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

Acrylonitrile ..... 11
Ammonia ..... 19
Benzene ..... 24
Beryllium and Beryllium Compounds ..... 35
1,3-Butadiene ..... 48
Cadmium and Cadmium Compounds ..... 59
Carbon Disulfide ..... 66
Carbon Tetrachloride ..... 81
Chlorinated Dibenzo-*p*-Dioxins and Chlorinated Dibenzofurans ..... 90
Chlorine ..... 106
Chlorine Dioxide ..... 114
Chloroform ..... 124
Chromium, Hexavalent (Soluble Compounds) ..... 138
Cresol Mixtures ..... 157
1,4-Dichlorobenzene ..... 165
1,1-Dichloroethylene ..... 170
Diethanolamine ..... 177
N,N-Dimethylformamide ..... 184
1,4-Dioxane ..... 189
Epichlorohydrin ..... 196
1,2-Epoxybutane ..... 200
Ethyl Chloride ..... 204
Ethylbenzene ..... 208
Ethylene Dibromide ..... 214
Ethylene Dichloride ..... 226
Ethylene Glycol ..... 234
Ethylene Glycol Monoethyl Ether ..... 240
Ethylene Glycol Monoethyl Ether Acetate ..... 248
Ethylene Glycol Monomethyl Ether ..... 253
Ethylene Glycol Monomethyl Ether Acetate ..... 260
Ethylene Oxide ..... 264
Fluorides *including* Hydrogen Fluoride ..... 270
Glutaraldehyde ..... 286
*n*-Hexane ..... 292
Hydrazine ..... 303
Hydrogen Chloride ..... 309
Hydrogen cyanide ..... 313
Hydrogen Sulfide ..... 321
Isophorone ..... 329
Isopropanol ..... 337
Maleic Anhydride ..... 348
Methanol ..... 354
METHYL BROMIDE ..... 360
Methyl Chloroform ..... 366
Methyl Isocyanate ..... 372
Methyl *t*-Butyl Ether ..... 383

Methylene Chloride ..... 389
4,4′-Methylene Dianiline ..... 399
Methylene Diphenyl Isocyanate ..... 407
Naphthalene ..... 413
Nickel and Nickel Compounds: Nickel Oxide ..... 420
Phenol ..... 429
Phosphine ..... 437
Phosphoric Acid ..... 446
Phthalic Anhydride ..... 453
Propylene ..... 460
Propylene Glycol Monomethyl Ether ..... 466
Propylene oxide ..... 471
Selenium and Selenium Compounds ..... 476
SILICA (CRYSTALLINE, RESPIRABLE) ..... 486
Styrene ..... 534
Sulfuric Acid ..... 551
Toluene ..... 560
2,4- and 2,6-Toluene Diisocyanate ..... 574
Trichloroethylene ..... 581
Triethylamine ..... 590
Vinyl Acetate ..... 596
Xylenes ..... 602

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

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

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

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

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

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

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

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

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

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

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

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

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

| Substance (CAS #) | Listed in CAPCOA (1993) | Chronic Inhalation REL ($\mu g/m^3$) | Oral REL (mg/kg Body Weight) | Hazard Index Target Organs | Human Data |
|---|---|---|---|---|---|
| Xylenes (m-, o-, p-) | ☑ | 700 | | Nervous system; respiratory system | ☑ |

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

*CHRONIC TOXICITY SUMMARY*

# ACRYLONITRILE

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

CAS Number: 107-13-1

## I. Chronic Toxicity Summary

| | |
|---|---|
| *Inhalation reference exposure level* | **5 µg/m$^3$** (2 ppb) |
| *Critical effect(s)* | Degeneration and inflammation of nasal epithelium in rats |
| *Hazard index target(s)* | Respiratory system |

## II. Chemical Property Summary (HSDB, 1994)

| | |
|---|---|
| *Description* | Clear, colorless to pale yellow liquid (technical grades) |
| *Molecular formula* | $C_3H_3N$ |
| *Molecular weight* | 53.1 g/mol |
| *Density* | 0.81 g/cm$^3$ @ 25$^o$C |
| *Boiling point* | 77.3$^o$C |
| *Melting point* | –82$^o$C |
| *Vapor pressure* | 100 torr @ 23$^o$C |
| *Solubility* | Soluble in isopropanol, ethanol, ether, acetone, and benzene |
| *Conversion factor* | 1 ppm = 2.17 mg/m$^3$ @ 25 $^o$C |

## III. Major Uses or Sources

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

## IV. Effects of Human Exposure

Many occupational epidemiology studies have investigated retrospectively the morbidity and mortality of acrylonitrile exposed workers. An increased incidence of lung cancer was associated with acrylonitrile exposure. No significant excess mortality has been observed for any noncarcinogenic endpoint. One early cross-sectional study (Wilson *et al.*, 1948) observed multiple deleterious effects in synthetic rubber manufacturing workers acutely exposed (20 to 45 minutes) to various concentrations of acrylonitrile (16 to 100 ppm, 34.7 to 217 mg/m$^3$). Mucous membrane irritation, headaches, feelings of apprehension, and nervous irritability were observed in the majority of workers. Other less common symptoms observed included low-grade anemia, leukocytosis, and mild jaundice. These effects were reported to subside with cessation of exposure. Human volunteers exposed for a single 8 hour period to acrylonitrile vapors exhibited no deleterious CNS effects at concentrations ranging from 5.4 to 10.9 mg/m$^3$ (2.4 to 5.0 ppm) (Jakubowski *et al.*, 1987).

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

Czeizel *et al*. (1999) studied congenital abnormalities in 46,326 infants born between 1980 and 1996 to mothers living within a 25 km radius of an acrylonitrile factory in Nyergesujfalu, Hungary. Ascertainment of cases with congenital abnormalities was based on the Hungarian Congenital Abnormality Registry plus review of pediatric, pathology and cytogenetic records. Particular attention was paid to indicators of germinal mutations (sentinel anomalies, Down's syndrome, and unidentified multiple congenital abnormalities) and to indicators of teratogens (specific pattern of multiple congenital abnormalities). Three congenital abnormalities: pectus excavatum in Tata, 1990-1992 (OR = 78.5, 95%CI = 8.4-729.6), undescended testis in Nyergesujfalu between 1980 and 1983 (8.6, 1.4-54.3) and in Esztergom, 1981-1982 (4. 2, 1.3-13.5) and clubfoot in Tata, 1980-1981 (5.5, 1.5-20.3) showed significant time-space clusters in the study area. The risk of undescended testis decreased with increasing distance from the factory. An unusual increase for the combination of oral cleft and cardiac septal defects was seen in multimalformed babies in Tatabanya in 1990. Unfortunately there were no data on levels of acrylonitrile or any other exposure.

## V. Effects of Animal Exposure

Quast *et al.* (1980) exposed Sprague-Dawley rats (100/sex/ concentration) 6 hours/day, 5 days/week for 2 years to concentrations of 0, 20, or 80 ppm acrylonitrile vapors (0, 43, or 174 $mg/m^3$). A statistically significant increase in mortality was observed in the first year among 80 ppm exposed rats (male and female). Additionally, the 80 ppm exposed group had a significant decrease in mean body weight. Two tissues, the nasal respiratory epithelium and the brain, exhibited treatment-related adverse effects due to acrylonitrile exposure. Proliferative changes in the brain glial cells (i.e., tumors and early proliferation suggestive of tumors) were significantly increased in the 20 ppm (8/100) and 80 ppm (20/100) females versus female controls (0/100), and in the 80 ppm males (22/99) versus male controls (0/100). Noncarcinogenic, extrarespiratory effects were observed in the nasal turbinate epithelium at both exposure concentrations, 20 and 80 ppm (see table below). Thus the LOAEL was 20 ppm. No treatment-related effects in the olfactory epithelium, trachea, or lower respiratory epithelium were observed at either concentration.

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

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

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

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

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

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

| Acrylonitrile | MCV (m/sec) | SCV (m/sec) | AMAP (mvolts) | ASAP (μvolts) |
|---|---|---|---|---|
| 0 ppm | 42.9 ± 0.9[a] | 53.3 ± 1.0 | 17.8 ± 1.2 | 186 ± 8 |
| 25 ppm | 41.6 ± 0.8 | 50.5 ± 0.8* | 16.1 ± 0.8 | 164 ± 11 |
| 50 ppm | 38.1 ± 0.9** | 49.1 ± 0.5*** | 15.7 ± 1.0 | 159 ± 5* |
| 100 ppm | 38.5 ± 1.2** | 48.4 ± 1.0*** | 17.4 ± 0.9 | 133 ± 11*** |

[a] Mean ± SEM; * $p<0.05$; ** $p<0.01$; ***$p<0.001$

In a developmental study, Murray *et al.* (1978) exposed rats to acrylonitrile vapors at 0, 40 ppm (87 mg/m$^3$), or 80 ppm (174 mg/m$^3$) for 6 hours/day during gestational days 6 to 15. In the 80 ppm exposed group, significant increases in fetal malformations were observed including short tail, missing vertebrae, short trunk, omphalocoele, and hemivertebra (Murray *et al.*, 1978). No differences in implantations, live fetuses, or resorptions were seen in the exposed (40 and 80 ppm) versus the control group. Maternal toxicity was observed as decreased body weight at both exposure levels. After adjustment to continuous exposure, this study identified a developmental NOAEL of 10 ppm and a LOAEL of 20 ppm (with maternal toxicity).

Saillenfait *et al*. (1993) studied the developmental toxicity of eight aliphatic mononitriles in Sprague-Dawley rats after inhalation exposure for 6 hr/day during days 6 to 20 of gestation. The range of exposure levels for acrylonitrile was 12, 25, 50, and 100 ppm; group sizes were 20-23 females. Embryolethality was observed after exposure to 25 ppm (54 mg/m$^3$) acrylonitrile in the presence of overt signs of maternal toxicity. Fetal weights were significantly lower at 25 ppm. Thus 12 ppm (26 mg/m$^3$) is a NOAEL for developmental toxicity using this study design.

## VI. Derivation of Chronic Reference Exposure Level

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

Sprague-Dawley rats (100/sex/concentration) were exposed 6 hours/day, 5 days/week for 2 years to 0, 20, or 80 ppm acrylonitrile (0, 43, and 174 mg/$m^3$, respectively). Significant degenerative and inflammatory changes were observed in the respiratory epithelium of the nasal turbinates at both exposure concentrations (20 and 80 ppm). This treatment-related irritation of the nasal mucosa appeared in the 20 ppm exposed male rats as either epithelial hyperplasia of the nasal turbinates, or as hyperplasia of the mucous secreting cells. In the 20 ppm exposed females it appeared as either focal inflammation in the nasal turbinates or flattening of the respiratory epithelium of the nasal turbinates. In 80 ppm exposed rats the effects were more severe, including suppurative rhinitis, hyperplasia, focal erosions, and squamous metaplasia of the respiratory epithelium. No treatment-related effects in the olfactory epithelium, trachea, or lower respiratory system were observed at either concentration. This study identified a LOAEL for pathological alterations in the respiratory epithelium of the extrathoracic region of the respiratory tract of 20 ppm (43 mg/$m^3$). The U.S. EPA (1994) based its RfC of 2 µg/$m^3$ on the same study but included a Modifying Factor (MF) of 10 for database deficiencies. The criteria for use of modifying factors are not well specified by U.S. EPA. Such modifying factors were not used by OEHHA.

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

mucous secreting cells in males, focal inflammation in the nasal turbinates in females, and flattening of the respiratory epithelium of the nasal turbinates in females. The resulting $BMC_{05}$ values (1.27, 1.33, 2.18, 1.35) were averaged to yield a value of 1.5 ppm. The RGDR adjustment and appropriate uncertainty factors were applied as indicated in the above table and resulted in a chronic REL of 5 $\mu g/m^3$.

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

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

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

## VII. Data Strengths and Limitations for Development of the REL

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

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

## VIII. Potential for Differential Impacts on Children's Health

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

## IX. References

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*CHRONIC TOXICITY SUMMARY*

# AMMONIA

*(Anhydrous ammonia; aqueous ammonia)*

**CAS Registry Number: 7664-41-7**

## I. Chronic Toxicity Summary

| | |
|---|---|
| *Inhalation reference exposure level* | **200 μg/m$^3$ (300 ppb)** |
| *Critical effect(s)* | Pulmonary function tests or subjective symptomatology in workers |
| *Hazard index target(s)* | Respiratory system |

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

| | |
|---|---|
| *Description* | Colorless gas |
| *Molecular formula* | $NH_3$ |
| *Molecular weight* | 17.03 g/mol |
| *Density* | 0.7710 g/L @ 0°C |
| *Boiling point* | -33.35° C |
| *Vapor pressure* | 7510 torr @ 25°C |
| *Solubility* | Soluble in water, alcohol, and ether |
| *Conversion factor* | 1 ppm = 0.71 mg/m$^3$ |

## III. Major Uses or Sources

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

## IV. Effects of Human Exposures

Comparisons were made between 52 workers and 31 control subjects in a soda ash plant for pulmonary function and eye, skin and respiratory symptomatology (Holness *et al.*, 1989). The pulmonary function tests included FVC (forced vital capacity – the total amount of air the subject can expel during a forced expiration), $FEV_1$ (forced expiratory volume in one second), $FEF_{50}$ (forced expiratory flow rate at 50% of the FVC) and $FEF_{75}$ (forced expiratory flow rate at

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

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

| Group | Exposure | Week 1 | Week 2 | Week 3 | Week 4 | Week 5 | Week 6 |
|---|---|---|---|---|---|---|---|
| A | ppm $NH_3$<br>hours | 25<br>2 | 50<br>4 | 100<br>6 | 25<br>2 | 50<br>4 | 100<br>6 |
| B | ppm $NH_3$<br>hours | 50<br>6 | 50<br>6 | 50<br>6 | 50<br>6 | 50<br>6 | 50<br>6 |
| C | ppm $NH_3$<br>hours | 100<br>6 | 50<br>4 | 25<br>2 | 25<br>6 | 50<br>4 | 100<br>2 |

Pulmonary function tests (respiration rate, FVC and $FEV_1$) were measured in addition to subjective complaints of irritation of the eyes and respiratory tract. The difficulty experienced in performing simple cognitive tasks was also measured, as was pulse rate. There were reports of transient irritation of the nose and throat at 50 or 100 ppm. Acclimation to eye, nose, and throat irritation was seen after two to three weeks (in addition to the short-term subjective adaption). No significant differences between subjects or controls on common biological indicators, in physical exams, or in performance of normal job duties were found. After acclimation, continuous exposure to 100 ppm, with occasional excursions to 200 ppm, was easily tolerated and had no observed effect on general health.

## V. Effects of Animal Exposures

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

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

Guinea pigs (10/group) and mice (20/group) were continuously exposed to 20 ppm (14.2 mg/m$^3$) ammonia for up to 6 weeks (Anderson *et al.*, 1964). Separate groups of 6 guinea pigs and 21 chickens were exposed to 50 ppm and 20 ppm ammonia for up to 6 and 12 weeks, respectively. All species displayed pulmonary edema, congestion, and hemorrhage after 6 weeks exposure, whereas no effects were seen after only 2 weeks. Guinea pigs exposed to 50 ppm ammonia for 6 weeks exhibited enlarged and congested spleens, congested livers and lungs, and pulmonary edema. Chickens exposed to 200 ppm for 17-21 days showed liver congestion and slight clouding of the cornea. Anderson and associates also showed that a 72-hour exposure to 20 ppm ammonia significantly increased the infection rate of chickens exposed to Newcastle disease virus, while the same effect was observed in chickens exposed to 50 ppm for just 48 hours.

Coon *et al*. (1970) exposed groups of rats (as well as guinea pigs, rabbits, dogs, and monkeys) continuously to ammonia concentrations ranging from 40 to 470 mg/m$^3$. There were no signs of toxicity in 15 rats exposed continuously to 40 mg/m$^3$ for 114 days or in 48 rats exposed continuously to 127 mg/m$^3$ for 90 days. Among 49 rats exposed continuously to 262 mg/m$^3$ for 90 days, 25% had mild nasal discharge. At 455 mg/m$^3$ 50 of 51 rats died. Thus 127 mg/m$^3$ (179 ppm) is a subchronic NOAEL for upper respiratory effects in rats. Coon *et al*. (1970) also found no lung effects in 15 guinea pigs exposed continuously to 40 mg/m$^3$ (28 ppm) ammonia for 114 days.

## VI. Derivation of Chronic Reference Exposure Level

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

The Holness *et al*. (1989) study was selected because it was a chronic human study and was published in a respected, peer-reviewed journal. It is also the only chronic study available. The USEPA (1995) based its RfC of 100 µg/m$^3$ on the same study but included a Modifying Factor

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

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

## VII. Data Strengths and Limitations for Development of the REL

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

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

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*CHRONIC TOXICITY SUMMARY*

# BENZENE

*(Benzol; Benzole; Cyclohexatriene)*

**CAS Registry Number: 71-43-2**

## I. Chronic Toxicity Summary

| | |
|---|---|
| *Inhalation reference exposure level* | **60 μg/m$^3$ (20 ppb)** |
| *Critical effect(s)* | Lowered red and white blood cell counts in occupationally exposed humans |
| *Hazard index target(s)* | Hematopoietic system; development; nervous system |

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

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

## III. Major Uses or Sources

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

## IV. Effects of Human Exposure

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

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

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

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

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

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

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

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

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

## V. Effects of Animal Exposure

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

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

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

A study on the chronic hematological effects of benzene exposure in C57 Bl/6 male mice (5-6 per group) showed that peripheral lymphocytes, red blood cells and colony-forming units (CFUs) in the bone marrow and spleen were significantly decreased in number after treatment with 10 ppm (32.4 mg/m$^3$) benzene for 6 hours/day, 5 days/week for 178 days (Baarson *et al.*, 1984).

Inhalation of 0, 10, 31, 100, or 301 ppm (0, 32.4, 100.4, 324, or 975 mg/m$^3$) benzene for 6 hours/day for 6 days resulted in a dose-dependent reduction in peripheral lymphocytes, and a reduced proliferative response of B- and T-lymphocytes to mitogenic agents in mice (Rozen *et al.*, 1984). In this study, total peripheral lymphocyte numbers and B-lymphocyte proliferation to lipopolysaccharide were significantly reduced at a concentration of 10 ppm (32.4 mg/m$^3$). The proliferation of T-lymphocytes was significantly reduced at a concentration of 31 ppm (100.4 mg/m$^3$).

Male and female mice (9-10 per group) exposed to 100 ppm (324 mg/m$^3$) benzene or greater for 6 hours/day, 5 days/week for 2 weeks showed decreased bone marrow cellularity and a reduction of pluripotent stem cells in the bone marrow (Cronkite *et al.*, 1985). The decrease in marrow cellularity continued for up to 25 weeks following a 16-week exposure to 300 ppm (972 mg/m$^3$) benzene. Peripheral blood lymphocytes were dose-dependently decreased with benzene exposures of greater than 25 ppm (81 mg/m$^3$) for 16 weeks, but recovered to normal levels following a 16-week recovery period.

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

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

Aoyama (1986) showed that a 14-day exposure of mice to 50 ppm (162 mg/m$^3$) benzene resulted in a significantly reduced blood leukocyte count.

The NTP (1986) conducted a bioassay in F344 rats and B6C3F1 mice of benzene by corn oil gavage. Doses were 0, 25, 50, and 100 mg/kg-day for females and 0, 50, 100, and 200 mg/kg-day for males. Dose-related lymphocytopenia and leukocytopenia were observed in both species in all dosed groups but not controls. Mice exhibited lymphoid depletion of the thymus and spleen and hyperplasia of the bone marrow.
Cronkite *et al*. (1989) exposed CBA/Ca mice to 10, 25, 100, 300, 400 and 3000 ppm benzene 6 hours/day, 5 days/week for up to 16 weeks. No effects were observed at the 10 ppm level. Lymphopenia was observed in the 25 ppm exposure group. Higher concentrations of benzene produced dose-dependent decreases in blood lymphocytes, bone marrow cellularity, spleen colony-forming units, and an increased percentage of CFU-S in S-phase synthesis.
Farris *et al*. (1997) exposed B6C3F$_1$ mice to 1, 5, 10, 100, and 200 ppm benzene for 6 hr/day, 5 days/week, for 1, 2, 4, or 8 weeks. In addition some animals were allowed to recover from the exposure. There were no significant effects on hematopoietic parameters from exposure to 10 ppm benzene or less. Exposure to higher levels reduced the number of total bone marrow cells, progenitor cells, differentiating hematopoietic cells, and most blood parameters. The replication of primitive progenitor cells was increased. The authors suggested that this last effect, in concert with the genotoxicity of benzene, could account for the carcinogenicity of benzene at high concentrations.

