>	Short <i>et al.</i> , 1988
<i>Study population</i>	Monkeys (3/sex/group)
<i>Exposure method</i>	Discontinuous inhalation exposure (0, 1.1, 3.3, or 9.8 mg/m ³)
<i>Critical effects</i>	Neutrophilic infiltration of the nasal epithelium, respiratory irritation in all species
<i>LOAEL</i>	1.1 mg/m ³
<i>NOAEL</i>	Not observed
<i>Exposure continuity</i>	6 hr/day, 5 days/week
<i>Average experimental exposure</i>	Relevant exposure assumed to be 1.1 mg/m ³ for repetitive acute exposures
<i>Human equivalent concentration</i>	Not determined (inadequate data for monkeys)
<i>Exposure duration</i>	6 months
<i>LOAEL uncertainty factor</i>	3
<i>Subchronic uncertainty factor</i>	1 (due to acute inflammatory character of response)
<i>Interspecies uncertainty factor</i>	10 (default since HEC could not be calculated)
<i>Intraspecies uncertainty factor</i>	10
<i>Cumulative uncertainty factor</i>	300
<i>Inhalation reference exposure level</i>	0.004 mg/m ³ (4 µg/m ³ , 0.001 ppm, 1 ppb)

C. Alternative analysis for chronic effects in the smaller group size monkey study

<i>Study</i>	Short <i>et al.</i> , 1988
<i>Study population</i>	Monkeys (3/sex/group)
<i>Exposure method</i>	Discontinuous inhalation exposure (0, 1.1, 3.3, or 9.8 mg/m ³)
<i>Critical effects</i>	Hyperplastic changes of the nasal epithelium
<i>LOAEL</i>	Not observed (1.1 mg/m ³ in rats and hamsters)
<i>NOAEL</i>	9.8 mg/m ³
<i>Exposure continuity</i>	6 hr/day, 5 days/week
<i>Average experimental exposure</i>	1.75 mg/m ³ for NOAEL group
<i>Human equivalent concentration</i>	Not determined (inadequate data for monkeys)
<i>Exposure duration</i>	6 months
<i>LOAEL uncertainty factor</i>	1
<i>Subchronic uncertainty factor</i>	10 (less than 8% of lifetime)

<i>Interspecies uncertainty factor</i>	10 (default since HEC could not be calculated)
<i>Intraspecies uncertainty factor</i>	10
<i>Cumulative uncertainty factor</i>	1,000
<i>Inhalation reference exposure level</i>	0.002 mg/m ³ (2 µg/m ³ , 0.0005 ppm, 0.5 ppb)

Comment 5. In sum, the strongest basis for a REL is the monkey data by Short et al., which leads to an inhalation REL of 0.06 mg/m³ (60 µg/m³). This is the preferred approach for maleic anhydride, which is highly reactive in nasal tissues, because of the strength of the Short monkey data and because the monkey respiratory system is more like that of humans than are rats or hamsters. RELs based on rats (5 µg/m³) or hamsters (6 µg/m³) are consistent between these two species and within an order of magnitude of the REL based on the monkey data. The rat and hamster values are lower primarily because they are nasal breathers and have a more tortuous architecture in their nasal cavities that tends to enhance the retention of reactive vapors and gases, factors not applicable to humans.

Response. The response to Comment 4 presented a reassessment by OEHHA with 3 alternative analyses that incorporate consideration of the lack of evidence of cumulative chronic effects or systemic toxicity differing substantially from acute irritative effects. These analyses using guidelines developed by USEPA and OEHHA resulted in possible chronic REL values of 1, 2, and 4 µg/m³. Because of the small size of the monkey group studied and several reports implicating maleic anhydride in asthmatic responses in sensitized individuals, OEHHA recommends the first reanalysis (A. Alternative analysis for the repeated acute effects of irritation and inflammatory responses among the larger experimental group size rodent study). This reanalysis resulted in a chronic REL for maleic anhydride of 1 µg/m³ to protect against both chronic and repetitively induced acute adverse effects.

Chemical Manufacturers Association - Olefins Panel

Comments on the chronic RELs for **ethylene** and **1,3-butadiene** 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. (Comments on propylene were dealt with previously.)

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.

I. Comments regarding the ethylene REL. OEHHA developed a chronic inhalation REL of 100 µg/m³ for ethylene based on the chronic REL of ethylene oxide, to which ethylene is metabolized.

Comment 1. OEHHA should not use an ethylene oxide study to establish the REL for ethylene. It is fundamental to sound science that, when sufficient data are available, the risk assessment for a chemical should be based on studies of the chemical itself. To do otherwise is scientifically unjustified and introduces unnecessary uncertainties into the risk assessment. Use of surrogates (e.g., structural analogue relationships or metabolite studies) may be appropriate if there is insufficient data on the chemical itself, but, even then, such approaches should be used with caution.

Although sufficient data exist to conduct a risk assessment for ethylene [discussed in more detail below], OEHHA has used data for ethylene oxide. Such an approach - using data on a metabolic product when data on the chemical are available - is highly unusual and is unprecedented in U.S. EPA and other agency evaluations of ethylene. The Panel strongly objects to this approach.

Response. OEHHA staff agree that such approaches are unusual and should be used with caution. However, when the chronic REL was developed, OEHHA staff wanted to base as many RELs as possible on human data. Since ethylene is metabolized to ethylene oxide, we originally decided to base the REL for ethylene on the REL for ethylene oxide, which was based on human data. However, based partly on critiques of the Schulte et al. by the Chemical Industry Institute of Toxicology (CIIT) and the Ethylene Oxide Industry Council of the CMA, we are revising the chronic REL for ethylene oxide and basing it on the report of neurotoxicity in EtO exposed workers by Klees et al. (1990).

Comment 2. The data do not support OEHHA's use of an ethylene oxide study to establish the ethylene REL. OEHHA's rationale for using the ethylene oxide data is that 1) ethylene is metabolized to ethylene oxide and 2) humans may be more sensitive to effects from ethylene oxide inhalation than are animals in experimental studies. The OEHHA summary states that, at the maximum rate of metabolism of ethylene in the rat, the theoretical ethylene oxide exposure is 5.6 ppm, which is below observed NOAEL levels in the rat of 10-50 ppm

ethylene oxide. OEHHA then speculates that humans may be more sensitive to ethylene oxide exposure than experimental animals, because "[n]on-cancer adverse effects (LOAELs) have been found at concentrations of 10 to 0.17 ppm (Zampollo *et al.*, 1984; Estrin *et al.*, 1987; Schulte *et al.*, 1995)." A comprehensive review of these studies shows they do not support this contention.

The 0.17 ppm value is taken from Schulte *et al.* (1995), which OEHHA also used as the basis for the ethylene oxide REL. The Schulte *et al.*, study is discussed extensively in comments which are being submitted separately by the Ethylene Oxide Industry Council, which are incorporated herein by reference. Those comments show: 1) the Schulte study is of questionable validity because of its small control population; 2) the effects noted by Schulte *et al.*, have not been demonstrated to have clinical significance -- that is, they are not adverse effects; and 3) the exposure assessment, which was acknowledged by the study authors to be a weakness of the study, did not account for peak exposures. Schulte *et al.* state in their paper that their results are not conclusive and may merely reflect chance physiological variation. Therefore, the Schulte *et al.*, study does not support 0.17 ppm as an adverse effect level in humans.

Zampollo *et al.* (1984) reported two cases of peripheral neuropathy in twelve nurses who removed objects from an ethylene oxide sterilizer and sorted the objects on a tray. The paper provides very little information on the collection of ethylene oxide concentration data, but does clearly state that values were 30 to 400 ppm in the vicinity of the sorting tray while a nurse sorted sterilized objects. Thus, this study does not support a human LOAEL of 10 ppm or less.

Estrin *et al.* (1987) measured nervous system function in 8 hospital workers that worked in proximity to ethylene oxide sterilizers and in 8 nonexposed controls. The authors report that, "Six exposed subjects reported olfactory detection of the gas on repeated occasions indicating exposures near or above the odor threshold of 700 ppm." In addition, industrial hygiene sampling records showed peak exposures in the employees' breathing zones in excess of the upper detection limit of 200 ppm. Estrin *et al.* (1987) note that, "Exposure to EtO [ethylene oxide] in hospitals generally occurs in predictable, relatively high, short-term peaks." Thus, although the average exposure may be low, the observed effects in studies of hospital workers quite possibly are due to the high peak concentrations and are not indicative of potential effects from chronic exposure to low levels of ethylene oxide. Thus, this study does not support a LOAEL of 10 ppm or less for human exposure to ethylene oxide.

Response. Because of the difficulties with the use of the study of Schulte *et al.* (1995) in the development of a REL for ethylene oxide (see CIIT and CMA comments and responses on the ethylene oxide REL), OEHHA has decided not to base the chronic inhalation REL for ethylene on that report.

Comment 3. In contrast to the Zampollo *et al.* and Estrin *et al.* case studies of workers exposed to high peak concentrations, Joyner (1964) conducted a retrospective morbidity study of 37 workers with 5 to 16 years of occupational exposure to ethylene oxide at 5 to 10 ppm.

There was no statistically significant increase in the incidence of neurological disorders compared to controls. After a review of the data for ethylene oxide, Golberg (1986) concluded that neurological effects were unlikely to occur at ethylene oxide exposures up to 100 ppm. Thus, the weight of evidence does not support OEHHA's proposition that humans are more sensitive to ethylene oxide exposure than are experimental animals.

Response. Other studies have been reported since Golberg made his conclusion in 1986. OEHHA staff believe that neurological effects may occur in workers due to chronic exposures to ethylene oxide below 100 ppm. Such studies are described in the ethylene oxide summary under effects of human exposure and include Estrin *et al.* (1987, 1990) and Klees *et al.* (1990). OEHHA is now proposing a revised chronic REL for ethylene oxide of 30 $\mu\text{g}/\text{m}^3$ based on nervous system effects in humans as reported by Klees *et al.* (1990).

Comment 4. Furthermore, even if there were evidence that humans are more sensitive than rodents to inhaled ethylene oxide, it would not follow that humans are most sensitive to effects from inhaled ethylene. The metabolism of a compound to a toxic metabolite occurs within the cells of metabolically-active tissues such as the liver. The effects of directly inhaling ethylene oxide, therefore, are not necessarily the same as the effects of ethylene oxide generated by metabolism of inhaled ethylene.

There are a number of endogenous sources of ethylene in the human organism: lipid peroxidation, oxidation of free methionine, oxidation of hemin in hemoglobin, and metabolism of intestinal bacteria (Filser *et al.*, 1992). In addition, natural exogenous sources of ethylene exist. It is a natural product of vegetation of all types and acts as an endogenous plant growth regulator. Sawada and Totsuka (1986) estimate that approximately 74 percent of ethylene emissions are from natural sources. Thus, humans evolved in the presence of both exogenous and endogenous sources of ethylene.

Studies being conducted by Dr. James Swenberg of the University of North Carolina demonstrate that humans have endogenous levels of significant quantities of ethylene oxide adducts. Dr. Swenberg has found that endogenous levels of the ethylene oxide-DNA adduct in the human liver are equivalent to levels produced in rats exposed to 10 ppm ethylene oxide or mice exposed to 33 ppm ethylene oxide. [Note: The level of 7-hydroxyethylguanine (7-HEG) in DNA from liver of nonexposed humans was 1.4 to 4.5 pmol/ μmol Guanine, with a mean value of 3.0 pmol/ μmol G. The mean level of 7-HEG in the liver of rats exposed to 10 ppm EtO was 3.3 pmol/ μmol G, and the mean level of 7-HEG in the liver of mice exposed to 33 ppm EtO was 3.75 pmol/ μmol G. A copy of a presentation by Dr. Swenberg that includes this data is provided as Appendix A.] Assuming equivalent concentrations of ethylene oxide produce equivalent concentrations of DNA adduct in humans and rodents, the humans were exposed endogenously at the rodent equivalent of 10 to 33 ppm inhaled ethylene oxide. Using a factor of 3 percent ethylene converted to ethylene oxide (human conversion saturation), the human endogenous exposure would be equivalent to an environmental exposure of 333 to 1100 ppm ethylene. Thus, OEHHA's proposed REL of 0.1 ppm ethylene appears to be some 3,300 to 11,000 times lower than what the human body spontaneously produces.

Response. OEHHA acknowledges that the body can produce ethylene. The body also produces the toxic chemical carbon monoxide (CO) from heme and uses nitric oxide (NO) as a hormone. Levels of hydrogen chloride, which can cause inflammation in other tissues, are normally present in the stomach. The relevant information of interest is the adverse effect(s) of exogenous ethylene which is inhaled.

Comment 5. The Panel therefore believes OEHHA is not justified in using ethylene oxide data to establish the REL for ethylene. Because adequate data exist to directly evaluate ethylene, OEHHA should base the REL on the ethylene studies. [Note: If OEHHA nevertheless persists in using ethylene oxide, then its analysis should be revised in accordance with the comments being separately submitted by the Ethylene Oxide Industry Council.]

Response. OEHHA has revised its chronic REL for EtO based in part on the comments from CMA's Ethylene Oxide Industry Council and those from the Chemical Industry Institute of Technology (CIIT). We will be discussing this with the Scientific Review Panel on Toxic Air Contaminants.

Comment 6. OEHHA should derive the REL for ethylene from the chronic study on ethylene. The toxicological database for ethylene includes both a comprehensive lifetime inhalation study in rats (Hamm *et al.*, 1984) and an inhalation reproductive/developmental study in rats (Aveyard and Collins, 1997). These studies provide an adequate and appropriate basis for deriving the REL for ethylene, especially since the pharmacokinetics of ethylene in rats and humans has been shown to be similar (Shen *et al.*, 1989). The existence of the reproductive/developmental study provides confidence that the chronic study did not miss potential sensitivity to reproductive or developmental effects. Because the route of exposure for both studies is inhalation, they are particularly relevant for derivation of the REL, which is an air concentration risk parameter.

Hamm *et al.* (1984) exposed rats to 300, 1000, or 3000 ppm ethylene for 6 hours/day, 5 days/week, for 24 months with no observed toxic effects. Hematology, blood chemistry, and urinalysis tests were performed at six-month intervals throughout the study. Over 24 months, no differences were observed between exposure groups with respect to mortality, clinical blood chemistry, urinalysis, body weights, organ weights or histopathology of a variety of tissues and organs. Inflammatory lesions typical of this strain of rat were distributed equally among all exposure groups. The NOEL in this study was 3000 ppm.

As discussed by OEHHA, a 13-week inhalation study of Sprague-Dawley rats found no treatment related effects at levels up to 10,000 ppm ethylene (Rhudy *et al.*, 1978). Parameters measured included body weight, total weight gains, food consumption, hematology, clinical chemistry, urinalysis, and histopathology.

Aveyard and Collins (1997) evaluated the potential effects of ethylene inhalation on male and female rat reproduction, growth and development using OECD Guideline 421

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(Reproduction/ Development Toxicity Screening Test). Administration of ethylene at nominal concentrations of 200, 1000, or 5000 ppm showed no evidence of toxicity. There were no adverse effects on male or female reproductive performance, fertility, pregnancy, maternal and suckling behavior, or growth and development of the offspring from conception to Day 4 post-partum. The general toxicity NOEL was 5000 ppm and the reproductive/developmental toxicity NOEL was 5000 ppm.

The Panel believes that OEHHA should derive the ethylene REL from the chronic rat study, as follows:

Study	Hamm et al. (1984)
Study population	Fischer 344 Rats (120/sex/group)
Exposure Method	Inhalation exposure at 300, 1000 or 3000 ppm
Critical effects	None
Exposure continuity	6 hr/d, 5 d/wk
Exposure duration	24 months
NOEL	3000 ppm
Average experimental exposure	535 ppm
Human equivalent conc.	535 ppm (gas with no extrathoracic effects, based on RGDR = 1.0 using default assumption that lambda (a) = lambda (h))
Subchronic uncertainty factor	1
LOAEL uncertainty factor	1
Interspecies uncertainty factor	10
Intraspecies uncertainty factor	10
Cumulative uncertainty factor	100
Inhalation reference exposure level (REL) for ethylene	5.4 ppm (6.2 mg/m ³)

Response. OEHHA staff agree that this is an acceptable approach to a REL and is considering basing the chronic REL for ethylene on the Hamm et al. report. However, since an HEC calculation has been made, an interspecies uncertainty factor of 3 can be used instead of 10. Unfortunately, no critical effect can be assigned from the study by Hamm et al. In the workplace ethylene is considered to be a “simple” asphyxiant. Thus its target organ could be considered to be the respiratory system and/or the blood since asphyxiants prevent oxygen from getting to hemoglobin. However ethylene has been used as an anesthetic in people (for example: Brumbaugh JD. 1928. Effects of ethylene-oxygen anesthesia on the normal human being. JAMA 91:462-465). Such use indicates effects other than asphyxiation. In addition ethylene can be metabolized to ethylene oxide which is a neurotoxicant. Thus, in humans there is evidence to consider ethylene as a gas with systemic effects.

Comment 7. The Panel notes that, given the fact that no effects have been detected in any studies of ethylene, even at very high air concentrations (1% ethylene), this REL is very conservative. The Occupational Safety and Health Administration (OSHA) does not regulate inhalation exposure to ethylene. The American Conference of Governmental and Industrial

Hygienists (ACGIH) has determined ethylene is essentially toxicologically inert. It has not set a threshold limit value (TLV) for ethylene, but has classified it as a "simple asphyxiant," defined as follows:

Simple Asphyxiants -- "Inert" Gases or Vapors. A number of gases and vapors, when present in high concentrations in air, act primarily as simple asphyxiants without other significant physiologic effects. A TLV may not be recommended for each simple asphyxiant because the limiting factor is the available oxygen.

Response: Ethylene is included because it is listed as a Hot Spots chemical. OEHHA admits that it is difficult to develop a reference exposure level for a simple asphyxiant. However several reports, which are cited in the revised chronic toxicity summary for ethylene, have indicated that ethylene has been used as an anesthetic. This implies that ethylene has neurotoxic effects and is not just a simple asphyxiant.

Comment 8: OEHHA's REL discussion should emphasize the lack of effects observed for ethylene, even at concentrations as high as 10,000 ppm in a subchronic study.

Response: The chronic REL summary states that "The available data indicate that ethylene has a low potential for non-cancer chronic toxicity in experimental animals." Also it states that no effects were seen in the 13 week study where 10,000 ppm were studied.

II. Comments regarding the 1,3-butadiene REL. OEHHA developed a chronic inhalation REL of 8 $\mu\text{g}/\text{m}^3$ for 1,3-butadiene based on ovarian atrophy in mice exposed by inhalation.

Comment 9. OEHHA should base the butadiene REL on rat data, because human metabolism of butadiene is more similar to the rat than the mouse. Ovarian atrophy in the mouse is not an appropriate endpoint for derivation of the REL. OEHHA notes in the draft Technical Support Document that "the animal species most sensitive to a substance is not necessarily the most similar to humans in developing adverse effects from a particular exposure." In the case of butadiene, use of the most sensitive species - the mouse - is not appropriate, because compelling evidence indicates that the rat is a more appropriate model for estimating risks to humans. The ovarian atrophy observed in the mouse has not been observed in the rat, even when exposed to butadiene at concentrations as high as 8000 ppm. This is due to differences in the metabolism of butadiene by the mouse and the rat. Studies show that human metabolism of butadiene is similar to that of the rat, and not of the mouse. Therefore, direct extrapolation from the mouse ovarian effects is inappropriate to derive a health effect level for human protection.

The Panel previously has submitted comments to OEHHA concerning the potential reproductive toxicity of 1,3-butadiene. For example, in December 1996 the Panel submitted comments on OEHHA's "Draft Prioritized Candidate Chemicals Under Consideration for

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Developmental/Reproductive Toxicity Evaluation," dated October 4, 1996. [Note: Letter from Langley A. Spurlock, Vice President, CHEMSTAR, to Cynthia Oshita, Senior Hazardous Materials Specialist, OEHHA, re: Draft Prioritized Candidate Chemicals Under Consideration for Developmental/Reproductive Toxicity Evaluation, October 4, 1996 (Dec. 2, 1996)] In October 1997, the Panel submitted comments in response to OEHHA's request for relevant information on chemicals under consideration for Proposition 65 listing via administrative mechanisms. [Note: *Comments of the Chemical Manufacturers Association Olefins Panel on the Possible Listing of 1,3-Butadiene as a Reproductive Toxicant Via Administrative Mechanisms*, submitted to Cynthia Oshita, OEHHA (Oct. 21, 1997)] Attachments to these comments include relevant excerpts from Panel comments to OSHA and testimony to OSHA by Dr. Mildred Christian, a leading authority on developmental and reproductive toxicity. The Panel urges OEHHA to review these comments and their attachments with respect to developing an REL for butadiene. Upon request, we will submit additional copies of the comments and attachments.

As explained in the previous comments to OEHHA, the mouse is unique in its sensitivity to butadiene. Ovarian atrophy or other reproductive effects have not been observed in the rat at butadiene exposure levels up to 8000 ppm administered by inhalation for two years (Owen *et al.*, 1987). In addition, no histopathologic changes were detected in the ovaries of rats, guinea pigs, rabbits, or dogs exposed to butadiene at concentrations up to 6700 ppm for eight months (Carpenter *et al.*, 1944, as discussed in Christian, 1996).

Dr. Glenn Sipes and his colleagues, of the University of Arizona, have developed data that explain the mechanism by which butadiene causes ovarian atrophy in the mouse (Doerr *et al.*, 1996). Their work shows that the monoepoxide metabolite of butadiene causes some ovarian effects in the mouse, but not in the rat. The diepoxide metabolite causes ovarian effects in both the mouse and rat, but is more potent in the mouse and is far more potent in the mouse than is the monoepoxide. In other words, the primary cause of the ovarian atrophy observed in mouse (and not observed in the rat) appears to be the diepoxide metabolite of butadiene.

Rats are much less efficient at metabolizing butadiene to monoepoxide than are mice, and primates - including humans - convert even less butadiene to the monoepoxide than do rats (Csanady, *et al.*, 1992; Schmidt and Loeser, 1986; Himmelstein, *et al.*, 1994; Himmelstein, *et al.*, 1995; Dahl, *et al.*, 1991). Workers exposed to butadiene showed at least 25-fold lower levels of the monoepoxide hemoglobin adduct per ppm-hour than rats, and more than 100-fold lower adduct levels than mice (Osterman-Golkar, *et al.*, 1993). Furthermore, the metabolism of the monoepoxide in the mouse proceeds largely by further epoxidation to the diepoxide (Himmelstein, *et al.*, 1997). In contrast, rats form very little diepoxide (Csanady, *et al.*, 1992; Thornton-Manning, *et al.*, 1995), and primates hydrolyze most of the monoepoxide, rather than convert it to diepoxide (Csanady, *et al.*, 1992; Dahl, *et al.*, 1991). Thus, diepoxide levels are much higher in mice than in rats or primates (Thornton-Manning, *et al.*, 1995; Sweeney, *et al.*, 1997; Seaton, *et al.*, 1995).