Reproductive and developmental effects have been reported following benzene exposure. Coate *et al*. (1984) exposed groups of 40 female rats to 0, 1, 10, 40, and 100 ppm (0, 3.24, 32.4, 129.6, or 324 mg/m$^3$) benzene for 6 hours/day during days 6-15 of gestation. In this study, teratologic evaluations and fetotoxic measurements were done on the fetuses. A significant decrease was noted in the body weights of fetuses from dams exposed to 100 ppm (324 mg/m$^3$). No effects were observed at a concentration of 40 ppm (129.6 mg/m$^3$).

Keller and Snyder (1986) reported that exposure of pregnant mice to concentrations as low as 5 ppm (16 mg/m$^3$) benzene on days 6-15 of gestation (6 hr/day) resulted in bone-marrow hematopoietic changes in the offspring that persisted into adulthood. However, the hematopoietic effects (e.g. bimodal changes in erythroid colony-forming cells) in the above study were of uncertain biological significance. In a similar later study, Keller and Snyder (1988) found that exposure of mice *in utero* to 20 ppm (64 mg/m$^3$) benzene on days 6-15 of gestation resulted in neonatal suppression of erythropoietic precursor cells and persistent,

enhanced granulopoiesis. This effect was considered significant bone-marrow toxicity by the authors. No hematotoxicity was seen in this study at 10 ppm (32 mg/m$^3$).

An exposure of 500 ppm (1,600 mg/m$^3$) benzene through days 6-15 gestation was teratogenic in rats while 50 ppm (160 mg/m$^3$) resulted in reduced fetal weights on day 20 of gestation. No fetal effects were noted at an exposure of 10 ppm (Kuna and Kapp, 1981). An earlier study by Murray *et al.* (1979) showed that inhalation of 500 ppm benzene for 7 hours/day on days 6-15 and days 6-18 of gestation in mice and rabbits, respectively, induced minor skeletal variations in the absence of maternal toxicity. Red and white blood cell counts in the adults of either species were measured by Murray *et al.* (1979) but were not significantly different from control animals. However, fetal mouse hematological effects were not measured.

Tatrai *et al.* (1980) demonstrated decreased fetal body weights and elevated liver weights in rats exposed throughout gestation to 150 mg/m$^3$ (47 ppm).

## VI. Derivation of Chronic Reference Exposure Level (REL)

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

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

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

## VII. Data Strengths and Limitations for Development of the REL

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

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

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

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

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

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

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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

| | |
|---|---|
| *Inhalation reference exposure level* | **0.007 μg Be/m$^3$** |
| *Critical effect(s)* | Beryllium sensitization and chronic beryllium disease in occupationally exposed humans |
| *Hazard index target(s)* | Respiratory system; immune system |
| *Oral reference exposure level* | 0.002 mg/kg-day |
| *Critical efect* | Small intestinal lesions in dogs |
| *Hazard index target(s)* | Gastrointestinal tract/liver |

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

| | *Metallic beryllium* | *Beryllium oxide* | *Beryllium hydroxide* | *Beryllium sulfate* |
|---|---|---|---|---|
| *Description* | Solid gray, hexagonal structure | White light, amorphous powder | White amorphous powder or crystalline | Colorless tetragonal crystals |
| *Molecular formula* | Be | BeO | $Be(OH)_2$ | $BeSO_4$ |
| *Molecular weight* | 9.012 g/mol | 25.01 g/mol | 43.03 g/mol | 105.07 g/mol |
| | | | | |
| *Solubility* | Insoluble in water | | | Soluble |
| *Conversion factor* | Not applicable | | | |

## III. Major Uses and Sources

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

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

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

## IV. Effects of Human Exposure

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

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

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

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

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

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

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

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

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

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

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

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

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

## V. Effects of Animal Exposure

Three chronic studies, two in rats (Vorwald and Reeves, 1959; Reeves *et al.*, 1967) and one in guinea pigs (Reeves *et al.*, 1970), observed adverse inflammatory and proliferative respiratory changes following inhalation exposure to beryllium compounds. Vorwald and Reeves (1959) observed inflamed lungs and fibrosis in rats exposed to 0.006 mg $Be/m^3$ (as BeO) for an unspecified duration. A later study exposed Sprague-Dawley CD rats for 72 weeks (7 hr/d, 5 d/wk) to 34.25 µg $Be/m^3$ from $BeSO_4$ (Reeves *et al.*, 1967). Gross and histological changes observed in exposed versus unexposed rats included increased lung weight, inflamed lungs, emphysema, arteriolar wall thickening, granulomas, fibrosis, and proliferative responses within the alveoli (LOAEL = 34.25 µg $Be/m^3$). Guinea pigs were exposed to 0, 3.7, 15.4, or 29.3 µg $Be/m^3$ (from the sulfate) for 6 hours/day, 5 days/week for up to 1 year (Reeves *et al.*, 1970). Respiratory alterations observed in the beryllium-exposed groups included increased tracheobronchial lymph node and lung wet weights, interstitial pneumonitis, and granulomatous lesions. These adverse respiratory effects were observed in all the beryllium dosed groups and indicated a chronic inhalation LOAEL of 3.7 µg $Be/m^3$.

Wagner *et al.* (1969) exposed monkeys, rats, and hamsters to 0.21 and 0.62 mg Be/m$^3$ as fumes from bertrandite or beryl ore, respectively, for 6 hours/day, 5 days/week for up to 17 months. Exposed animals displayed severe effects, including (1) bronchial lymphocytic infiltrates, abscesses, consolidated lobes, and granulomatous lesions after exposure to 0.21 mg Be/m$^3$ from bertrandite ore, and (2) inflamed lungs, fibrosis, and granuloma after exposure to 0.62 mg Be/m$^3$ from beryl ore. Lung inflammation was observed in the exposed monkeys, and a few granulomatous lung lesions were observed in the hamsters after similar exposure conditions (up to 23 months).

Immunological effects have been observed in a few subchronic studies (Schepers, 1964; Schepers *et al.*, 1957; Stiefel *et al.*, 1980). Schepers (1964) exposed monkeys (*Macacus mullata*) to three soluble forms of beryllium ($BeF_2$, $BeSO_4$, $BeHPO_4$) daily for 6 hours/day over 7 to 30 days. Increased lung weight, inflammation, emphysema, and fibrosis of the lung were observed after 17 days at 0.198 mg Be/m$^3$ (as $BeSO_4$). Histological examination found pleuritis, congestion, emphysema, consolidation, and edema of the lung. Immunological effects were seen as hyperplasia of the lymph nodes typical of immune activation after 7 to 18 days exposure to either 0.198 or 0.184 mg Be/m$^3$ as the sulfate or fluoride. A subchronic inhalation study reported immunological effects as increased, beryllium-specific stimulation of T-lymphocytes *in vitro* from Wistar rats and guinea pigs exposed daily (6 hours/day) over 10 weeks (LOAEL = 0.5 mg/m$^3$) (Stiefel *et al.*, 1980). However, a subchronic inhalation study in Wistar and Sherman rats (Schepers *et al.*, 1957) observed multiple lung alterations including granulomas (LOAEL = 35 µg Be/m$^3$) but did not find any accompanying immunological effects after 30 days discontinuous exposure (5-6 d/wk, 4-8 hr/d) to beryllium fumes from $BeSO_4$.

## VI. Derivation of Chronic Reference Exposure Levels

Derivation of Inhalation Reference Exposure Level

| | |
|---|---|
| *Key study* | Kreiss *et al*., 1996 |
| *Study population* | 8 beryllium-sensitized workers among 136 employees in a beryllia ceramics plant |
| *Exposure method* | Workplace |
| *Critical effects* | Beryllium sensitization (chronic beryllium disease) |
| *LOAEL* | 0.55 µg/m$^3$ (median exposure of sensitized workers) |
| *NOAEL* | Not observed |
| *Exposure continuity* | Workplace |
| *Average experimental exposure* | 0.2 µg/m$^3$ for LOAEL group (0.55 x 10/20 x 5/7) |
| *Human equivalent concentration* | 0.2 µg/m$^3$ |
| *Exposure duration* | 6.1 years (5 mo – 10 yr) |
| *LOAEL uncertainty factor* | 10 (low incidence but serious, irreversible chronic disease) |
| *Subchronic uncertainty factor* | 1 |
| *Interspecies uncertainty factor* | 1 |
| *Intraspecies uncertainty factor* | 3 (sensitized may not be only sensitive subpopulation) (see below) |
| *Cumulative uncertainty factor* | 30 |
| *Inhalation chronic REL* | 0.007 µg/m$^3$ |

| | |
|---|---|
| *Supportive study* | Eisenbud *et al.* (1949) |
| *Study population* | Approximately 10,000 individuals within 2 miles of a beryllium manufacturing plant |
| *Exposure method* | Environmental exposure |
| *Critical effects* | Pulmonary berylliosis in 11 residents |
| *LOAEL* | 0.03 µg/m$^3$ (geometric mean of range of measured exposures associated with berylliosis of 0.01 to 0.1 µg/m$^3$) |
| *NOAEL* | Not observed |
| *Exposure continuity* | Continuous |
| *Average exposure* | Estimated to be approximately 0.3 µg/m$^3$ (historical exposures estimated to be 10-fold higher than measured values) for LOAEL group |
| *Human equivalent concentration* | 0.3 µg/m$^3$ for LOAEL group |
| *Exposure duration* | Up to 7 years |
| *LOAEL uncertainty factor* | 10 |
| *Subchronic uncertainty factor* | 3 |
| *Interspecies uncertainty factor* | 1 |
| *Intraspecies uncertainty factor* | 3 |
| *Cumulative uncertainty factor* | 100 |
| *Inhalation chronic REL* | 0.003 µg/m$^3$ |

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

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

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

## VII. Data Strengths and Limitations for Development of the REL

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

## VIII. Potential for Differential Impacts on Children's Health

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

### *Derivation of Chronic Oral Reference Exposure Level*

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

| | |
|---|---|
| *Study* | Morgareidge *et al*., 1976 |
| *Study population* | Male and female dogs (5/sex/group) |
| *Exposure method* | Diet containing 0, 1, 5, 50 or 500 ppm Be as beryllium sulfate tetrahydrate |
| *Critical effects* | Small intestinal lesions |
| *LOAEL* | 500 ppm |
| *NOAEL* | 50 ppm (1.2 mg/kg bw-day) |
| *Exposure continuity* | Continuous |
| *Exposure duration* | Up to 3 years, 4 months |
| *Average experimental exposure* | 1.2 mg/kg bw-day (males, 1.1; females, 1.3) |
| $BMD_{05}$ | 0.244 mg/kg-day |
| *LOAEL uncertainty factor* | Not needed in BMD approach |
| *Subchronic uncertainty factor* | 1 |
| *Interspecies uncertainty factor* | 10 |
| *Intraspecies factor* | 10 |
| *Cumulative uncertainty factor* | 100 |
| *Oral reference exposure level* | 0.002 mg/kg-day |

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

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

USEPA used the same study to derive its RfD of 0.002 mg/kg-day. The U.S. EPA stated its confidence in the RfD as: study - medium; database – low to medium, and RfD - low to medium. USEPA used a $BD_{10}$ approach and included a database UF of 3. OEHHA used a $BD_{05}$ approach (specifically a Weibull model in the USEPA's BMDS software) and did not include a database UF since the criteria for use of modifying factors such as this are not well specified by U.S. EPA. However, the final value for the oral chronic REL was the same as the USEPA's RfD.

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

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

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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

| | |
|---|---|
| *Inhalation reference exposure level* | **20 µg/m$^3$** (8 ppb) |
| *Critical effect(s)* | Increased incidence of ovarian atrophy in mice |
| *Hazard index target(s)* | Female reproductive system |

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

| | |
|---|---|
| *Description* | Colorless gas |
| *Molecular formula* | $C_4H_6$ |
| *Molecular weight* | 54.09 g/mol |
| *Boiling point* | –4.4$^o$C |
| *Melting point* | –108.9$^o$C |
| *Vapor pressure* | 910 torr at 20$^o$C |
| *Solubility* | Very slightly soluble in water (735 mg/L); soluble in ethanol, ether, acetone, benzene and organic solvents |
| *Conversion factor* | 1 ppm = 2.21 mg/m$^3$ at 25$^o$C |

## III. Major Uses and Sources

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

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

## IV. Effects of Human Exposure

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

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

## V. Effects of Animal Exposure

The few available chronic animal inhalation studies have focused on the potential carcinogenicity of 1,3-butadiene. The National Toxicology Program (NTP) has sponsored two chronic inhalation studies in $B6C3F_1$ mice (NTP, 1984; Melnick *et al.*, 1990; NTP, 1993), while Hazelton Laboratories Europe (HLE) Ltd. conducted a chronic inhalation study in Sprague-Dawley rats (HLE, 1981; Owen *et al.*, 1987; Owen and Glaister, 1990).

The two $B6C3F_1$ mice inhalation studies sponsored by NTP (Huff *et al.*, 1985; Melnick *et al.*, 1990; NTP, 1984; NTP, 1993), although focused on carcinogenicity, identified other adverse chronic effects. The earlier NTP (1984) study in mice administered 0, 625 or 1250 ppm 1,3-butadiene for 6 hours/day, 5 days/week for up to 61 weeks. Nonneoplastic changes observed were elevated testicular and ovarian atrophy at both doses (625 and 1250 ppm); liver necrosis in male mice at both doses and in female mice at 1250 ppm; and nonneoplastic lesions in the nasal cavity at 1250 ppm. At the highest dose, adverse changes in the nasal cavity included chronic inflammation, fibrosis, cartilaginous metaplasia, osseous metaplasia, and atrophy of the sensory epithelium. No nasal or respiratory lesions were seen in the controls. This study identified a chronic LOAEL of 625 ppm for gonadal atrophy in both sexes.

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

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

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

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

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

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

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

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

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

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

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

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

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

## VI. Derivation of Chronic Reference Exposure Level (REL)

| | |
|---|---|
| *Study* | NTP (1993) |
| *Study population* | $B6C3F_1$ mice (70/sex/group) |
| *Exposure method* | Discontinuous inhalation (0, 6.25, 20, 62.5, 200, 625 ppm) over 2 years |
| *Critical effects* | Increased incidence of ovarian atrophy |
| *LOAEL* | 6.25 ppm |
| *NOAEL* | Not observed |
| $BMC_{05}$ | 1.40 ppm |
| *Exposure continuity* | 6 hr/d, 5 d/wk |
| *Exposure duration* | 103 weeks |
| *Average experimental exposure* | 0.25 ppm for $BMC_{05}$ (1.40 ppm x 6/24 hr/day x 5/7 days/week) |
| *Human equivalent concentration* | 0.25 ppm (gas with systemic effects, based on RGDR = 1.0 using default assumption that lambda (a) = lambda (h)) |
| *LOAEL uncertainty factor* | Not needed in the BMC approach |
| *Subchronic uncertainty factor* | 1 |
| *Interspecies uncertainty factor* | 3 |
| *Intraspecies uncertainty factor* | 10 |
| *Cumulative uncertainty factor* | 30 |
| *Inhalation reference exposure level* | 8 ppb (0.008 ppm; 0.02 $mg/m^3$; 20 $\mu g/m^3$) |

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

observed at 200 and 625 ppm (442 or 1381 mg/m$^3$, respectively). After 15 months, ovarian atrophy was observed at exposure levels of 20 ppm (44.2 mg/m$^3$) and above. In mice exposed for up to 2 years (103 weeks), the incidence of ovarian atrophy increased at all exposure concentrations relative to controls, which establishes a chronic LOAEL of 6.25 ppm (13.81 mg/m$^3$) for reproductive toxicity.

Presentation of the ovarian atrophy data in quantal form (see Table 1) allows the use of the benchmark concentration (BMC) approach to determine the REL. A log-normal probit analysis (U.S. EPA, National Center for Environmental Assessment, benchmark dose software, version 1.20) using only the control group and the log-dose of the three lowest butadiene exposure groups provided the lowest chi-square value (i.e., the best line fit to the data points). The proportion of mice developing ovarian atrophy in the two highest exposure groups did not increase appreciably with increasing exposure concentration, and therefore, deviated from the log-normal probit plot. The significantly shortened survival rate in these two groups may be one reason for this deviation. Another possible cause is that a relatively resistant subgroup of mice (to ovarian atrophy) is revealed at the two highest doses following 2-year exposure to 1,3-butadiene. Thus, it may be biologically plausible to remove these resistant subgroups when using a BMC approach. The maximum likelihood estimate (MLE) for a 5% response was 1.53 ppm. The resulting 95% lower confidence limit at the MLE provided a $BMC_{05}$ of 1.40 ppm. A $BMC_{05}$ is considered to be similar to a NOAEL in estimating a concentration associated with a low level of risk.

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

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

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

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

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

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

## VII. Data Strengths and Limitations for Development of the REL

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

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*CHRONIC TOXICITY SUMMARY*

# CADMIUM AND CADMIUM COMPOUNDS

**CAS Registry Number: 7440-43-9**

## I. Chronic Toxicity Summary

| | |
|---|---|
| *Inhalation reference exposure level* | **0.02 μg/m$^3$** (respirable) |
| *Critical effect(s)* | Kidney effects (proteinuria) and respiratory effects (reduction in forced vital capacity and reduction in peak expiratory flow rate) in occupationally exposed humans |
| *Hazard index target(s)* | Kidney; respiratory system |

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

| | |
|---|---|
| *Description* | Blue-white solid |
| *Molecular formula* | Cd |
| *Molecular weight* | 112.41 g/mol |
| *Density* | 8.642 g/cm$^3$ @ 20ºC |
| *Boiling point* | 765ºC (CRC, 1994) |
| *Melting point* | 320.9ºC |
| *Vapor pressure* | 1 torr @ 394ºC |
| *Conversion factor* | Not applicable |

## III. Major Uses or Sources

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

## IV. Effects of Human Exposure

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

proteinuria indicative of renal impairment. The workers in E1 had been exposed for a mean of 4.08 years to 31 $\mu g/m^3$ total cadmium (1.4 $\mu g/m^3$ respirable cadmium). The 27 workers in E2 had been exposed for a mean of 8.6 years to 134 $\mu g/m^3$ total cadmium (88 $\mu g/m^3$ 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 $\mu g/m^3$ total cadmium (21 $\mu g/m^3$ 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 $\mu g/m^3$ for workers exposed for 28 years and a NOAEL of 1.4 $\mu g/m^3$ 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 $\beta_2$-microglobulin) was consistently observed when the worker's liver cadmium burden exceeded 40 ppm and the time-weighted cumulative exposure index exceeded 400-500 $\mu g$ years/$m^3$.

A detailed investigation of renal function in 75 male cadmium-exposed workers identified significant increases in urinary excretion of several low- and high molecular weight proteins, including $\beta_2$-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 $\mu g/m^3$, 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 $\beta_2$-microglobulin. Of these, the most sensitive parameter, urinary $\beta_2$-microglobulin, demonstrated an inflection point at 1108 $\mu g$ years/$m^3$ with a 95% lower confidence limit of 509 $\mu g$ years/$m^3$. The endpoint selected is indicative of defects in tubular reabsorption of proteins.

Diminished sensitivity of smell has also been observed in cadmium exposed workers (Rose *et al.*, 1992). Cadmium body burden, $\beta_2$-microglobulin levels, and olfactory function were measured in a group of 55 male workers exposed to cadmium fumes in a brazing operation. A group of 15 control workers was also tested. Exposed workers exhibited high urinary cadmium levels, tubular proteinuria, and a significant, selective defect in odor detection threshold.

## V. Effects of Animal Exposure

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

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

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

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

## VI. Derivation of Chronic Reference Exposure Levels (REL)

***Derivation of Chronic Inhalation Reference Exposure Level***

| | |
|---|---|
| *Study* | Lauwerys *et al.*, 1974 |
| *Study population* | Humans (22 exposed men and 22 unexposed men in LOAEL group; 31 exposed women and 31 non-exposed women in NOAEL group) |
| *Exposure method* | Occupational exposures |
| *Critical effects* | Kidney effects - proteinuria in 68% of LOAEL group<br>Respiratory effects – reduction in forced vital capacity (FVC), forced expiratory flow in 1 second ($FEV_1$); reduction in peak expiratory flow rate |
| *LOAEL* | 21 $\mu g/m^3$ respirable cadmium |
| *NOAEL* | 1.4 $\mu g/m^3$ respirable cadmium |
| *Exposure continuity* | Assumed to be 5 days/week for 8 hours/day during which 10 $m^3$ air is breathed |
| *Average occupational exposure* | 0.5 $\mu g/m^3$ for NOAEL group (1.4 x 10/20 x 5/7) |
| *Human equivalent concentration* | 0.5 $\mu g/m^3$ for NOAEL group |
| *Exposure duration* | Average of 4.1 years (1 to 12 years) for NOAEL group |
| *LOAEL uncertainty factor* | 1 |
| *Subchronic uncertainty factor* | 3 |
| *Interspecies uncertainty factor* | 1 |
| *Intraspecies uncertainty factor* | 10 |
| *Cumulative uncertainty factor* | 30 |
| *Inhalation reference exposure level* | 0.02 $\mu g/m^3$ |

## VII. Data Strengths and Limitations for Development of the REL

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

A similar evaluation of the LOAEL group led to an alternate estimate for an inhalation reference exposure level of 0.05 $\mu g/m^3$. The LOAEL group had an average occupational exposure of 5.0 $\mu g/m^3$ and an average exposure duration of 27.8 years (21 to 40 years). Default uncertainty

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

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

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

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

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

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

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

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

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

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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

| | |
|---|---|
| *Inhalation reference exposure level* | **800 µg/m$^3$** (300 ppb) |
| *Critical effect(s)* | CNS/PNS (reduction in motor nerve conduction velocities in occupationally-exposed humans) |
| *Hazard index target(s)* | Nervous system; reproductive system |

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

| | |
|---|---|
| *Description* | Clear, colorless or faintly yellow liquid |
| *Molecular formula* | $CS_2$ |
| *Molecular weight* | 76.14 g/mol |
| *Boiling point* | 46.5$^o$C |
| *Melting point* | –111.5 $^o$C |
| *Vapor pressure* | 297 torr @ 20$^o$C |
| *Solubility* | Slightly soluble in water (2.94 g/L); miscible in anhydrous methanol, ethanol, ether, benzene, chloroform, and carbon tetrachloride |
| *Conversion factor* | 3.1 mg/m$^3$ per ppm at 25$^o$C |

## III. Major Uses and Sources

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

## IV. Effects of Human Exposure

A primary target of carbon disulfide ($CS_2$) toxicity is the nervous system. The major neurotoxic action of carbon disulfide is the development of mental disturbances. These include change of personality, irritability, and forgetfulness, often with accompanying neurophysiological and neuropathological changes after prolonged exposure. Such changes include decreased peripheral nerve impulse conduction, motor and/or sensory neuropathies, cerebral or cerebellar atrophy, and neuropsychological organic changes (Aaserud *et al.* 1988, 1990, 1992; Foa *et al.*, 1976; Hirata *et al.* 1992; Ruijten *et al.* 1990, 1993). Alterations in behavioral indices have historically been associated with high levels of $CS_2$, often in excess of 20 ppm (Foa *et al.* 1976; Hanninen *et al.*, 1978).

Studies have identified alterations in the nerve conduction of workers chronically exposed to lower $CS_2$ levels (Hirata *et al.*, 1992a; Johnson *et al.*, 1983; Ruijten *et al.*, 1990; Ruijten *et al.*, 1993). A cross-sectional study of Japanese spinning workers identified alterations in the central nervous system as measured by brain stem auditory evoked potential (BAEP) (Hirata *et al.*, 1992). The latencies of the three main BAEP components increased significantly in workersexposed to $CS_2$ for more than 20 years when compared to controls. $CS_2$ exposures ranged from 3.3 to 8.2 ppm (mean = 4.76 ppm). Ruijten *et al.* (1993) identified mild presymptomatic nerve impairment (decreased conduction velocities and response amplitudes) in 44 $CS_2$-exposed workers with an average cumulative exposure range from 192 to 213 ppm-year (mean duration = 26.1 years).

A NIOSH occupational study evaluated the effects of $CS_2$ on the peripheral nervous system. Johnson *et al.* (1983) identified a significant dose related reduction in the maximum motor nerve conduction velocities (MCV) in the calves and ankles of male viscose rayon workers exposed to high (median = 7.6 ppm) $CS_2$ levels versus a comparison group exposed to low concentrations (median = 0.2 ppm). The workers were all employed in artificial fiber production in the same plant. Since these reduced MCVs were still within the normal range, the authors considered the measured difference an indication of minimal neurotoxicity. The mean exposure concentration for all exposed workers (n = 145) ranged from 0.6 to 16 ppm (mean = 7.6 ppm) with a mean duration of 12.1 years. This study identified a chronic LOAEL of 7.6 ppm for minor neurological effects (decreased peroneal nerve MCV and sural nerve conduction velocity).

Another epidemiological study evaluated a group of 111 Belgian viscose rayon factory workers exposed to 4 to 112 $mg/m^3$ $CS_2$ (time-weighted average 1 to 40 $mg/m^3$) (Vanhoorne et al., 1995). Among four categories of cumulative exposure (0, 1 to 300, 301 to 600, and greater than 600 $mg/m^3$-years), a clear dose-response effect was observed for reduced mean peroneal motor nerve conduction velocities in both fast and slow fibers. Unfortunately, the data are incompletely reported, and the mean duration of exposure is not given. Subgroups of workers whose exposures ever exceeded 10 ppm (n=64) and never exceeded 10 ppm (n=30) each showed significantly reduced fibular nerve motor conduction velocities compared with non-exposed workers.

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

ischemic heart disease in $CS_2$ exposed workers (Hernberg *et al.*, 1970; MacMahon and Monson, 1988; Tiller *et al.*, 1968; Tolonen *et al.*, 1979). A 2.5-fold excess in mortality from coronary heart disease in workers exposed to $CS_2$ was first reported by Tiller *et al.* (1968). A subsequent prospective study by Hernberg *et al.* (1970) found a 5.6-fold increased risk in coronary heart disease mortality and a 3-fold increased risk of a first nonfatal myocardial infarction in $CS_2$ exposed workers.

Male workers (n=177) in a Polish fiber plant were exposed to $CS_2$ for an average of 14 years (range of 5 to 38 years). Controls were 93 healthy male workers from other factories that did not use carbon disulfide. Carbon disulfide exposed workers had higher rates (42%) of 24-hour electrocardiographic abnormalities than non-exposed workers (24%, p=0.006) (Bortkiewicz et al., 2001). The most common abnormalities were ventricular extrasystoles and repolarization disturbances, the latter occurring most often in workers with the longest $CS_2$ exposures. Long-term blood pressure monitoring did not reveal any differences between exposed and control groups.

Male workers in a Belgian viscose rayon factory (n=85) were estimated by personal active sampling to have exposures of 2 to 32 mg/m$^3$ $CS_2$. Controls were 37 non-exposed workers from factories that did not use $CS_2$. Exposed workers had reduced common carotid artery distensibility as measured with ultrasound sonography, while the carotid artery compliance coefficient was not significantly affected. Also, blood pressures and cholesterol levels were not significantly different than observed among control workers (Kotseva et al., 2001a). Differences in carotid artery distensibility remained significant after adjustment for age, smoking, alcohol consumption, ethnicity, body mass index, heart rate, and systolic blood pressure.

Egeland *et al.* (1992) and Vanhoorne *et al.* (1992) have reported that human exposure to $CS_2$ for more than one year causes increases in biochemical changes often associated with cardiovascular disease - diastolic blood pressure, low density lipoprotein cholesterol, and apolipoproteins A1 and B. Egeland *et al.* (1992) used cross sectional data on 165 $CS_2$-exposed workers (245 controls) collected in 1979 by Fajen *et al.* (1981). The affected workers were exposed for at least 1 year in a viscose rayon factory to an estimated median TWA (8-hour) of 7.6 ppm. The Egeland *et al.* (1992) study indicated that modest $CS_2$ exposure (range = 3.4 to 5.1 ppm, median = 4.1 ppm) was associated with increased low density lipoprotein cholesterol (LDLc), the type of increase associated with atherosclerotic heart disease. No significant differences were seen between controls and the low $CS_2$ exposed group (range = 0.04 to 1.02 ppm, median = 1.00 ppm). Study NOAEL and LOAEL for increased LDLc and diastolic blood pressure were thus 1.0 ppm and 4.1 ppm , respectively. Vanhoorne *et al.* (1992) identified increased LDL-cholesterol, apolipoprotein B, systolic and diastolic blood pressure as indicative of an increased coronary risk in workers from a Belgian viscose rayon factory (115 exposed and 76 controls). $CS_2$ concentrations ranged from 1 to 36 ppm. Duration of exposure was not indicated. Even though these biochemical changes were observed, no significant increases in cardiovascular disease, such as angina, myocardial infarction, or ischemia, were determined by ECG changes.

Workers (n=141) with a minimum of 1 year employment in viscose rayons factories were compared with 141 age and gender-matched plastic industry workers. Current exposures were estimated as 1 to 30 mg/m$^3$ (03 to 10 ppm). Exposed workers were categorized as group 1 or

group 2, with cumulative exposures of less than or greater than 100 mg/m$^3$-years, respectively. Group 2 ($p<0.001$) but not group 1 workers had increased mean total cholesterol (5.3 and 4.5 mmol/l) compared with controls (4.6 mmol/l) (Kotseva, 2001b).

$CS_2$ causes reproductive toxicity in both males and females. Lancranjan *et al.* (1969), Lancranjan (1972), Cirla *et al.* (1978), and Wagar *et al.* (1983) studied male reproductive effects of occupational exposure to $CS_2$ and showed significant adverse effects on spermatogenesis, levels of serum FSH and LH, and libido; these effects persisted in 66% of the workers subject to follow-up. Zhou *et al.* (1988) investigated pregnancy outcomes and menstrual disturbances in 265 women occupationally exposed to $CS_2$ in five facilities and 291 controls. The $CS_2$-exposed women had a significantly higher incidence of menstrual disturbances versus the control group (overall 34.9% vs. 18.2%). $CS_2$ levels varied between the five facilities (exposure category means of low = 3.1 mg/m$^3$, intermediate = 6.5 mg/m$^3$, and high = 14.8 mg/m$^3$), but all workers from these $CS_2$ facilities had significantly higher incidences of menstrual disturbance. Irregularity of menstruation was the most common disturbance, followed by abnormal bleeding. No evidence was observed to indicate an adverse effect on the term and outcome of pregnancy.

An abstract of an epidemiological study of birth defects among female workers occupationally exposed to $CS_2$, was reported by Bao et al. (1991). Exposures were at rayon factories in four Chinese provinces and began at least 6 months prior to pregnancy and continued during pregnancy. An increased rate of birth defects (2.6% vs. 1.3%) among 682 exposed women was noted compared to 745 women in the control group. The most common defects were congenital heart defects, inguinal hernia, and CNS defects. However, there was no significant difference in birth defects between those with estimated exposures greater than 10 mg/m$^3$ compared to those with lower exposures. There were no differences in rates of stillbirth, low birth weight, or neonatal or perinatal deaths among any of the groups.

The possibility of determining LOAEL and/or NOAEL values for the major $CS_2$-related adverse effects from epidemiology studies, which predominately use workers from the viscose rayon industry, is limited. The limitations include incomplete historical exposure measurements, concurrent exposure to other chemicals (including hydrogen sulfide or methylene chloride), lack of personal exposure determinations, and a high variability of individual exposures due to decreases of plant $CS_2$ concentrations over time.

## V. Effects of Animal Exposure

Studies investigating the potential for $CS_2$ toxicity in animals have usually been limited by intermediate or subchronic duration (less than 1 year) and a lack of multiple dose or exposure groups. The neuropathologic changes consistently observed in rodents following $CS_2$ exposure include axonal swelling, demyelination, swelling at neuromuscular junctions, muscle atrophy and degeneration, damage to terminal axons, and nerve fiber breakdown (Clerici and Fechter, 1991; Colombi *et al.* 1981; Eskin *et al.*, 1988; Jirmanova and Lukas, 1984; Maroni *et al.*, 1979; Szendzikowski *et al.*, 1973). These adverse effects have been observed over a range of

exposures (250 to 800 ppm), but few studies have attempted to establish a dose response for this $CS_2$-induced neurotoxicity.

In a 90 day subchronic inhalation study, Sprague-Dawley and Fischer 344 rats exposed discontinuously (6 hours/day, 5 days/week) to $CS_2$ developed morphological alterations in nerves including axonal swelling and myelin degradation (Gottfried *et al.*, 1985). This study established a subchronic NOAEL of 50 ppm and a LOAEL of 300 ppm for morphological changes in nerves. A longer inhalation study in Wistar rats observed impairment in the conduction velocity of the sciatic and tibial nerves after 6 and 12 months of intermittent exposure to 289 ppm $CS_2$ (LOAEL of 289 ppm) (Knobloch *et al.*, 1979).

In a 13-week subchronic study, male and female F344 rats inhaled 0, 50, 500, or 800 ppm $CS_2$ discontinuously (6 h/day, 5 days per week) (Sills *et al.*,1998). Development of distal axonopathy in the muscular branch of the posterior tibial nerve (MBPTN) and spinal cord was examined. After 13 weeks, giant swollen axons were observed with thin myelin sheaths as well as some degenerated and regenerated axons. Axonal swelling was noted in the spinal cords of rats exposed to 500 or 800 ppm $CS_2$. In the 800 ppm group, additional axonal swelling was observed in the muscular branch of the posterior tibial nerve, . Neurofilament deposits were found in swollen axons in the spinal cord and MBPTN. The NOAEL for axonal swelling was 50 ppm.

Wronska-Nofer (1973) showed a positive relationship between the level of triglycerides, the rate of cholesterol synthesis, and $CS_2$ exposure in Wistar rats exposed to 0, 73.8, 160, 321, or 546 ppm $CS_2$ for 5 hours/day, 6 days/week over 8 months. This study found a subchronic LOAEL of 73.8 ppm for disturbances in lipid metabolism (increase in serum cholesterol and serum triglycerides).

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

Hepatic toxicity has also been induced in rats exposed to relatively high doses of $CS_2$, usually following pretreatment with liver inducers such as phenobarbital. Bond *et al.* (1969) showed that high doses of $CS_2$ to rats produced an increase in periportal liver fat, and decreases in hepatic cytochrome P450 content and in microsomal mixed function oxidase (MFO) activity. After phenobarbital induction, exposed rats exhibited more severe hepatoxicity characterized by hydropic degeneration and necrosis. Other hepatotoxic effects seen after $CS_2$ exposures greater

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

The 24-hr lethal ip $LD_{50}$ values for $CS_2$ were estimated in 1-, 5-, 10-, 20-, 30- and 40-day-old rats (sample size not specified) (Green and Hunter, 1985). 1-day-old rats ($LD_{50}$ 583 mg/kg, ip) were about 3-times more susceptible than 20-day-old rats ($LD_{50}$ 1545 mg/kg, ip).

$^{14}C$- and $^{35}S$-labelled $CS_2$ was given ip to 1-, 5-, 10-, 20-, 30-, and 40-day-old rats (Snyderwine and Hunter, 1987). Thirty- and forty-day-old rats (sample size not reported) metabolized significantly more $CS_2$ to $CO_2$ and expired significantly less $CS_2$ than 1- to 20-day-old rats. Twenty-four hr after administration, up to 13 times more $^{35}S$ -label (radioactivity per g of tissue) were present in organs from 1-day-old rats than in similar organs from 40-day-old rats. The study does not specifically address the toxicological implications of the metabolic differences, and did not include fully mature animals. However, inability to detoxify $CS_2$ would lead to higher tissue concentrations and thus, potentially, increased toxicity.

The metabolite responsible for $CS_2$ hepatotoxicity is believed to be reactive sulfur atoms that covalently bind to cellular macromolecules (Dalvi, 1988). Similarly, the correlation between increased lethality (Green and Hunter, 1985) and increasing binding of $^{35}S$ –label (Snyderwine and Hunter, 1987) in younger $CS_2$ -exposed animals is consistent with a role for reactive sulfur. Neurotoxicity of $CS_2$ results from the formation of thiourea lysine cross-links between neurofilament proteins (DeCaprio et al., 1992; Valentine et al., 1997; Erve et al., 1998).

New Zealand white rabbits (24 per group) inhaled 0, 60, 100, 300, 600 or 1200 ppm $CS_2$ for 6 h/d on gestation days 6 to 18 (Pathology Associates, 1991). Developmental toxicity (NOAEL = 300 ppm; 930 mg/m$^3$) was noted at concentrations lower than those associated with significant maternal toxicity (NOAEL = 600 ppm; 1860 mg/ m$^3$) (Pathology Associates, 1991). The adults did have some slight hematological changes at the 600 ppm level, but the authors questioned the biological significance of these marginal findings. Reduced fetal body weights were noted at 600 and 1200 ppm. Cumulative malformations were increased in the 1200 (3720 mg/m$^3$) but not 600 ppm group, though there were no significant increases in any specific malformation in any group. Maternal effects at 1200 ppm included decreased body weight, ataxia, wheezing, and tremors. In an initial range-finding study, exposure to 3000 ppm was associated with significant lethality.

Rats were exposed to 100 mg/m$^3$ (32 ppm) for 4 hr/d on gestation days 7 and 8, and the embryos explanted to culture medium at day 9.5. Growth of explants of 10 treated and 17 control embryos was monitored for 44 hours. $CS_2$ at this concentration induced growth retardation in treated embryos relative to controls (Zhao et al., 1997).

In a two-generation study, Tabacova et al. (1983) exposed pregnant Albino rats (30-32 pregnant females per group) to 0.03, 10, 100, or 200 mg/m$^3$ (0.01, 3, 32, or 64 ppm) $CS_2$. The two highest dose levels were both teratogenic and maternally neurotoxic. There were no significant adverse effects in the F1 generation at the 2 low dose levels. However, significant increases in teratogenicity were found in the F2 generation at 10 mg/m$^3$, as well as increased postnatal

neurological effects including hypoactivity, mild ataxia and gait disturbances, hind-limb weakness, spinning and tremor (Tabacova et al., 1983). While the overall rate of malformations (club foot, hydrocephalus, microcephalus, generalized edema) exhibited a dose-response trend, with increased effects in the F2 generation, the specific malformations exhibited a less-consistent pattern. For example, while club foot was the predominant malformation in the F1 fetuses (occurring at 100 and 200 mg/m$^3$); much lower rates of club foot were noted in the F2 generation (including none in the 200 mg/m$^3$ group). Limitations of the study include a lack of information on chemical purity and exposure methods, lack of concurrent controls, lack of clear dose-response trend, and incomplete reporting on the statistical significance of reported behavioral effects.

Wistar albino rats (32 animals per group) were exposed to 50, 100, or 200 mg/m$^3$ (16, 32, or 64 ppm) $CS_2$ for 8 hours per day throughout gestation. There were no statistically significant results in the 50 mg/m$^3$ group. In the 100 and 200 mg/m$^3$ groups, there were statistically significant increases in reduced fetal body weights, and reduced post natal body weights for 21 days, which subsequently disappeared. There was an increase in external malformations (hydrocephalus, club foot, and tail deformations) at the two higher doses (Tabacova et al., 1978).

Behavioral effects were examined in the offspring of Lati:CFY rats (8 per group) exposed to 0, 10, 700, or 2000 mg/m$^3$ $CS_2$ (3, 230, or 640 ppm) for 6 hours per days over days 7 to 15 of gestation. The two high doses caused significant perinatal mortality. Avoidance conditioning was tested using a bell as a conditional stimulus prior to an electric shock. The animals learned to avoid the shock by jumping onto a pole at the sound of the bell. The latency to jump onto the pole and errors were measured as a means to evaluate avoidance conditioning in the treated versus control animals. The authors reported that there was a dose-related change in avoidance conditioning among male pups over the first 15 days (Lehotsky et al., 1985). While the magnitude of the effect on avoidance conditioning was greater at all doses relative to controls, and at 2000 mg/m$^3$ compared with 700 mg/m$^3$, the effect was virtually identical between the 10 and 700 mg/m$^3$. This lack of dose-response effect raises some question about the significance of this finding.

Effects of low (0.03 and 10 mg/m$^3$; 0.01 and 3 ppm) prenatal exposures (8 hours per day throughout gestation) of $CS_2$ were studied in Wistar albino rats. No congenital malformations or significant prenatal effects were found in the 9-11 litters evaluated at each dose. Mortality during postnatal days 10 through 21 was increased in the 10 mg/m$^3$ group. Delays in the development of visual and auditory function were reported in the higher dose group (Tabacova and Balabaeva, 1980). There was no mention of maternal toxicity in this study.