In summary, the diepoxide metabolite of butadiene appears to be responsible for the ovarian atrophy observed in the mouse. Very little diepoxide, if any, is produced through

metabolism in rats, and no atrophy is observed in rats exposed to butadiene. Even less diepoxide is produced in human tissues. Therefore, the data in mice are not relevant to assessment reproductive effects in humans, and the mouse ovarian atrophy is an inappropriate basis for the establishment of an REL.

Response. OEHHA staff agree that the mouse ovary may be more (or much more) sensitive to butadiene due to butadiene's metabolism to the diepoxide and that people are more like the rat in their formation of epoxides from butadiene. The diepoxide could be much more rapidly destroyed in rats than in mice. (In a somewhat analogous situation both mice and rats form a reactive carcinogenic epoxide from aflatoxin. Mice metabolize the aflatoxin epoxide via glutathione much more rapidly than rats, so that the rat is about 1000x as sensitive as the mouse to aflatoxin-induced carcinogenesis.)

Unique may not be an appropriate term in the case of butadiene if mice are really at one end of the spectrum in sensitivity to butadiene. Unique is probably better applied to situations such as male rat kidney tumors due to accumulation of alpha_{2u} globulin which only accumulates in the kidneys of male rats. OEHHA staff still propose using an interspecies uncertainty factor of 3 for this endpoint with butadiene because we believe that pharmacodynamic differences between mice and men are still not adequately counted for.

Comment 10. OEHHA should develop an REL based on rat data. Apart from reproductive toxicity, the mouse NTP study relied upon by OEHHA gave a NOAEL of 200 ppm, based on nonneoplastic hematotoxic effects (NTP, 1993). As for ovarian atrophy, however, these effects in the mouse do not appear applicable to other species. In the chronic study of Sprague-Dawley rats, blood was evaluated from 20 animals of each sex per group after 3, 6, 12, and 18 months of exposure to 0, 1000, or 8000 ppm of butadiene (IISRP, 1981; Owen *et al.*, 1987). Any changes of hematological parameters that occurred were within normal values for the strain and laboratory, and the study authors did not consider them to be toxicologically significant.

Cowles *et al.* (1994) conducted retrospective mortality, prospective morbidity, and hematological analyses of male workers employed in butadiene monomer production from 1948 to 1989. Hematology data was available for 429 of these workers. No hematological differences were seen for any butadiene-exposed employees, including a group exposed to an estimated time-weighted average of 10 ppm, as compared to employees not exposed to butadiene. This is consistent with Checkoway and Williams (1982), who reported minimal changes in the hematology of a subgroup of 8 workers in a styrene-butadiene rubber manufacturing plant, exposed to 20 ppm butadiene, versus 145 workers exposed to less than 2 ppm butadiene. The statistical significance of the changes is questionable due to the very small population and the failure to account for confounding factors such as race, smoking, body size, exercise, and ethanol intake. Checkoway, *et al.* (1984) concluded that the hematologic parameter values for the subgroup of 8 were within the normal range. Both Checkoway, *et al.*, (1984) and IARC (1992) concluded that the changes could not be interpreted as an effect on the bone marrow.

This difference in the hematological effects seen in the mouse study versus rat and human studies is in keeping with the metabolic differences discussed above. *In vitro* and *in vivo* evidence indicates that hematopoietic effects such as macrocytic megaloblastic anemia induced in mice by butadiene exposure are due to the epoxide metabolites, especially the monoepoxide (Colagiovanni, et al., 1993; Irons, et al., 1995). Mice, but not rats or humans, have a subpopulation of primitive hematopoietic progenitor cells which are very sensitive to the monoepoxide metabolite. Species differences in the metabolism of butadiene to the epoxides, as well as the different susceptibility of the hematopoietic system, indicate that the mouse is not the most appropriate species for deriving a chronic REL.

Because human metabolism of butadiene is more similar to that of the rat than that of the mouse, the Panel believes that the REL is more appropriately based on rat data than on mouse data. A suitable study is the two-year chronic inhalation study (IISRP, 1981; Owen, *et al.*, 1987). That study provided a NOEL of 1000 ppm, which can be converted to an REL as follows:

Study	IISRP, 1981; Owen, <i>et al.</i> , 1987
Study population	Sprague-Dawley rats (100/sex/group)
Exposure method	Discontinuous whole body inhalation exposure (0, 1000, or 8000 ppm)
Critical effects	Minor clinical effects (eye and nose excretions, slight ataxia); increased liver and kidney weights; nephrosis.
LOAEL	8,000 ppm
NOEL	1,000 ppm
Exposure continuity	6 hr/d, 5 d/wk
Exposure duration	2 years
Average experimental exposure	178.6 ppm
Human equivalent concentration	178.6 ppm (gas with no extrathoracic 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	10
Intraspecies uncertainty factor	10
Cumulative uncertainty factor	100
Inhalation reference exposure level	1.8 ppm (4 mg/m ³)

Response. The commentator has provided a plausible alternative to the chronic REL calculated by OEHHA. However, since there is a 200 ppm NOAEL in mice for a hematological toxicity, the use of the rat 1000 ppm NOAEL may not be appropriate. Yet since no hematologic effects were seen in 2 epidemiologic studies at 10 and 20 ppm butadiene, the use of the hematologic endpoint may also not be appropriate. As stated below, OEHHA prefer to use the mouse data because of the sensitivity of the endpoint.

Comment 11. If OEHHA uses mouse data, it should apply a pharmacokinetic adjustment. For the reasons discussed above, the Panel believes the rat provides a better model for conducting a human health risk assessment of butadiene than does the mouse. If OEHHA nevertheless chooses to base the REL on mouse data, it should use a physiologically-based pharmacokinetic (PBPK) model to adjust that data, due to the great differences in mouse metabolism of butadiene from that of rats and humans. Use of the 200 ppm NOAEL for hematological effects and applying OEHHA's standard adjustments would result in an REL of 0.36 ppm. Without adjustment for the metabolic differences between mice and humans, however, that REL would be extremely conservative.

Extensive work has been done and is continuing to develop and refine PBPK models for butadiene (Himmelstein, *et al.*, 1997; ECETOC, 1997). Upon request, the Panel would be pleased to provide technical support to OEHHA to apply appropriate PBPK adjustments to mouse data for the development of a REL, if OEHHA declines to use rat data for the REL.

Response. OEHHA appreciates the offer of technical support by the commentator. OEHHA staff have some experience in pharmacokinetic modeling of butadiene (Brown, J.P., and Collins, J.F.: Use of microcomputers to apply butadiene metabolic data to public health risk assessment. *FASEB J.* 7:A1130, 1993). A credible approach might be to use an interspecies uncertainty factor less than the default of 10 (or 3 after an HEC adjustment) for mouse to man since in the case of butadiene humans are not up to (3 to) 10 times more sensitive than mice. Pharmacokinetic information indicates that mice are not less sensitive than people to 1,3-butadiene. However we still need to account for pharmacodynamic differences. Thus we use an interspecies UF of 3 after the HEC adjustment.

OEHHA staff note that USEPA has used a benchmark dose approach to develop a (proposed) reproductive/developmental RfC for butadiene of 0.15 ppb (0.3 $\mu\text{g}/\text{m}^3$) based on the dominant lethal effect of decreased litter size in mice at birth.

Chemical Manufacturers Association – Phthalate Ester Panel

Comments on the chronic RELs for **phthalic anhydride** 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 phthalic anhydride of 10 µg/m³ based on eye and respiratory irritation, asthma, and bronchitis in 23 workers occupationally exposed for a mean of 13.3 years (Nielsen *et al.* (1988; 1991)). (Comments of the Panel on DEHP were dealt with previously.)

Comment 1: *Phthalic anhydride.* OEHHA should base an interim REL for phthalic anhydride on the ACGIH TLV, and should emphasize in its discussion phthalic anhydride's solid nature and its low oral toxicity.

Response: OEHHA has not based chronic RELs on ACGIH TLVs. USEPA, OEHHA, and even ACGIH have all determined ACGIH TLVs should not be used in developing health-based exposure guidance for general populations including the elderly and children. TLVs lack a consistent basis and are intended to protect only healthy workers from discontinuous exposures, rather than the public from continuous exposures. Many TLVs are not health-based and/or are intended to reduce rather than eliminate the occurrence of adverse health effects.

Comment 2: OEHHA should emphasize in the REL discussion the fact that phthalic anhydride is a solid at ambient temperatures, and that it has very low systemic toxicity when ingested.

Response: OEHHA noted the crystalline form of phthalic anhydride at ambient temperatures and its low vapor pressure. Mass concentration units (µg/m³) were not converted to volume concentration units (ppb) in the Chronic Toxicity Summary. However as noted above for DEHP, particulate air contaminants may exist at levels hazardous to human health. The particulate nature of phthalic anhydride in inhalation exposure studies in animals administered by Sarlo and Clark (1992) and Sarlo and associates (1994) was clearly presented. Text is being added at several locations in the document to emphasize the particulate nature of DEHP in human and animal exposure studies.

Comment 3: OEHHA based the proposed REL for phthalic anhydride on a pair of studies by Nielsen *et al.* (1988; 1991). Those studies do not support a correlation between phthalic anhydride exposure and the purported critical effects. The reported effects were minimally adverse and reversible, are commonly reported by workers, and could have been due to colds, allergies, or exposure to other chemicals.

Response: Several categories of response were significantly increased in heavily exposed workers compared with those with limited exposures. The effects noted (asthma, chronic bronchitis, conjunctivitis, and rhinitis) were consistent with a hypersensitization response among repeatedly exposed workers. A similar hypersensitization response was noted in animals exposed to phthalic anhydride dust (Sarlo *et al.*, 1994). The induction of asthma and

bronchitis would not be categorized as a “minimally adverse” response. Reversibility of adverse effects is not a sufficient reason to ignore the finding; among other reasons, the RELs are intended to protect the public from continuous lifetime exposure. That effects noted in occupationally exposed workers may be due, in least in part, to exposure to other substances is a reasonable concern. However, as noted above, the immunologically-based effects noted are consistent with those noted among rats exposed only to phthalic anhydride. As for the contention that effects noted among the heavily exposed workers might be due to colds or allergies, there is no reason to anticipate the heavily exposed workers should be more affected than lightly exposed workers.

Comment 4: No existing chronic or subchronic inhalation studies of phthalic anhydride are appropriate for the derivation of an REL, so OEHHA should not establish a final REL for phthalic anhydride.

Response: As described in the response to comment 8, OEHHA still concludes that the data of Nielsen et al. (1988; 1991) are adequate for the purposes of deriving a chronic REL. As is the case for all chemicals reviewed, additional data would be desirable and will be considered if such data should become available in the future.

Comment 5: As an interim measure, OEHHA should base an interim REL on the ACGIH TLV, adjusted for continuous exposure and variation in sensitivity.

Response: OEHHA has not based chronic RELs on ACGIH TLVs. USEPA, OEHHA, and even ACGIH have all determined ACGIH TLVs should not be used in developing health-based exposure guidance for general populations including the elderly and children. TLVs lack a consistent basis and are intended to protect only healthy workers from discontinuous exposures rather than the public from continuous exposures. Many TLVs are based on feasible control technology, not health, and are intended to reduce rather than eliminate the occurrence of adverse health effects.

Chloropicrin Manufacturers' Task Force (CMTF)

The Chloropicrin Manufacturers' Task Force (CMTF) submitted comments on January 29, 1998 regarding the draft chronic reference exposure level for **chloropicrin** presented in the OEHHA *Air Toxics "Hot Spots" Risk Assessment Guidelines Part II. Technical Support Document for Determining Chronic Reference Exposure Levels*. The members are Ashta Chemicals, Holtrachem Manufacturing, Niklor Chemical, Trinity Manufacturing, Agrevo Canada, Angus Chemical, Dow AgroSciences, Great Lakes Chemical Corp. and Trical Products. OEHHA developed a chronic REL of 4 µg/m³ based on respiratory system effects (nasal rhinitis) in rats.

Comment 1. OEHHA's proposed REL for chloropicrin is based on a chronic inhalation oncogenicity study performed by whole-body exposure to rats (Burleigh-Flayer and Benson, 1995). OEHHA identified increased mortality, increased lung and liver weights and rhinitis as effects of chloropicrin inhalation exposure in their summary of the Burleigh-Flayer and Benson study. CMTF disagrees that liver weights were affected by chloropicrin treatment in the chronic rat study. Tables 17-22 of the study final report (Burleigh-Flayer and Benson, 1995) present organ weight data that show male rat liver weights, both absolute and relative to body and brain weight, were unaffected by exposure to chloropicrin. The absolute liver weight of female rats was statistically-significantly depressed in the mid-dose group (as was this group's body weight) but not in the low or high-dose groups. The liver weight of the female animals as compared to their body or their brain weight, i.e., relative liver weight, was not affected by chloropicrin treatment in any dose level in the study. The decrement in absolute liver weight but not relative liver weight observed in the mid-dose female rats is a reflection of the body weight diminution experienced by these animals and is not indicative of a toxic effect in the liver. The study director concluded this, and on page 19 of the study report writes: "Some statistically-significant changes in absolute kidney and liver weight for female animals from the low and/or mid groups were believed to be the result of their lower final body weight and were not believed to be exposure related."

Response. OEHHA reexamined this issue and accepts the commentator's correction that the liver findings involve decreased liver and body weights in the mid-dose female rats, lack a monotonic dose-response relationship, and are not evidence of a direct toxic effect to this organ. Therefore the identification of liver effects as an endpoint is being removed.

Comment 2. OEHHA adjusted the No Observed Adverse Effect Level (NOAEL) from the Burleigh-Flayer and Benson study for continuous exposure (to 0.018ppm) and applied an uncertainty factor of 3 for interspecies uncertainty and an additional factor of 10 for intraspecies uncertainty. The CMTF believes that, because the critical effects that support the derivation of the OEHHA REL are limited to respiratory system irritation and are not progressive, there is no need for an interspecies uncertainty factor. The nonspecific irritation effects seen at the portal of entry and target organ following overexposure to chloropicrin are equivalent across all species tested (Chun and Kintigh, 1993; Yoshida, 1987; Schardein, 1994; Schardein, 1993a and 1993b; Burleigh-Flayer, 1994; NCI, 1978; Wisler, 1995; Ulrich, 1995). Nonspecific irritation at the site of contact was seen in all species evaluated, including dogs,

rabbits, rats and two strains of mice. There is no basis to conclude that humans will respond differently from these mammalian species.

Response. The available data do indicate that chloropicrin is highly reactive and causes effects at the immediate sites of contact. But the effects noted can be more severe than irritation. Kane and associates (1979) noted exfoliation, erosion, ulceration, and necrosis of respiratory and olfactory epithelium of mice exposed to 7.9 ppm chloropicrin for 6 hours per day for 5 days. Fibrosing peribronchitis and peribronchiolitis were noted in the lower respiratory tract. Furthermore there is no evidence comparing the relative toxicity of chloropicrin between rodents and humans. Most notably, increased mortality was observed in the Burleigh-Flayer and Benson study. Secondly, similar effects are commonly noted among different species exposed to the same chemical but the magnitude of exposures causing equivalent response may differ substantially.

Comment 3. Likewise, there is no basis to presume that human respiratory tissue will be differentially susceptible to chloropicrin irritation. Therefore, a 10-fold factor for intraspecies uncertainty is not justified for chloropicrin.

Response. The intraspecies factor is intended to protect sensitive subgroups, such as the elderly and children, and those with preexisting medical conditions that may increase the susceptibility to adverse effects following exposure to chloropicrin. Variability in response among individuals to the same toxic stimuli has been noted in virtually all toxicity studies, although the degree of variability may differ for different chemicals and different endpoints. On the basis of currently available data, OEHHA believes a 10-fold intraspecies uncertainty factor is warranted.

Comment 4. Because the respiratory effects of chloropicrin are concentration and not dose-dependent, duration of exposure is not a factor in producing effects, nor in preventing effects. Accordingly, the OEHHA adjustment of the Burleigh-Flayer exposure to a continuous exposure is unnecessary. According to the American Conference of Governmental Industrial Hygienist (ACGIH), exposure to chloropicrin at a concentration of 0.1 ppm will not result in eye or respiratory irritation, but irritation does occur at concentrations of 0.3 to 0.37 ppm (ACGIH, 1991). Concentration-dependent chemicals are defined as fast-acting chemicals whose toxic effects are immediate, and correlate more closely to concentration than dose. Included in this category are sensory irritants, and chemicals that are corrosive or vesicant in their action. In contrast, the effects of dose-dependent chemicals are a function of both concentration and duration of exposure" (Craig, 1995). Chloropicrin at low levels (0.15-0.3 ppm) produces a clear warning of exposure. At higher exposure levels (1 ppm or more), chloropicrin produces a consistent pattern of pulmonary injury in humans and test animals. The protective warning properties of chloropicrin occur at airborne concentrations of 0.15 ppm. Exposure to chloropicrin below this concentration has no effect and an application of safety, or uncertainty, factors is without rationale. Because the short-term effects, i.e. sensory irritation, are the overriding effects from chloropicrin exposure, chronic toxicity data from animal studies should not be used to establish chloropicrin exposure criteria.

Response. The commentator did not provide any direct evidence to support the contention that effects following chloropicrin exposure are completely independent of exposure duration. Were a large subchronic uncertainty factor applied, the commentator's point might have greater relevance. But in this case no subchronic uncertainty factor was used. Thus the degree to which exposure duration may have lesser importance for this chemical is already reflected in the data collected in the chronic exposure study. The commentator's main point may be thus directed at the approximately 5.6-fold adjustment used to account for the discontinuous (6 hr/day, 5/day per week) exposures. There are no data demonstrating that there would be no difference between continuous and discontinuous exposures in this case, so some adjustment is warranted. In the Kane study, recovery was observed three days after the completion of a 5 day exposure period, indicating that continuous exposure may result in more severe effects than discontinuous exposure (where some recovery will be taking place).

Comment 5. In response to the statement in the draft REL indicating that adequate reproductive toxicity data is a major area of uncertainty in the chloropicrin data base, the CMTF would like to point out the existence of a chloropicrin multi-generation reproductive toxicity study (Schardein, 1994).

Response. OEHHA thanks the commentator for providing information about this unpublished study. As of June 1999 it has not appeared in the peer-reviewed literature. However, OEHHA would like to obtain a copy for review.

Elementis Chromium

Comments on the chronic REL for **chromium VI** were made by R.J. Barnhart, Ph.D., Vice President-Technical, of Elementis Chromium, Corpus Christi, Texas in a letter dated January 27, 1998. OEHHA proposed a chronic REL of 0.0008 µg/m³ for respiratory effects based on a study by Lindberg and Hedenstierna (1983) of workers exposed to chromic acid.

Comment 1. Page A-161. The table listing specific compounds. The chemical formulas for potassium chromate, sodium chromate, potassium dichromate and sodium dichromate are wrong. Hydrogen atoms should not be included in these formulas.

Response. Comment noted. The hydrogen atoms will be removed. OEHHA regrets the error.

Comment 2. Page A-162. Physical and Chemical Properties. The properties listed are not valid for all the compounds identified on page A-161. These properties are reasonably accurate for chromic acid but not for the other compounds.

Response. The title will be changed to reflect this comment.

Comment 3. Page A-162. Section III. Second paragraph. Chromates are no longer used in cooling towers or automobiles to inhibit corrosion in recirculating water.

Response. Chromates have been phased out over the last several years. The reference cited was published in 1988. The California Air Resources Board banned this use in 1989. We will revise the text accordingly.

Comment 4. Page A-162. Section IV. First two paragraphs. In both of these studies the effects of poor personal hygiene practices are probably significant. This is noted in Lucas and Kramkowski (1975). Although personal hygiene practices were not specifically discussed in the Lindberg and Hedenstierna (1983) publication, another study done on chrome plating workers by the same group (Lindberg and Vesterberg, 1983) noted that more than a third of the workers studied (33/91) had "yellow hands" or chrome sores. These are obvious signs of very poor personal hygiene practices that can easily result in the direct transfer of chromic acid to the outer nasal passages and septum. Also, in electroplating the normal operations involve putting objects to be plated in the baths, removing these objects from the baths and adjusting the operating conditions of the bath. These procedures usually require short periods where the operator is directly above the bath subjected to high exposures and long periods away from the bath at much lower exposure. This would produce high peak exposures even though average exposures would be much lower. In fact in Lindberg and Hedenstierna (1983) the following statement is made:

The observation that damage to the nasal septum correlated better with short-term peak exposure than with 8-hr mean concentrations of chromic acid clearly underscores the detrimental effects of high peak concentrations of chromic acid.

Consequently, many of the effects reported are very likely the result of poor personal hygiene or high peak exposures rather than the reported average exposures. When studies of electroplating workers are used for regulatory purposes, these limitations should be recognized.

Response. The poor hygiene practices of the workers in the Lindberg and Hedenstierna (1983) study is unfortunate, both for the workers and for the use of the study as the basis of the chronic REL. Epidemiological studies usually have many complicating factors. However, epidemiological studies of chromium VI workers in other industries exposed to species other than chromic acid have also reported toxicity of the upper respiratory system. Other lung symptoms reported in the key study, such as a diminished forced expiratory flow between Monday morning and Thursday afternoon, are not likely to have resulted from poor personal hygiene.

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 a Hazard Index exceeds 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 5. Page A-163. Section VI. The use of Lindberg and Hedenstierna (1983) for the derivation of a Chronic Reference Exposure Level (REL) for all hexavalent chromium compounds is not appropriate. This study involves workplace exposure to chromic acid. Although chromic acid is a hexavalent chromium compound, it is very unlikely to be a significant component of the hexavalent chromium content of ambient air. Chromic acid is very acidic and highly oxidizing and therefore has very low stability in the environment (Barnhart, 1997). When exposed to the environment it will either react and be chemically reduced to the trivalent chromium state or be neutralized to a dichromate or chromate salt. Under certain conditions these chromate salts can be stable in the environment and therefore regulatory levels for ambient air should be based on these compounds (Finley *et al.*, 1993).