Several other studies yielded either no teratogenic effects or effects only at maternally toxic exposures. Saillenfait et al. (1989) exposed rats via inhalation to 0, 100, 200, 400, or 800 ppm $CS_2$ for 6h/d during days 6-20 of gestation. Lower exposures (100 or 200 ppm; 310 or 620 mg/m$^3$) were not associated with maternal toxicity or adverse effects on the developing embryo or fetus. Higher concentrations (400 or 800 ppm; 1240 or 2480 mg/m$^3$) yielded a significant reduction of maternal weight gain as well as reductions of fetal body weight and a low incidence of club foot. Significant increases in unossified sternebrae were reported following 800 ppm (2480 mg/m$^3$) exposures. Nemec et al. (1993) reported no teratogenicity or maternal,

developmental, or reproductive toxicity among pregnant CD rats and their offspring following exposure to 125 or 250 ppm (388 or 775 mg/m$^3$) from 2 weeks prior to mating through gestation day 19. At 500 ppm, dams had decreased body weight gain and food consumption; decreased litter viability but no teratogenic effects were noted. $CS_2$ was not found to be teratogenic or embryotoxic following intraperitoneal administration to rats on days 1-15 of gestation (Beliles et al., 1980; Hardin et al., 1981). No significant effects were noted in animal inhalation exposures (20 to 40 ppm; 62 to 125 mg/m$^3$ $CS_2$) with either rats on days 1-19 of gestation or rabbits on days 1-24 of gestation.

## VI. Derivation of Chronic Reference Exposure Level

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

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

**Table 1. Peroneal MCV data used for benchmark dose modeling**

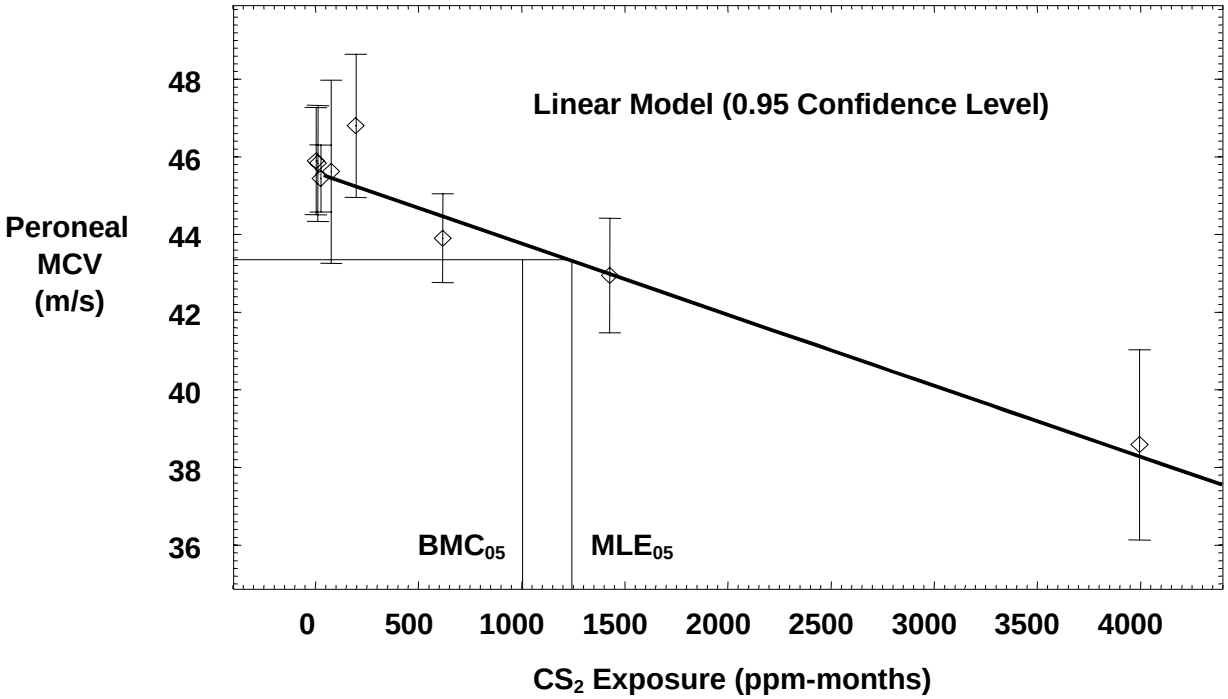
| ***Exposure (ppm-months)*** | ***Subjects*** | ***Peroneal MCV (m/s)*** | |
|---|---|---|---|
| | | ***Mean*** | ***Std. Dev.*** |
| 3.8 (2 - 6) | 32 | 45.9 | 3.8 |
| 13.1 (6 - 16) | 61 | 45.8 | 5.8 |
| 26.5 (16-44) | 140 | 45.4 | 5.2 |
| 77.3 (44-122) | 17 | 45.6 | 4.6 |
| 197 (122 – 336) | 17 | 46.8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

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

Table 2. Benchmark dose modeling results

| Model | ***$MLE_{05}$ (ppm-mo)*** | ***$BMC_{05}$ (ppm-mo)*** | ***p value*** |
|---|---|---|---|
| Linear | 1245 | 1005 | 0.84 |
| Polynomial | 1100 | 736 | 0.78 |
| Hill | 1092 | 670 | 0.65 |
| Power | 1245 | 1005 | 0.58 |

The $BMC_{05}$ from the best-fitting linear model was used. An occupational $BMC_{05}$ of 6.9 ppm was derived by dividing the 1005 ppm-month value by the average exposure duration of 145 months (12.1 years). The time-weighted average value was thus 2.5 ppm (6.9 ppm x 10/20 x 5/7).




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

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

## VII. Data Strengths and Limitations for Development of the REL

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

## VIII. Potential for Differential Impacts on Children's Health

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

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

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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

| | |
|---|---|
| *Inhalation reference exposure level* | **40 μg/m$^3$** (6 ppb) |
| *Critical effect(s)* | Increased liver weight and hepatic fatty infiltration in guinea pigs |
| *Hazard index target(s)* | Alimentary system; development (teratogenicity); nervous system |

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

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

## III. Major Uses or Sources

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

## IV. Effects of Human Exposure

Kazantzis *et al.* (1960) evaluated 17 employees of a quartz processing factory who were occupationally exposed to 45-100 ppm (284-630 mg/m$^3$) carbon tetrachloride ($CCl_4$) vapor. Fifteen of the 17 workers complained of symptoms including nausea, anorexia, vomiting, flatulence, epigastric discomfort or distention, depressive symptoms, headache or giddiness for up to 4 months prior to the evaluation. A week after $CCl_4$ concentrations were reduced to 0-9 ppm with control measures, workers were symptom-free.

## V. Effects of Animal Exposure

Adams *et al.* (1952) chronically exposed albino Wistar rats, guinea pigs, albino rabbits and rhesus monkeys to 0, 5, 10, 25, 50, 100, 200 and 400 ppm $CCl_4$ for varying duration. For each exposure group, two control groups were devised (unexposed and air-exposed controls) consisting of animals similar in age, sex, weight and number. The 2 control groups responded similarly to the experimental protocol.

In the 100, 200 and 400 ppm exposure groups (Adams *et al*., 1952), mortality was excessive with moderate to severe liver cirrhosis and other various pathological changes in all the species tested. Fifteen male and 15 female rats were exposed to 50 ppm $CCl_4$ 134 times for 187 days. They experienced decreased body weight gain and liver weight increase as well as moderate fatty degeneration and slight to moderate liver cirrhosis. Females showed kidney weight increase and four rats showed slight to moderate swelling of the kidney tubular epithelium. Guinea pigs (8 males and 8 females; 143 exposures in 200 days) showed depressed growth in the first two weeks, enlarged livers, moderate fatty degeneration and liver cirrhosis, and increased levels of liver total lipids, neutral fat, esterified cholesterol and plasma prothrombin clotting time.

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

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

At 25 ppm $CCl_4$, 15 male and 15 female rats were exposed 137 times for 191 days. Early growth depression in males was observed, although final body weights did not significantly differ from the controls. Significant liver weight increase and slight to moderate fatty degeneration occurred. Liver lipid content was nearly twice the level of the controls and esterified cholesterol was five times that of the controls. For this exposure, phospholipid and neutral fat were not measured. Five male guinea pigs were exposed 133 times over 185 days and 5 female guinea pigs were exposed 93 times over 126 days. Symptoms included growth depression, liver weight

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

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

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

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

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

During the 61 mg/m$^3$ (9.7 ppm) $CCl_4$ continuous exposure study, 3 guinea pigs died (one each after 47, 63, and 71 days). All the monkeys were emaciated and experienced hair loss. Depressed body weight increases were seen in all exposed animals compared to the controls. Autopsies showed enlarged and/or discolored livers in a high percentage (not given) of monkeys, guinea pigs, rabbits, and rats. Rats and guinea pigs showed hepatic fatty acid changes, and a moderate reduction in succinic dehydrogenase activity was also evident in guinea pigs. Varying but lesser degrees of these changes were also seen in the other species tested.

The low concentration of 6.1 mg/m$^3$ (1 ppm) $CCl_4$ was attained by diluting the $CCl_4$ to 10% of the above concentration with *n*-octane, resulting in a solution of 6.1 mg/m$^3$ $CCl_4$ in 61 mg/m$^3$ of *n*-octane (Prendergast *et al.,* 1967)). The level of *n*-octane used was shown to be nontoxic by an *n*-octane control, which yielded no effects. (The current TLV for n-octane is 1400 mg/m$^3$ (300 ppm) (ACGIH, 1992).) No animals died during this study, and no signs of toxicity were noted. All exposed animals except the rats showed reduced weight gain when compared to the controls, and all species exhibited nonspecific inflammatory lung changes. Guinea pig liver lipid contents and serum urea nitrogen concentrations were similar to the control values. In several animals there were some nonspecific inflammatory changes in the liver, kidney and heart, but the authors did not attribute these to the chemical exposure. There was no other observed hematologic or histopathologic toxicity at this level.

Shimizu *et al.* (1973) exposed groups of 4 female Sprague-Dawley rats to 10, 50 and 100 ppm of $CCl_4$ vapor for 3 hours a day, 6 days a week for up to 6-8 weeks. The rats were terminated two days after the last inhalation. Accumulation of $CCl_4$ occurred in the adipose tissue and was measured after 1 and 3 weeks of exposure. For the 10 ppm group, accumulation was gradual, reaching a level of 1/3 the amount found in the 50 ppm group after 6 weeks. A slight increase of triglycerides in the liver (6.2-6.4 mg/g) was observed in the 10 ppm group, but no control group was used for comparison.

The intermittent exposure caused a more pronounced and higher number of change indices to occur (34 as opposed to the 17 change indices of the monotonous regimen), indicating a greater intensity of liver damage. Changes included a significant decrease in hippuric acid synthesis, presence of mitochondrial enzymes (glutamate dehydrogenase and ornithine carbonyl transferase) in the blood (indicating severe damage to hepatocytes), significant increase in cytoplasmic enzyme activity, and a decrease in the level of cytochrome P-450 in liver tissue. The effects seen in the monotonous group were the same variety as those in the intermittent group, but were less intense. The content of $CCl_4$ in the blood was similar for both the intermittent and monotonous exposure groups. Another test was performed over a period of 27 days varying the regimen, and therefore the concentration, of intermittent exposure while keeping the TWA level of $CCl_4$ stable. Increasing the concentration threefold or fivefold with five 10 minute peaks did not potentiate the toxic effects. Varying the regimen tenfold to five 5-minute peaks (peak exposure 402 mg/m$^3$ (63.8 ppm)) with a time weighted average exposure of 6.5 ppm (41$\pm$1 mg/m$^3$) did, however, result in more severe liver damage.

Sakata *et al.* (1987) exposed 10-15 male Sprague-Dawley rats to <10 ppm $CCl_4$ vapor for 15 minutes a day, twice a week for 8 weeks. All the rats had chronic liver damage involving

nodular liver surfaces and extensive fibrosis. Researchers also found similar results in rats after 8 weeks of subcutaneous injections of 0.1 mL of 50% $CCl_4$ solution in olive oil twice a week.

Ideura *et al.* (1993) exposed male Wistar rats to $CCl_4$ vapor for 7 minutes, 3 times a week for 6-10 weeks (concentration unspecified). Six experimental groups of 4-5 rats were used, two of which were exposed for 10 weeks, another two for 6 weeks, and two unexposed control groups. Following the last exposures, rats were injected with varying amounts of endotoxin (1.0 mL lipopolysaccharide (LPS)). The rats were sacrificed 24 hours after the injection and processed for histological examination. Examination of the rats' left kidneys and livers revealed liver cirrhosis with destruction of normal structure and massive ascites retention after 10 weeks of exposure as compared to the controls. Those exposed for 6 weeks exhibited an increase in fibrous tissue. The control groups displayed normal liver structure. Researchers found that rats previously resistant to endotoxin became susceptible following $CCl_4$ exposure, which was manifested as induced acute renal tubular necrosis in cirrhotic rats.

Yoshimura *et al.* (1993) performed a similar experiment to that of Ideura *et al.* (1992) by exposing male Wistar rats for 6 (5 rats) and 10 weeks (5 rats) to 99% $CCl_4$ vapor for 3 minutes a day. A control group of 5 rats was given phenobarbitone for 10 weeks. After 24 hours following the final exposure, rats were injected with endotoxin. Six weeks of $CCl_4$ exposure caused liver fibrosis with bridging fibrosis, while 10 weeks of exposure to $CCl_4$ caused liver cirrhosis and destruction of the normal liver architecture.

Pregnant rats were exposed to 0, 300, or 1000 ppm (0, 1938, or 6460 mg/m$^3$) carbon tetrachloride for 7 hours/day on days 6-15 of gestation (Schwetz *et al*., 1974). Significant fetal growth retardation, measured by decreased crown-rump length and body weight, was observed in the offspring of the exposed groups (n = 22 litters) compared with controls (n = 43 litters). Subcutaneous edema was observed in the 300 ppm group but not in the 1000 ppm group. Sternebral anomalies were observed in the 1000 ppm group.

Effects of Chronic $CCl_4$ Exposure (Adams *et al.*, 1952)

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

Data from Guinea Pigs and Rats Exposed to 5 ppm $CCl_4$ for 7 Months (Adams *et al*., 1952)

| | | | ←----- g organ weight/g body weight---→ | | | |
|---|---|---|---|---|---|---|
| Group | *n* | *BW (g)* | *Lung* | *Heart* | *Liver* | *Kidneys* |
| Rats, male | | | | | | |
| Unexposed controls | 11 | 336 | 0.65 | 0.32 | 2.38 | 0.65 |
| Air-exposed controls | 16 | 322 | 0.62 | 0.31 | 2.25 | 0.66 |
| 5 ppm $CCl_4$ | 13 | 336 | 0.62 | 0.31 | 2.23 | 0.65 |
| Rats, female | | | | | | |
| Unexposed controls | 14 | 204 | 0.86 | 0.38 | 2.41 | 0.73 |
| Air-exposed controls | 17 | 209 | 0.76 | 0.37 | 2.76 | 0.76 |
| 5 ppm $CCl_4$ | 18 | 214 | 0.81 | 0.38 | 2.58 | 0.73 |
| Guinea pigs, male | | | | | | |
| Air-exposed controls | 7 | 695 | 0.79 | 0.27 | 3.07 | 0.63 |
| 5 ppm $CCl_4$ | 8 | 669 | 0.82 | 0.27 | 3.14 | 0.65 |
| Guinea pigs, female | | | | | | |
| Air-exposed controls | 9 | 611 | 0.81 | 0.27 | 2.58 | 0.59 |
| 5 ppm $CCl_4$ | 6 | 636 | 0.78 | 0.26 | 2.82* | 0.57 |

* p =0.004

## VI. Derivation of Chronic Reference Exposure Level (REL)

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

Of the 2 adequate chronic inhalation studies available on $CCl_4$, the Adams *et al.* (1952) study was chosen over the Prendergast *et al.* (1967) study as the key reference for the carbon tetrachloride chronic REL. The Adams *et al.* (1952) experiment was conducted over a longer

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

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

## VII. Data Strengths and Limitations for Development of the REL

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

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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

| | |
|---|---|
| *Inhalation reference exposure level* | **0.00004 µg/m$^3$ (40 pg/m$^3$)** |
| *Oral reference exposure level* | **1 x 10$^{-8}$ mg/kg/day (10 pg/kg/day)** |
| *Critical effect(s)* | Increased mortality, decreased weight gain, depression of erythroid parameters, increased urinary excretion of porphyrins and delta-aminolevulinic acid, increased serum activities of alkaline phosphatase, gamma-glutamyl transferase and glutamic-pyruvic transaminase, gross and histopathological changes in the liver, lymphoid tissue, lung and vascular tissues in rats. |
| *Hazard index target(s)* | Alimentary system (liver); reproductive system; development; endocrine system; respiratory system; hematopoietic system |

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

| | |
|---|---|
| *Description* | All are white crystalline powders at 25° C. |
| *Molecular Formula* | $C_{12}H_4C_{l4}O_2$ (TCDD) |
| *Molecular Weight* | 321.97 g/mol (TCDD) |
| *Density* | 1.827 g/ml (estimated for TCDD) |
| *Boiling Point* | 412.2°C (estimated for TCDD) |
| *Melting Point* | 305-306°C (TCDD) |
| *Vapor Pressure* | 1.52 x 10$^{-9}$ torr at 25°C (TCDD) |
| *Solubility* | In water: 19.3 ng/L at 22°C (TCDD) |
| *Log $K_{ow}$* *(octanol/water partition coefficient)* | 6.15-7.28 (6.8 for TCDD) |
| *Log $K_{oc}$* *(organic-carbon distribution coefficient)* | 6.0-7.39 |
| *Henry's Law Constant* | 8.1 x 10$^{-5}$ ATM-m$^3$/mol |

## III. Major Uses and Sources

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

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

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

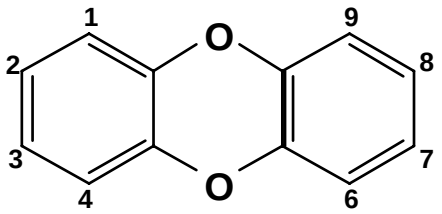
Ah receptor as well as on relative toxicity of different congeners. Values for the international system of toxic equivalents are provided in Table 1 (U.S. EPA, 1989).

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

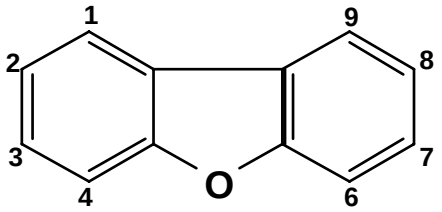
| **Compound[1,2]** | **I-TEF** |
|---|---|
| Mono-, Di-, and Tri-CDDs and CDFs | 0 |
| TetraCDD | |
| 2,3,7,8-substituted | 1.0 |
| Others | 0 |
| PentaCDD | |
| 2,3,7,8-substituted | 0.5 |
| Others | 0 |
| HexaCDD | |
| 2,3,7,8-substituted | 0.1 |
| Others | 0 |
| HeptaCDD | |
| 2,3,7,8-substituted | 0.01 |
| Others | 0 |
| OctaCDD | 0.001 |
| TetraCDF | |
| 2,3,7,8 | 0.1 |
| Others | 0 |
| PentaCDF | |
| 1,2,3,7,8-PentaCDF | 0.05 |
| 2,3,4,7,8-PentaCDF | 0.5 |
| others | 0 |
| HexaCDF | |
| 2,3,7,8-substituted | 0.1 |
| Others | 0 |
| HeptaCDF | |
| 2,3,7,8-substituted | 0.01 |
| Others | 0 |
| OctaCDF | 0.001 |

[1] CDD designates chlorinated dibenzo-p-dioxin
[2] CDF designates chlorinated dibenzofuran

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




Dibenzo-p-dioxin




Dibenzofuran

## IV. Effects of Human Exposure

The information available on possible chronic toxic effects in humans is complicated by the relative insensitivity of epidemiological studies, the limited ability of case studies of exposed individuals to establish cause and effect relationships, the heterogeneous nature of human populations, the broad spectrum of exposures to other toxic agents in the human environment, and the episodic exposure of many of the exposed human populations which have been studied (e.g., Seveso, Italy). As a result, a limited number of effects have been associated with exposure to dioxins in humans. The meaning of these effects in terms of toxicity in most cases remains to be clarified. The majority of information comes from cross-sectional medical studies. Chloracne is the most widely recognized effect of exposure to 2,3,7,8-TCDD and TCDD-like PCDDs and PCDFs. Chloracne is a persistent condition, which is characterized by comedones, keratin cysts and inflamed papules and is seen after acute and chronic exposure to various chlorinated aromatic compounds (Moses and Prioleau, 1985). Other dermal effects include hyperpigmentation and hirsutism or hypertrichosis (Jirasek *et al.*, 1974; Goldman, 1972; Suskind *et al.*, 1953; Ashe and Suskind, 1950); both appear to resolve themselves more quickly over time than chloracne, making them more of an acute response rather than a chronic response (U.S. EPA, 1994a). Epidemiological data available for 2,3,7,8-TCDD have not allowed a determination of the threshold dose required for production of chloracne (U.S. EPA, 1994b). Case studies suggest that there may be a relationship between 2,3,7,8-TCDD exposure and hepatomegaly (Reggiani, 1980; Jirasek *et al.*, 1974; Suskind *et al.*, 1953; Ashe and Suskind, 1950) and hepatic enzyme changes (Mocarelli *et al.*, 1986; May, 1982; Martin 1984; Moses *et al.*, 1984). Nevertheless, cross sectional epidemiological studies of trichlorophenol (TCP) production workers (Suskind and Hertzberg., 1984; Bond *et al.*, 1983; Moses *et al.*, 1984; Calvert *et al.* 1992), Vietnam veterans (Centers for Disease Control Vietnam Experience Study, 1988; Roegner *et al.*, 1991) and Missouri residents (Webb *et al.*, 1989; Hoffman *et al.*, 1986)

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

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

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

## V. Effects of Animal Exposure

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

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

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

acutely toxic to early life stages of fish and birds with fish being most sensitive ($LD_{50}$ of 0.4 µg/kg for rainbow trout sac fry eggs and $LD_{50}$ of 34 ng/kg for lake trout eggs); some species of birds are also relatively sensitive ($LD_{50}$ of 0.25 µg/kg for chicken eggs) (Peterson *et al.*, 1993). 2,3,7,8-TCDD has been documented to increase the incidence of prenatal mortality in a number of species of laboratory animals including the Rhesus monkey, Guinea pig, rabbit, rat, hamster, and mouse (Peterson *et al.*, 1993). Exposure to 2,3,7,8-TCDD during gestation produces a characteristic set of fetotoxic responses in most laboratory animals which includes: thymic hypoplasia, subcutaneous edema, and decreased growth (Peterson *et al.*, 1993). More species specific responses include cleft palate formation in the mouse at doses below maternal toxicity (Moore *et al.*, 1973; Smith *et al.*, 1976; Couture *et al.*, 1990), intestinal hemorrhage in the rat (Sparschu *et al.*, 1971), hydronephrosis in the mouse and hamster (Moore *et al.*, 1973; Smith *et al.*, 1976; Couture *et al.*, 1990; Birnbaum *et al.*, 1989; Olson *et al.*, 1990), and extra ribs in the rabbit (Giavini *et al.*, 1982). Female rats have also been found to be affected by perinatal exposure to 2,3,7,8-TCDD with clefting of the clitoris, incomplete or absent vaginal opening and a smaller vaginal orifice after a dose of 1 µg/kg to the mother on day 15 of gestation (Gray *et al.*, 1993).

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

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

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

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

## VI. Derivation of Chronic Reference Exposure Level (REL)

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

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

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

## VII. Data Strengths and Limitations for Development of the REL

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

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

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*CHRONIC TOXICITY SUMMARY*

# CHLORINE

**CAS Registry Number: 7782-50-5**

## I. Chronic Toxicity Summary

| | |
|---|---|
| *Inhalation reference exposure level* | **0.2 µg/m$^3$ (0.08 ppb)** |
| *Critical effect(s)* | Hyperplasia in respiratory epithelium in female rats |
| *Hazard index target(s)* | Respiratory system |

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

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

## III. Major Uses and Sources

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

## IV. Effects of Human Exposure

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

employed for less than 10 years. Both groups of workers were exposed to a range of 2.60-11.0 mg/m$^3$ (0.37-1.75 ppm) chlorine. The control group's average age was 39.7 years (ranging from 26-55 years), and it consisted of 192 workers not exposed to chlorine, but working within the same plant. For all the groups, respiratory symptoms and smoking habits were evaluated as well as clinical examinations, ENT examinations, chest x-rays and pulmonary function tests. Groups A and B showed 3-8 times higher incidence of upper airway complaints than the control workers. Current smokers in groups A and B experienced the highest incidence of pulmonary symptoms and group A workers had a higher prevalence of rhino-pharyngeal signs than the control workers. Abnormalities in chest x-rays were seen in 8.6% of group A workers and in 2.8% of group B workers, compared to 2.3% of the control workers. Groups A and B showed significantly impaired pulmonary function in tests of V50/H and $FEF_{25-75}$ (forced expiratory flow between 25 and 75% of forced vital capacity (FVC), the total amount of air the subject can expel during a forced expiration) - compared with the control group, and group A showed reduced $FEV_1$ (forced expiratory volume in 1 second) results compared to the control group.

Kennedy *et al.* (1991) compared 321 pulpmill workers (189 of whom were exposed to chlorine or chlorine dioxide "gassings") to a control group of 237 rail yard workers in similar working conditions but not exposed to chlorine (79% and 84% respective participation rates). The workers had been employed for an average of 13 years at the pulpmill and 12.7 years at the rail yard. Chlorine gas and chlorine dioxide levels were measured together over a 4 week period during mainly a 12 hour shift. Time weighted averages (TWA) were <0.1 ppm, with the highest of <0.1-0.3 ppm. A significantly higher prevalence of wheezing was seen in pulpmill workers (both smokers and nonsmokers) who had reported more than one episode of chlorine "gassing" as compared to the rail yard workers and pulpmill workers with no chlorine gas exposure. More airflow obstruction was observed in exposed workers in spite of their nonsmoking and ex-smoking status, correlating to significantly lower average values for MMF (maximal mid-expiratory flow) and for the $FEV_1$ to FVC ratio. Comparison of pulpmill workers exposed to chlorine and /or chlorine dioxide with those pulpmill workers not exposed, suggests that chronic respiratory health impairment is associated with exposure to chlorine and/or chlorine dioxide. These researchers hypothesized that after the first high exposure incident, an inflammatory response occurred in small airways and that this reaction did not resolve in those workers who were continuously or repeatedly exposed to the irritant. It was also suggested that chronic airflow obstruction caused by repeated minor exposures led to chronic respiratory disability in some of the workers.

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

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

Bherer *et al.* (1994) conducted a follow up study of the Quebec pulp mill research done by Courteau and associates over a time interval of 18-24 months after the incidents of repeated exposures. Fifty-eight of the original 289 exposed workers from the moderate to high risk group were studied for developing reactive airways dysfunction syndrome (RADS). Workers at a moderate risk were defined as having shortness of breath after their most significant exposure, but not at the time of the initial study by Courteau *et al.* Moderate risk workers also had a record of other significant medical conditions and/or were 50 years of age or older. High risk workers were defined as those experiencing shortness of breath that continued one month after the exposure and/or abnormal lung sounds. Ninety percent of the follow up group completed questionnaires which revealed a 91% incidence of respiratory symptoms. Spirometry assessments and methacholine inhalation tests were conducted on 51 of the 58 workers. Twenty-three percent of the 58 workers still experienced bronchial obstruction and 41% continued to have bronchial hyper-responsiveness. Lower baseline $FEV_1$ was seen in those with a lower $PC_{20}$, and 52% of these workers showed an $FEV_1 < 80\%$ predicted.

Enarson *et al.* (1984) compared 392 pulpmill workers exposed to chlorine (unspecified duration) to a comparable group of 310 rail yard workers living in the same community, but not exposed to chlorine. In the pulpmill areas surveyed that predominantly had significant chlorine gas levels (machine room and bleach plant), workers were exposed to either an average of 0.02 ppm or 0.18 ppm $Cl_2$ respectively. Of the machine room workers, 23.2% experienced a cough as did 32.8% of those in the bleach plant, compared to 22.3% of the control rail yard workers. Chest tightness occurred in 31.5% of the machine room workers and 39.6% of the bleach plant workers as compared to 21.3% of the control. Only data from Caucasian subjects were reported.

Chester *et al.* (1969) evaluated 139 workers occupationally exposed to <1 ppm chlorine for an unspecified duration. Fifty-five of the 139 workers were exposed to additional accidental high concentrations of chlorine, which were severe enough to require oxygen therapy. Ventilation was affected by chlorine inhalation, with a decrease in the maximal midexpiratory flow (MMF). Smokers in this group had significantly reduced FVC, $FEV_1$ and MMF compared to nonsmokers. Fifty-six of the 139 subjects showed abnormal posteroanterior chest films, 49 of which had parenchyma and/or hilar calcifications consistent with old granulomatous disease and 11 of which had multiple, bilateral and diffuse calcifications. Researchers suggest that the first ventilation function affected in obstructive airway disease is MMF.

## V. Effects of Exposure to Animals

Wolf *et al.* (1995) exposed male and female B6C3F1 mice and F344 rats to chlorine gas concentrations of 0 ppm, 0.4 ppm, 1.0 ppm and 2.5 ppm. The exposures were carried out for 104 weeks at 6 hr/day 3 days/week for female rats and 6 hr/day 5 days/week for mice and male rats. Based on previous studies, the authors determined that female rats could not tolerate 5 days/week exposure to chlorine. Each treatment group contained 320 male and 320 female mice. The rats were studied in groups of 70, yielding 280 per gender per species. For the first 13 weeks of observation, body weights and clinical observations were noted weekly, and for the remainder of the study, they were recorded once every two weeks. After 52 weeks, 10 rats were euthanized and autopsied. Organ weights were recorded, and hematologic and clinical chemistry parameters were determined. These same measurements were performed on all of the surviving mice and rats at the conclusion of the 104 weeks. Male mice exposed to 1.0 and 2.5 ppm $Cl_2$ showed decreased weight gain compared to controls while only female mice exposed to 2.5 ppm $Cl_2$ showed decreased weight gain. Male rats showed decreased weight gain at all levels of exposure while female rats showed the same result at only 1.0 and 2.5 ppm $Cl_2$ exposures. Various nonneoplastic nasal lesions were seen in all the airway epithelial types in the nose and at all levels of exposures for both species. These lesions were evaluated against background lesions found in the control animals. A statistically significant incidence of fenestration was seen in all three exposure concentrations of $Cl_2$. Statistically significant responses were seen in the traditional and respiratory epithelial regions of all exposed rats and mice. Statistically significant damage to olfactory epithelium occurred in all exposed rats and female mice and also in the 1.0 and 2.5 ppm exposed groups of male mice.

Klonne *et al.* (1987) exposed 32 male and female rhesus monkeys to chlorine gas for one year to measured concentrations of 0, 0.1, 0.5, and 2.3 ppm $Cl_2$. These monkeys were exposed to chlorine for 6 hours/day, 5 days/week. The monkeys were evaluated periodically on the basis of body weight, electrocardiograms, neurologic examinations, pulmonary function, hematologic parameters, serum chemistry, urinalysis, and blood gas and pH levels. Results were compared to the same test measurements recorded prior to the study. No significant difference was seen in body weight at any point in the experiment. Ocular irritation (tearing, rubbing of the eyes, reddened eyes) was observed after 6 weeks of exposure in the 2.3 ppm group. No exposure-related differences were seen in neurologic examinations, electrocardiograms, clinical chemistry, urinalysis, hematology or blood gas levels. Also, no exposure-related changes were observed in the parameters of ventilation distribution. Pulmonary function evaluations yielded a statistically significant trend for increasing pulmonary diffusing capacity and distribution of ventilation values for males and females in the 2.3 ppm exposure group. Both males and females of the 2.3 ppm group exhibited statistically significant increased incidence of respiratory epithelial hyperplasia. A mild form of the lesions was also seen in the 0.5 ppm group, 0.1 ppm group (females only) and one male in the control group. Two parasitic infections occurred, affecting the respiratory tract and resulting in 11 monkeys housing parasites and/or ova. Additionally, 16 monkeys displayed histologic changes characteristic of the presence of the parasites. However, the parasitic induced lesions were not associated with lesions in the respiratory epithelium.

## VI. Derivation of Chronic Reference Exposure Level (REL)

| | |
|---|---|
| *Study* | Wolf *et al.*, 1995 |
| *Study population* | Female F344 rats (70 per group) |
| *Exposure method* | Discontinuous whole-body inhalation exposure (0, 0.4, 1.0 or 2.5 ppm) |
| *Critical effects* | Upper respiratory epithelial lesions (see following table) |
| *LOAEL* | 0.4 ppm |
| *NOAEL* | Not established |
| *$BMC_{05}$* | 0.14 ppm |
| *Exposure continuity* | 6 hours/day, 3 days/week (MWF) |
| *Average experimental exposure* | 0.015 ppm |
| *Human equivalent concentration* | 0.0024 ppm (gas with extrathoracic respiratory effects, RGDR = 0.16 based on BW = 229 g, MV = 0.17 L/min, SA(ET) = 15 $cm^2$) |
| *Exposure duration* | 2 years |
| *LOAEL uncertainty factor* | (not needed in the BMC approach) |
| *Subchronic uncertainty factor* | 1 |
| *Interspecies uncertainty factor* | 3 |
| *Intraspecies uncertainty factor* | 10 |
| *Cumulative uncertainty factor* | 30 |
| *Inhalation reference exposure level* | 0.08 ppb (0.20 $\mu g/m^3$) |

A benchmark dose analysis was performed using a log-normal probit analysis (Tox-Risk, version 3.5; ICF-Kaiser Inc., Ruston, LA) of the female rat data. Using the data for glandular epithelial eosinophilic proteinaceous accumulation (see Table 1 below) to derive the $BMC_{05}$ resulted in a 3-fold lower value than the LOAEL of 0.4 ppm, or $BMC_{05}$ = 0.14 ppm. (Adequate benchmark dose estimates could not be obtained for the other nasal lesions due to high background rates and shallow dose-response relationships.) A $BMC_{05}$ is considered to be similar to a NOAEL in estimating a concentration associated with a low level of risk.

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

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

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

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

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

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

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

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

## VII. Data Strengths and Limitations for Development of the REL

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

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*CHRONIC TOXICITY SUMMARY*

# CHLORINE DIOXIDE

*(anthium dioxide; alcide; chlorine oxide; chlorine peroxide; chloryl radical; doxcide 50)*

CAS Registry Number: 10049-04-4

## I. Chronic Toxicity Summary

| | |
|---|---|
| *Inhalation reference exposure level* | **0.6 μg/m$^3$** (0.2 ppb) |
| *Critical effect(s)* | Vascular congestion and peribronchiolar edema; hemorrhagic alveoli and congested capillaries in the lung in rats |
| *Hazard index target(s)* | Respiratory system |

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

| | |
|---|---|
| *Description* | Yellow to red liquid or gas |
| *Molecular formula* | $ClO_2$ |
| *Molecular weight* | 67.45 g/mol |
| *Density* | 1.642 g/cm$^3$ @ 0°C (liquid) |
| *Boiling point* | 9.9-11°C |
| *Melting point* | –59.5°C |
| *Solubility* | Soluble in water, alkaline and sulfuric acid solutions |
| *Conversion factor* | 1 ppm = 2.76 mg/m$^3$ |

## III. Major Uses or Sources

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

## IV. Effects of Human Exposures

Case reports of human occupational exposure to chlorine dioxide have shown that 19 ppm was fatal to one worker and 5 ppm was definitely irritating (Elkins, 1959). Seven out of 12 workers exposed regularly to chlorine dioxide at levels generally below 0.1 ppm (0.28 mg/m$^3$) reported symptoms of ocular and respiratory irritation leading to slight bronchitis (Gloemme and

Lundgren, 1957). However, the authors ascribed the bronchitis to occasional acute excursions of chlorine dioxide levels above 0.1 ppm due to technical problems such as equipment leakage. Concurrent exposure to chlorine and chlorine dioxide in pulp mill workers resulted in an increase in the reporting of subjective symptoms of irritation (Ferris *et al.*, 1967). In this study, the chlorine dioxide concentrations ranged from trace levels to 0.25 ppm (0.69 mg/m$^3$). No differences were found between these workers and controls by pulmonary function tests.

## V. Effects of Animal Exposures

Eight rats (sex unspecified) were exposed for 5 hours/day, 5 days/week, for 2 months to 0 or 1 ppm (2.8 mg/m$^3$) chlorine dioxide (Paulet and Debrousses, 1972). The number of control animals was not specified. Microscopic evaluation of the lungs revealed vascular congestion and peribronchiolar edema in all animals exposed to chlorine dioxide. The subchronic LOAEL for respiratory effects was therefore 1 ppm (2.8 mg/m$^3$).

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

Rats exposed to 5, 10, or 15 ppm (13.8, 27.6, or 41.4 mg/m$^3$) chlorine dioxide for 15 minutes, 2 or 4 times/day, for 1 month showed an increase in congested lungs, nasal discharge, and catarrhous lesions of the alveoli beginning at 10 ppm (Paulet and Debrousses, 1974). No significant changes in these parameters were seen at 5 ppm.

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

## VI. Derivation of Chronic Reference Exposure Level (REL)

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

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

OEHHA earlier developed a chronic REL for chlorine of 0.2 μg/m$^3$ (0.08 ppb) based on hyperplasia in respiratory epithelium in female rats. Based on chemical reactivity, the REL for chlorine dioxide might be expected to be lower than that for chlorine. However, there are much less toxicologic data available for chlorine dioxide than for chlorine.

## VII. Data Strengths and Limitations for Development of the REL

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

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*CHRONIC TOXICITY SUMMARY*

# CHLOROBENZENE

*(monochlorobenzene; benzene chloride; benzene monochloride; chlorbenzene; chlorbenzol; phenyl chloride)*

**CAS Registry Number: 108-90-7**

## I. Chronic Toxicity Summary

| | |
|---|---|
| *Inhalation reference exposure level* | **1000 μg/m$^3$** (300 ppb) |
| *Critical effect(s)* | Increased liver weights, hepatocellular hypertrophy, renal degeneration and inflammation, and testicular degeneration in rats |
| *Hazard index target(s)* | Alimentary system; kidney; reproductive system |

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

| | |
|---|---|
| *Description* | Colorless, neutral liquid |
| *Molecular formula* | $C_6H_5Cl$ |
| *Molecular weight* | 112.56 g/mol |
| *Boiling point* | 132$^o$C |
| *Melting point* | –45.2$^o$C |
| *Vapor pressure* | 11.8 torr at 25$^o$C |
| *Solubility* | Practically insoluble in water (0.049 g/100 ml); soluble in alcohol, benzene, chloroform, diethyl ether |
| *Conversion factor* | 1 ppm = 4.60 mg/m$^3$ at 25 $^o$C |

## III. Major Uses and Sources

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

## IV. Effects of Human Exposure

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

Two small studies utilizing volunteers exposed to single doses of monochlorobenzene have reported central nervous system effects (Ogata *et al.*, 1991; Tarkhova, 1965). An exposure chamber study of five volunteers exposed up to 60 ppm monochlorobenzene (276 $mg/m^3$) for a single 7 hour exposure described acute subjective symptoms such as drowsiness, headache, eye irritation, and sore throat (Ogata *et al.*, 1991). One other human volunteer study described altered electrical activity of the cerebral cortex in four individuals exposed to 43.4 ppm monochlorobenzene vapors for 2.5 minutes (Tarkhova, 1965).

## V. Effects of Animal Exposure

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

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

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

up to day 20 of gestation for 2 generations. Nair *et al*. found dose-related changes in the livers, kidneys, and testes in both generations of males ($F_0$ and $F_1$). Hepatotoxicity occurred as hepatocellular hypertrophy and increased liver weights (mean and absolute) at concentrations greater than 50 ppm (LOAEL = 150 ppm). At this concentration (150 ppm), renal changes included tubular dilation, interstitial nephritis, and foci of regenerative epithelium. Testicular degeneration of the germinal epithelium occurred in both generations of exposed males, but no chlorobenzene-induced adverse effects on reproductive performance or fertility were seen.