Response. Neither OEHHA nor US EPA agrees that the study of Lindberg and Hedenstierna (1983) is not appropriate. Hexavalent chromium is toxic. It is preferable from the point-of-view of protecting public health to use the data available on the most toxic species present. It would be helpful to know the half-life of the chromium VI ion in the air if that is what the comment about the very low stability of chromic acid in the environment implies. OEHHA's REL is for all chromium VI ions, not just those from chromic acid. In the Air Toxics Hot Spots Program, facilities do not speciate their chromium VI emissions. A 1988 report by the Research Triangle Institute (The fate of hexavalent chromium in the atmosphere. ARB Contract A6-096-32) indicated an average experimental half-life of 13 hours. Since emissions are continuous, there is the potential for continuous exposure. Reports of high percentages of

chromium VI above abandoned hazardous waste sites, as well as notable measurements of CrVI in ambient air and soil near chrome plating facilities, also seem inconsistent with a short half-life for chromium VI.

Comment 6. Page A-164. First paragraph. Both the principal author of the cited study (Lindberg, 1986) and the USEPA (USEPA, 1990) concluded that at average exposures to chromic acid of < 1 µg/m³ no effect in the respiratory tract was seen. Therefore, even if this study is considered, the use of an average exposure level of 0.24 µg/m³ Cr (VI) and a LOAEL uncertainty factor of 10 is not justified.

Response. Lindberg’s conclusion might be applicable for healthy workers, not for sensitive individuals. Workers that were exceptionally sensitive to respiratory irritation might choose to work in a different setting. Despite its 1990 conclusion, US EPA developed a RfC for chromic acid mists and Cr VI aerosols based on the Lindberg and Hedenstierna (1983) report. A LOAEL factor of 10 (or possibly greater) is certainly justified by the nasal ulceration and/or perforation seen in 11 of 24 workers exposed to levels above 2 µg/m³ (Table 3 below). The subjective irritation (reported by 4 of 19 workers exposed to levels below 2 µg/m³) could justify a UF of 3. However, the atrophy of the nasal mucosa seen below 2 µg/m³ in 4 in of 19 workers is considered by OEHHA staff to be a serious adverse effect.

Table 3 (from Lindberg and Hedenstierna, 1983). – Conditions of the Nose and Subjective Symptoms in Groups with Different Mean Values of Exposure and with Different Highest Exposure Values Measured Near the Baths where the Exposed Worker had worked During Some Part of the Day

	8-hr Mean Value of Exposure		Highest Exposure Value		
	≤1.9	2-20	0.2-1.2	2.5-11	20-46
CR(VI) µg/m ³	19	24	10	12	14
N	4	11	0	8	4
Subjective irritation	4	8	1	8	0
Atrophy	0	8*	0	0	7#
Ulceration	0	3	0	0	3
Perforation only					

* Two of 8 also had a perforation.

Two of 7 also had a perforation.

Comment 8. Based on these comments I recommend that the REL of 0.0008 µg/m³ proposed for hexavalent chromium in this draft not be accepted and that all relevant information including animal studies be considered in developing an appropriate REL. Additionally the use of the benchmark dose method (Malsch, *et al.*, 1994) should be considered since it allows the use of a larger database in deriving this value.

Response: OEHHA thanks the commentator for his comments. We have considered relevant information, including animal studies. In the Hot Spots program facilities do not speciate their emissions of chromium VI into aerosols, mists, and particulates. Thus to protect public health OEHHA concentrates on the most toxic species.

US EPA developed 2 RfCs for chromium VI. Neither RfC was based on a benchmark dose approach. The first RfC was 0.008 $\mu\text{g}/\text{m}^3$ for chromic acid mists and chromium VI aerosols based on the study by Lindberg and Hedenstierna (1983). OEHHA has reviewed the documentation on IRIS for that RfC and disagrees with some of the interpretations made by USEPA, including whether or not nasal atrophy is a severe effect (OEHHA believes that it is) and the exposure concentration selected as the basis of the REL. In addition, for this RfC USEPA decided that the multiplication of 2 intermediate UFs of 3 (which is actually the square root of 10) resulted in 9, not 10.

The second RfC, with the higher value of 0.1 $\mu\text{g}/\text{m}^3$ for chromium VI particulates, was based on the same studies in rats (Glaser et al., 1985; 1990), which were used by Malsch et al. to develop their value of 0.34 $\mu\text{g}/\text{m}^3$ by the benchmark approach, a value close to the value USEPA derived using the NOAEL/UF approach. The BC derived by Malsch et al. used the 95% lower confidence limit of the EC₁₀ (designated a Maximum Likelihood Estimate) rather than of the EC₀₅ preferred by OEHHA. Use of the LCL on an EC₀₅ would result in a value even closer to the US EPA value of 0.1.

References cited by commentator:

Barnhart, J. (1997). Occurrences, Uses, and Properties of Chromium. *Regulatory Toxicology and Pharmacology* 26(1): 3-7.

Finley, B. L., D. M. Proctor, and D. J. Paustenbach (1992). An Alternative to the USEPA's Proposed Inhalation Reference Concentrations for Hexavalent and Trivalent Chromium. *Regulatory Toxicology and Pharmacology* 16:161-176.

Lindberg, E. (1986). *Health Hazards in Chrome Plating*. Department of Environmental Hygiene, Karolinska Institute, Stockholm.

Lindberg, E., and G. Hedenstierna (1983). Chrome Plating: Symptoms, Findings in the Upper Airways, and Effects on Lung Function. *Archives of Environmental Health*. 38(6): 367-374.

Lindberg, E., and O. Vesterberg. (1983). Urinary excretion of proteins in chromeplaters, exchromeplaters, and referents. *Scand J. Work Environ. Health*. 9: 505-510.

Lucas, J. B. and R. S. Kramkowski (1975). Health Hazard Evaluation Determination Report Number 74-87-221. U.S. Department of Health, Education, and Welfare, Center for Disease Control, National Institute for Occupational Safety and Health.

Responses to Comments on the October 1997 Draft on Noncancer Chronic RELs

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Malsch, P. A., D. M. Proctor, and B. L. Finley (1994). Estimation of a Chromium Inhalation Reference Concentration Using the Benchmark Dose Method: A Case Study. *Regulatory Toxicology and Pharmacology* 20: 58-82.

United States Environmental Protection Agency (1990). Noncarcinogenic Effects of Chromium. Update to Health Assessment Document. Publ. No. EPA/600/8-87/048F. Office A Research and Development, U.S. Environmental Protection Agency, Washington, DC.

Union Carbide Corporation - Isophorone

Comments on the **isophorone** chronic REL were made by J. M. Cleverdon, Project Safety Manager for Union Carbide Corporation, in a letter dated December 17, 1997. The proposed chronic REL for isophorone (1,1,3-trimethyl-3-cyclohexene-5-one) was based on a probe and final study of inhalation teratology conducted by Bio/dynamics for Exxon Biomedical Sciences in 1983 and 1984. Mice and rats were exposed for 6 hours per day during gestation. Reduced crown-rump length were noted in female rat fetuses at 115 ppm, but not at 50 ppm. Thus a time-weighted gestational exposure NOAEL of 12.5 ppm, an interspecies uncertainty factor of 3, and an intraspecies uncertainty factor of 10 were used to derive a REL of 0.4 ppm (2,000 µg/m³). Exencephaly was noted in 4 fetuses of animals exposed to 150 ppm in the probe study (a finding not reproduced in the final study) and this effect was also cited in the summary of critical endpoints observed.

Comment 1. Union Carbide Corporation would like to thank you and your group for allowing us to comment on the draft document Air Toxics Hot Spots Program Risk Assessment Guidelines Part III: Technical Support Document for the Determination of Noncancer Chronic Toxicity Reference Exposure Levels, and specifically on Appendix A.69, Chronic Toxicity Summary - Isophorone. In general, we feel that the Air Toxicology and Epidemiology Section has done a credible job in developing methodologies for determining RELs and that the application of this methodology has been used appropriately in deriving a value of 2,000 µg/m³ for isophorone.

We would, however, take exception with the characterization of isophorone as "teratogenic". In the Chronic Toxicity Summary document, it is correctly pointed out on page A-424 (1st full paragraph, 10th sentence) that in a probe study a malformation, exencephaly, was observed in a late resorption in one rat litter from the high exposure concentration group (150 ppm), and in two litters of mice exposed to the high concentration group (in one late resorption from 1 litter, and in two live fetuses from a second litter). The document goes on to state on page A-425, sentence 10: "However, exencephaly is included as a critical effect in this summary because it is considered a serious teratogenic effect that was present at a dose only slightly higher than the LOAEL of the primary study (115 ppm)." We take exception to that statement because it fails to take into consideration the unconventional design of this teratology probe study and the outcome of the definitive developmental toxicology study.

Response. OEHHA has revisited the data bearing on the teratogenicity of isophorone. In this case, as in many other cases examined for this document, there remains considerable uncertainty, and substantial arguments can be made on both sides of the issue. This debate will only be adequately resolved with the acquisition of better data. The number of animals tested, on which the issue rests, is small but the effect observed, exencephaly, is of great concern. The authors of the original report suggested that the exencephaly was likely unrelated to isophorone exposures, but the data are inadequate to obviate concern.

Comment 2. It is very important to keep in mind that this probe study (copy attached) did not employ the typical design of a Segment II developmental toxicity study. Normally, such

studies involve sacrifice shortly before birth. There is a considerable historical database on developmental effects observed shortly before birth, by which time organogenesis is complete. That procedure was not followed in this case, however. Here, the probe study was conducted by exposing female rats and mice on days 6 through 15 of gestation. The mothers were sacrificed on gestation day 16 and the fetuses weighed, measured and examined for external malformations. This examination took place on approximately 4 days (mice) and 6 days (rats) prior to parturition and a critical time period of organogenesis. There is no historical database on which to evaluate the results observed in this probe study at gestation day 16. Thus, it is very difficult to evaluate the biological significance of the findings on day 16.

Response. While there may be limited comparable historical data, the probe study had a 12 member control group, which in any case are the best data on which to compare the exposed groups. In addition, it is unlikely that the results of the exencephaly would be different if the fetuses had been examined at day 20 or 22 of gestation.

Comment 3. This difficulty is compounded by the fact that the definitive study, conducted using substantially more females than in the probe study (22 per group for versus 12 per group in the probe study), found no exencephaly and no significant differences from controls in internal or external malformations at gestation day 20 (rats) and gestation day 18 (mice). If the effect observed in the probe study had been of biological significance, it would likely have appeared in the definitive study; but it did not.

Response. The definitive study, like the probe study, had relatively few exposed individuals. Assuming for the sake of argument that the exencephaly was actually induced by isophorone, the fact that such an endpoint affecting only a minority of individuals would be observed clustered in only one of a series of two small studies is not particularly surprising. The exencephaly may have been a chance occurrence unrelated to isophorone exposure or it may be an effect that occurs with a low incidence rate. Only additional study can resolve this issue.

Comment 4. In addition, in any developmental toxicity study (and in particular in this probe study) there is uncertainty in the exact timing of conception to within a twelve to twenty-four hour period (based upon vaginal smears and/or discovery of a plug). Hours and even minutes are critical in these early stages of embryo development. Observed landmark events can very well be dependent on the precise time of conception relative to terminal sacrifice. The stage of development in the late resorptions is even more uncertain since the exact time of death in these embryos could not be determined.

Response: The experimental control group was subject to the same uncertainties and yet no exencephaly was noted in those animals. Presumably, the initiating events producing exencephaly occur in the early stages of neural tube development. The comment does not seem to consider the irreversible course of events leading to exencephaly.

Comment 5. Considering the arguments above, it is not unreasonable to anticipate that various malformations, including exencephaly, might be observed in a probe study of this design. However, such findings should not be construed to indicate that the material is a teratogenic substance, particularly given the fact that exencephaly was not seen in the definitive study conducted by a more appropriate design where fetal examinations were conducted at term. Indeed, in the definitive study no significant differences from controls were seen for any malformations. The study authors concluded that the exencephaly found in the probe study was not related to the test material in light of the results of the definitive study.

Response: The studies raise a serious concern that can not be discounted on the basis of the issues raised by the commentator. The commentator does raise the legitimate argument that the effects noted could be unrelated to isophorone exposure. Again, this debate will only be resolved with better data relevant to this issue.

Comment 6. The fact that the malformations observed in the probe study were isolated to the high concentration group may be related to the evidence of delays in development identified in the definitive study.

Response: The clustering of malformations in the high-dose group would also be consistent with a dose-response effect by an agent causing the endpoint.

Comment 7. We do not contest the fact that fetal toxicity and delays in development were noted in that study. This finding in the definitive study is consistent with fetal toxicity and delayed development observed in many Segment II developmental toxicity studies conducted with solvents and other chemicals and seen in association with mild maternal toxicity.

Response: The chronic REL document for isophorone cited these effects as the primary finding used to derive the REL. Since there are uncertainties involved in the interpretation of the exencephaly noted in the probe study, the reference to teratogenicity will be removed from Sections I and VI. However, the discussion of the concern that this effect could be related to isophorone exposure will remain as a point of discussion in the document.

Comment 8. In addition to this specific comment on the isophorone, your letter of October 31, 1997 requested comments on a proposal to limit the degree of accuracy of chronic inhalation reference exposure levels to one significant figure. We feel that when significant figures are used in a real sense, accuracy is probably reasonably good to two significant digits, e.g., 95. mg/m³, 9.5 mg/m³, 0.095 mg/m³, but not, for instance, to four significant digits 95.25 mg/m³, 9.525 mg/m³ or 0.09525 mg/m³. We believe that expressing values to one significant digit would not necessarily reflect the accuracy of some measurements in this discipline.

Response: Uncertainty factors as used by OEHHA and USEPA for the development of chronic reference exposure levels are generally based on estimates of the most appropriate

Responses to Comments on the October 1997 Draft on Noncancer Chronic RELs

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value to the nearest order-of-magnitude (10-fold difference) or at best a 3-fold difference.

While we may have more precise information on some components of the risk assessment, the final REL can be no more certain than the weakest link in the chain of data used to derive it.

Thus, for example, we can not place any greater confidence in a REL estimate of 9.5 or 9.9 mg/m³. OEHHA is still considering whether to use one or two significant figures.

Vinyl Acetate Toxicology Group

Comments on the chronic REL for **vinyl acetate** were made by Robert J. Fensterheim, Executive Director of the Vinyl Acetate Toxicology Group, Inc. ("VATG"). The VATG represents all of the North American manufacturers of vinyl acetate and some of the major users of vinyl acetate which include: AT Plastics, Inc.; Borden, Inc.; Celanese Limited; E. I. Du Pont de Nemours and Company; Exxon Biomedical Sciences, Inc.; Millennium Petrochemicals; Rohm and Haas Company; and Union Carbide Corporation. OEHHHA proposed use of the US EPA RfC of 200 µg/m³ as the chronic REL for vinyl acetate.

Comment 1: OEHHHA has proposed an inhalation reference exposure level of based on a two year bioassay by Owen 1988. That study was sponsored by the vinyl acetate industry. In proposing the REL for vinyl acetate, OEHHHA elected to make use of the Reference Concentration (RfC) developed by U.S. EPA which is presented in their Integrated Information Risk System (IRIS) database. The VATG support OEHHHA's determination to rely on the EPA Reference Concentrations for purposes of establishing RELs, but the RfC must be based on the latest science and be up-to-date. In order to ensure continued consistency, we believe that OEHHHA should adopt a provision for presumptive and automatic updating of the REL whenever the EPA RfC is revised. Vinyl acetate, like several other compounds involved in active research and risk assessment activities, will be reevaluated in the near future. On January 2, 1998 (63 FR 75), EPA announced their decision to update the IRIS databases for several compounds including vinyl acetate. This update, which will include a reevaluation of the RfC, is scheduled to start in FY 1998. That update will be partially based on the considerable mechanistic research that the VATG has sponsored. We suggest that in developing the RELs that OEHHHA make reference to the IRIS database so that updates to the EPA RfCs can be readily incorporated into the OEHHHA RELs program.

Response: The USEPA RfC for vinyl acetate has been in place since 1990. 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. Use of RfCs as chronic RELs was one action that OEHHHA took to implement Governor's Executive Order W-137-96, which concerned the enhancement of consistency and uniformity in risk assessment between Cal EPA and USEPA. 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 review and possible endorsement. OEHHHA intends to harmonize with USEPA as much as possible, but not uncritically and not automatically.

Response to Comments on the Scientific Review Panel Draft of the

***Air Toxics Hot Spots Risk Assessment Guidelines Part I:
Determination of Acute Reference Exposure Levels
for Airborne Toxicants***

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Comments on the chloropicrin acute REL submitted by the Chloropicrin Manufacturers' Task Force (CMTF) in a letter from Stephen Wilhelm dated November 30, 1998

Comment 1: In regard to physical and chemical properties, a more complete description of the metabolites would be that chloropicrin photodegrades into phosgene, which is hydrolyzed to CO₂, HCl, NO_x, and monatomic chlorine.

Response: Comment noted. OEHHA did not attempt a complete description of all the fates of the chemicals studied, but appreciates the commentator's extension of our description.

Comment 2: In regard to major user or source, the draft document does not make clear that chloropicrin's primary use is as a preplant soil fumigant by itself or in formulations with other products. In addition it is no longer used for grain fumigation

Response: OEHHA will revise the draft to incorporate the comment.

Comment 3: A substantial body of recently completed chloropicrin studies was not included in the draft summary. Many are inhalation studies and should be considered in evaluations of chloropicrin inhalation toxicity. These studies are cited in the references to this document and include those by Chun and Kintigh, 1993; Yoshida, 1987; Schardein, 1994; Schardein 1993a and 1993b; Burleigh-Flayer, 1994; NCI, 1978; Wisler, 1995; Ulrich, 1995.

Response: The comment lists several studies to consider. Only Yoshida, 1987 is a study reported in the peer-reviewed literature and is a subchronic study. The others are a 1978 NTP carcinogenicity bioassay and unpublished studies (both inhalation and oral) from the Bushy Run Research Center (BRRC) and the International Research and Development Corporation (IRDC). None appear to be acute studies, but data in the Schardein developmental studies may be usable to develop an acute REL protective against a severe effect. OEHHA conducts a literature search before developing an REL. OEHHA prefers to use studies from the peer-reviewed literature, such as the Kane *et al.* paper used as the key study. In the interests of space and time, we only describe key studies used in deliberating the REL. Both J.L. Schardein of IRDC (64 papers listed on Medline in December 1998) and H. Burleigh-Flayer of BRRC (11 papers), who are listed as authors of the unpublished reports, publish in the peer-reviewed literature. Neither has published their work on chloropicrin. In fact Medline lists only 25 papers with the key word chloropicrin published since 1965. Publication of these recent studies would be a valuable addition to the toxicologic literature on chloropicrin and might be useful in protecting the public.

Comment 4: The Task Force believes that in developing the acute REL, OEHHA has inappropriately applied uncertainty factors to chloropicrin toxicity data. The draft acute REL for chloropicrin is derived with the use of a 10-fold dose reduction factor for interspecies uncertainty and an additional 10-fold dose reduction for intraspecies uncertainty. The 10-fold interspecies UF proposed for the acute REL is inconsistent with the 3-fold UF proposed by OEHHA for the chloropicrin chronic REL.

Response: The 10-fold interspecies UF proposed for the acute REL should not be directly compared with the 3-fold UF proposed by OEHHA for the chloropicrin chronic REL. The latter 3-fold factor was used instead of 10 because a correction had been made in the chronic REL derivation for the differences between human and animal respiratory tracts, a correction developed by USEPA for Reference Concentrations (RfCs). No such correction was made in the acute REL development. Therefore, the full 10-fold uncertainty factor is applied.

Comment 5: The most appropriate model for the use of uncertainty factors with chloropicrin is presented on page 43 of the draft. OEHHA explains situations where UFs of less than 10 can be used in the development of the REL. The example cited by OEHHA is acrolein, an acute respiratory irritant like chloropicrin. No UF was used for interspecies extrapolation because human data were cited, and a factor of 3 was used for the uncertainty of extrapolating from a LOAEL to a NOAEL. The CMTF believes that because the critical effects supporting the derivation of the chloropicrin OEHHA REL are limited to sensory and respiratory irritation and are not progressive, there is no need for an interspecies uncertainty factor. Nonspecific irritation effects seen at the portal of entry and target organ following exposure to chloropicrin are equivalent across all species tested (cites above). Nonspecific irritation at the site of contact was seen in all species evaluated, including dogs, rabbits, rats and two strains of mice. There is no basis to conclude that humans will respond differently from these mammalian species. Likewise, there is no basis to conclude that human respiratory tissue will be differentially susceptible to chloropicrin irritation.

Response: The UF of 3 used for acrolein cited in the comment was for LOAEL to NOAEL extrapolation. In the key study used for developing the chloropicrin REL, an animal NOAEL was available. In the case of acrolein, no interspecies extrapolation was necessary because the study was conducted in humans. The use of 1 as an interspecies UF when the study is conducted in animals contradicts most experience in toxicology and would have to be done on a case by case basis. While there is merit to the argument that nonspecific irritation at the site of contact might occur at similar concentrations across mammalian species, more data are needed before assuming that is the case in evaluating public health impacts. It would be useful (1) to sponsor studies of people exposed to varying airborne concentrations of chloropicrin for time periods up to 1 hour, so that the human and animal data could be directly compared or at least (2) to summarize the available data supporting the commentator's contention that an interspecies factor of 1 is adequate to protect public health.

Comment 6: Although the draft suggests that acute exposures to airborne toxicants follow a graded response (OEHHA, 1998), exceptions are known and acknowledged by OEHHA. Airborne exposures to chloropicrin stimulate the trigeminal nerve in the nose. This system is protective and responds on an all-or-none basis to chemicals such as CO₂, acetic acid, and H₂S in addition to chloropicrin. Human data for chloropicrin exposure are cited by OEHHA and support the position that an UF for interspecies differences in chloropicrin responsiveness is not justified. Likewise a 10-fold factor for intraspecies variability is not justified.

Response: It is not clear from the comment why human data cited in the OEHHA document support the position that an UF for interspecies differences in chloropicrin responsiveness is not justified. The human data are relatively limited. Grant (1986) reports that exposure to 1 ppm (6.7 mg/m³) causes immediate lacrimation and eye irritation. Eye irritation and lacrimation were observed in humans exposed to 0.3 ppm for 10 minutes (Prentiss, 1937). In the report cited by the commentator, Krieger (1996) indicates that Flury and Zernick (1931) report intensive irritation for 3-30 second exposures to 0.3-0.37 ppm chloropicrin. These levels bracket the observed NOAEL for decreased respiratory rate in mice in Kane *et al.*, 1979. The data suggests that eye irritation in humans is a more sensitive measure than respiratory decrease in mice based on the NOAEL in mice of 0.6 ppm.

OEHHA uses a 10-fold intraspecies uncertainty factor to account for variability in human response. The commentator provides no information why an uncertainty factor for variability in human response is not appropriate.

Comment 7: Because the respiratory effects of chloropicrin are concentration and not dose dependent, duration of exposure is not a factor in producing effects or in preventing effects. RELs are intended to protect against mild adverse effects, severe adverse effects and life threatening adverse effects. By definition, the duration of exposure for these effects is one hour. Chloropicrin is well-known for its exposure warning properties and the likelihood of a one-hour exposure at a level that would cause any degree of adverse effect is quite low. According to the document, An Assessment of Implied Worker Exposure and Risk Associated with Chloropicrin Loading, Application, and Field Tarping Activities Following Application, and Implied Exposure and Risk of Off-Field Concentrations Resulting From Soil Fumigation (Kreiger, 1996), “the inherent human and animal warning response to chloropicrin occurs at low levels (0.15-0.3 ppm) of exposure in air. Adverse effects of higher levels (1 ppm or more) of chloropicrin have revealed remarkably similar patterns of pulmonary injury in humans and test animals. Protective reflex responses and adverse effects represent two distinct responses of humans and animals to chloropicrin inhalation.” The protective warning properties of chloropicrin occur at airborne concentrations of 0.15ppm. Adverse effects as defined by OEHHA, “any effects resulting in functional impairment and/or pathological lesions that may affect the performance of the whole organism, or that reduce an organisms’ ability to respond to an additional challenge” will not occur at the chloropicrin concentrations that provoke the common chemical sense, i.e., the warning property. Exposure to chloropicrin below this concentration has no effect and an application of safety, or uncertainty, factors is without rationale. The California acute REL should therefore be established at 0.1 ppm.

Response: OEHHA considers irritancy an adverse health effect. The chloropicrin REL is based on measures of irritancy in an animal model that may not be a particularly sensitive measure of irritant effects. There are not adequate data in humans to characterize chloropicrin irritant effects well. As such, for the purposes of protecting sensitive members of the population, we use uncertainty factors. No data are provided that would substantiate that an individual will not be irritated at the “warning level” of 0.15 ppm. In fact, Flury and Zernick, 1931 report intensive eye irritation and lacrimation upon very short-term (3 to 30 second) exposures to 0.3 – 0.37 ppm

chloropicrin. In addition, the qualitative observation of similar “patterns” of toxicity cited in the comment are not helpful for quantitative evaluation of the REL.

OEHHA plans to update the guidance periodically. If human (or more animal) data become available which indicate that the proposed REL should be reassessed, OEHHA can reevaluate the REL in a future update.

Comment 8: OEHHA relies on an application of Haber’s Law to establish a time-concentration relationship for exposure to chloropicrin and effects of that exposure. Despite the statement on page 51 of the draft OEHHA document acknowledging the National Academy of Sciences position that Haber’s Law does not apply to some irritants, discussion is presented about the application of various “chemical-specific parameters” (n) in the Haber’s Law equation. The discussion suggests that the value for n be greater than 1 for chemicals in which the toxicity is determined more by exposure concentration than by duration of exposure. That is, n should be greater than 1 for chemicals like chloropicrin. The example for this case in the draft OEHHA document is ammonia and the range of values for n given in the draft document is 0.8-4.6. Table 1 presents a series of calculations of Haber’s Law for chloropicrin using several values for n and values for exposure time that are realistic. “Normalizing the time of exposure to 60 minutes and employing a value for n that is not greater than 1 can inflate the REL calculation by a factor of 60 to nearly 16,000.” The value for n used by OEHHA for the development of the chloropicrin REL was 1. Additional uncertainty factors for species extrapolation are not needed. [The comment also contains a Table 1 in which no UFs were applied.]

Response: OEHHA has suggested a modified Haber’s Law for use in time extrapolation. This modification allows for an exponent, n , to be applied to concentration other than one. As the exponent increases in value, the implication is that concentration is more important than time. Values greater than 3 or so reflect almost complete concentration dependence. There are a number of values of “ n ” that have been derived by ten Berge *et al.* (1986), OEHHA, and USEPA listed in Table 12. The values vary even for the same chemical using different datasets. While it is theorized that the value of “ n ” for chemical irritants should be greater than one, the data don’t always reflect that. For example, for the irritant chemical, chlorine, analysis of different datasets have produced values of “ n ” ranging from 1 to 3.5 (see Table 12, p. 52).

The comment supplies a table of extrapolated one-hour concentrations using assumptions of $n=1$, 2, or 3. Unfortunately, the extrapolations shown are for 10 second or 1 minute exposures extrapolated to one-hour exposures. We would not recommend using a modified Haber’s Law for extrapolating such short duration exposures as 10 seconds or even one minute. Thus, the comment that the extrapolation varies tremendously when using different values of “ n ” is not really appropriate for the extrapolation conducted by OEHHA, which was from ten minutes to one hour. When using an exponent of 2, the value of the OEHHA REL changes from 1 to 2.5 ppb. When using a value of n of 3, the REL would be 3.3 ppb. Thus, while there is definitely a difference in evaluating the REL using different values of n , the difference is not orders of magnitude as implied by the comment.

**Comments from the Ethylene Glycol Ethers Panel
submitted by Courtney Price of the CMA**

Comment 1: EGBE is not a primary reproductive or developmental toxicant. The comprehensive EGBE toxicology data base (including the Tyl 1984 rabbit developmental study relied on by OEHHA) has been reviewed by many expert groups. None have found the compound likely to be a human reproductive or developmental hazard. The National Institute of Occupational Safety and Health's (NIOSH) 1990 Criteria Document, for example, after noting (at p. 45) that maternal toxicity occurred at 200 ppm in the Tyl study on which OEHHA relies for its proposed REL, concludes (at p. 65): "Data obtained from animal studies indicate that EGBE and EGBEA do not cause adverse reproductive or developmental effects." Government agencies have not set guidelines based on reproductive/developmental effects. EGBE is not listed as a reproductive or developmental toxicant under Proposition 65.

OEHHA presents a contradictory assessment of EGBE developmental studies. Its assessment interprets the Tyl (1984) rat study as have other reviewers including NIOSH; it finds (at p. C-109) that it is not clear whether the high dose reproductive findings (delayed skeletal ossification) are direct effects of EGBE or secondary effects of concurrent maternal toxicity. On the other hand, OEHHA's assessment (at p. C-109) of the concurrent Tyl rabbit study notes the maternal toxicity at 200 ppm, but ignores that finding in determining that the acute REL will be based on "developmental effects" found at the same dose. The proper assessment of both Tyl studies (rat and rabbit), as NIOSH and others have found, is that EGBE is not a direct reproductive nor a developmental toxicant in rodents. Therefore, acute human exposure level guidelines for EGBE should not be based on such effects. OEHHA reaches a second unjustified conclusion about the Tyl rabbit study.

Response: OEHHA agrees with the comments. We have revised the proposed REL based on reproductive/developmental effects and have instead used the human data described in Carpenter *et al.* (1956) and Johansen *et al.* (1991).

Comment 2: The draft (at p. 110) acknowledges, as have other reviewers, that hematological effects contributed to the high dose adverse developmental outcomes in rats. The draft, however, argues that the high dose reproductive and fetal toxicity in the rabbit study was not secondary to hematological effects and that rabbits do not appear to be susceptible to EGBE-induced susceptibility (at p. C-110). To the contrary, although Tyl (1984) took no blood measurements during exposure that could have detected hemolysis (blood was only analyzed 11 days after the cessation of exposure), she reports red urine in the cages (57 Env. Health Perspect. at p. 60). Rabbits, like rats and mice, have been found susceptible to EGBE-induced hemolysis. Indeed, OEHHA itself notes on the previous page (C-109) that "rabbit erythrocytes resemble rat erythrocytes and are therefore also sensitive to the hemolytic effects of EGBE (Ghanayem *et al.*, 1992)." See also: Carpenter 1956; Tyler, 1984; Truhaut, 1979 (EGBE acetate); and Allen 1993a and 1993b, all reporting hematologic effects in rabbits by inhalation, oral or dermal exposures. Particularly pertinent to interpretation of the Tyl blood results 11 days after cessation of exposure

are the findings reported in Tyler, 1984 of hemoglobinuria in rabbits during exposure, but with recovery after 14 days of non-exposure, indicating that recovery occurs and thus explains why the Tyl study did not detect hemolysis 11 days after exposure.

Response: OEHHA agrees with the comment and has revised this proposed REL based on the conclusion that hemolysis did occur in the rabbits as pointed out in the comment. We have instead used human data on irritation as the basis for the REL.

Comment 3: The acute REL should be based on human data. The draft determines (at p. C-111) an acute REL of 3.8 ppm (19 mg/m³) based on a LOAEL for mucous membrane irritation of 113 ppm in Carpenter (1956) and uncertainty factors of 10 for intra-species and 3 for the LOAEL (to NOAEL extrapolation). The acute REL to be derived from the Carpenter data should be increased at least three-fold. A ten-fold uncertainty factor for intra-human variability is unwarranted. The OSHA PEL has been 50 ppm for many years (although OSHA proposed reducing it to 25 ppm to conform to the ACGIH TLV) and the European Union Occupational Exposure Limit is 20 ppm. No reports of irritation have occurred at these limits. For irritation effects of EGBE, an uncertainty factor of 3 should be fully adequate. Thus, the REL should be 11.4 ppm (55 mg/m³).

Response: In the Carpenter *et al.* (1956) report the study subjects were 2 male volunteers, one 34 years old, the other 44, who were presumably in good health. Subjects were exposed to 113 ppm for 4 hours. Subjects reported irritation of the eyes and nose. No NOAEL was noted in this study at this exposure level and time. In Johansen *et al.* (1991), 7 healthy males were exposed to 20 ppm for 2 hours, with no apparent effect. The absence of reports of irritation at the various occupational exposure limits in the working population is encouraging. However, the intraspecies factor is designed to address the variability in the general human population. Since the sample sizes are so small (n = 2 and n = 7), a factor of at least 10 is needed to protect women, infants, children, the elderly, those less “healthy”, those too infirm to work, etc.

Comments from Elizabeth Margosches, Ph.D.,

Comment 1: P. 13 The level protective against severe adverse effects is the REL when the most sensitive endpoint found is a severe adverse effect. The REL then might not be protective against mild effects.

Response: The REL is protective against essentially all effects even when the endpoint is derived for a severe effect. The reason is that the most sensitive endpoint is used, that is, the endpoint that occurs at the lowest experimental concentration is used as the basis of the REL, and that effect might be classified as severe (e.g., teratogenic effects) rather than mild (e.g., mild irritant effects).

Comment 2: p. 14 of the document states that “It is OEHHA’s intent that, to the maximal extent possible, the levels will protect nearly all individuals.” This is so vague as to suggest you cannot succeed.

Response: We have tried to convey the idea that we would like to protect as many people as is feasible. However, there are individuals who may exhibit idiosyncratic responses to chemicals which would not show up in typical animal or human studies. In addition, it is difficult at best to quantify what percentile of the population one is protecting at a specified concentration since there are too many uncertainties in human response to accurately ascertain that value. Hence we cannot be confident in stating what percentile of the population we believe are being protected from a given REL.

Comment 3: p. 14 Section 1.6 I would include some language indicating that some kind of manipulation of the exposures observed or administered in the basis studies is needed to be able to make inferences about one-hour exposures and whether these will be elaborated elsewhere in the document.

Response: We will indicate that time-extrapolation is needed when the exposure duration is not one-hour, and that this is described later in Section 3.4.

Comment 4: If 35 of 51 RELs are based on human data, why write in Section 1.6.1 that your choices are driven by what you get from animal toxicology? More elaboration is needed.

Response: Section 1.6.1 deals with the issue of how people are exposed in real time and contrasts this with how animals are exposed in laboratory settings. The same could be said of chamber exposures of humans. We will add that into the paragraph that describes the differences between experimental exposures and real-life exposure patterns.

Comment 5: Section 2.4.1.1.1 shouldn't refer to negative epidemiological studies unless you wish to denote ones that may indicate protective effects. Even then, you can see the ambiguity of your terminology.

Response: Section 2.4.1.1.1 states "Negative epidemiological studies present an additional difficulty in interpretation. Estimating the power of the study to detect an effect can be useful in providing an indication of the maximum incidence consistent with the failure to show that the exposed group was statistically different from the control group." It is not clear why the commentator objects to this or why the terminology is ambiguous.

**Comments of Ernest V. Falke, Ph.D.,
U.S.EPA, Office of Pollution Prevention and Toxics**

Comment 1: You have put together a good document. I hope you leave the door open for frequent revisions as you gain experience. The biggest impedance to any progress is the adherence to established procedures after they become obsolete. I also note that you have not used dosimetry corrections and believe that is a good decision.

Response: Comment is noted and appreciated.

Comment 2: I work on the National Advisory Committee for Acute Exposure Guideline Levels so many of my comments are related to that effort. As an overall comment I suggest you include the following statement which is in the AEGL SOP. "NAC/AEGL Committee reasonableness test: The committee generally evaluates the resultant AEGL values within the context of other supporting data to determine the reasonableness of the extrapolated values. A consensus of the committee favors the use of uncertainty factors that result in an AEGL value that best fits the supporting data." The reasonableness test is also referred to as the laugh test. Look at the bottom line. Do the numbers make sense? If they don't, then adjust the uncertainty factors. Do not rigidly adhere to rules which give a nonsensible number.

Response: Comment noted. We have attempted to be flexible in the use of UFs where the data indicate that such flexibility is appropriate. Where it is most difficult to know if the numbers make sense are in those cases where there is the least information available - where there is little to compare the number against. Comparisons with occupational standards are generally not helpful unless the underlying basis of the standard is known and relevant; unfortunately, that is generally not the case.

Comment 3: Regarding your definition of mild adverse effects. Will the person experience 'slight' irritation at or below the level? How does odor detection enter into this equation? Can odor be perceived below the mild effects level? What if the odor is objectionable? How does this enter into the equation?

Response: Very few (those with an idiosyncratic reaction) should experience any irritation below the level protective against mild adverse effects. Depending on the chemical, odor may be detected below the level protective against mild adverse effects. The odor may even be objectionable but by itself an odor is a nuisance but not an adverse health effect. Many people find normal odors objectionable, such as those from garlic and other foodstuffs, but the perception of the odor is not usually considered an adverse health effect. However, when the perception of odor is accompanied by physiological responses such as headache and nausea, OEHHA considers such an effect an adverse health effect.

Comment 4: On page 12, you include reproductive/developmental effects in the 'severe' level. Should developmental effects be in the 'life-threatening' level since many times the consequence of chemical exposure can be fetal death?

Response: Developmental effects such as fetal death could be considered life-threatening whereas malformations are generally severe adverse health effects. This needs to be addressed on a case by case basis. We have not used fetal death as an endpoint to extrapolate from in deriving RELs.

Comment 5: You present values in terms of mg/m^3 . You should also express them in ppm. Most publications use ppm and having the REL level presented in both units will facilitate REL comparisons to published literature toxicity values.

Response: In the chemical summaries (appendix C) both are presented. Some materials (metals) cannot be expressed as ppm. In the Hot Spots program the RELs are compared to ground level concentrations expressed as mg/m^3 or $\mu\text{g}/\text{m}^3$ in a hazard index approach. Thus these units are much more useful than ppm or ppb for our program.

Comment 6: On page 28 (table 7), you mention specific decrements in pulmonary function tests as severe. What is the basis for this? What is the normal variability seen in humans? In the latest SAB review of the EPA ARE guidelines there was criticism of the use of a RAW decrement which was considered within normal variation.

Response: A severe effect based on pulmonary function tests would have a clinically significant change in specific airway resistance (100% increase) or airway conductance (50% decrease) plus a $\geq 20\%$ drop in FEV1 or other symptoms consistent with bronchoconstriction. This combination is consistent with reactive airways disease/asthma which is a serious, occasionally life-threatening condition. This is described more fully on the following page of the TSD.

Comment 7: In the text, you refer to Appendix D for Categorical Regression as a methodology. This method came under extensive criticism at the recent Scientific Advisory Board review of the ARE guidelines. It has a number of problems which preclude its use at this time. What is extremely useful is assigning effects to categories and plotting them. This allows one to visualize the entire data set in one chart. It provides a very useful tool to identify data trends, outliers, and how well the REL levels chosen fit against the entire spectrum of toxicity data on a chemical. With all of the emphasis on mathematical models people tend to overlook the incredible capacity of the human brain to intuitively make associations from patterns that no statistical model can approach.

Response: The Categorical Regression Methodology is included in an appendix for completeness. We have not used it to derive any values.

Comment 8: On page 31, in the discussion of BD, you cite the work on developmental toxicity in arriving at conclusions. This is not valid to extrapolate to acute outcomes. The developmental toxicity analysis has very complex algorithms to account for litter effects among other things.

Response: The discussion is included for completeness. We acknowledge that the algorithms are complex. Staff recognizes that there are differences in how well the benchmark dose (BD) (or benchmark concentration, BC, in our case) approach works for different endpoints. We did not arrive at conclusions for other endpoints from the developmental toxicity work cited in the document.

Comment 9: In the discussion of BD, you cite Fowles and Alexeeff (1996) as support for the choice of the 5% incidence level. This is an abstract. Your choice of the response level and 'model' is the most important conclusion you draw with respect to the use of the BD. How broad is the spectrum of chemicals used in drawing this conclusion? What were the endpoints? LC50?

Response: The study examined 18 chemicals from 29 studies. The endpoints included lethality in animals, eye irritation in animals and people, respiratory irritation in animals and people, and CNS effects in people. The most acutely lethal compounds included phosgene and methyl isocyanate while the least acutely lethal included vinyl chloride.

Comment 10: On page 33, in comparing the log-normal probit with the Weibull model you talk of the statistical fit. The 'fit' applies only to the data region. The model is used to extrapolate outside the data region where the validity of the 'model' is questionable. The EPA Benchmark software (beta version) has about 5 different models which seem to fit the data reasonably well in the data range and even make similar predictions at the 10% level but diverge wildly at the 1% response level. It would be interesting to compare the divergence at the 5% response level. The 5% level may indeed be a good compromise. You mention that the log-normal probit works the best for steep dose response curves. If you have a steep dose response curve, why not use a ruler? What will you do with a shallow dose response curve? The 5% response level is disturbingly close to a probable biological response. You should compare your predicted 5% response with actual observed NOAELs to give the reader a better feel for how well your methodology fits the data and the confidence one can have in using the model.

Response: The problem of extrapolating beyond the observed range has been a long-term criticism of cancer and noncancer risk assessment. Unfortunately we have no choice but to extrapolate in order to protect public health. In regard to comparing the 5% response rate predictions (BC_{05}) and the NOAEL, Fowles and Alexeeff (1996) examined studies of 16 chemicals in animals and people for 4 acute endpoints and found that both the 1% and the 5% BCs were within a factor of 2 of the NOAEL. Thus the NOAEL was generally between the 1% and 5% BC which is one reason to place the BC below the NOAEL in Figure 6. The BC_{05} is not always below an identified NOAEL. The BC_{05} is a more accurate estimate based on linear regression of at least one dose-response curve (sometimes more) than the NOAEL which is constrained by the investigator's choice of dose levels. Thus, the comparison to the NOAEL is compromised by the imprecision of the NOAEL estimate and should not necessarily be used to engender confidence in the BC_{05} . If anything, it should be the other way around – the BC_{05} should engender confidence in the NOAEL. Fowles and Alexeeff (1996) also evaluated two models, the probit and the Weibull models. The results from the two models were not substantially different at the BC_{05} level.

Comment 11: Ideally if one were going to use statistical models one would fit an infinite number of curves ('models') against the data and choose the one with the best fit for each chemical. Pragmatically if one is going to do statistical modeling the log-normal and 5% response is probably a reasonable fit. However, consideration may in the future be given to using something like the EPA benchmark dose software to model a number of different curves and picking the one with the best fit in the data range to extrapolate to the response of interest. Just because Hattis effectively modeled some human data in the data range with a specific model does not mean it is the best model available. The choice of the best model to use to predict in the non-data range is almost a leap of faith. Also why is the log-normal biologically plausible - what are you getting at here?

Response: Comment noted. The log-normal is biologically plausible when several factors work together to produce the toxic response. In addition, many biological parameters are lognormally distributed probably because multiple factors influence the end result. Finally, our analysis and Crump's original analysis indicated that the results do not substantially differ at the response level we are using.

Comment 12: The best, most valid use of the benchmark dose is to predict a NOAEL from a LOAEL. However, the MLE should be used as the estimate of dose response and the statistical variability around that estimate used in the consideration of the selection of uncertainty factors - along with the entire body of supporting evidence.

Response: Comment noted. We disagree with the use of the MLE in the benchmark analysis because it does not utilize all of the available information. OEHHA has used the entire body of evidence to decide on what uncertainty factors we propose applying.

Comment 13: On page 34 Table 8, I disagree with the blind lowering of uncertainty factors (UF) because a benchmark dose analysis was performed. Conversely the blind use of an UF of 10 for intraspecies variability when animal studies are used is not productive. The benchmark dose is a tool to aid the evaluator. Once you extrapolate outside the data range you go beyond science - its use is not necessary more accurate since 'more accuracy' is a hypothesis you have proposed but not proven with data. Statistics is a 'precise methodology' within the data range of a specific experiment. Once you go outside that range or consider the entire body of evidence then other factors become important. The entire body of supporting evidence, including mechanism of action, should be considered when setting UFs with the benchmark concentration being only one component of the equation. The blind application of UFs in a rigid paradigm cuts out the powerful capacity of the human brain to interpret information and draw conclusions.

Response: The problem of extrapolating beyond the observed range has been a long-term criticism of both cancer and noncancer risk assessment. Unfortunately in risk assessment we have no choice but to extrapolate given the practical limits on the number of animals that can be tested and the ethical wrong of exposing people to harmful levels of chemicals. At some point we resort to scientific judgment (the human brain) and risk management. The lowering of the UF because a BC approach has been used is not entirely blind since one must first have better data than one

would have in, for example, the worst case of a free-standing NOAEL. In addition, more of the data (e.g., the entire dose-response curve and in some cases multiple dose-response curves) is being used to determine the BC thus addressing the uncertainty of using only a NOAEL (free-standing or otherwise). OEHHA has used judgment and data in assigning the uncertainty factors. There is support in the scientific literature for a 10-fold UF for intraspecies uncertainty (see Section 3.3.4.2). Where we felt there were sufficient information on sensitive subpopulations we reduced the intraspecies uncertainty factor of 10.