## VI. Derivation of Chronic Reference Exposure Level (REL)

| | |
|---|---|
| *Study* | Nair *et al.* (1987) |
| *Study population* | Sprague-Dawley rats (30/sex/group) |
| *Exposure method* | Discontinuous inhalation exposures (0, 50, 150, and 450 ppm) |
| *Critical Effects* | Increases in absolute and relative liver weights ($F_0$ and $F_1$ both sexes), hepatocellular hypertrophy ($F_0$ and $F_1$ males), renal degeneration and inflammation ($F_0$ and $F_1$ both sexes), testicular degeneration ($F_0$ and $F_1$ males). |
| *LOAEL* | 150 ppm |
| *NOAEL* | 50 ppm |
| *Exposure continuity* | 6 hours/day, 7 days/week |
| *Exposure duration* | 11 weeks |
| *Average experimental exposure* | 13 ppm for NOAEL group (50 x 6/24) |
| *Human equivalent concentration* | 26 ppm (gas with systemic effects, based on RGDR = 2.0 for lambda (a) : lambda (h)) (Gargas *et al*., 1989) |
| *LOAEL uncertainty factor* | 1 |
| *Subchronic uncertainty factor* | 3 |
| *Interspecies uncertainty factor* | 3 |
| *Intraspecies uncertainty factor* | 10 |
| *Cumulative uncertainty factor* | 100 |
| *Inhalation reference exposure level* | 0.3 ppm (300 ppb; 1.0 mg/m$^3$, 1000 µg/m$^3$) |

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

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

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

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

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

## VII. Data Strengths and Limitations for Development of the REL

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

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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

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

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

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

## III. Major Uses and Sources

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

## IV. Effects of Human Exposure

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

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

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

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

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

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

## V. Effects of Animal Exposure

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

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

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

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

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

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

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

* $p < 0.05$

## VI. Derivation of Chronic Reference Exposure Level (REL)

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

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

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

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

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

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

## VII. Data Strengths and Limitations for Development of the REL

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

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*CHRONIC TOXICITY SUMMARY*

# CHLOROPICRIN

*(trichloronitromethane; nitrochloroform; nitrochloromethane)*

**CAS Registry Number: 76-06-2**

## I. Chronic Toxicity Summary

| | |
|---|---|
| *Inhalation reference exposure level* | **0.4 µg/m$^3$** (0.05 ppb) |
| *Critical effect(s)* | Nasal rhinitis and bronchiectasis in mice |
| *Hazard index target(s)* | Respiratory system |

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

| | |
|---|---|
| *Description* | Colorless to faint yellow liquid |
| *Molecular formula* | $CCl_3NO_2$ |
| *Molecular weight* | 164.4 g/mol |
| *Boiling point* | 112°C |
| *Melting point* | –64°C (CRC, 1994) |
| *Vapor pressure* | 5.7 torr @ 0°C (Fries and West, 1921); 3.2 kPa (24 torr) @ 25°C (Tomlin, 1994) |
| *Solubility* | 1.6 g/L water @ 25°C; 2.272 g/L water @ 0°C 1.9 g/L water @ 20°C; miscible with benzene, ethanol, carbon disulfide, ether, carbon tetrachloride, acetone, methanol, acetic acid |
| *Conversion factor* | 6.72 µg/m$^3$ per ppb at 25ºC |

## III. Major Uses and Sources

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

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

## IV. Effects of Human Exposure

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

## V. Effects of Animal Exposure

Burleigh-Flayer and Benson (1995) conducted a chronic inhalation bioassay with CD rats (50-60 per sex per dose) exposed discontinuously to 0 (air), 0.1, 0.5, or 1.0 ppm 99.6% pure chloropicrin vapor 6 hours/day for 5 consecutive days/week over 107 weeks. Clinical signs (such as hypoactivity and decreased startle response) were increased in both sexes, primarily at 1.0 ppm. Increased mortality was noted in males at 0.5 and 1 ppm and in females at 1 ppm. Absolute and relative increased lung and liver weights and increased nasal rhinitis were reported in both sexes at the 1 ppm level. However, no effects were seen at 0.1 ppm. Thus this study yielded a NOAEL of 0.1 ppm (0.67 mg/m$^3$) for chronic non-cancer effects in rats.

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

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

*p<0.05; **p<0.01

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

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

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

**p<0.01

Yoshida *et al*. (1987) exposed groups of 12 male Fischer 344 rats intermittently to 0, 0.37, 0.67, 1.58, or 2.93 ppm chloropicrin vapor 6 h/day, 5 days/week for 13 weeks. Mean body weights were reduced in the highest 2 exposure groups, and red blood cell count, hematocrit, and hemoglobin concentration were significantly increased in the 2.93 ppm group. The treatment-related histological lesions reported were degeneration and necrosis of the bronchial and bronchiolar epithelia at 2.93 ppm and hypertrophy of these epithelia at 1.58 ppm. Thus the primary target organ was the respiratory tract and the subchronic NOAEL was 0.67 ppm (4.5 $mg/m^3$). (Eyelid closure and decrease in motor activity were seen in all exposure groups only during exposure. No morphological changes were seen at 0.67 ppm, so the authors deemed the behavior changes minor and not toxicologically important.)

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

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

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

## VI. Derivation of Chronic Reference Exposure Level (REL)

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

The data on bronchiecstasis incidence in male and female mice were combined and the chronic REL for chloropicrin was developed using the BMC approach. Of the several models tested, the Gamma MultiHit Model gave the best fit to the combined bronchiecstasis data (p = 0.9750). The $MLE_{05}$ was 0.070 ppm and the $BMC_{05}$ was 0.042 ppm. Use of time extrapolation to equivalent continuous exposure, an RGDR adjustment for the area of the respiratory tract affected, and a total uncertainty factor of 30 resulted in a chronic REL of 0.05 ppb (0.4 $\mu g/m^3$).

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

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

## VII. Data Strengths and Limitations for Development of the REL

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

## VIII. Potential for Differential Impacts on Children's Health

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

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*CHRONIC TOXICITY SUMMARY*

# CHROMIUM, HEXAVALENT (SOLUBLE COMPOUNDS)

| *Molecular Formula* | *Molecular Weight* | *Synonyms* | *CAS Registry Number* |
|---|---|---|---|
| $CrO_3$ | 99.99 g/mol | Chromic trioxide, chromium oxide, chromium trioxide, chromium (VI) oxide. (In acid aqueous solutions, exists as $H_2CrO_4$ – "chromic acid") | 1333-82-0 |
| $K_2CrO_4$ | 194.20 g/mol | Potassium chromate, dipotassium chromate, potassium (VI) chromate, dipotassium monochromate, chromate of potash | 7789-00-6 |
| $Li_2CrO_4$ | 129.87 g/mol | Lithium chromate, chromium lithium oxide, chromic acid dilithium salt, lithium chromate (VI) | 14307-35-8 |
| $Na_2CrO_4$ | 161.97 g/mol | Sodium chromate, chromic acid disodium salt, chromium disodium oxide, sodium chromate (VI), chromate of soda | 7775-11-3 |
| $K_2Cr_2O_7$ | 294.20 g/mol | Potassium dichromate, dichromic acid dipotassium salt, bichromate of potash | 7778-50-9 |
| $Na_2Cr_2O_7$ | 261.96 g/mol | Sodium dichromate, bichromate of sodium, dichromic acid disodium salt, chromium sodium oxide | 10588-01-9 |

## I. Chronic Toxicity Summary

### A. Soluble Hexavalent Chromium Compounds (except chromic trioxide)

| | |
|---|---|
| *Inhalation reference exposure level* | **0.2 µg Cr(VI)/m$^3$** |
| *Critical effect(s)* | Bronchoalveolar hyperplasia in lungs of rats |
| *Hazard index target(s)* | Respiratory system |
| *Oral reference exposure level* | **0.02 mg Cr(VI)/kg/day** |
| *Critical effect(s)* | Red blood cell effects (decreased mean corpuscular volume (MCV) and mean corpuscular hemoglobin (MCH)) in mice |
| *Hazard index target(s)* | Hematopoietic system |

**B. Chromic Trioxide** (as chromic acid mist)

| | |
|---|---|
| *Inhalation reference exposure level* | **0.002 µg Cr(VI)/m$^3$** |
| *Critical effect(s)* | Respiratory effects (nasal atrophy, nasal mucosal ulcerations, nasal septal perforations, transient pulmonary function changes) in human occupational study |
| *Hazard index target(s)* | Respiratory system |

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

| | |
|---|---|
| *Description* | $CrO_3$: dark red or brown crystals, flakes, or powder, exists as chromic acid ($H_2CrO_4$) in solution;<br>$K_2CrO_4$, $Na_2CrO_4$: yellow crystals;<br>$K_2Cr_2O_7$, $Na_2Cr_2O_7$: orange-red crystals;<br>$Li_2CrO_4$: yellow crystalline powder |
| *Molecular formula* | See above |
| *Molecular weight* | See above |
| *Density* | $CrO_3$: 2.70 g/cm$^3$ @ 25°C |
| *Boiling point* | $CrO_3$: decomposes (temperature not available);<br>$K_2Cr_2O_7$: 500 °C with decomposition;<br>$Na_2Cr_2O_7$: 400 °C |
| *Melting point* | $CrO_3$: 197 °C;<br>$K_2CrO_4$: 975 °C;<br>$Na_2CrO_4$: 792 °C;<br>$K_2Cr_2O_7$: 398 °C;<br>$Na_2Cr_2O_7$: 356.7 °C |
| *Vapor pressure* | Not applicable |
| *Solubility* | $CrO_3$: soluble in water, ethyl alcohol, ethyl ether, sulfuric and nitric acid;<br>$K_2CrO_4$, $K_2Cr_2O_7$, $Na_2Cr_2O_7$: soluble in water, insoluble in ethyl alcohol;<br>$Na_2CrO_4$: soluble in water, slightly soluble in ethyl alcohol;<br>$Li_2CrO_4$: soluble in water and ethyl alcohol |
| *Conversion factor* | Not applicable for particulates and mists |

## III. Major Uses or Sources

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

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

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

## IV. Effects of Human Exposure

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

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

Kleinfeld and Rosso, 1965; Gomes, 1972; Sorahan *et al*., 1998). However, workers in the chromate extraction and ferrochromium industry, exposed to particulates containing soluble Cr(VI) compounds, have also reported nasal lesions (Mancuso, 1951; Federal Security Agency, 1953; Machle and Gregorius, 1948; Wang *et al*., 1994; Walsh, 1953). Other less frequent mucous membrane injuries have been reported in workers exposed to chromate dust and chromic acid including sinusitis, laryngitis, conjunctivitis, and oral ulcerations (Mancuso, 1951; Federal Security Agency, 1953; Johansen *et al*., 1994). Nasal lesions include perforated septum, ulcerated septum, nasal atrophy, nosebleed, and inflamed mucosa following exposure to air chromium levels of about 0.1 to 5.6 $mg/m^3$. Exposure duration, when reported, ranged from 2 weeks to 25 years. However, there were problems in quantifying the effect for the above studies. The difficulties were primarily lack of adequate methods or data for determining exposure duration and/or exposure levels. The occupational studies summarized below provide the most reliable estimates of inhalation durations and concentrations resulting in chronic toxicity.

Workers exposed to $\geq$2 $\mu g/m^3$ Cr(VI) as chromic acid exhibited an increased incidence of nasal atrophy, nasal mucosal ulcerations, and nasal septal perforations as compared to controls (Lindberg and Hedenstierna, 1983). Workers exposed to less than 2 $\mu g/m^3$ (expressed as $\leq$ 1.9 $\mu g/m^3$) exhibited an increased incidence of irritated nasal mucosa and nasal atrophy compared to controls. The median exposure time of exposed workers was 2.5 years (range = 0.2-23.6 years). Frequency of throat and chest symptoms was similar to that of controls. The same study reported statistically significant decreases in forced expiratory volume in 1 second ($FEV_1$), forced vital capacity (FVC), and mean forced expiratory flow during the middle of the FVC in 1 second ($FEF_{25-75}$) measurements taken on a Thursday afternoon as compared to those taken on a Monday morning in nonsmoking workers exposed to 2 $\mu g/m^3$ Cr(VI) or more. Similar changes were observed in the smokers although only the difference in the FVC measured on a Thursday was statistically significant. No significant differences were observed between pulmonary function measurements of exposed and unexposed workers taken on a Monday morning (prior to a work week of exposure). Thus the authors infer that the observed pulmonary function changes are transient.

Nasal lesions were observed in 35 of 37 chrome platers exposed to a mean breathing zone concentration of 7.1 μg/m3 (range = 1.4-49.3 μg/m3) total chromium for an average of 2.2 years (range = 1.2 weeks-11 years) (Cohen et al., 1974). Actual exposure to Cr(VI) averaged 2.9 μg/m3 (range = 0.09-9.1 μg/m3). Workers employed more than one year had significantly greater nasal pathology than workers employed one year or less. Due to poor personal hygiene habits of the exposed workers, a 'direct contact' etiology may explain some of the nasal lesions.

Urinary levels of $\beta_2$-microglobulin in 24 chrome platers increased in dose-dependent fashion with increasing intensity of exposure to Cr(VI), indicating a nephrotoxic effect resulting from inhalation of Cr(VI) (Lindberg and Vesterberg, 1983). The 8-hr mean Cr(VI) levels ranged from 2 to 20 $\mu g/m^3$ and averaged 6 $\mu g/m^3$. Total exposure times ranged from 0.1 to 26 years and averaged 5.3 years. Most of the 24 chrome workers had irritation symptoms of the airways. As a group, the chrome platers had significantly higher levels of urinary $\beta_2$-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 $\beta_2$-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 $\beta_2$-microglobulin levels. Urinary albumin levels remained unchanged in the Cr(VI)-exposed group. The results suggest that the nephrotoxic effects are reversible at the exposure levels studied.

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

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

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

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

## V. Effects of Animal Exposure

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

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

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

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

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

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

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

a All BALF parameters are mean + SD, n = 10/group
* $p < 0.05$; ** $p < 0.001$: comparison of exposed groups vs. controls

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

Nasal septal perforation, hyperplastic and metaplastic changes in the larynx, trachea, and bronchus, and emphysema were observed in mice exposed two days per week for 12 months to $CrO_3$ mist (Adachi, 1987; Adachi *et al.*, 1986). Chromic acid concentrations were either 3.63 mg/m$^3$ for 30 minutes per day or 1.81 mg/m$^3$ for 120 minutes per day. An additional 20 mice exposed to 1.81 mg/m$^3$ were necropsied 6 months after the last exposure. Lesions of the nasal septum, trachea, and lungs were still evident in some mice.

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

Groups of eight male and eight female Sprague-Dawley rats were supplied with drinking water containing 0-11 ppm (0-11 mg/L) Cr(VI), as $K_2CrO_4$, for 1 year (Mackenzie *et al.*, 1958). The control group (10/sex) received distilled water. A second experiment involved three groups of 12 male and 9 female rats. One group was given 25 ppm (25 mg/L) Cr(VI); a second received 25 ppm chromium in the form of chromic chloride; and the controls received distilled water. For rats treated with 0-11 ppm (in the diet), hematological determinations (red and white blood cell counts, differential white cell counts, and hemoglobin) were performed monthly, and tissues (livers, kidneys and femurs) were examined at 6 months and 1 year. Spleens were also examined

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

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

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

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

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

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

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

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

## VI. Derivation of Chronic Reference Exposure Levels (RELs)

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

| | |
|---|---|
| *Study* | Glaser *et al*., 1990 |
| *Study population* | Male Wistar rats (30 per group) |
| *Exposure method* | Discontinuous whole-body inhalation (0, 54, 109, 204, or 403 µg Cr(VI)/m$^3$ as sodium dichromate aerosol) |
| *Critical effects* | Bronchoalveolar hyperplasia |
| *LOAEL* | 50 µg/m$^3$ |
| *NOAEL* | Not observed |
| *$BMC_{05}$* | 12.50 µg/m$^3$ |
| *Exposure continuity* | 22 hr/day, 7 days/week |
| *Exposure duration* | 90 days |
| *Average exposure* | 11.46 µg/m$^3$ Cr(VI) (12.50 x 22/24) |
| *Human equivalent concentration* | 24.47 µg/m$^3$ Cr(VI) (2.1355 [RDDR] x 11.46) |
| *LOAEL uncertainty factor* | Not needed in the BMC approach |
| *Subchronic uncertainty factor* | 3 |
| *Interspecies uncertainty factor* | 3 |
| *Intraspecies uncertainty factor* | 10 |
| *Cumulative uncertainty factor* | 100 |
| *Inhalation reference exposure level* | 0.2 µg/m$^3$ (0.0002 mg/m$^3$) |

The study by Glaser *et al*. (1990) provides the best available inhalation data that demonstrate a dose-response relationship for various pulmonary toxicity endpoints. The $BMC_{05}$ of 12.50 µg/m$^3$ was derived from quantal data for bronchoalveolar hyperplasia. The presence of bronchoalveolar hyperplasia in exposed rats is supported by other indicators of lung inflammation, including increased total protein, LDH, and albumin in BALF (see Table 1). A quantal-linear model analysis (U.S. EPA, National Center for Environmental Assessment, benchmark dose software, version 1.20) of the quantal data provided the most reasonable line fit and resulted in the lowest $BMC_{05}$. A $BMC_{05}$ is considered to be similar to a NOAEL in estimating a concentration associated with a low level of risk. Lung histiocytosis (macrophage accumulation) was present in nearly all exposed animals, but this quantal data set was only suitable for a NOAEL/LOAEL approach and was not considered as direct an indicator of lung injury as bronchoalveolar hyperplasia.

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

The U.S. EPA (1998) RfC of 0.1 µg/m$^3$ is also based on data from Glaser et al. (1990), but derived a $BMC_{10}$ (16 µg/m$^3$), as developed by Malsch *et al*. (1994), from continuous data of

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

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

For comparison with the proposed REL, the occupational study by Huvinen et al. (1996) established a NOAEL of 0.5 µg/m3 for lack of pulmonary findings. However, this study is deficient for REL purposes due to the lack of a LOAEL. Unfortunately, other occupational studies suffered from lack of adequate methods or data for determining exposure duration and/or exposure levels. Use of an occupational time adjustment (10/20 m3 inhaled/day, 5/7 days/week) and an interspecies UF of 10 for the Huvinen et al. (1996) study would result in an estimated REL of 0.02 µg/m3. Average exposure duration was 16 years, so a subchronic UF of 1 was sufficient.

B. Derivation of Chronic Inhalation Reference Exposure Level for CrO3 as Chromic Acid

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

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

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

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

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

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

| | |
|---|---|
| *Study* | Mackenzie *et al.*, 1958 |
| *Study population* | 8 male and 8 female Sprague-Dawley rats |
| *Exposure method* | Drinking water |
| *Critical effects* | No adverse effects seen |
| *LOAEL* | None |
| *NOAEL* | 2.4 mg/kg-day (converted from 25 mg/L of chromium as $K_2CrO_4$) |
| *Exposure continuity* | Continuous |
| *Exposure duration* | 1 year |
| *Average experimental exposure* | 2.4 mg/kg-day (0.11 ppm Cr(VI)) |
| *LOAEL uncertainty factor* | 1 |
| *Subchronic uncertainty factor* | 1 |
| *Interspecies uncertainty factor* | 10 |
| *Intraspecies uncertainty factor* | 10 |
| *Cumulative uncertainty factor* | 100 |
| *Oral reference exposure level* | 0.02 mg/kg bw-day |

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

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

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

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

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

## VII. Data Strengths and Limitations for Development of the REL

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

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

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

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*CHRONIC TOXICITY SUMMARY*

# CRESOL MIXTURES

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

## I. Chronic Toxicity Summary

| | |
|---|---|
| *Inhalation reference exposure level* | **600 µg/m$^3$** (100 ppb) |
| *Critical effect(s)* | Neurotoxicity |
| *Hazard index target(s)* | Nervous system |

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

| | |
|---|---|
| *Description* | Colorless in pure form; yellowish, brownish-yellow, or pinkish liquid |
| *Molecular formula* | $C_7H_8O$ |
| *Molecular weight* | 108.14 g/mol |
| *Boiling point* | 191.0°C (o-cresol)<br>202°C (m-cresol)<br>201.9°C (p-cresol) |
| *Melting point* | 29.8°C (o-cresol)<br>11.8°C (m-cresol)<br>35.5°C (p-cresol) |
| *Solubility* | Soluble in 50 parts water; miscible with alcohol, benzene, ether, glycerol, petroleum ether; soluble in vegetable oils, glycol |
| *Conversion factor* | 4.42 µg/m$^3$ per ppb at 25ºC |

## III. Major Uses and Sources

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

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

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

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

## IV. Effects of Exposures to Humans

Brief exposure to 6 mg cresol/$m^3$ resulted in irritation of the throat and nose, nasal constriction, and dryness in 8 of 10 subjects (Uzhdavini *et al.*, 1972).

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

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

## V. Effects of Exposures to Animals

The effects of inhaled o-cresol were examined in several species (Uzhdavini *et al.*, 1972, as reported in ATSDR, 1992 and U.S. EPA, 1982). Cats exposed for 30 minutes to 5-9 mg o-cresol/$m^3$ showed signs of respiratory irritation as indicated by increased parotid gland secretions. Exposure of mice for 2 hrs/day for 1 month to 50 mg o-cresol/$m^3$ did not have an effect on mortality, however, heart muscle degeneration and degeneration of nerve cells and glial elements were observed.

Uzhdavini *et al.* (1972) exposed rats (both sexes, numbers not stated) by inhalation to 9.0 ± 0.9 mg o-cresol/$m^3$, first for 2 months (6 hours/day, 5 days/week), then for 2 more months (4 hours/day, 5 days/week). Endpoints examined in rats included elementary conditioned defensive reflex, white blood cell levels, bone marrow elements, and liver function (as indicated by increased susceptibility to hexobarbital narcosis). Both cresol-exposed and control animals showed some loss of the defensive reflex; the effect occurred in all exposed animals before the end of the second month and in control animals at later times. White blood cell counts were elevated in male animals, peaked at the end of the exposure period, and returned to normal one month after cessation of exposure. Exposed animals also showed a statistically significant change in the leukoid-to-erythroid ratio in the bone marrow. Liver toxicity was suggested by an extension in the duration of hexobarbital narcosis in treated animals. Although guinea pigs were similarly evaluated for changes in blood cell counts and ECG, scant reporting of experimental detail limits the usefulness of this portion of the study.

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

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

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

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

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

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

The National Toxicology Program (NTP) sponsored reproductive toxicity tests of cresol isomers in Swiss CD-1 mice using the risk assessment by continuous breeding (RACB) protocol (Heindel *et al*., 1997a, 1997b). For o-cresol the exposure concentrations in the continuous cohabitation task were 0.05%, 0.2%, and 0.5% in feed (approximately 60, 220, and 550 mg/kg/day (Heindel *et al*., 1997a). At these doses o-cresol was not a reproductive toxicant. When a m-/p-cresol mixture was used at concentrations of 0.25, 1.0 and 1.5% in feed (approximately 370, 1500, and 2100 mg/kg/day), the m/p mixture was a reproductive toxicant, since (1) fewer $F_1$ pups per litter were produced, (2) both generations showed reduced pup weights, and (3) reproductive organs showed weight reductions. Unfortunately the responses were not dose-dependent and the mixture was judged not to be a selective reproductive toxicant. Oral gavage administration of o-, m-, or p-cresol, separately, in rats did not produce selective reproductive toxicity; i.e., for each of the cresol isomers, in the absence of parental toxicity, there was no reproductive toxicity. The NOEL for reproductive toxicity for each isomer was 175 mg/kg/day (Tyl 1989a, 1989b, 1989c).

## VI. Derivation of Inhalation Chronic Reference Exposure Level

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

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

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

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

As noted above, the inhalation study conducted by Kurliandskii *et al.* (1975) suggests that adverse health effects occur in experimental animals at exposure levels considerably below those reported by Uzhdavini *et al.* (1972) (9 mg/$m^3$ vs. 0.05 mg/$m^3$). The report from which the lower level is drawn has limitations. Human subjects exposed briefly to levels below the LOAEL have reported respiratory irritation.

## VII. Data Strengths and Limitations for Development of the REL

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

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*CHRONIC TOXICITY SUMMARY*

# 1,4-DICHLOROBENZENE

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

CAS Registry Number: 106-46-7

## I. Chronic Toxicity Summary

| | |
|---|---|
| *Inhalation reference exposure level* | **800 µg/m$^3$** (100 ppb) |
| *Critical effect(s)* | General effects (reduced body weights and food consumption) in rats<br>CNS effects (tremors) in rats<br>Respiratory/dermal effects (nasal and ocular discharge) in rats<br>Liver effects (increased liver weight) in rats, and<br>Kidney effects (increased kidney weight) in rats. |
| *Hazard index target(s)* | Nervous system; respiratory system; alimentary system; kidney |

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

| | |
|---|---|
| *Description* | White crystals, monoclinic prisms |
| *Molecular formula* | $C_6H_4Cl_2$ |
| *Molecular weight* | 147.01 g/mol |
| *Boiling point* | 174$^o$C |
| *Melting point* | 52.7$^o$C |
| *Vapor pressure* | 10 torr @ 54.8$^o$C |
| *Solubility* | Soluble in chloroform, carbon disulfide, alcohol, ether, acetone, benzene |
| *Conversion factor* | 1 ppm = 6.0 mg/m$^3$ at 25ºC |

## III. Major Uses and Sources

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

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

## IV. Effects of Human Exposure

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

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

## V. Effects of Animal Exposure

Rats, rabbits and guinea pigs were exposed to 0, 96, 158, 341 or 798 ppm (0, 577, 950, 2050 or 4800 mg/m$^3$) 1,4-DCB by inhalation 7 hours/day, 5 days/week for 6-7 months (Hollingsworth *et al.*, 1956). High dose animals showed marked tremors, weakness, loss of weight, eye irritation and unconsciousness. Liver and kidney changes included cloudy swelling and centrilobular cellular degeneration (liver). In another inhalation study in rats animals were exposed to 0, 75 or 500 ppm (0, 451 or 3006 mg/m$^3$) for 5 hours/day, 5 days/week for 76 weeks (Riley *et al.*, 1980). The authors found increased kidney and liver weights in the high dose group. Thus 75 ppm was a NOAEL. Studies with oral exposure to 1,4-DCB, including the NTP (1987) chronic bioassay study (maximum dose of 300 mg/kg-day), have also found an increased incidence of renal and hepatic lesions (cellular degeneration and focal necrosis).

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

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

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

## VI. Derivation of Chronic Reference Exposure Level

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

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

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

## VII. Data Strengths and Limitations for Development of the REL

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

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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

| | |
|---|---|
| *Inhalation reference exposure level* | **70 μg/m$^3$** (20 ppb) |
| *Critical effect(s)* | Increased mortality; hepatic effects (mottled livers and increases in liver enzymes) in guinea pigs |
| *Hazard index target(s)* | Alimentary system |

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

| | |
|---|---|
| *Description* | Colorless liquid |
| *Molecular formula* | $C_2H_2Cl_2$ |
| *Molecular weight* | 96.95 g/mol |
| *Boiling point* | 31.7$^o$C |
| *Melting point* | –122.5$^o$C |
| *Vapor pressure* | 500 torr @ 20$^o$C |
| *Solubility* | Soluble in water (2.5 g/L); miscible in organic solvents |
| *Conversion factor* | 3.97 μg/m$^3$ per ppb at 25 $^o$C |

## III. Major Uses and Sources

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

## IV. Effects of Human Exposure

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

## V. Effects of Animal Exposure

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

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

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

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

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

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

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

## VI. Derivation of Chronic Reference Exposure Level (REL)

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

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

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

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

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

## VII. Data Strengths and Limitations for Development of the REL

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

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CHRONIC TOXICITY SUMMARY

# DIETHANOLAMINE

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

**CAS Registry Number: 111-42-2**

## I. Chronic Toxicity Summary

| | |
|---|---|
| *Inhalation reference exposure level* | **3 μg/m$^3$** ( 0.6 ppb) |
| *Critical effect(s)* | Laryngeal lesions in rats |
| *Hazard index target(s)* | Respiratory system; cardiovascular system |

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

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|---|---|
| *Description* | Colorless crystals |
| *Molecular formula* | $C_4H_{11}NO_2$ |
| *Molecular weight* | 105.14 g/mol |
| *Density* | 1.097 g/cm$^3$ @ 20°C |
| *Boiling point* | 268.8°C |
| *Melting point* | 28°C |
| *Vapor pressure* | 0.00014 torr @ 25°C |
| *Solubility* | Soluble in alcohol, water, acetone |
| *Conversion factor* | 1 ppm = 4.3 mg/m$^3$ @ 25°C |

## III. Major Uses and Sources

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

## IV. Effects of Chronic Exposures to Humans

There have been no controlled or epidemiological studies of chronic diethanolamine exposure in humans. There is a single case report of occupational asthma determined to be due to the patient's handling of a cutting fluid containing diethanolamine (Piipari *et al*., 1998). Specific bronchial provocation tests were done with the cutting fluid containing DEA and with DEA aerosol at two concentrations (0.75 mg/m$^3$ and 1.0 mg/m$^3$) below the occupational limit of 2.0 mg/m$^3$. DEA caused asthmatic airway obstruction at both concentrations, but IgE-antibodies specific for DEA were not found.

## V. Effects of Exposures in Animals

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

Gamer *et al*. (1996*)* exposed groups of 26 Wistar rats (13 male and 13 female) head-nose to a liquid aerosol of DEA for six hours per working day for 90 days at target concentrations of 15, 150, and 400 mg/m$^3$. Three of each sex were used for whole animal perfusion studies and the remaining 20 animals were examined for pathology. The study found no functional or morphological evidence of neurotoxicity. Retardation of body weight increase was observed in animals exposed to high concentrations. No systemic effects occurred at the low dose, but systemic effects in the liver, kidney, male reproductive system, and red blood cell occurred in the high concentration dose group. In the mid-dose group, mild liver and kidney effects were present. Local irritation of the larynx and trachea was found in the high and mid dose groups; irritating laryngeal effects were also detected in the low dose group. Based on this study 15 mg/m$^3$ is a NOAEL for liver and kidney effects and a LOAEL for irritation of the larynx. The equivalent continuous exposure at the LOAEL is 2.7 mg/m$^3$ (15 x 6/24 x 5/7).

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

| Aerosolized diethanolamine | Chronic inflammation of the larynx | Squamous hyperplasia | Focal squamous metaplasia of laryngeal epithelium at base of the epiglottis |
|---|---|---|---|
| 0 | None* | None | None |
| 15 mg/m$^3$ | 4/20 | 0/20 | 20/20 |
| 150 mg/m3 | 20/20 | 13/20 | 20/20 |
| 400 mg/m$^3$ | 20/20 | 17/20 | 20/20 |

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

In an abstract Hartung *et al*. (1970) reported that inhalation by male rats of 6 ppm (25.8 mg/m$^3$) DEA vapor 8 hours/day, 5 days/week for 13 weeks resulted in depressed growth rates, increased lung and kidney weights, and even some mortality. Rats exposed continuously for 216 hours (nine days) to 25 ppm (108 mg/m$^3$) DEA showed increased liver and kidney weights, elevated

blood urea nitrogen (BUN), and increased serum glutamate oxaloacetate transferase (SGOT), an indicator of liver damage (Hartung et al., 1970). In studies at lower DEA levels, Eastman Kodak (1967) exposed dogs, weanling and adult rats, and guinea pigs to 0.26 ppm (1.1 mg/m$^3$) DEA for 90 days and found no pathology attributable to DEA. In a 45-day study with 0.5 ppm (2.2 mg/m$^3$) DEA they also found no pathology attributable to DEA except for a possible slight retardation in rat growth rate.

Gamer *et al*. (1993) exposed groups of 25 pregnant Wistar rats on gestation days 6-15 to a (nose-only) liquid aerosol of DEA at 10, 50 and 200 mg/m$^3$. Maternal toxicity, indicated by vaginal hemorrhage in 8 of the dams on gestation day 14, and fetotoxicity, evidenced by a statistically significant ($p<0.05$) increased incidence of total fetal skeletal variations, were observed at 200 mg/m$^3$. No teratogenic effects were seen at any level. Thus 50 mg/m$^3$ was a NOAEL for maternal toxicity and for embryo-fetal effects.

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

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

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

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

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

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

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

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

## VI. Derivation of Chronic Reference Exposure Level (REL)

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

No chronic inhalation studies with diethanolamine were located in the peer-reviewed literature. Thus the 90 day study by Gamer *et al*., which found a LOAEL of 15 mg/m$^3$ for irritation of the

rat larynx, was used to derive the REL. All 20 of the rats in the 15 mg/m$^3$ exposure group showed focal squamous metaplasia of the laryngeal epithelium at the base of the epiglottis, and 4 of the 20 had inflammatory cells present in the larynx. The former lesion seemed to be very limited and did not justify use of the full LOAEL uncertainty factor of 10.

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

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

## VII. Data Strengths and Limitations for Development of the REL

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

## VIII. Potential for Differential Impacts on Children's Health

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

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*CHRONIC TOXICITY SUMMARY*

# N,N-DIMETHYLFORMAMIDE

*(N-formyldimethylamine)*

**CAS Registry Number: 68-12-2**

## I. Chronic Toxicity Summary

| | |
|---|---|
| *Inhalation reference exposure level* | **80 µg/m$^3$** (30 ppb) |
| *Critical effect(s)* | Liver dysfunction and respiratory irritation in humans |
| *Hazard index target(s)* | Alimentary system, respiratory system |

## II. Chemical Property Summary (HSDB, 1994)

| | |
|---|---|
| *Description* | Colorless to very slightly yellow liquid |
| *Molecular formula* | $C_3H_7NO$ |
| *Molecular weight* | 73.09 g/mol |
| *Boiling point* | 153°C |
| *Melting point* | –61°C |
| *Vapor pressure* | 3.7 torr @ 25°C |
| *Solubility* | Soluble in alcohol, ether, acetone, benzene, and chloroform; miscible with water |
| *Conversion factor* | 2.99 µg/m$^3$ per ppb at 25ºC |

## III. Major Uses and Sources

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

## IV. Effects of Human Exposure

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

noted (Cirla *et al.*, 1984). Other changes, that were not statistically significant, included increased SGOT and SGPT and enlarged livers. The mean time-weighted average concentration of DMF was 22 mg/m$^3$ (range = 8-58 mg/m$^3$). Symptoms of irritation occurring only during work at statistically significantly higher incidences included watery eyes, dry throat, and coughing. Also, the exposed workers reported a reduced sense of smell and dry coughs at home with a statistically significant difference as compared to controls. Several of the DMF exposed workers also reported alcohol intolerance characterized by a disulfiram-type reaction (facial flushing and palpitations following alcohol ingestion). Alcohol consumption, a potential confounder, was controlled for in the study design.

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

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

Wang *et al*. (1991) investigated the prevalence of liver injury associated with DMF exposure in 183 of 204 (76%) employees of a synthetic leather factory by performing medical examinations, liver function tests, and creatine phosphokinase (CPK) determinations. Air concentrations were measured with personal samplers and gas chromatography. The concentration of DMF in air to which each worker was exposed was categorized as high (DMF exposure index 2: 25-60 ppm; 75-180 mg/m$^3$), medium (index 1: 10-40 ppm), and low (index 0: <10 ppm). High exposure concentrations were significantly associated with elevated alanine aminotransferase (ALT) levels (i.e., greater than or equal to 35 International Units/liter), a result that did not change after stratification by hepatitis B carrier status. Logistic regression analysis indicated that exposure to high DMF levels was associated with elevated ALT ($p = 0.01$), whereas hepatitis B surface antigen (HBsAg) was slightly but independently associated with elevated ALT ($p = .07$). Workers with normal ALT values had significantly higher mean ALT and aspartate

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

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

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

## V. Effects of Animal Exposure

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

A developmental toxicity study using three species (mice, rabbits, and rats) and four routes of administration (oral, inhalation, dermal, and intraperitoneal) identified the rabbit as the most sensitive of the three species. Groups of 15 pregnant rabbits were exposed for 6 hours per day on days 8-20 of gestation to 50, 150, or 450 ppm (150, 449, or 1350 mg/m$^3$) DMF (Hellwig *et al.*, 1991). Slight maternal toxicity, as indicated by non-statistically significant decreases in maternal body weight gain, was observed in the 450 ppm exposure group. An increased number of total malformations per litter was observed in the 450 ppm exposure group. Malformations observed at statistically higher incidences compared to controls included hernia umbilicalis, external variations, pseudoankylosis of the forelimbs, and skeletal variation and retardation. The authors conclude that there was a clear teratogenic effect in rabbits following maternal exposure to 450 ppm DMF and a marginal effect following exposure to 150 ppm DMF. A NOAEL of

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

## VI. Derivation of Chronic Reference Exposure Level

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

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

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

## VII. Data Strengths and Limitations for Development of the REL

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

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*CHRONIC TOXICITY SUMMARY*

# 1,4-DIOXANE

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

**CAS Registry Number: 123-91-1**

## I. Chronic Toxicity Summary

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

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

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

## III. Major Uses and Sources

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

## IV. Effects of Human Exposure

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

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

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

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

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

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

## V. Effects of Animal Exposure

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

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

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

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

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

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

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

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

## VI. Derivation of Chronic Reference Exposure Level (REL)

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

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

## VII. Data Strengths and Limitations for Development of the REL

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

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

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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

| | |
|---|---|
| *Inhalation reference exposure level* | **3 μg/m$^3$** (0.8 ppb) |
| *Critical effects* | Histological changes in nasal turbinates in rats |
| *Hazard index target(s)* | Respiratory system; eyes |

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

| | |
|---|---|
| *Description* | Colorless liquid |
| *Molecular formula* | $C_3H_5ClO$ |
| *Molecular weight* | 92.52 g/mol |
| *Density* | 1.181 g/cm$^3$ @ 20° C |
| *Boiling point* | 117° C |
| *Melting point* | –26°C |
| *Vapor pressure* | 13 torr @ 20° C |
| *Solubility* | Slightly soluble in water, soluble in most organic solvents |
| *Conversion factor* | 1 ppm = 3.78 mg/m$^3$ @ 25° C |

## III. Major Uses and Sources

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

## IV. Effects of Exposures to Humans

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

## V. Effects of Exposures in Animals

Rats were exposed for 136 weeks (6 hours/day, 5 days/week) to 0, 10, 30, or 100 ppm (0, 38, 113, or 380 mg/m$^3$) epichlorohydrin (Laskin *et al.*, 1980). Kidney damage in the form of renal tubular degeneration and dilatation was observed in rats exposed to 30 ppm or greater. The observation of severe inflammation in the nasal passages of 90% of the control animals, as well as in the treated animals, prevented comparison of this effect between the two groups.

A subchronic exposure of rats to 9, 17, 27, 56, or 120 ppm (34, 64, 102, 212, or 454 mg/m$^3$) for 6 hours/day, 5 days/week for 11-19 exposures showed evidence of extrarespiratory effects. These included liver congestion and necrosis and tubular atrophy in the kidneys at the highest concentration (Gage, 1959). Lethargy and weight loss were observed at 56 ppm.

A study on the effects of epichlorohydrin exposure for 10 weeks (6 hours/day, 5 days/week) on male and female fertility in rats and rabbits showed that male rats, exposed to 50 ppm (189 mg/m$^3$), were significantly less fertile than controls, as measured by successful matings to unexposed females (John *et al.*, 1979; 1983a). No histological changes were observed in the testes of the male rats at the end of exposure. No significant effects on fertility occurred in the exposed female rats. Degenerative changes in the nasal epithelium were observed in the female rats exposed to 25 ppm (94.5 mg/m$^3$), and in both sexes at 50 ppm.

A teratology study was carried out in rats and rabbits exposed to 0, 2.5, or 25 ppm (0, 9.5, or 95 mg/m$^3$) epichlorohydrin 7 hours/day during the critical days of gestation. There were no significant differences between controls and treated animals in the incidence of developmental defects, in maternal toxicity, or in histopathology of the lungs, nasal turbinates, or trachea (John *et al.*, 1983b).

Mice and rats (10/sex/concentration/strain) were exposed to 0, 5, 25, or 50 ppm (0, 19, 95, or 190 mg/m$^3$) epichlorohydrin for 6 hours/day, 5 days/week for 90 days (Quast *et al.*, 1979). Animals were observed for clinical signs of toxicity and were measured biweekly for body weight changes. Body weight measurements, clinical chemistry, hematology, and urinalysis were conducted. Gross and histopathological examinations were performed at the end of the experiment. Exposures of rats to 25 and 50 ppm epichlorohydrin resulted in inflammation, focal erosions, hyperplasia, and metaplasia in the nasal turbinates. No adverse effects were observed in rats exposed to 5 ppm (19 mg/m$^3$). Mice similarly showed focal erosion, hyperplasia and metaplasia in the epithelium of the nasal turbinates when exposed to 25 ppm epichlorohydrin or greater.

## VI. Derivation of Chronic Reference Exposure Level

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

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

## VII. Data Strengths and Limitations for Development of the REL

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

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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

| | |
|---|---|
| *Inhalation reference exposure level* | **20 μg/m$^3$** (6 ppb) |
| *Critical effect(s)* | Degenerative lesions of the nasal cavity in mice |
| *Hazard index target(s)* | Respiratory system; cardiovascular system |

## II. Physical and Chemical Properties (HSDB, 1997)

| | |
|---|---|
| *Description* | Colorless liquid with disagreeable odor |
| *Molecular formula* | $C_4H_8O$ |
| *Molecular weight* | 72.12 g/mol |
| *Density* | 0.837 g/cm$^3$ @ 17°C |
| *Boiling point* | 63.3°C |
| *Melting point* | Not available (CRC, 1994) |
| *Vapor pressure* | 176 torr @ 25°C |
| *Solubility* | Soluble in ethanol, ether, acetone, water |
| *Odor threshold* | Unknown |
| *Conversion factor* | 1 ppm = 2.95 mg/m$^3$ |

## III. Major Uses or Sources

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

## IV. Effects of Human Exposure

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

## V. Effects of Animal Exposure

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

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

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

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

Male and female mice exposed to 800 ppm (2360 mg/m$^3$) EBU for 6 hours/day, 5 days/week, for 13 weeks were listless after the first exposure (NTP, 1988). Animals from this group all died by the end of the 13-week exposure. Renal tubular necrosis, and thymic and splenic atrophy were seen in mice exposed to 800 ppm; decreased liver weights were observed following exposure of mice to 400 ppm (1180 mg/m$^3$) or more. Inflammation of the nasal turbinates was seen in female mice exposed to 100 ppm (295 mg/m$^3$) or more. No inflammation was observed in controls.