Comment 14: On page 36, Figure 6, you place the BC below the NOAEL. This is not necessarily so and fails to take into account the different spectrum of data one gets on different chemicals. With steep dose-response curves one could easily have a BC above the NOAEL. The more shallow the dose-response curve the more uncertain the extrapolation of the BC into a non-data range where mechanisms may differ. If you are going to propose this relationship (BC < NOAEL) and use it as a cornerstone to your methodology you should at least demonstrate that the relationship holds for most chemicals - including chemicals with steep dose-response curves and shallow dose-response curves - and across a number of chemical classes.

Response: We agree with the commentator that the BC could occur below the NOAEL, at the NOAEL or above the NOAEL. We selected the first possibility for illustrative purposes. The figure was not meant to be exhaustive.

Comment 15: On page 37, you justify lowering the UF in humans to 3 if a BC analysis is performed on data on human subjects. This should not be done in a rote manner. The mechanism of action of the chemical should be considered along with the body of data. A higher UF may be called for. Conversely, the use of NOAELs should not automatically entail the use of an UF of 10. The entire body of supporting data should be used when selecting UFs.

Response: Comment noted. As stated above, the lowering of the UF because a BC approach has been used is not entirely blind since to use the BC approach one must first have better data than one would have in the worst case of a free-standing NOAEL or LOAEL, and more of the data is being used in the BC approach thus addressing the uncertainty of using only a NOAEL or LOAEL (free-standing or otherwise). OEHHA has considered the body of evidence for each chemical before deciding on which UF to use. Staff agree that there could be cases in which the UF used with the BC₀₅ might be greater than 3, but it seems less likely when enough subjects have been exposed such that the BC approach can be used. Of course there is always the possibility that there are people with severe idiosyncratic reactions at low levels in the population.

Comment 16: On page 37, the formaldehyde example, you state 'vinyl chloride for 3 hours'. Do you mean hydrogen chloride? As for time scaling, the use of n=2 is based on lethality data but you are modeling a mild irritation endpoint. Irritation tends to be more concentration dependent. In this case the response occurs at a threshold concentration regardless of time of exposure. With irritants the body should be able to handle a specific level of chemical exposure at a steady state with no discomfort.

Response: The document should say “formaldehyde for 3 hours.” We have made the appropriate change to the text of the document. The best way to deal with the time and concentration aspects of irritant effects is a topic of ongoing discussion and research.

Comment 17: Page 39 contains an excellent example of addressing all of the supporting evidence and relying on a rigid paradigm.

Response: Comment noted.

Comment 18: On page 40, Alexeeff *et al.*, 1997 is not in the references.

Response: Staff will add the reference to the revised TSD.

Comment 19: In Table 9, unless the data base is so poor as to be useless, composite UFs of 1000 should not be used. If the data base is that bad it should not be used to set levels. Multiplying worst case by worst case by worst case to get 1000 is unrealistic and will lead to numbers too low to have any relevant meaning.

Response: Staff agree that the use of high composite UFs is troubling. For chronic RELs USEPA limits the maximum composite UF to 3,000. If a chemical is known to be acutely toxic, protection of public health indicates that an attempt be made to attempt to determine health guidance values. Additional experimental data may later lead to revision of the REL.

Comment 20: On page 48, a sentence implies children are ALWAYS more sensitive than adults. This is not necessarily so.

Response: Comment noted. However, the word always does not appear in the statement.

Comment 21: On page 45, the reference Gillis *et al.*, 1997 is not in the references.

Response: Staff will include the reference in its final revision of the TSD.

Comment 22: These are useful guidelines on page 46 (table 10) but should be viewed as such. Rigorous, unthinking application of these uncertainty factors without considering all of the supporting information can lead to numbers too conservative or not conservative enough.

Response: Comment noted. Staff agree that rigorous, unthinking application of such UFs without considering all of the supporting information can lead to numbers too conservative or not conservative enough. We have internally debated the application of the UF in developing each REL in this document.

Comment 23: On page 52 (Table 13), state why you use n=2 one way and n=1 the other way. Currently the AEGL Committee is using an experimentally derived n where available and n=2 where it is not available but is beginning to consider using n=1 or n=2 according to the direction of extrapolation.

Response: The value of 2 is explained in the last sentence on page 49. The value of 1 was chosen as a value protective of public health since adequate experimental data to justify any other value were not available. Staff is revising the text to provide better explanation of why we chose $n=1$ in Haber's Law when extrapolating from less than one hour to one-hour exposures.

Comment 24: On page 53, Item 6, you may want to start listing international planning levels also. We are becoming more and more involved with the international community.

Response: Comment noted.

Comments from Chemical Manufacturer's Association Isopropanol Panel

Comment 1. Isopropanol (IPA) should not be regulated as an air toxic. An extensive toxicological database exists on the toxicity of IPA and demonstrates that this chemical is of low toxicological concern. It is not regulated at the federal level based on toxicity concerns and the OSHA PEL of 400 ppm confirms that it is relatively nontoxic. IPA has relatively low photoreactivity and has been approved as a substitute for ozone-depleting substances. Thus, the removal of IPA from California's air toxics list would facilitate pollution prevention efforts. The panel has submitted a petition to CARB requesting that IPA be removed from the air toxics list.

Response: Isopropanol is a listed substance under the Air Toxics Hot Spots Act and is emitted in fairly large amounts in California. The REL is based on toxicity information and IPA is judged to be sufficiently toxic to justify the development of the REL by OEHHA.

Comment 2: OEHHA should not finalize an acute REL for IPA until the panel has an opportunity to complete additional studies. The proposed acute REL is based on Nelson *et al.*, 1943 where ten human volunteers exposed for three to five minutes were asked to report subjective symptoms of irritation. IPA at 400 ppm produced mild eye, nose and throat irritation in an unspecified number of subjects. The use of naïve subjects, short duration of exposure, and reliance on subjective responses do not provide a sufficient basis for distinguishing between odor perception and sensory irritation. The Panel is sponsoring a new study with human volunteers to identify the sensory irritation thresholds for IPA. The study will be completed in 1999. CMA encourages OEHHA not to finalize the REL until the results of this research can be considered.

Response: OEHHA has used the best current human data available to develop the REL. The process of REL development is an iterative process. As new data become available, OEHHA can update these guidelines. OEHHA intends to conduct approximately annual updates. OEHHA welcomes the additional study and will carefully consider the data when it becomes available.

Comment 3. Although improved, the revised REL for isopropanol remains inappropriately low. OEHHA took into account the 1995 comments of the CMA isopropanol panel in choosing a NOAEL of 200 ppm from the Nelson study, rather than starting with 400 ppm as a LOAEL. Also, OEHHA now uses 4 minutes rather than 3 minutes as the exposure duration from which to start the time extrapolation. While the Panel appreciates these changes, we continue to believe that the proposed value is not scientifically appropriate. The revised REL is more than 300 times lower than the ACGIH 8-hour TLV and OSHA PEL (400 ppm). It is more than 380 fold lower than the ACGIH and OSHA 15-minute STEL of 500 ppm. The revised REL is more than 10 times lower than the odor threshold.

An uncertainty factor of 15.4 is unnecessary to account for the short duration of exposure of the Nelson study. The use of Haber's Law for time extrapolation is not appropriate for chemicals such as isopropanol whose effects are based primarily on concentration. Where the physiologic effect is primarily concentration-dependent, use of Haber's Law will produce incorrect values

because it assumes that the triggering of the physiologic effect is based on both concentration and time. OEHHA should therefore not use Haber's Law for these substances. The comment goes on to compare IPA with acetone and MEK that, it is stated, do not produce irritation in a time-dependent but only concentration-dependent fashion. No correction factor is needed because time is not relevant to triggering the effect. The Panel's study is "expected to include assessments of both brief and occupationally-relevant exposure durations, and therefore should provide definitive data on this issue".

Response: Comparison of occupational standards with the REL developed for the general public is problematic because of the greater sensitivity of members of the general public relative to healthy workers. The general public includes infants and children, the elderly, pregnant women, the infirm, and other sensitive subpopulations. Frank health effects are also known to occur at the TLV in some instances. Thus, comparison of the TLV or STEL to the REL does not provide much information.

Use of time extrapolation does have associated uncertainties. It is true that some effects are primarily concentration-dependent and less dependent on time. As such, we are using a modification of Haber's Law, which reflects the dependency on concentration where data are available. The exponent, n , in the equation $C^n \times T = K$ goes to infinity as the effect becomes entirely concentration dependent and not time dependent. For example, ammonia has an exponent " n " of 4.6 in the equation $C^n \times T = K$, which indicates that the irritancy is largely concentration dependent and only a little time-dependent. However, empirical information is not available to develop a data-derived value for the exponent, n , for isopropanol. Hence, we used a default value of 1 to extrapolate from less-than one hour to one-hour exposures. When the Panel completes its study, and if it shows that time extrapolation should be using a larger exponent if appropriate for irritancy from isopropanol exposure, OEHHA can use this information in an update of the REL for isopropanol.

Comment 4. The revised REL inappropriately includes eye, nose, and throat irritation with pulmonary irritation under the category of respiratory irritation. OEHHA continues to use a hazard index approach for risk characterization. The comment is concerned that adding the other irritants will in effect decrease the REL for IPA. OEHHA improperly groups chemicals whose effects are probably not additive. Numerous airborne chemicals stimulate different nerve endings in the respiratory tract. The mechanism of action and severity of effect may differ significantly. The comment supplies a table from Alarie that refines the types of irritant effects on the respiratory tract. The comment is concerned that the lumping of IPA as a respiratory irritant might lead the public to believe that IPA causes pulmonary irritation when it only causes eye, nose, and throat irritation. The hazard index should group only those chemicals which effect the same portion of the respiratory tract or have the same mechanism of action.

Response: OEHHA has indeed grouped chemicals which may act with different mechanisms on different portions of the respiratory tract. Since chemicals usually act on more than one cell type in the respiratory tree while perhaps one region is more affected than another, we are suggesting designating the entire respiratory system as one target organ. This simplistic grouping is health protective in that it is unknown whether irritation of the upper and lower airway simultaneously

by two different chemicals is additive or synergistic or less than additive. Overall, we assume that the effect on the whole organism would be at a minimum additive. There is no reason to assume the actions of an irritant acting on the upper airway primarily would be antagonistic to an irritant acting mostly on the lower airway. If there were data to the contrary, we would be interested in seeing the data and including it in our risk assessment approach.

Comment 5. The proposed Level II REL for isopropanol is not consistent with other established values and is not scientifically appropriate. OEHHA proposes a level II REL of 12 ppm. It is not justifiable to say that concentrations above 12 ppm are likely to be disabling or produce long-lasting effects. The level II REL is based on effects in the rat. OEHHA identifies a LOAEL based on slight but statistically significant decreases in motor activity observed in male but not female rats at 1500 ppm and similar effects observed in a chronic study. These mild effects in rats do not provide a defensible basis for setting a level II value for humans. OEHHA should return to its original proposal of 400 ppm based on the Nelson *et al* study.

Response: OEHHA has utilized information from two studies in rats, Gill *et al.* (1995) and Burleigh-Flayer *et al.* 1994, which examined effects on motor activity of exposure to up to 10,000 ppm isopropanol. The Gill *et al* study identified a NOAEL of 500 ppm for CNS effects (as decreased motor activity). An uncertainty factor of 10 was applied for interspecies extrapolation and another factor of 10 was applied for intraspecies extrapolation. A time adjustment based on modified Haber's Law with $n=2$ brings the REL to 12 ppm (about 31 mg/m³). Effects on the CNS are considered serious effects.

The ACGIH and the NRC did not have these studies available to them at the time the TLV and EEGL were established. In addition, in developing the EEGL, NAS did not extrapolate from the 3-5 minute exposure of the Nelson study out to one hour. If this were done, then they would have derived an EEGL of 20 ppm. This number is consistent with the 12 ppm we have derived from the animal data.

**Comments on the Methyl Bromide Acute REL Submitted By
Courtney Price of the CMA CHEMSTAR Panel.**

Comment 1: OEHHA proposes a REL of 1 ppm (3.9 mg/m³) for methyl bromide. If accepted, this REL would be based on a NOAEL of 103 ppm from a study in beagle dogs exposed to methyl bromide for 23-24 days (Pharmaco-LSR, 1994). Dogs exposed to 103 ppm showed minimal evidence of neurotoxicity, primarily characterized by decreased activity on Day 9 of the study. OEHHA declines to use the standard lognormal time extrapolation because the limited number and size of the distinct dose groups in the study was deemed insufficient for analysis using this model. Rather than using the NOAEL derived from the acute exposure study, OEHHA inappropriately proposes to apply a 100-fold safety factor to the NOAEL observed after a 7-hour/day exposure for 8 days. This approach is inconsistent with OEHHA's standard procedure.

The acute neurotoxicity study in rats (Driscoll and Hurley, 1993) is the appropriate acute toxicity endpoint study for calculation of a 1-hour REL for methyl bromide. The selection of this study is consistent with procedures currently used by USEPA for acute toxicity hazard assessment.

Response: The acute REL is based on the Pharmaco LSR (1994) unpublished study submitted to the Department of Pesticide Regulation (DPR) and reviewed by DPR and OEHHA scientists. Groups of dogs were exposed for 7 hours to between 103 and 394 ppm methyl bromide for varying numbers of days. The critical endpoints were CNS and pulmonary effects, and lacrimation. The REL is based on effects observed after the first day of exposure. The 103 ppm exposure level was identified as a NOAEL for the one-day exposure. The statement in the comment that OEHHA based the NOAEL on an 8-day exposure is incorrect.

After much discussion with Department of Pesticide Regulation staff and outside experts at University of California, Davis, it was decided not to extrapolate to a one-hour concentration due to the limited nature of the database for evaluating time-concentration relationships, as well as the complicated acute toxicity of methyl bromide when exposures occur close together. The concentration required to induce adverse effects decreases with repeated exposures. This complicates application of a one-hour REL to the real world where the REL is compared to a "maximum" modeled one-hour concentration that might be experienced in consecutive hours or days. An uncertainty factor of 100 was used for interspecies and intraspecies extrapolation, yielding an REL of 1 ppm.

The 1993 study by C.D. Driscoll and J.M. Hurley entitled "Methyl bromide: single exposure vapor inhalation neurotoxicity study in rats" is an unpublished report from the Bushy Run Research Center. The commentator did not submit a copy of the unpublished report with the comments. If the commentator wishes to submit the report, the study can be considered in future updates.

Comment 2: The (Driscoll and Hurley) study also meets the requirements for numbers of animals and dose groups necessary for using the standard log-normal model with extrapolation for exposure time.

Response: Without the study in hand staff cannot evaluate whether the data are adequate.

Comment 3: The NOAEL in the Driscoll and Hurley study for a six-hour exposure was 100 ppm for neurobehavioral effects. Since effects produced by methyl bromide are both time and concentration dependent, the 100 ppm 6 hour NOAEL was extrapolated (by the commentator) to a one-hour NOAEL. "In other words, the 100 ppm/6-hour exposure is equivalent to a 600 ppm/1-hour exposure". Based on the following calculations:

Concentration x MW conversion (ppm to mg/m³) x inhalation volume/hour x hours = Total Dose to animal

Animal total dose x MW conversion (mg/m³ to ppm) x 1/human inhalation volume/hour = human equivalent ppm

a 6-hour exposure in rats is equivalent to a human 1-hour exposure of 2182 ppm. Application of a 100X Margin of Safety to this value yields a 1-hour REL of 21.82 ppm. This value is supported by the results shown in several methyl bromide acute endpoint toxicity studies in rats, mice, rabbits and dogs. (The commentator supplied a table of RELs calculated in the same manner from different studies.)

Response: Unless OEHHA is provided a copy of the study, we cannot evaluate the study. However, if the study by Driscoll and Hurley is well-conducted, the following analysis could be considered. According to p. 6 of the comment letter, Driscoll and Hurley obtained a NOAEL of 100 ppm for a 6 hour exposure of rats to methyl bromide. If time extrapolation is not done, the NOAEL can be divided by a UF of 100 (10 each for inter- and intraspecies uncertainty) to yield an acute REL of 1 ppm, the same value proposed by OEHHA based on the dog study. If time extrapolation is done using Haber's equation with the default value of n=2, we obtain an equivalent 1 hour NOAEL of 245 ppm, and an acute REL of 2.45 ppm which is rounded to 2 ppm, again very close to the OEHHA proposed value.

The commentator obtained a value of 21.82 ppm by using a combination of 2 methods - (1) a log-normal time extrapolation model and (2) an inhalation exposure calculation for methyl bromide used to convert a one-hour animal exposure to a one-hour human exposure as described in the comment. (1) The text of the letter indicates that the time extrapolation used is the modified Haber's Equation using n=1. We discuss this in the response to comment 1 above. (2) For animal to human extrapolation, the USEPA Human Equivalent Concentration (HEC) methodology results in a human HEC equal to or lower than the animal exposure concentration. The methodology submitted by the commentator results in a human equivalent concentration at least 10 times greater than the animal concentration for all the datasets presented in the comment (Table 2 in the comment letter), an unusual result. While these methods may have merit, the commentator would need to present much more information to show that they are scientifically

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preferable to those used by USEPA for calculating the human equivalent concentration and by OEHHA for calculating the one-hour REL.

**Comments on the Acute Reference Exposure level for Nickel and Nickel Compounds by
Neil J. King of Wilmer, Cutler & Pickering on behalf of NiPERA, NiDI, and Inco**

Comment 1: OEHHA calculated the acute REL for Ni and Ni compounds on the basis of Cirila *et al.* (1985) in which a sensitive population of metal platers with occupational asthma were exposed to nickel sulfate hexahydrate, a soluble nickel compound, and evaluated for atopy and pulmonary function challenge. The critical effect was an FEV₁ decrement > 15%, a mild adverse effect that is reversible following removal from exposure. Because the Cirila *et al.* (1985) study involved a sensitive human population, there was no need to apply an interspecies or an intraspecies uncertainty factor. However, since the critical endpoint was a LOAEL (33 µg as extrapolated to a one-hour concentration), OEHHA's calculation reflects application of a LOAEL uncertainty factor of 3, which produced a 1-hour acute REL of 11 µg Ni/m³.

We believe OEHHA correctly selected this human study to derive the acute REL for nickel sulfate and other soluble nickel compounds which may release nickel ions that bind to cellular proteins to produce an inflammatory response in the respiratory tract. It probably is not appropriate, however, to apply a REL derived from a study of soluble nickel sulfate to metallic (elemental) nickel, which undoubtedly would have a much higher acute inhalation REL (assuming it could be acutely toxic at all). An acute REL associated with exposure to soluble nickel also would be lower than an acute REL derived from studies where exposure to insoluble nickel compounds, since they are far less likely to produce an inflammatory response. Thus the Acute REL that OEHHA has derived from the Cirila *et al.* study of nickel sulfate-exposed asthmatics can be viewed as a "worst-case" value -- to the extent it is applied to nickel compounds generally.

Response: The commentator's statements are plausible, but unfortunately are not backed by available data. For this reason, we would not consider the REL a worst-case value. Furthermore, without data on more nickel species we are only theorizing about relative acute toxicity. We derive RELs with the data available. Data were available in the Cirila *et al.* study for nickel sulfate. It may be possible in the future to speciate nickel compounds for the purposes of developing more than one REL. However, it would then require facilities in the Hot Spots program to speciate their nickel emissions, a potentially costly prospect for most. Facilities currently just report their total nickel emissions. However, risk managers may weigh such statements about toxicity and the type of processes occurring at a facility when dealing with a hazard index exceeding 1.

Comment 2: We also agree with OEHHA's application of a LOAEL uncertainty factor of 3 rather than 10, since the adverse effect in the study by Cirila *et al.* -- a small reversible decrement in airway function as evidenced by FEV₁ measurements -- is caused by mild irritation of the respiratory tract. Accordingly, we support the Acute 1-hour REL of 11 µg Ni/m³ that OEHHA has calculated for soluble nickel sulfate. We believe, however, that its application should be limited to nickel compounds and that it should be identified as a "worst-case" value when applied to insoluble or sparingly soluble nickel species.

Response: As indicated above, we are not aware of sufficient data to draw the distinction between soluble and insoluble compounds as suggested by the comment. Further while we have classified the effects as mild, it is on the borderline of severe and mild. The study documents FEV₁ changes >15%. We generally categorized effects < 20% as mild. Thus, some of the subjects may have responded in the severe range. Further as suggested by the Scientific Review Panel, the UF for mild effects was changed to 6 from 3 based on available data and analyses of the LOAEL to NOAEL ratios. Consequently, the REL has decreased by 50%.

Comment 3: Accordingly, OEHHA should modify the heading of the Acute Toxicity Summary for “Nickel and Nickel Compounds” by limiting it to nickel compounds.

Response: Until we see specific data documenting that elemental nickel is not acutely toxic, we will retain the current heading. Staff note that metallic mercury has toxic effects and that elemental lead was included with lead compounds when the California ARB identified lead as a toxic air contaminant.

Comment 4: In addition, OEHHA should correct one confusing entry in the Acute Toxicity Summary. Section I of that Summary shows the Acute REL to be 11 µg Ni/m³, as does the derivation calculation in Section VII of the Summary. But the initial line in Section VII shows the REL to be 3.3 µg Ni/m³. That entry should be corrected.

Response: The value of 3.3 µg Ni/m³ was incorrectly listed on the initial line of Section VII. The value of 11 µg Ni/m³ was based on the use of 3 for the LOAEL to NOAEL uncertainty factor when the effect is mild irritation. Based on a comment by the Scientific Review Panel at the December 2, 1998 meeting we are changing the LOAEL to NOAEL uncertainty factor to 6 and the nickel REL to 6 µg Ni/m³.

Comment from Dr. Kathy Norlein, Minnesota Department of Health

Comment: California must be commended on the work completed to date on the acute values. The commentator expressed the concern that when a study was available that tested asthmatics no additional uncertainty factors were used to account for sensitive subpopulations. While it is reasonable to assume that asthmatics are a potentially sensitive subpopulation, the group of asthmatics that would be accepted for study is a “healthy” subpopulation of all asthmatics. To ethically be able to test asthmatics, they need to be adults who are in good health. Subjects with other health ailments are generally rejected for study (smokers, drug/alcohol users, very young, very old. Etc.) A factor of 10 would not be necessary because a somewhat sensitive subpopulation was tested. Rather than using a factor of “1” assuming that a sensitive subpopulation has been tested, a factor of 3 or 2 would be more prudent.

Response: The comment is an interesting one. When we chose an intraspecies uncertainty factor of 1 for chemicals tested in asthmatics, it is because we know asthmatics in particular are more sensitive to the chemical in question. There may be cases where a different group represents a sensitive subpopulation (lead and children for example). Then, a test in asthmatics would not be a test in sensitive subjects. The other point of the comment is a bit harder to argue, namely that because most asthmatics in a study are relatively healthy, there should be an additional uncertainty factor of 2 or 3 to protect less healthy individuals. We believe that there may be situations where it would be appropriate to use an intraspecies uncertainty factor of 2 or 3 when tests were conducted on a sensitive subpopulation. Determination of the most appropriate additional factor is problematic due to a lack of data on which to base such a factor. However, we think we have covered the most important groups fairly well in our analyses and REL derivations to date. We thank the commentator for the suggestion and will make use of it in future deliberations.

Comments from Mr. Ted Holcombe, Pacific Gas and Electric

Comment 1: The commentator is concerned with RELs which have large uncertainty factors, and notes that in Table 9 five compounds have UF of 1000, and fifteen compounds have a UF between 100 and 300. The comment also states that “OEHHA reduces LOAEL data by time factor multiplication and then by uncertainty factor multiplication”. The commentator suggests that the time adjustment factor should be included as an uncertainty factor. The comment also notes that “successive multiplication of these time and uncertainty adjustments factors leads to large differentials between LOAELS and proposed RELs”.

Response: The uncertainty factors are designed to provide a factor for interspecies extrapolation, intraspecies variability, and use of a Lowest-Observed-Adverse-Effect Level rather than a NOAEL. There are a number of studies indicating that humans are more sensitive than laboratory animals to a number of toxicants on a mg/kg-day basis. This is due to toxicokinetic differences (generally faster metabolism and clearance of the toxicant in the smaller lab animals) and can also be due to toxicodynamic differences (differences in how the toxicant interacts at the receptor). When data are available to define these differences, they are used in REL development. However, for the most part, these data are unavailable. There are also a number of papers that evaluate the range of human sensitivity to different toxicants. It can be several-fold to orders of magnitude. A ten-fold factor is adequate for most compounds and is thus the default. If data are available to refine this, then these data are utilized in the REL calculations (e.g., when sensitive subgroups are the study population). Use of large uncertainty factors reflects a relatively poor database for that chemical and endpoint.

Time adjustment does not always result in a “lowering of the LOAEL” as indicated in the comment. The purpose is to adjust from varied exposure durations to a one-hour exposure. It is not an uncertainty factor per se. Instead, it is the best scientific method we are aware of for adjusting for the toxicologic relationship between concentration and time.

Comment 2: The proposed acrolein REL is 41 times below the level of detection of the best available source test technique used in the 1996 risk assessment for the Kettleman Compressor Station.

Response: While this information is interesting, it does not necessarily mean that the proposed REL for acrolein is not valid. It might indicate that source test methods may be inadequate to evaluate the public health impacts of acrolein. Also, it appears that the test method limit of detection is above the concentrations evaluated in human subjects.

Comment 3: Citing a 1988 paper, the comment states that the proposed arsenic REL of 0.39 $\mu\text{g}/\text{m}^3$ is 25-times lower than the suggested arsenic intake level of 16 to 50 $\mu\text{g}/\text{day}$ as an essential nutrient.

Response: An element is considered essential if a diet deficient in the element leads to adverse health effects. Uthus and co-workers (1983) and the EPA (1984) have summarized studies demonstrating adverse effects of arsenic-deficient diets in goats, mini-pigs, chicks, and rats, where arsenic-deficiency affected manganese metabolism. However, further study is needed to resolve whether an arsenic-deficient diet is adverse to humans. No one has claimed that inhalation of arsenic is necessary to maintain good health. A trace element may also be classified as essential if the amount of the element in the body is maintained by biological processes. By this criterion, arsenic is nonessential (Liebscher and Smith, 1968). Neither a specific receptor nor a physiological role has been identified in humans.

It should also be noted that for many metals toxicity by the inhalation route is greater than toxicity by the oral route. Thus, it may not be appropriate to compare dietary exposures or even essentiality with inhalation exposures to the same element.

Comment 4: “Arsine gas is generally recognized as one of the more hazardous arsenic compounds, while pentavalent arsenic is generally recognized as less hazardous. Yet OEHHA’s methodology leads it to propose an REL for trivalent arsine gas of $160 \mu\text{g}/\text{m}^3$, while all other arsenic compounds are assigned a REL of $0.39 \mu\text{g}/\text{m}^3$,” which is based upon trivalent arsenic. “Pentavalent arsenic is more deserving than arsine of being assigned a separate REL.”

Response: Arsine gas has its own peculiar toxicity, lysis of red blood cells, and data are available to evaluate an REL for this compound. While we may be able to evaluate specific pentavalent arsenic compounds in future updates to this document, at the present time, we chose to use trivalent arsenic compounds as the basis for the REL. As a practical matter, most facilities report emissions of arsenic without speciating into trivalent or pentavalent. Thus, it is more health protective to have an REL based on trivalent compounds, since in general they are more toxic than pentavalent arsenic compounds.

Comment 5: PG&E appreciates the effort OEHHA has put into uncertainty estimation and does not dispute that each individual step OEHHA contemplates has a plausible justification. OEHHA does not adequately explain why it multiplies these uncertainty factors by one-another rather than adding them first. Adding the factors would yield far more believable RELs. The comment goes on to give examples of acrolein REL determined by dividing by the sum of the uncertainty factors rather than the product and noting that such a REL would unlikely to be exceeded for most combustion sources.

Response: The uncertainty factors are designed to account for specific uncertainties. We do not have data that indicates accounting for one also accounts for another, for example we do not know if a 10-fold uncertainty factor for interspecies differences also accounts for some or all of the intrahuman variability. Therefore, it is most prudent to treat the factors separately, which is what one does in using a multiplicative scheme.

Comment 6: The commentator disagrees with only providing one REL for a chemical to use in risk assessment. The comment suggests developing RELs by dividing a known effect level by uncertainty factors that have been added together rather than multiplied. The comment suggests

retaining our current approach but renaming that REL an “Uncertainty Elimination Level”, and suggests that the risk assessment guidelines include hazard indices that use both a “known effect level” and an “uncertainty elimination level” as the reference points to divide into the modeled ground level concentration. These three points (“uncertainty elimination level”, “reference exposure level” using additive Ufs, rather than multiplicative, “known effect level”) would provide the public with more information than just using an REL.

Response: The commentator’s suggestion to provide more information to the public and risk managers by having three levels to compare the ground level concentration to is an interesting one. In fact, we have attempted to provide the risk manager with information on not only the REL which is designed to protect against all adverse effects, but also with information on levels that would protect against severe adverse effects and life-threatening effects. The purpose of this is to allow the risk managers to see what adverse effects occur above the REL, and to judge the seriousness of that exceedance. As noted in the above response, we do not agree that the REL should be based on a method which adds the uncertainty factors before dividing the LOAEL by those factors, rather than multiplying the uncertainty factors. This would not be likely to protect sensitive subpopulations. In addition, an interested party can go into our documents (they are on the Internet on our Webpage) to learn how the REL was developed and see what the LOAEL is from the key study used in the calculations.

References used in the response:

Uthus, EO *et al.* (1983) Consequences of arsenic deprivation in laboratory animals. In: *Arsenic: Industrial, Biomedical, Environmental Perspectives*, Lederer WH and Fensterheim RJ eds. New York: Van Nostrand and Reinhold Company, pp. 173-189.

U.S EPA (1984) Health assessment document for inorganic arsenic: Final report. Office of Research and Development. Research Triangle Park, NC 27711 (EPA-600/8-83-021F).

Liebscher K and Smith H (1968) Essential and nonessential trace elements: A method of determining whether an element is essential or nonessential in human tissue. *Arch Environ Health* 17:881-890.

**Comments from Courtney Price, Phenol Regulatory Task Force,
Chemical Manufacturers Association**

Comment 1: The task group agrees with OEHHA's decision to withdraw and revise its original proposed REL of 0.38 ppm for phenol. As the Task Group pointed out in its prior comments, the originally proposed REL was based on a animal study and the application of highly conservative uncertainty factors. The Task Group agrees with the OEHHA decision to rely on human data, but believes that the proposed REL for phenol of 1.5 ppm still is unduly conservative and does not accurately reflect phenol's acute inhalation risks. The proposed value is inconsistent with standards established by other regulatory bodies.

OEHHA based its proposed REL for phenol on a study designed to evaluate absorption of phenol across the lung and through the skin, not to evaluate phenol's toxicity. Since no adverse affects were noted in the study, OEHHA took the highest concentration tested and called that a NOAEL. The Task Group does not believe that the NOAEL should be without reference to other data. The most direct and relevant measure of phenol's potential irritating effects can be found in Ruth (1986) in which the human irritancy threshold for phenol was determined to be 47 ppm. Therefore, the true human NOAEL for irritancy should be higher than 5.2 ppm but not higher than 47 ppm.

Animal data also support a higher REL for phenol's respiratory effects. The comment cites a study in which no phenol was detected in blood of rats exposed to phenol at 25 ppm. The comment states that these data indicate that inhaled phenol is readily conjugated and detoxified. The comment cites another ongoing study sponsored by CMA which does not show toxic effects at exposures of 25 ppm for up to two weeks.

OEHHA is urged in the comment to consider the rat data and revise the REL upward.

Response: The comment is correct in noting that the REL is based on a "free-standing" NOAEL from Piotrowski, 1971. However, the REL was developed after looking at the Ruth (1986) review. A measured irritancy threshold of 47 ppm is not inconsistent with an REL of 1.5 ppm after including time extrapolation and uncertainty factors. The time extrapolation was conducted because the exposure in the Piotrowski study was for 8 hours. Thus the 1-hour equivalent concentration was 15 ppm. Application of an uncertainty factor of 10 to account for sensitive subpopulations leads to a proposed REL of 1.5 ppm.

The information cited by the commentator that there were no adverse effects in rats at 25 ppm or that phenol could not be detected in rat blood at 25 ppm is not compelling. The phenomenon of irritancy would not be tested by measuring phenol concentrations in the blood. In addition, there is no indication given that objective measures of irritancy were taken in the ongoing study in rats cited in the comment. It is difficult to know when a laboratory animal is experiencing irritation until it is rather pronounced.

Comment 2: OEHHA should not apply an uncertainty factor of 10 to account for potential variability in human response to phenol's mild irritating properties. The Task Group believes that, in light of the endpoint at issue (mild irritancy effects) and the entire toxicological database, OEHHA's use of an uncertainty factor of 10 is overly conservative and yields an artificially low REL for phenol. The RAAC recommended that OEHHA delineate situations where uncertainty factors less than 10 could be used in the REL development process. The RAAC also recommended that OEHHA consider the appropriateness of the existing data and severity of the effect in establishing the uncertainty factors. The NOAEL used for the REL already represents a conservative estimate of the human threshold for irritation effects by phenol. OEHHA did not use an uncertainty factor of 10 for ammonia, formaldehyde, hydrochloric acid, hydrogen sulfide, nitric acid, nitrogen dioxide, sulfates, and sulfur dioxide.

Response: OEHHA has consistently used an uncertainty factor of 10 for intraspecies variability when the test subjects did not include sensitive individuals. There is no evidence that the human variability in response to mild irritancy is less than that associated with other toxicological endpoints. There is therefore no a priori reason to use an uncertainty factor less than 10 for intraspecies variability in response. The examples cited by the commentator were either examples where a benchmark dose calculation was involved (thus decreasing the need for a 10-fold UF) or where sensitive subpopulations were included in the studies upon which the REL is based.

Comment 3: The Task Group urges OEHHA to consider other existing standards for phenol. Existing standards are significantly higher than the level OEHHA seeks to establish. The OSHA PEL for phenol is 5 ppm. NIOSH recommends an 8-hour exposure limit of 5 ppm. Most relevant here is the ERPG-1 value of 10 ppm for phenol. The ERPG-1 level is similar in concept to the OEHHA REL. The ERPG-1 level is the maximum airborne concentration below which it is believed that nearly all individuals could be exposed for up to one hour without experiencing other than mild transient adverse health effects or without perceiving a clearly defined objectionable odor.

Response: OEHHA evaluated all available existing standards in developing the RELs. The occupational standards lack a consistent basis for derivation, are not designed for or recommended for protection of the general public, and in many cases may not prevent adverse health effects among workers. The ERPG-1 level is designed for emergency response, not routine predictable releases. The ERPG-1 level definition indicates that mild transient effects may occur at this level. For the Air Toxics Hot Spots program, OEHHA is interested in protecting against all effects including mild transient effects in a residential setting due to routine and predictable releases, not emergency situations. Thus the ERPG-1 is not directly applicable to the Air Toxics Hot Spots program.

**Comments from Robert Reynolds, Air Pollution Control Officer,
Lake County Air Pollution Control District in a letter to Dr. John Froines**

Comment 1: There is an ambient air quality standard for H₂S that was adopted which has been reviewed formally and informally on several occasions over nearly thirty years of existence. The latest formal review that I am aware of occurred in 1984. The standard is presently set at 0.03 ppm which is utilized by all air districts. This standard is considerably lower than the proposed REL forwarded to you by OEHHA staff for your consideration of adoption. CAPCOA guidelines set the original acute REL and AAQS at 42 µg/m³. OEHHA staff proposes a value of 142 µg/m³, but in Table A-1 of the referenced report a value of 100 µg/m³ is indicated.

Field observations and review of public complaints historically received by the Air Districts would indicate a “no observed effects level” (NOEL) at or below the AAQS. The public has complaints on record to the air districts of both nausea and headaches at or below the AAQS of 35 µg/m³. These are the same symptoms reported in the laboratory study utilized to adjust a reported “lowest observed effects level” (LOEL) to the proposed 142 µg/m³ REL. There is no scientific data to refute the argument of a NOEL that is lower than that proposed and there is valid data in the AAQS H₂S review record to indicate a lower value.

Response: OEHHA is revisiting the H₂S REL and has obtained records of complaint and air concentration from the Air District. OEHHA intends to revise the REL back to the AAQS based on the physiological responses of headache and nausea at levels substantially above the detection of H₂S odor.

Comment: The referenced human exposure studies were for 30 minute exposures and adjusted for a one-hour exposure by dividing the identified LOEL by two. There is no scientific evidence to indicate a linear time exposure relationship, or that a one-hour averaged exposure that allows markedly higher peaks than the hourly REL value is appropriate. From field responses and other exposure studies it appears that H₂S is unique in that a few minutes of exposure may induce a noted effect such as nausea. In the 1984 review of the AAQS it was noted that some districts had adopted a shorter term standard (i.e., 3 minutes) in addition to the state AAQS. A shorter than one-hour AAQS was recommended by our District. It was further noted that ambient air monitoring documented peak values as high as 22.5 times the hourly value, and peaks several fold the hourly average were common.

Response: Comments noted. OEHHA agrees with the commentator and is revising the proposed REL for H₂S to base it on the AAQS.

Comment: The OEHHA utilized study was performed on a sensitive population and no safety factor for a more sensitive population was used for this reason. There is no indication that the noted effect is the most sensitive health effect, nor that other sub-populations found in the general population such as asthmatic children, pregnant women, infants, or the respiratory impaired are not more sensitive to H₂S. I would suggest that the frequently noted effects judged from public

complaints are in fact odor annoyance with the corresponding physiological effects of nausea and headache. Likely the most sensitive sub-populations are pregnant women or respiratory impaired children.

Response: Comment noted. OEHHA's REL was to be based on respiratory irritation. This was in part because we were using the REL in a hazard index approach with respiratory irritation as an endpoint. The revised REL will be the AAQS. However, it will not be used in a hazard index approach for respiratory irritation, but rather will be used in a hazard index approach with odor-induced headache and nausea as the endpoint. As such, it will likely be in a class of its own.

Comment: In the case of the acute REL OEHHA should at a minimum confer with the California Air Districts and assess the complaints received from the public over the years to determine a NOEL prior to reaching a conclusion and making a final recommendation not based on direct scientific evidence.

The acute REL should remain at the AAQS value until such time as a NOEL with a direct scientific basis different than the AAQS is conclusively established.

Response: The commentator's concerns have been taken into consideration and OEHHA is now proposing to go back to the original proposed acute REL, namely the AAQS.

**Comments from Dr. Judy Strickland, U.S.EPA,
National Center for Environmental Assessment, Research Triangle Park**

Comment 1: In general, I found the Technical Support Document to be thorough in explaining definitions of adversity, level of severity, populations of concern, identification of key studies, weight of evidence, and strength of evidence. These concepts are difficult to convey to the reader, but the TSD provides the best concise treatment I've seen.

Response: The comment is noted and much appreciated.

Comment 2: Page 1, paragraph 3, line 1: The recommendation from the NAS should be supported with a citation from the reference.

Response: We will add the citation.

Comment 3: Page 3, Figure 1 – This is the only place in the whole document where dosimetric adjustments and HEC are mentioned. The document should provide some discussion of dosimetric adjustments and guidance on how they are to be made. An appropriate section for this discussion would be 3.3.4.1 which discusses uncertainties for animal to human extrapolations. If dosimetric adjustments will not be used to extrapolate from animals to humans, “dosimetric adjustments” and “HEC” should be removed from the figure.

Response: OEHHA agrees this is a bit confusing. We did not use dosimetric adjustments and HEC calculations in this set of compounds presented in the document. However, we may want to use it in the future and that is why we put it into the figure. We will indicate in the text in Section 3.3.4.1 that while we did not do any HEC adjustments in deriving the RELs in Appendix C, we may use dosimetric adjustments in the future.

Comment 4: Page 13, Section 1.5 – This is a good discussion on sensitive subpopulations. Some of our internal reviewers requested a discussion like this in our acute methodology (U.S.EPA, 1998).

Response: Comment noted and appreciated.

Comment 5: Page 24, paragraph 5, lines 5-6 – this is the only mention of an inadequate toxicology database. The document should explain the type of data required for a chemical-specific database to be adequate in terms of the types of toxicological endpoints studies during acute exposures. For example, is a database complete if no reproductive or developmental data are available for short-term exposures? The answer may be yes for a chemical that acts at the point of contact (an irritant), or no for a chemical which acts systemically. We have not made a decision (at EPA) regarding what types of endpoints are the minimum requirements for developing an acute RfC. We do have such requirements for RfC development (USEPA, 1994a).

Response: This is an interesting point, and one we have not completely addressed. We have not set out what exactly is required for an acute database to be considered complete. Rather, on a case-by-case basis, we have evaluated the literature and set RELs based on available data if the studies were adequate to do so. We have not, for example, included an additional uncertainty factor for missing reproductive/developmental studies. However, for the most part, the chemicals we have evaluated have enough information to know what the key toxic effects of that chemical are.

Comment 6: Page 29, paragraph 3, line 7 – I’m having trouble matching up the criteria for mild effects in this text with those in Table 7. Does “inhalation challenge” here refer to methacholine challenge in the table or does it refer to challenge with the chemical of interest? Please clarify.

Response: In this context (paragraph 2, page 28 in the hard copy version), the inhalation challenge is with the chemical of interest. We will clarify that in the text.

Comment 7: Page 31, paragraph 4, line 5-7 – Table 7 indicates that these criteria correspond to severe effects in a methacholine challenge test, not as a response to inhalation of an airborne chemical. Please clarify. Would the criteria in Table 7 for a methacholine challenge apply to a histamine challenge as well?

The criterion for the FEV₁/FVC ratio should be added to Table 7 also.

Response: Table 7 categorizes the adverse effects on pulmonary function into severity categories using methacholine challenge as the example in row 2. When evaluating the effects of a chemical, for instance SO₂, it is the effects of the inhalation challenge with the chemical that we are rating in comparison to Table 7 using methacholine challenge results. We would have to research the histamine challenge question, but the point of the table is really how much of an effect on the various pulmonary function measures is mild, severe, or life-threatening.

Comment 8: Page 35, paragraph 1, lines 8-9 – the USEPA, 1997, reference needs to be listed in the reference section.

Response: We have added it to the reference section.

Comment 9: We will be posting our own benchmark dose software on our web page. This software includes seven models for dichotomous data, several models for continuous data and a few nested models for developmental data. During the Science Advisory Board’s review of the acute reference exposure methodology, the Board was divided on whether to recommend the use of one default benchmark model or the use of several models to determine the best fit to the data.

Response: Comment noted.

Comment 10: page 53, Table 12 – All these chemicals have at least two effects listed in the table. One is in parentheses and one is not. A note in the table or text should explain the significance of the effects in parentheses and denote which effect was used to calculate the n.

Response: The parenthetical refers to whether the chemical is a locally acting irritant or whether it acts systemically. The endpoint is given first, and then the general statement on the mechanism (local v. systemic) is given in parentheses. We will clarify that in the table.

Comment 11: Hydrogen sulfide REL - I had also characterized the >30% decreased airway resistance in the two subjects as an adverse effect but was admonished for doing so during the SAB review of the acute exposure methodology. Dr. Mark Utell insisted that this magnitude of decrease in airway resistance is within the range of normal variation.

Response: We would disagree with the SAB member in that regard. We characterized the effect as a mild adverse effect in the two individuals.

Comments from Western Independent Refiners Association

Comment 1: OEHHA has not considered the ACGIH Threshold Limit Values. TLVs are limits that refer to airborne concentrations of substances and represent conditions under which it is believed that nearly all workers may be repeatedly exposed day after day without adverse effect (ACGIH, 1997). It does not appear that OEHHA ever considered the TLVs in deriving their RELs. We believe that the draft OEHHA RELs should be compared to the TLV and any major differences reconciled.

Response: As noted in the methodology section of the document, pp. 15-18, OEHHA evaluated existing guidelines including TLVs as sources of information during the REL development process. However, TLV values lack a consistent basis for derivation, are not designed for use with the general public and in fact are not recommended for use for the general public by ACGIH. In addition, in many cases, they do not prevent adverse health effects among workers (Roach and Rappoport, 1990).

Comment 2: The RELs do not consider sensory irritation effects associated with background, or ambient, exposure level. Sensory irritation studies are difficult to interpret because they are based on subjective human responses. Many studies report that subjects exposed to clean air have reported eye, nose, and throat irritation in up to 22% of the volunteers. We recommend that OEHHA begin the analysis of the dose-response relationship for sensory irritation at concentrations that effect at least 20% of the experimental subjects to avoid incorporating data that represents background or variable irritant effects due to factors unrelated to the test chemical.

Response: Although no reference was supplied by the commentator, the comment is apparently referring to a review by Paustenbach *et al.* (1997) which points out that many studies of irritancy of formaldehyde report greater than 0% response rate in the clean air exposed controls. The effect noted in the comment (controls feeling sensory irritation) may be real. However, it would be inappropriate to assume that in each human study of irritation, 20% of the people would have been irritated by clean air anyway and only response levels above 20% should be considered. Of the 7 studies of formaldehyde eye irritancy described in Paustenbach *et al.* (1997) which indicated a percent response for eye irritation in controls (0 ppm formaldehyde group), 3 had 0% response, one had 5% response and the others reported 22, 27, and 39 % response. OEHHA is striving to use the best available information and emphasizing human studies. The chemicals that irritate the eye and respiratory tract are known to be irritating from a number of reports, not just the reports we used as the basis of the REL. The basis of the commentator's statement that only those responses above 20% should be considered is not substantiated in the comment or in the paper by Paustenbach *et al.* (1997).

Comment 3: Uncertainty factors used to derive sensory irritants concentrations should be smaller than those used to establish safe exposure levels for systemic toxicity. Most irritant gases act directly on the mucous membranes or on the lungs and the intensity of effect is usually primarily dependent on the maximum concentration in air. This is unlike other adverse health

endpoints. We recommend that OEHHA consider the available data on susceptible populations for each chemical and use safety factors appropriate to the mechanism of toxic action. Further the size of the safety factor should vary according to the severity of the most sensitive adverse effect and the anticipated diversity of susceptibility. A safety factor of 2 is adequate for reversible eye and upper respiratory irritants. Higher safety factors should be used when the effect is not reversible.

Response: The uncertainty factor of 10 for intraspecies variability is not meant to reflect the severity of a response. Rather, it is meant to protect sensitive subpopulations by encompassing the wide variability in response of humans to toxicants. In fact, a 10 fold uncertainty factor might not be adequate for some compounds (Calabrese, 1990). The commentator does not provide data to substantiate the statement that a safety factor of 2 is appropriate for irritants or that a smaller uncertainty factor is justified based on mechanism of action.

Comment 4: OEHHA improperly used animal data to set RELs when human data were available. OEHHA selected critical endpoints using animal data when human data was available. WIRA believes that OEHHA should set RELs on human studies when they are available.

Response: As stated in our document, OEHHA prefers the use of human data when it is available and adequate. The comment provides no specific instances in referring to use of animal data when there were human data available.

Comment 5: In setting many RELs, OEHHA used studies in which repeated daily exposures to the chemical under study was for 4 to 8 hours per day over an extended time period. When setting short-term limits, studies with an exposure duration of about one-hour should be used and in no cases should studies where exposure durations exceed 8-hours be used.

Response: OEHHA has largely used studies with exposure durations less than 8 hours and down to ten minutes to generate one-hour RELs. OEHHA did use repeated dose studies of reproductive/developmental toxicity for several chemicals. Developmental and reproductive toxicants produce their effects during critical developmental periods that can be quite short (on the order of hours). Toxicity studies of necessity expose the dams throughout pregnancy since it is not known necessarily which time point is the most critical. To expose sets of dams for a given one-hour period or even 8 hour period throughout the pregnancy would not be logistically feasible, and would be very costly. Thus, for these types of toxicants, we only have repeated exposures studies available to us. OEHHA makes the assumption that a one-hour exposure sometime during development could produce a developmental or reproductive effect. We extrapolate from the daily exposure, which is generally 6 to 8 hours/day, to a one-hour exposure using a modified Haber's Law to derive the REL. This is justifiable given the mechanism of action of many reproductive/developmental toxicants.

December 10, 1998

Comments from ChemRisk on behalf of the Western States Petroleum Association

Comment 1: OEHHA should use the dose-response literature to develop one-hour RELs rather than rely upon a single study. The comment goes on to recommend using the method described in Guth *et al* (1992) to develop a dose-response curve based on an aggregate of all various high quality studies. This method was recently performed for formaldehyde (Paustenbach *et al.*, 1997). The dose –response analysis for formaldehyde was then adopted as the basis for the TLV by ACGIH. Approaches using a single NOAEL neither integrate information across the entire exposure-duration range, nor allow for the use of all data at a particular duration. Also, the NOAEL method does not allow for consideration of the shape of the dose-response curve, the number of subjects in each group and the statistical variation in the response and its measurement.

Response: OEHHA is well-aware of the limitations of the NOAEL approach and they are discussed in our document. However, the approach used by Guth 1992, categorical regression, is very data intensive and is not useful for the vast majority of chemicals. We have acknowledged the method (see Appendix D), but have not applied it in this document. The analysis alluded to in the comment was not supplied and we do not know how uncertainty factors were applied to the analysis, or if they were applied. Also, the TLV is not useful for the general public and is not recommended for use by ACGIH for that purpose. In addition, U.S.EPA has been developing the categorical regression analysis and has yet to finalize their approach or develop reference levels using that approach.

Comment 2: Sensory irritation studies are difficult to interpret because they are based on subjective human responses. Subjects exposed to clean air have reported eye, nose, and throat irritation in up to 22% of the volunteers (Anderson *et al.*, 1974; Sauder *et al.*, 1986; Kulle *et al.*, 1987; Green *et al.*, 1987; Kulle, 1993). These studies clearly show that symptoms of sensory irritation are often due to factors unrelated to exposure to the chemical. We recommend that OEHHA begin the analysis of the dose-response relationship for sensory irritation at concentrations that effect at least 20% of the experimental subjects to avoid incorporating data that represents background or variable irritant effects due to factors unrelated to the test chemical.

Response: OEHHA staff recognize that there is uncertainty in any experimental design. The effect noted in the comment (controls feeling sensory irritation) may be real. However, it would be inappropriate to assume that in each human study of irritation, 20% of the people would have been irritated by clean air anyway and only response levels above 20% should be considered. It should also be noted that of the 7 studies described in Paustenbach *et al.*, 1997 that indicated a percent response for eye irritation in controls (0 ppm formaldehyde group), 3 had 0% response, one had 5% response and the others reported 22, 17, and 39 % response. OEHHA is striving to use the best available information and emphasizing human studies. The chemicals that irritate the eye and respiratory tract are known to be irritating from a number of reports, not just the reports we used as the basis of the REL. The basis of the commentator's statement that only those responses above 20% should be considered is not substantiated in the comment or in the paper by Paustenbach *et al.* (1997) referred to later in these comments.

Comment 3: Uncertainty factors used to derive sensory irritants concentrations should be smaller than those used to establish safe exposure levels for systemic toxicity. Most irritant gases act directly on the mucous membranes or on the lungs and the intensity of effect is usually primarily dependent on the maximum concentration in air. Most other adverse health endpoints, such as developmental or neurotoxic effects, are primarily determined by the pharmacokinetics of the chemical. When attempting to prevent systemic toxicity from occurring in an exposed population, the type, number and size of uncertainty factors should be different than that used to predict an acceptable level of exposure to a sensory irritant (Paustenbach , 1997). We recommend that OEHHA consider the available data on susceptible populations for each chemical and use safety factors appropriate to the mechanism of toxic action. Further the size of the safety factor should vary according to the severity of the most sensitive adverse effect and the anticipated diversity of susceptibility. A safety factor of 2 to 5 should be adequate for reversible eye and upper respiratory irritants. Higher safety factors should be used when the effect is not reversible.

Response: The uncertainty factor of 10 for intraspecies variability is not meant to reflect the severity of a response. Rather, it is meant to protect sensitive subpopulations by encompassing the wide variability in response of humans to toxicants. In fact, a 10 fold uncertainty factor might not be adequate for some compounds (Calabrese, 1990). The commentator does not provide data to substantiate that a safety factor of 2 is appropriate for irritants.

Comment 4: At times, OEHHA selected a critical endpoint in animals when other quality human data were available that reported a dose-response relationship for the most sensitive adverse effect. We suggest that OEHHA set RELs primarily on appropriate human studies. Otherwise, RELs will quite often be overly stringent due to the repeated, and unnecessary application of uncertainty factors. For example, OEHHA selected a decrease in fetal body weight as the critical adverse effect for developing a one-hour limit for toluene to protect against severe adverse effects. Human data were available that demonstrate neurological impairment following acute exposure to toluene. While our recommended NOAEL of 1,875 mg/m³ toluene is the same as OEHHA's selected NOAEL based on animal weights, OEHHA's ultimate "severe adverse effects level" represents an overly conservative estimate because an additional uncertainty factor of 10 for animal to human extrapolation is incorporated. We have reviewed the published papers regarding developmental effects and find that toluene is only a developmental risk at doses that produce frank toxicity. These doses are much greater than those recommended here, and they also illustrate the inappropriateness of using studies that use repeated daily exposures for setting acute exposure limits.

Response: The chemical toluene is a reproductive and developmental toxicant under Proposition 65. Animals studies show clear evidence of developmental and reproductive toxicity. We used human studies of the CNS effects of toluene to develop the REL and have used reproductive/developmental effects as the basis of the level protective against severe adverse effects. The REL is used in risk assessments. The level protective against severe adverse effects is provided for the risk manager to help in deciding what steps need to be taken when the REL is

exceeded. The commentator does make an important point that when available and adequate, human data should be used. We have attempted to follow that guideline in developing our RELs. Studies of reproductive/developmental toxicity in humans are quite rare and usually derive from occupational exposures. As such, appropriate data on this endpoint necessarily come from animal studies. To ignore this effect of toluene because human studies are not available for developmental/reproductive toxicity would not be protective of public health. At the same time, OEHHA acknowledges the uncertainty of extrapolating from the repeated exposures studies to a one-hour exposure.

Comment 5: OEHHA should use appropriate exposure studies with relevant durations of exposure as the basis for determining RELs based on sensory irritation. In setting many RELs, OEHHA used studies in which repeated daily exposures to the chemical under study was for 4 to 8 hours per day over an extended time period. When setting short-term limits, studies with an exposure duration of at least 15 minutes but no greater than 4 to 8 hours should be used for setting exposure limits, particularly for the sensory irritants.

Response: OEHHA has largely used studies with exposure durations less than 8 hours and down to ten minutes to generate one-hour RELs, particularly for irritants. The comment apparently is referring to the use of reproductive/developmental toxicity studies that are always longer than one day. Developmental toxicants produce their effects during critical developmental periods that can be quite short. Toxicity studies of necessity expose the dams throughout pregnancy since it is generally not known which time point is the most critical. To expose sets of dams for a given one-hour period or even 8 hour period throughout the pregnancy would be logistically difficult to impossible, and would be very costly. Thus, for these types of toxicants, we only have repeated exposures available to us. OEHHA makes the assumption that a one-hour exposure sometime during development could produce a developmental effect, and thus extrapolate from a 6 or 8 hour exposure in each day of gestation. This is justifiable given the mechanism of action of many developmental toxicants. To ignore developmental toxicity from short-term exposures is imprudent.

Comment 6: OEHHA should appropriately consider information for setting ambient and emergency air limits from the ACGIH TLV and Ceiling values. During the past 15 years, whenever community ambient air limits have been developed for both acute and chronic exposure, most regulatory agencies have at least consulted the ACGIH TLVs to determine whether they contained information that might be helpful. Often, some fraction of the 8 hr TLV or the STEL or Ceiling Value was adopted as the chronic or acute ambient air limit (Paustenbach, 1997). By definition, TLVs are limits that refer to airborne concentrations of substances and represent conditions under which it is believed that nearly all workers may be repeatedly exposed day after day without adverse effect (ACGIH, 1997). It does not appear that OEHHA reviewed the Documentation for the TLV. The purpose of the short-term exposure values is virtually identical to the objectives OEHHA wishes to achieve. We believe the draft RELs should be compared to the STELs or CV and any major differences reconciled.

Response: OEHHA evaluated TLVs in our examination of existing guidelines during the REL development process. However, these values lack a consistent basis for derivation, are not

designed for use with the general public and in fact are not recommended for use for the general public by ACGIH. In addition, in many cases, they do not prevent adverse health effects among workers (Roach and Rappoport, 1990). ACGIH documentation has been consulted to identify potentially relevant studies.

In addition, the purpose of the short-term exposure values set for occupational settings is not identical to OEHHA's objectives as stated in the comment. OEHHA is attempting to protect nearly all people in a population including sensitive individuals. The occupational standards are set for healthy largely male workers, not the general population.

Specific comments on individual chemicals from ChemRisk on behalf of WSPA:

Comment on Acrolein: We recommend a one-hour REL of 0.046 mg/m^3 (0.02 ppm) for acrolein to protect against mild irritant effects in the community. This is based on the eye irritation threshold of 0.46 mg/m^3 (0.2 ppm) (NRC, 1981) and a safety factor of 10 to account for variability in susceptibility to acrolein.

Response: OEHHA based the REL on a study in 36 healthy humans that examined eye irritation by acrolein (Darley *et al.*, 1960). The study reported a LOAEL of 0.06 ppm, lower than the NRC estimate of the threshold used by the commentator. The basis for the designation of an eye irritant threshold by NRC is unclear. The NRC document is a secondary source of information. OEHHA applied an uncertainty factor of 3 for the LOAEL to NOAEL extrapolation and an additional uncertainty factor of 10 for intraspecies variability, for a cumulative uncertainty factor of 30. The resultant REL was 0.17 ppb. At the SRP meeting, we were given direction that for mild effects, we should be using an uncertainty factor of 6 for the LOAEL to NOAEL extrapolation. Therefore, the REL may change to 0.08 ppb ($0.17 \text{ } \mu\text{g/m}^3$).

Comment on Ammonia: As delineated in our 1995 comments, WSPA still believes that datasets from individual studies should not be combined and modeled simultaneously for developing a one-hour REL for ammonia. Normally, the benchmark concentration approach (BMC) involves modeling studies individually and selecting the best dataset most relevant to human exposure effects. In addition, no clean air controls were evaluated in the studies incorporated in OEHHA's BMC approach. As a result, the background effects of ammonia for irritation are assumed to be zero. We recommend that OEHHA consider modeling the BMC approach on the individual studies and selecting the most appropriate dataset relevant to one-hour exposure to the community.

Response: It is unclear why the commentator recommends against combining individual datasets in the benchmark concentration approach. One of the advantages of the benchmark approach is that you can use information from multiple appropriate studies available on that endpoint. This same commentator in comment number one suggests using the methodology of Guth *et al.*, 1992, which develops a dose-response curve based on an aggregate of all of the various high quality studies. The categorical regression analysis recommended in comment # 1 combines datasets and conducts a regression analysis on the combined data points.

Comment on Arsenic: OEHHA used the Nagymajtenyi *et al.*, 1985, study on developmental toxicity of arsenic oxide in mice as the basis for their REL. The ATSDR (1997) interpreted the Nagymajtenyi study to show that high levels of arsenic can cause developmental effects, but does not provide a clear basis for estimating a level of concern in humans. In addition, Ide and Bullough (1988) and Perry *et al.* (1948) show that no respiratory tract irritation is observed in occupational workers exposed to inorganic arsenic at a concentration of 0.11 mg/m³ for two months. ATSDR identified 0.11 mg/m³ as a NOAEL for respiratory irritation, which is the most sensitive adverse endpoint in humans.

The acute toxicity of organic, inorganic and metallic forms of arsenic is significantly different and is primarily attributed to the extent of absorption in the lungs. For example, arsenic sulfide and lead arsenate are cleared from the lungs slowly, indicating the rate of absorption may be lower if the inhaled arsenic is a highly insoluble form (Marafonte and Vahter, 1987). Therefore, a one-hour REL for total arsenic compounds will overestimate the amount of biologically available arsenic and will result in an overly conservative REL for anticipated exposures of the general population. We recommend that OEHHA consider developing one-hour RELs for the different forms of organic, inorganic, and metallic arsenic compounds. The one-hour REL to protect against severe adverse effects of developmental toxicity should not be considered when there is insufficient data available to support fetotoxic effects at low concentrations of exposure anticipated in the community. As a result, OEHHA has developed a one-hour REL based on an inappropriate critical endpoint when other more sensitive toxicity data endpoints are available in humans.

Response: OEHHA does not agree with ignoring the developmental effects of arsenic because there are insufficient data in humans. Arsenic compounds are fetotoxic and teratogenic in several laboratory animals. Epidemiological studies in Sweden (Nordstrom, 1978a,b; Beckman, 1978, Nordstrom *et al.*, 1979) indicate an increase in congenital malformations and adverse pregnancy outcome in smelter workers exposed to arsenic and other toxic substances. It is quite difficult to conduct epidemiological studies, particularly studies of people exposed to lower environmental levels. The suggestion by the commentator to wait until data are available in humans would be imprudent from a scientific and public health standpoint. The lack of adequate data in humans regarding reproductive endpoints is not a reason to ignore the reproductive and developmental toxicity of arsenic.

The arsenic REL is not intended to be used with organic arsenicals. The toxicity summary heading is "Arsenic and Inorganic Arsenic Compounds". We also recognize that there are differences in the potency of the arsenic compounds as indicated on page C-22. We have based the REL on a trivalent arsenic compound, arsenic trioxide. Trivalent arsenic compounds tend to be more potent toxicants than pentavalent compounds. Arsenic oxide is not necessarily the most potent trivalent arsenic compound, though. We used a LOAEL from a reproductive/developmental study in mice as the point of extrapolation, and as such do apply an uncertainty factor of 1000. While this creates a higher degree of uncertainty than using human data on respiratory irritation as suggested in the comment, we do not think it appropriate to ignore the fact that arsenic compounds are developmental toxicants.

It may be possible to research separate arsenic compounds and develop some compound-specific RELs. However, as a practical matter, facilities in the Air Toxics Hot Spots program report their emissions as total arsenic and do not speciate. If facilities were to speciate, it would provide incentive to develop compound-specific RELs. At this point, OEHHA does not plan to do so.

Comment on Benzene: OEHHA based the one-hour REL of 0.24 ppm for benzene on decreased inflammatory cell numbers in the spleen of mice following repeated daily exposures (Rosenthal and Snyder, 1985) (NOAEL of 10 ppm). Two dosing schedules were followed and it was only under the second regimen involving exposure 6 h/d for 5 days, pathogen challenge, and an additional exposure for 7 days that the animals exhibited decreased immunocompetence. We do not think it is appropriate to base a NOAEL on a regimen that uses a pre-exposure and continuing exposure. The commentator argues for a NOAEL of 100 ppm based on increased bacterial counts on day 4 post-infection. Since there was no overall functional impairment by Day 7 after infection in any exposure group, the commentator argues for a NOAEL of 300 ppm with no observed LOAEL. It appears that OEHHA believes that any delay in the immune response, however slight, should be taken as an indicator of a toxic effect. Due to the transient nature of the effect and since the effect was seen only after sub-chronic exposure, the combined inter- and intraspecies modifying factors of 100 on the NOAEL should be decreased by a factor of at least 3 to 10 to account for these departures from a true single exposure study.

We also recommend that OEHHA consider other available studies that involve acute single exposures.

The OEHHA level protective against severe adverse effects for benzene of 3.25 mg/m³ is based on decreased mean fetal birth weights following repeat exposures. There is insufficient evidence to indicate that benzene is teratogenic or embryotoxic in animals or humans at concentrations of 10 ppm for 8 hr/day (Schwetz, 1983). Instead, we recommend that OEHHA consider more appropriate human data available in the literature involving the health effects of benzene following acute or intermittent exposure. We suggest a one-hour REL of 7.1 ppm, which is below the average odor threshold of 61 ppm to protect against mild transient effects. This is based on a NOAEL of 25 ppm from a human study showing no effects following a single 8-hour exposure (NRC, 1986; Gerarde, 1960). The NOAEL is adjusted with a time extrapolation based on a modified Haber's Law with $n = 2$ and divided by an uncertainty factor of 10.

We recommend a one-hour "severe adverse effect level" of 71 ppm and believe that such a limit would be adequate to protect against irreversible or severe adverse effects. The recommended level is based on a 250 ppm NOAEL for hematopoietic effects in human workers (Paustenbach *et al.*, 1992). The NOAEL is adjusted using a modified Haber's Law with $n = 2$ and then divided by an uncertainty factor of 10 for human variability. We suggest OEHHA consider studies reporting acute human effects of benzene following a single, short-term exposure, rather than effects associated with repeated-dose subchronic exposure in a developmental toxicity study conducted in experimental animals.

Response: The commentator points out an important issue in developing acute one-hour reference exposure levels. When a sensitive endpoint is studied using repeated exposures, how

does one use that data to develop a one-hour REL? We have already discussed our position with respect to developmental/reproductive toxicity in response to earlier comments. Since all developmental/reproductive toxicity studies use repeated exposures, it is only possible to use those repeat exposure regimens to address this important and sensitive toxicological endpoint. Since we do not know at which point in time the developmental effect is exerted, we use the daily exposure as a starting point for time extrapolation.

In the case of benzene immunotoxicity, we again are faced with a study that used repeat exposures over 5 days. OEHHA used the Rosenthal and Snyder (1985) study in mice which evaluated the immune response to *Listeria monocytogenes* infection following exposure to benzene at 0, 10, 30, 100, and 300 ppm 6 h/d for 5 days, with or without continuing exposure for 7 days post-infection. The commentator indicated that effects (decreased immunocompetence) were only observed in the animals continually exposed after the infection. This is not the case. Lymphocyte proliferation in response to the infection was suppressed in all groups exposed to 30 ppm or higher benzene for 6 hours/day for 5 days pre-infection, as well as in all groups who were benzene-exposed for 7 days after the infection. We identified a NOAEL of 10 ppm based on the numbers of lymphocytes in the spleen as well as on the number of *L. monocytogenes* in the spleen at 4 days post-infection, in both the groups exposed to benzene prior to infection only and those exposed both prior to and for 7 days after infection. Hence the argument that the NOAEL should be 100 ppm for those not continuing exposure post-infection based on increased bacterial counts in the spleen ignores the effects on the hosts' immune system cells. Rosenthal and Snyder conclude that their study suggests a suppressive effect of benzene on T-cell function and/or number. The study authors also state that the observation of no significant changes at Day 1 of infection suggests that benzene exposure does not affect the ability of non-T-cell activated macrophages to eradicate *Listeria* cells during the early phase of the immune response.

The commentator makes the point that the effect was transient, and that the uncertainty factors should be decreased due to the transient nature of the effect and because the exposures were repeated over 5 days. The authors of the paper note that neither exposure regimen (5 days pre-infection or continued for 7 days post-infection) suppressed the immune response enough to enable the bacteria to persist through to Day 7. The increased numbers of *L. monocytogenes* at Day 4 of infection suggests a delay in the immune response to this infection. Rosenthal and Snyder state that the reason for the apparent recovery is not known but may be related to the mechanism of resistance in *L. monocytogenes*-resistant C57Bl/10 mice used in the study. After sublethal challenge, *L. monocytogenes*-resistant mice show an increase in the number of monocytes during infection and a progressive influx of macrophages into infective foci, whereas this chemotactic and inflammatory response is absent in *L. monocytogenes*-susceptible mice. It is not clear where humans would stand on the *L. monocytogenes* susceptibility scale. The extrapolation of multiple 6 hour/day exposures to a one-hour exposure is more uncertain than if the exposure were for only one 6 hour period. OEHHA agrees that this is an uncertainty. Unlike reproductive/developmental studies, where it is largely agreed that a short exposure at a key time in gestation will produce a developmental/reproductive adverse effect, we are unsure if a one-hour exposure prior to infection in the mouse model would produce the same effect as the 6 hour/day for 5 day exposures. We acknowledge that there are several studies showing adverse impacts on the hematopoietic system in animals after relatively short-term exposures. However,

because of the uncertainty in extrapolating for this endpoint from repeated exposures to a one-hour exposure, we are revising the REL and basing it on the studies of Kuna and Kapp (1981), and Coates *et al.* (1984) on reproductive/developmental toxicity in rats. The resultant REL of 0.4 ppm is very close to the original REL of 0.24 ppm.

The level protective against severe adverse effects is based on a reproductive/developmental study of benzene exposure in rats. A 40 ppm NOAEL was observed in this study (Coate *et al.*, 1984). Kuna and Kapp (1981) found teratogenic effects in rats at 500 ppm, and lower fetal weights at 50 ppm. The NOAEL in this study was 10 ppm. We applied a 100-fold uncertainty factor to the higher NOAEL of 40 ppm for interspecies and intraspecies variability. The level protective against severe adverse effects is thus 0.4 ppm. We propose to use this level as the REL. The commentator's suggestion of using a NOAEL for hematopoietic effects in humans based on the Paustenbach *et al.*, 1992 study ignores the potential for benzene to result in adverse reproductive/developmental effects. We do not think that would be a prudent choice.

Comment on Formaldehyde: OEHHA proposed a one-hour REL of 0.25 ppm formaldehyde based on a benchmark concentration approach from the study of Kulle *et al.*, 1987. OEHHA's assessment resulted in a one-hour acute exposure level similar to those developed by other agencies, but the methodology used in the OEHHA assessment was quite different. OEHHA did not consider other available human studies, especially exposures of susceptible subpopulations such as asthmatics. As a result, OEHHA incorporated an additional level of conservatism in their approach by a factor of 3 accounting for variability of susceptible individuals to formaldehyde. While the Kulle *et al.* study presented reasonable dose-response data, alone it only represents what was seen in a small group of individuals. Many other studies were considered by an expert committee which was asked to identify a proposed occupational exposure limit (Paustenbach *et al.*, 1997). The group evaluated 150 journal articles and used data from them to build a dose-response curve for human sensory irritation. The data indicated that eye irritation did not become significant until a concentration of at least 1 ppm. The data indicate irritation was time-independent since exposures to 0.3 ppm did not produce irritation above background following either 10 minute or 6 hour exposures.

The reliance on one study is not warranted when such a rich database is available. Further the application of conservative uncertainty factors to this single study is not justified because of the large database of many human studies. OEHHA applied an uncertainty factor of 3 to account for variability of individuals susceptible to formaldehyde exposure. However, several studies have investigated the human response to formaldehyde in so-called sensitive individuals, like asthmatics. These studies concluded that asthmatics were no more sensitive to airway effects of formaldehyde than non-asthmatics and that bronchoconstriction will only occur at concentrations greater than 2.5 mg/m³ (Green *et al.*, 1987; Sauder *et al.*, 1986; Sauder *et al.*, 1987; Sheppard *et al.*, 1986; Witek *et al.*, 1987).

ACGIH set a ceiling value for formaldehyde of 0.37 mg/m³ based on irritation. AIHA set an ERPG-1 of 1.23 mg/m³. We recommend that OEHHA reevaluate the dose-response relationship for formaldehyde and review the paper by Paustenbach *et al.*, 1997. OEHHA should omit any additional uncertainty factors accounting for variability of individuals susceptible to formaldehyde

since several studies have already established that sensitive individuals such as asthmatics respond no differently than the general population.

Response: As indicated in the introduction to our document, OEHHA conducts an extensive review of the literature before developing an REL. This was the case for formaldehyde as well as the other chemicals in this document. In the interests of space and time, the acute toxicity summaries only discuss key studies used in the analysis. OEHHA conducted a benchmark concentration analysis of the data on mild and moderate eye irritation in Kulle *et al.*, 1987. The lower confidence limit on the 5% response rate was determined to be 0.44 ppm for a 3 hour exposure. Using a modified Haber's Law equation with the exponent, n , set to 2 we estimated a one-hour BC_{05} as 0.76 ppm. We divided this number by an uncertainty factor of 3 to account for sensitive subpopulations. The commentator indicates that because asthmatics are not more sensitive to formaldehyde than nonasthmatics we should not have an uncertainty factor in our analysis. However, the uncertainty factor is not meant to account solely for the response of asthmatics. It is meant to account for human interindividual variability in response. In some cases the asthmatic is more susceptible to the effects of an irritant chemical while in other cases it may be that there is simply a wide variability in the threshold of irritation in the human population. In Paustenbach *et al.* (1997) it is noted that, from the Andersen and Molhave study (1983) (and others), there appears to be a relatively wide variation in individual susceptibility to irritation from formaldehyde. That is what we are attempting to account for in applying the intraindividual uncertainty factor for formaldehyde, and not for the susceptibility of asthmatics.

While many studies fail to demonstrate an increased sensitivity of asthmatics to formaldehyde (Sheppard *et al.*, 1984), other studies indicate that people can become sensitized to formaldehyde (with occupational exposures) and that these people will develop asthmatic symptoms in response to challenge with formaldehyde (Burge *et al.*, 1985; Nordman *et al.*, 1985; Hendrick and Lane, 1977). This is noted in our document on page C-131 - 132.

The commentator refers us to an analysis published by Paustenbach and coworkers (1997) that describes the results of the deliberations of a panel sponsored by the Formaldehyde Institute that was charged with evaluating data to determine an adequate occupational exposure limit. The Kulle study is included in the analysis. The results of the panel's deliberations were applied to an occupational setting. In the panel's deliberations they concluded that reports of eye irritation below 0.3 to 0.5 ppm were not reliable. This appears to be due to reports of irritation in clean air controls in some studies. OEHHA does not agree with applying this conclusion across the board based on what could be poorly controlled environments in some of the studies' controls. The analysis did not consider protecting the general population which, unlike the healthy worker population, includes infants, children, the elderly, and the ill, and others who due to their sensitivity to irritants would likely not be working in an industry where exposure to irritants occurs. In fact, the cited review indicates that the panel acknowledges that the results of the studies involving generally healthy, relatively young volunteers may not reflect the range of results that would be observed if perhaps 100 workers of varying age and health status underwent the same testing. One could take that statement further that a worker population does not provide an adequate population to extrapolate to the general population. Hence, we believe our benchmark

concentration analysis is more appropriate for developing a REL applicable to the general population than the occupational exposure limit described in Paustenbach *et al.* (1997).

Comment on Hydrogen Sulfide: OEHHA identified a one-hour REL of 0.14 mg/m³ (0.1 ppm) to protect against mild adverse effects based on the human study of Jappinen *et al.* (1990). OEHHA based the REL on increased airway resistance in 2 of 10 subjects following a 30-minute exposure to 2 ppm H₂S. However, Jappinen reported no statistically significant changes in airway conductance for the entire group. And thus we recommend that OEHHA consider 2 ppm as a NOAEL rather than a LOAEL. This is further supported by Jappinen *et al.*, 1990, which demonstrated no significant pulmonary function changes or bronchial responsiveness to histamine in a group of smokers, workers with allergies, and atopic individuals exposed to 1-11 ppm H₂S. Using 2 ppm as a NOAEL, the commentator recommends a REL of 1 ppm (1.4 mg/m³) to protect against mild adverse effects.

Response: OEHHA has revisited the hydrogen sulfide REL development. Other commentators pointed out that the Ambient Air Quality Standard (AAQS) of 42 µg/m³ was not merely an odor threshold. Some individuals experience headache, nausea and even vomiting upon exposure to odorous concentrations of H₂S. There is extensive documentation of this problem from local air pollution control districts. It is hard to argue that headache and nausea are not adverse health effects. While the toxicological mechanism may not be easy to understand or explain, the physiological effects are real. Therefore, OEHHA is proposing to use the one-hour AAQS of 42 µg/m³ as the one-hour REL. The toxicological endpoint is thus headache and nausea. The value will not be used in assessing respiratory irritation, which occurs at levels significantly above the AAQS.

Comment on Nickel: OEHHA did not consider the different forms of nickel in the toxicity assessment of a one-hour REL. OEHHA based the REL on pulmonary function changes in workers with occupational asthma exposed to nickel sulfate. The REL was estimated by converting to nickel equivalents. This reduces the observed LOAEL of 0.3 mg/m³ to 0.067 mg/m³.

The acute toxicity of soluble and insoluble forms of nickel will differ significantly due to the extent of absorption of nickel across the lung. A REL based on soluble nickel will overestimate the amount of biologically available nickel and overestimate the potential health risk for less soluble forms of nickel. Ideally, separate RELs should be developed for water-soluble nickel and for relatively insoluble nickel compounds. We recommend a one-hour REL of 0.07 mg/m³ for nickel sulfate to protect against changes in pulmonary function. This is based on a LOAEL of 0.3 mg/m³ adjusted by a modified Haber's Law with n = 2 to 0.212 mg/m³. This value is further adjusted with a safety factor of 3 to account for extrapolation from a LOAEL to a NOAEL, resulting in an REL of 0.07 mg/m³ for nickel sulfate.

Response: OEHHA used a study by Cirila *et al.*, 1985 which evaluated changes in lung function of metal plating workers with occupational asthma. The significant effect was >15% decrease in FEV₁. The volunteers were exposed for 30 minutes to 0.3 mg NiSO₄·6H₂O. The equivalent concentration of nickel is 67 µg/m³. Extrapolating to a one-hour exposure using an exponent, n,

set to 1, the resulting nickel concentration is $33 \mu\text{g}/\text{m}^3$. This was then divided by an uncertainty factor of 3 for extrapolating from a LOAEL to a NOAEL for mild respiratory effects which results in a REL of $11 \mu\text{g}/\text{m}^3$.

The commentator objects to using the nickel equivalents of nickel sulfate hexahydrate as the LOAEL. Nickel is the element that has caused the effect on FEV₁ in the study population, not the sulfate or water moieties of the nickel sulfate hexahydrate. While it may be true that other nickel salts may have different potencies for impacting lung function, in the Hot Spots program, facilities report their emissions as total nickel. Therefore, it is prudent to use a study of soluble nickel compounds as the basis of the REL and apply the nickel proportion of that compound to the derivation of the REL. It may be possible in the future to speciate nickel compounds for the purposes of developing an REL. However, it would then require facilities to speciate their emissions, a potentially costly prospect for most.

Comment on Toluene: There are questions as to the appropriateness of the two studies used by OEHHA to develop the REL for toluene and the “severe adverse effect level”. Based on an analysis of the available data, sensory irritation is the most sensitive endpoint following acute exposure to toluene and more serious adverse effects such as neurological depression occur at higher concentrations following acute exposure.

OEHHA based the REL on Anderson *et al.*, 1983; the stated purpose of this study was to evaluate neurobehavioral effects. Observations on sensory irritation were reported as incidental findings. These data do not provide a sound health risk-based limit for toluene, and OEHHA should not rely on subjective reports of “sensory irritation” for developing a one-hour REL. The Anderson *et al.* study reported incidental observations of headache, dizziness, and feelings of intoxication in individuals exposed to 100 ppm and not the lower dose (40 ppm). The commentator recommends an REL based on Echeverria *et al.*, 1989, a study designed to evaluate sensory irritation. A NOAEL of 75 ppm for a 7 hour exposure was adjusted to a one-hour exposure using an exponent of $n = 2$ in a modified Haber’s Law calculation to derive a NOAEL of $741 \text{ mg}/\text{m}^3$. This was then divided by an uncertainty factor of 10 to arrive at a suggested REL of $74 \text{ mg}/\text{m}^3$ (20 ppm). We recommend that OEHHA consider incorporating data from other representative studies to establish a dose-response relationship for sensory irritation.

OEHHA based the one-hour level protective against severe adverse effects on a study intended to evaluate developmental defects in animals. OEHHA based the REL on a NOAEL of 500 ppm for decreased fetal body weights following repeated exposures to toluene up to 36 days. For many chemical agents, the toxic effects of a single exposure may be quite different than the toxic effects produced by repeated exposures. We recommend that OEHHA consider, as an alternative, available studies on short-term human exposures to toluene concentrations that produce marked adverse effects, such as neurological impairment (Gamberle and Hultengren, 1972).

Response: OEHHA based the REL on Anderson *et al.* (1983), a study of 16 young healthy subjects evaluating nasal mucus flow, lung function, subjective response, and psychometric performance during 6-hour exposures to clean air, 10, 40 or 100 ppm toluene. No effects were noted at 10 and 40 ppm, but at 100 ppm irritation was experienced in the eyes and nose. The test

battery investigated visual perception, vigilance, psychomotor functions, and higher cortical functions. The test battery included five-choice, rotary pursuit, screw-plate, Landolt's rings, Boudon Weisma, multiplication, sentence comprehension, and word memory tests. No statistically significant effects occurred at $p < 0.05$. For three tests (multiplication errors, Landolt's rings, and the screw plate test) there was a borderline significance ($0.05 < p < 0.1$). The subjects reported headache, dizziness, and a feeling of intoxication at 100 ppm. This study was well-controlled and well-conducted. The study was inclusive of irritation and other "subjective" symptoms. The volunteers were asked to rate the following on a continuous scale: their estimate of air temperature, humidity, air movement, light intensity, noise level, air quality, odor level; whether they felt fatigue, sleepiness, work strain, difficulty of work, effort, speed of reaction, irritation of the eyes, nose, throat and lower airway, cough, headache, feeling of intoxication, dizziness, and nausea. Thus, this study was designed to evaluate irritation, contrary to what is implied in the comment. OEHHA believes therefore that this study is useful for developing the REL. It is interesting to note that the authors conclude there is a wide variability in irritation, and that throughout the day there was no adaptation to the irritation. The REL for 6-hours was extrapolated to a one-hour REL using a modified Haber's Law exponent = 2. An uncertainty factor of 10 was applied for interindividual variation in response to derive an REL of 9.8 ppm.

The Echeverria study cited by the commentator, in contrast to the comment, does not identify a higher NOAEL (75 ppm) than the Anderson *et al.*, 1983 study, and so is not useful for developing an REL. In this study, 42 students were exposed to 0, 75, or 150 ppm toluene and changes in CNS function and symptoms were recorded. Verbal and visual memory, perception, psychomotor skill, manual dexterity, mood, fatigue, and verbal ability were evaluated over the course of the seven hour exposures. As in the Anderson study, each subject was their own control. An analysis of variance and test for trend was performed on the difference and score for each concentration where each subject was their own control. Adverse performance was found for a number of tests at 150 ppm, and headache and eye irritation increased in a dose-dependent fashion. The incidence of subjects sleeping also increased in a dose-dependent fashion. The comment above indicates that a NOAEL of 75 ppm is observed in this study. However, the authors of the study indicate that subtle neurological effects were found at 75 ppm. In particular, the pattern recognition latency score for the control group differed significantly by the Scheffe test from the 75 ppm and 150 ppm groups. The authors also note that the incidence of subjects sleeping and of headache and eye irritation increased in a dose-dependent fashion with a positive test for trend. The authors conclude that "this study supports a lowering of the PEL because acute subjective and objective effects have been found at 75 and 150 ppm, bracketing the TLV of 100 ppm". Therefore it is not correct to identify 75 ppm as a NOAEL from this study.

The level protective against severe adverse effects is based on a reproductive/developmental toxicity study. Studies of reproductive/developmental toxicity of necessity involve repeated exposures. It is generally not known at what stage in gestation a developmental defect or an impact on the reproductive capacity of the animal can occur. Thus we have used the one-day exposure concentration (generally 6 or 7 hr/day) in these cases to extrapolate back to a one-hour concentration.

Comment on Xylenes: OEHHA developed a one-hour REL based on findings in Nelson *et al.* (1943) of reported symptoms of eye, nose, and throat irritation at 870 mg/m³ xylenes. Although these results are consistent with other studies, the exposure duration of 3 minutes is a significant shortcoming in its use in developing a one-hour REL. There are several other human studies available with exposure durations closer to one-hour that provide a more appropriate basis for developing a one-hour REL for xylenes. We recommend that the one-hour REL be based on the human studies by Carpenter *et al.* (1975; 1976) and Hastings (1984).

Since irritation is generally time-independent after about 15 minutes of exposure, the concentration of xylenes becomes the important factor in determining the threshold for irritation response. We recommend that OEHHA use an n=2 in the time extrapolation calculation.

In the Carpenter (1975) study, 460 mg/m³ was considered the NOAEL because the number of volunteers that reported irritation was not significantly greater than the control group. In another study, Carpenter *et al.* (1976) found increased observations of eye irritation in all of the volunteers exposed to 930 and 1,800 mg/m³ xylene for 15 minutes, but only observed irritation in one volunteer (10% of total) at concentrations of 220 and 450 mg/m³. The NOAEL is identified as 450 mg/m³ based on eye irritation. Hastings *et al.* (1984) exposed 150 volunteers to 0, 430, 860, and 1,720 mg/m³ mixed xylenes for 30 minutes and found eye irritation in 56% of controls, 60%, 70% and 90% of the volunteers exposed at 430, 860, and 1730 mg/m³ respectively. The xylene concentration of 430 mg/m³ was considered as the NOAEL because reports of eye irritation were the same as controls. This study shows a similar NOAEL to the other studies but uses a 30 minute exposure period. We recommend an REL of 30.4 mg/m³ to protect against eye irritation based on Nelson *et al.*, 1943; Carpenter *et al.*, 1975, 1976; Hastings *et al.*, 1984. The basis of the REL is a NOAEL of 430 mg/m³ for eye irritation. The NOAEL was adjusted from the 30 minute exposure to a one-hour REL with a time extrapolation where n=2, and adjusted by an uncertainty factor of 10 to account for human variability in sensitivity to irritation. The one-hour REL (derived by the commentator) is thus 30.4 mg/m³ for xylene.

Response: OEHHA originally used the study of 10 healthy human volunteers exposed to 100 or 200 ppm xylenes for 3 to 5 minutes. Subjects reported eye, nose, and throat irritation at 200 ppm but not 100 ppm. Thus, this study provides a NOAEL of 100 ppm for extrapolation. OEHHA applied a time adjustment factor to extrapolate from the 3 minute exposure to one-hour equivalent exposure using a value of 1 for the exponent, n, in a modified Haber's Law. The resulting concentration was then divided by an uncertainty factor of 10 for intraspecies variability. The resultant REL is 0.5 ppm or 2.2 mg/m³.

The comment points out one shortcoming of the study in that exposure durations were quite brief. This does present more uncertainty in extrapolating to an equivalent one-hour concentration, than if the duration were much closer to one-hour. The studies cited by the commentator provide a NOAEL for 15 to 30 minute exposures very similar to the NOAEL for a 3 minute exposure in Nelson *et al.* If OEHHA extrapolates using an exponent of 1 from a 3 minute to a 60 minute concentration, the resulting "equivalent" concentration is 10 fold higher than if the extrapolation runs from 30 minutes to 60 minutes. OEHHA agrees that this other data should be taken into account, but prefers to use an exponent of 1 for extrapolating from exposure durations of less

than one hour to a one hour equivalent concentration. Using a NOAEL of 430 mg/m³ for 30 minute exposure, and extrapolating to a 60 minute exposure results in an equivalent concentration of 50 ppm (220 mg/m³). Applying an uncertainty factor of 10 for intraspecies variability yields an REL of 5 ppm (22 mg/m³).

**CALIFORNIA ENVIRONMENTAL PROTECTION AGENCY
OFFICE OF ENVIRONMENTAL HEALTH HAZARD ASSESSMENT**

NOTICE TO INTERESTED PARTIES

NOTICE OF PUBLIC COMMENT PERIOD

ON

AIR TOXICS "HOT SPOTS" PROGRAM RISK ASSESSMENT GUIDELINES

SEPTEMBER 17, 1999

The Office of Environmental Health Hazard Assessment (OEHHA) is releasing the second part of a revised draft document, *Air Toxics Hot Spots Program Risk Assessment Guidelines Part III: Technical Support Document for the Determination of Noncancer Chronic Reference Exposure Levels* to solicit public comment on the revision and to obtain review by the ARB's Scientific Review Panel. This draft document is part of a series of Risk Assessment Guidelines that are being developed by OEHHA for use in implementing the Air Toxics "Hot Spots" Program mandated by the Air Toxics Hot Spots Information and Assessment Act of 1987, as amended. The original draft document was released in October 1997. More than forty sets of comments were received from the public. Staff have reviewed the comments, responded to the comments in writing, and revised the draft document.

The original 1997 document contained a description of the methodology and toxicity summaries and Reference Exposure Levels for 120 compounds. To facilitate review, OEHHA decided to release the chemical toxicity summaries in batches of 40. In June 1999 a revised draft document including the methodology (Introduction), toxicity summaries for the first 40 chemicals (based primarily on their emissions in California), and public comments with staff responses to the methodology and these 40 chemicals were distributed for review. This notice pertains to the toxicity summaries for the second set of 40 chemicals, which will be distributed for public review (along with the responses to comments received during the first public comment period) by September 27, 1999. The document will be available on the OEHHA Home Page at <http://www.oehha.ca.gov>. The distribution of the document will commence a 30-day public review period that will end on October 27, 1999. We are soliciting public input on the second batch of toxicity summaries and Reference Exposure Levels during this public comment period.

Please direct any inquiries concerning technical matters or availability of the document to Dr. James Collins at (510) 622-3146. Please direct your written comments regarding the revised draft document to Dr. Melanie A. Marty, Chronic RELs, 1515 Clay St., 16th Floor, Oakland, CA 94612. Information about dates and agenda for meetings of the Scientific Review Panel can be obtained from the ARB web page at <http://www.arb.ca.gov/srp/srp.htm>.