Miller *et al*. (1981) exposed rats and mice of either sex to 0, 75, 150, or 600 ppm (0, 221, 442, or 1770 mg/m$^3$) EBU 6 hours/day, 5 days/week, for 13 weeks. In this study, no treatment-related effects were noted except for histological lesions in the nasal mucosal epithelium and reduced specific gravity in the urine of rats treated with 600 ppm.

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

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

## VI. Derivation of Chronic Reference Exposure Level

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

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

## VII. Data Strengths and Limitations for Development of the REL

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

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*CHRONIC TOXICITY SUMMARY*

# ETHYL CHLORIDE

*(Chloroethane; monochloroethane; ether hydrochloric)*

**CAS Registry Number: 75-00-3**

## I. Chronic Toxicity Summary

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

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

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

## III. Major Uses or Sources

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

## IV. Effects of Human Exposure

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

## V. Effects of Animal Exposure

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

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

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

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

## VI. Derivation of Reference Exposure Level

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

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

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

## VII. Data Strengths and Limitations for Development of the REL

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

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*CHRONIC TOXICITY SUMMARY*

# ETHYLBENZENE

*(Phenylethane; NCI-C56393)*

**CAS Registry Number: 100-41-4**

## I. Chronic Toxicity Summary

| | |
|---|---|
| *Inhalation reference exposure level* | **2000 µg/m$^3$ (400 ppb)** |
| *Critical effect(s)* | Liver, kidney, pituitary gland in mice and rats |
| *Hazard index target(s)* | Alimentary system (liver); kidney; endocrine system |

## II. Physical and Chemical Properties (HSDB, 1994)

| | |
|---|---|
| *Description* | colorless liquid |
| *Molecular formula* | $C_8H_{10}$ |
| *Molecular weight* | 106.16 g/mol |
| *Boiling point* | 136.2°C |
| *Melting point* | -95°C |
| *Vapor pressure* | 10 torr @ 25.9°C |
| *Density* | 0.867 g/cm$^3$ @ 20°C |
| *Solubility* | Soluble in ethanol and ether, low solubility in water (0.014 g/100 ml at 15°C) |
| *Conversion factor* | 1 ppm = 4.35 mg/m$^3$ |

## III. Major Uses or Sources

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

## IV. Effects of Human Exposure

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

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

**V. Effects of Animal Exposure**

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

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

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

Rats (17-20 per group) were exposed to 0, 600, 1200, or 2400 mg/m$^3$ for 24 hours/day on days 7 to 15 of gestation (Ungvary and Tatrai, 1985). Developmental malformations in the form of "anomalies of the uropoietic apparatus" were observed at the 2400 mg/m$^3$ concentration.

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

Rabbits exposed by these investigators to the same concentrations as the rats on days 7 to 15 of gestation, exhibited maternal weight loss with exposure to 1000 mg/m$^3$ ethylbenzene. There were no live fetuses in this group for which abnormalities could be evaluated. No developmental defects were observed in the lower exposure groups.

Rats (78-107 per group) and rabbits (29-30 per group) were exposed for 6 or 7 hours/day, 7 days/week, during days 1-l9 and 1-24 of gestation, respectively, to 0, 100, or 1000 ppm (0, 434, or 4342 mg/m$^3$) ethylbenzene (Andrew *et al.*, 1981; Hardin *et al.*, 1981). No effects were observed in the rabbits for maternal toxicity during exposure or at time of necropsy. Similarly, no effects were seen in the fetuses of the rabbits. The only significant effect of ethylbenzene exposure in the rabbits was a reduced number of live kits in the 1000 ppm group. A greater number and severity of effects were seen in rats exposed to 1000 ppm ethylbenzene. Maternal rats exposed to 1000 ppm exhibited significantly increased liver, kidney, and spleen weights compared with controls. Fetal rats showed an increase in skeletal variations at the 1000 ppm concentration, but the results of the 100 ppm exposure were not conclusive.

Clark (1983) found no significant effects on body weight, food intake, hematology, urinalysis, organ weights or histopathology in rats (18 per group) exposed to 100 ppm (434 mg/m$^3$) ethylbenzene for 6 hours/day, 5 days/week, for 12 weeks.

Degeneration of the testicular epithelium was noted in guinea pigs and a rhesus monkey exposed to 600 ppm (2604 mg/m$^3$) for 6 months (Wolf *et al.*, 1956). No effects were reported for female monkeys exposed to the same conditions.

Cragg *et al.* (1989) exposed mice and rats (5/sex/group) to 0, 99, 382, and 782 ppm (0, 430, 1659, and 3396 mg/m$^3$) 6 hours/day, 5 days/week for 4 weeks. Some evidence of increased salivation and lacrimation was seen in the rats exposed to 382 ppm. No other gross signs of toxicity were observed. Both male and female rats had significantly enlarged livers following exposure to 782 ppm. Female mice also showed a significant increase in liver weight at this concentration. No histopathological lesions were seen in the livers of these mice.

Dose-dependent induction of liver cytochrome P450 enzymes in rats by ethylbenzene was observed by Elovaara *et al.* (1985). Rats (5 per group) were exposed to 0, 50, 300, or 600 ppm (0, 217, 1302, or 2604 mg/m$^3$) ethylbenzene for 6 hours/day, 5 days/week for 2, 5, 9, or 16 weeks. Cytochrome P450 enzyme induction, and microscopic changes in endoplasmic reticulum and cellular ultrastructure were evident at all ethylbenzene concentrations by week 2, and persisted throughout the exposure. Liver weights were not elevated in these studies.

## VI. Derivation of the Chronic Reference Exposure Level

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

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

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

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

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

## VII. Data Strengths and Limitations for Development of the REL

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

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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

| | |
|---|---|
| *Inhalation reference exposure level* | **0.8 μg/m$^3$** (0.1 ppb) |
| *Critical effect(s)* | Decreased sperm count/ejaculate, decreased percentage of viable and motile sperm, increased semen pH, and increased proportion of sperm with specific morphological abnormalities in human males |
| *Hazard index target(s)* | Reproductive system |

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

| | |
|---|---|
| *Description* | Colorless, heavy, nonflammable liquid with a mildly sweet, chloroform-like odor. |
| *Molecular formula* | $C_2H_4Br_2$ |
| *Molecular weight* | 187.88 g/mol |
| *Boiling point* | 131-132°C |
| *Melting point* | 9.9°C |
| *Vapor pressure* | 0.11 torr at 20°C |
| *Solubility* | Slightly soluble in water (3400 mg/L water at 20°C). Miscible with most organic solvents. |
| *Conversion factor* | 7.68 μg/m$^3$ per ppb at 25°C |

## III. Major Uses and Sources

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

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

## IV. Effects of Human Exposures

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

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

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

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

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

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

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

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

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

## V. Effects of Animal Exposures

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

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

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

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

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

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

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

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

In a long-term inhalation exposure study, F344 rats and $B6C3F_1$ mice were exposed to 0, 10, or 40 ppm EDB 6 hr/day, 5 days/week for up to 103 weeks (NTP, 1982). In male and female rats, the high dose groups had reduced body weights and increased mortality that began at about week 60. The treatment-related non-neoplastic pathology included hepatic necrosis (both sexes), epithelial hyperplasia and suppurative inflammation throughout the respiratory system (both sexes), and nephropathy (males only). Toxic nephropathy and mineralization were also seen in high dose female rats. Testicular degeneration and atrophy occurred with greater frequency in exposed rats and may be related to observed testicular tumors. Spermatic granulomas were also more frequently seen in high-dose males. Degeneration of the adrenal cortex appeared to be dose-related in females, but only one incidence each was seen in low and high dose males. Increased incidence of retinal atrophy was observed in exposed females. In mice, body weights were reduced at the high dose in both males and females. Many of the high dose animals exhibited a progressive weakness of the limbs or body during the second year. Increased mortality occurred in a dose-related manner in females and was significantly greater in low dose males. Non-neoplastic pathology included epithelial hyperplasia throughout the respiratory system and serous and suppurative inflammation of the nasal cavity in exposed mice. In all male mice, the principal cause of death was urinary bladder inflammation. However, bladder epithelial hyperplasia was only seen in exposed animals. An increased incidence of suppurative inflammation of the prostate was present but was also seen in controls. Dose-related spleen hematopoiesis was observed in females.

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

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

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

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

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

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

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

## VI. Derivation of Chronic Reference Exposure Level (REL)

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

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

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

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

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

## VII. Data Strengths and Limitations for Development of the REL

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

## VIII. Potential for Differential Impacts on Children's Health

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

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*CHRONIC TOXICITY SUMMARY*

# ETHYLENE DICHLORIDE

*(1,2-dichloroethane)*

**CAS Registry Number: 107-06-2**

## I. Chronic Toxicity Summary

| | |
|---|---|
| *Inhalation reference exposure level* | **400 µg/m$^3$** (100 ppb) |
| *Critical effect(s)* | Hepatotoxicity; elevated liver enzyme levels in serum of rats. |
| *Hazard index target(s)* | Liver |

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

| | |
|---|---|
| *Description* | Clear, colorless, oily liquid |
| *Molecular formula* | $C_2H_4Cl_2$ |
| *Molecular weight* | 98.97 g/mol |
| *Density* | 1.2351 g/cm$^3$ @ 20ºC |
| *Boiling point* | 57.4ºC |
| *Melting point* | –96.9ºC |
| *Vapor pressure* | 64 torr @ 20ºC |
| *Solubility* | Slightly soluble in water (0.869 g/100 ml at 20ºC); miscible with alcohol; soluble in ordinary organic solvents |
| *Conversion factor* | 1 ppm = 4.05 mg/m$^3$ |

## III. Major Uses or Sources

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

## IV. Effects of Human Exposure

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

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

## V. Effects of Animal Exposure

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

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

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

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

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

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

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

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

In developmental toxicity studies summarized by Zhao *et al.* (1997), rats were exposed to 0, 24.8, and 207.6 mg/m$^3$ (equivalent to 0, 6, and 51 ppm) EDC for 6 hr/day from two weeks before mating and throughout gestation. Statistically significant increases in pre-implantation loss and decreased male pup weights were observed at the highest dose. Gross skeletal and visceral malformations were not found.

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

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

In a two-generation study conducted by Lane *et al.* (1982), ICR Swiss mice were administered 30, 90, or 290 mg/L EDC in drinking water (equivalent to about 5, 15, or 50 mg/kg bw/day) starting five weeks before mating of the $F_0$ generation. No treatment-related effects on fertility, gestation, viability, weight gain, or lactation indices were noted. EDC exposure did not result in teratogenic or dominant lethal effects.

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

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

## VI. Derivation of Chronic Reference Exposure Level (REL)

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

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

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

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

## VII. Data Strengths and Limitations for Development of the REL

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

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

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*CHRONIC TOXICOLOGY SUMMARY*

# ETHYLENE GLYCOL

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

**CAS Registry Number: 107-21-1**

## I. Chronic Toxicity Summary

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

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

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

## III. Major Uses and Sources

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

## IV. Effects of Human Exposure

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

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

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

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

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

## V. Effects of Animal Exposure

A chronic feeding study in rats and mice was conducted by DePass *et al.* (1986a). In this study, rats (130 per sex per group) and mice (80 per sex per group) were exposed to 0, 0.04, 0.2, or 1 g/kg/day for up to 2 years. All male rats in the high dose group died by 475 days. A large number of effects were observed in this group, including: reduced body weight, increased water intake, increased blood urea nitrogen and creatinine, reduced erythrocyte counts, reduced hematocrit and hemoglobin, increased neutrophil count, and increased urine volume. Heart, kidney, lung, parathyroid, stomach, and other vascular mineralization and hyperplasia were observed histologically in the high dose group of the male rats. Female rats exhibited fatty changes and granulomas in the liver at the high dose. Liver effects were not reported for the

males. The NOAEL in rats for chronic oral ethylene glycol toxicity was 200 mg/kg/day. No effects were observed in mice. Therefore, the NOAEL for mice was 40 mg/kg/day.

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

| *Mortality in Coon et al. (1970)* | | | *Number died/number exposed* | | | | |
|---|---|---|---|---|---|---|---|
| Ethylene glycol mg/m$^3$ | Exposure duration | Equivalent continuous concentration | Rat | Guinea pig | Rabbit | Dog | Monkey |
| 0 (control) | 90 days | 0 | 4/123 | 0/73 | 0/12 | 0/12 | 0/8 |
| 10±1 | 6 wk | 2.4 | 0/15 | 0/15 | 0/3 | 0/2 | 0/2 |
| 57±14 | 6 wk | 13.6 | 0/15 | 0/15 | 0/3 | 0/2 | 0/2 |
| 12±2 | 90 days | 12.0 | 1/15 | 3/15 | 1/3 | 0/2 | 0/3 |

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

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

A similar experiment in mice using nose-only exposures was conducted by these researchers (Tyl *et al.*, 1995a) to determine the role of dermal absorption and/or ingestion on the effects

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

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

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

## VI. Derivation of Chronic Reference Exposure Level

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

The subchronic study by Wills *et al.* (1974) represents the only human inhalation data for ethylene glycol toxicity. The experiment showed a concentration-response relationship, with onset of irritation occurring at 188 mg/m$^3$ and intense and intolerable irritation occurring at 308

mg/m$^3$. The volunteers were followed for 6 weeks without any apparent long-term effects from the exposures. Although the irritation experienced in the human subjects appears to be an acute phenomenon and not a cumulative lasting effect, the subchronic uncertainty factor of 10 was retained to protect against other systemic effects associated with ethylene glycol such as kidney damage which may occur over a long-term exposure.

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

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

## VII. Data Strengths and Limitations for Development of the REL

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

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*CHRONIC TOXICITY SUMMARY*

# ETHYLENE GLYCOL MONOETHYL ETHER

*(2-ethoxyethanol; EGEE)*

**CAS Registry Number: 110-80-5**

## I. Chronic Toxicity Summary

| | |
|---|---|
| *Inhalation reference exposure level* | **70 µg/m$^3$ (20 ppb)** |
| *Critical effect(s)* | Testicular degeneration and decreased hemoglobin in rabbits |
| *Hazard index target(s)* | Reproductive system; hematopoietic system |

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

| | |
|---|---|
| *Description* | Colorless liquid; sweet, pleasant, ether-like odor |
| *Molecular formula* | $C_4H_{10}O_2$ |
| *Molecular weight* | 90.12 |
| *Boiling point* | 135°C |
| *Vapor pressure* | 3.8 torr @ 20°C; 5.31 torr at 25°C |
| *Solubility* | Miscible with water and organic solvents |
| *Conversion factor* | 3.69 µg/m$^3$ per ppb at 25ºC |

## III. Major Uses and Sources

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

## IV. Effects of Human Exposure

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

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

Welch and Cullen (1988) evaluated shipyard painters exposed to ethylene glycol ethers (EGEE and EGME). Air concentrations at the workplace were estimated based on 102 samples over six shifts in Sparer *et al*. (1988). Time –weighted average (TWA) exposures to EGEE ranged from 0 to 80.5 $mg/m^3$ with a mean of 9.9 $mg/m^3$. TWA exposures to EGME ranged from 0 to 17.7 $mg/m^3$ (mean = 2.6 $mg/m^3$). The authors note that during the time period of measurement, painting activities were unusually low and previous NIOSH analyses indicated considerably higher exposures. Ninety-four painters and 55 controls answered a medical and environmental exposure questionnaire including work history and provided blood, urine, and in some cases semen samples. Mean hemoglobin levels, total cell counts and differential counts did not differ between exposed and control. However, the authors found that the lowest quartile of hemoglobin was mostly painters and the lowest polymorphonuclear leukocyte counts were in painters. Nine painters were considered anemic and five were considered granulocytopenic. The authors note that the absence of a significant difference in the group as a whole and the inability to detect a dose-response pattern in the exposed group make a strong conclusion unwarranted.

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

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

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

## V. Effects of Animal Exposure

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

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

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

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

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

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

## VI. Derivation of Reference Exposure Level

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

The reproductive effects observed in the subchronic inhalation study of Barbee *et al.* (1984) were determined by the US EPA (U.S. EPA, 1990) to be the most sensitive endpoints due to EGEE exposure and resulted in a reference concentration (RfC) of 0.2 mg/m$^3$ (0.06 ppm). OEHHA staff concurred regarding the basis of the U.S. EPA RfC but differed in the application of the interspecies uncertainty factor. Reduced testes weight and testicular degeneration were found in rabbits exposed to EGEE at 403 ppm for 13 weeks. Changes in hematological parameters including decreased hemoglobin, hematocrit, and erythrocyte count were also observed at this dose. A gas:extrarespiratory effect ratio of 1.0 was used to calculate a human equivalency concentration (HEC) in the absence of information relating the effect in rabbits relative to humans.

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

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

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

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

## VII. Data Strengths and Limitations for Development of the REL

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

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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

| | |
|---|---|
| *Inhalation reference exposure level* | **300 µg/m$^3$ (60 ppb)** |
| *Critical effect(s)* | Teratogenicity and fetotoxicity in rabbits |
| *Hazard index target(s)* | Development |

## II. Chemical Property Summary (HSDB, 1996)

| | |
|---|---|
| *Description* | Colorless liquid |
| *Molecular formula* | $C_6H_{12}O_3$ |
| *Molecular weight* | 132.16 g/mol |
| *Boiling point* | 156°C |
| *Vapor pressure* | 2 torr @ 20°C |
| *Solubility* | Soluble in water (229 g/l at 20°C); sol. in alcohol, ether, acetone; miscible with olive oil, aromatic hydrocarbons |
| *Conversion factor* | 5.41 µg/m$^3$ per ppb at 25ºC |

## III. Major Uses and Sources

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

## IV. Effects of Human Exposure

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

Ten male volunteers were exposed to EGEEA by inhalation. Five were exposed to 14, 28, and 50 mg EGEEA/$m^3$ and five to 28 mg/$m^3$ for 4 hours (Groeseneken *et al.*, 1987a). Twenty-two percent of the absorbed dose was eliminated in the urine as ethoxyacetic acid within 42 hours. In another study, male volunteers exposed to EGEEA by inhalation under various conditions were found to eliminate some in the form of ethylene glycol monoethyl ether (EGEE) (Groeseneken *et al.*, 1987b).

## V. Effects of Animal Exposure

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

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

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

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

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

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

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

## VI. Derivation of Chronic Reference Exposure Level (REL)

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

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

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

## VII. Data Strengths and Limitations for Development of the REL

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

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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

| | |
|---|---|
| *Inhalation reference exposure level* | **60 µg/m$^3$ ( 20 ppb)** |
| *Critical effect(s)* | Testicular toxicity in rabbits |
| *Hazard index target(s)* | Reproductive system |

## II. Physical and Chemical Properties (HSDB, 1995)

| | |
|---|---|
| *Description* | Colorless liquid |
| *Molecular formula* | $C_3H_8O_2$ |
| *Molecular weight* | 76.09 |
| *Density* | 0.965 g/cm$^3$ @ 20° C |
| *Boiling point* | 125°C |
| *Melting point* | -85.1°C |
| *Vapor pressure* | 6.2 torr @ 20°C |
| *Solubility* | Miscible with water, alcohol, benzene, ether, acetone |
| *Conversion factor* | 1 ppm = 3.1 mg/m$^3$ @ 25°C |

## III. Major Uses and Sources

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

## IV. Effects of Human Exposure

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

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

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

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

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

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

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

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

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

## V. Effects of Animal Exposure

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

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

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

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

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

Pregnant mice were exposed to 100, 150, or 200 mg/kg/day EGME on days 10-17 of gestation (Holladay *et al.*, 1994). Thymic atrophy and inhibition of fetal thymocyte maturation were observed in EGME-treated offspring examined on day 18 of gestation. Also, the ability of the EGME-treated fetal mouse liver cells to repopulate the spleen of irradiated mice was significantly impaired as compared to that of control fetal mouse liver cells.

## VI. Derivation of Reference Exposure Level

| | |
|---|---|
| *Study* | Miller *et al.*, 1983; U.S. EPA, 1995 |
| *Study population* | Rats and rabbits |
| *Exposure method* | Inhalation (0, 30, 100, or 300 ppm) |
| *Critical effects* | Decreased testes weight and degenerative changes in the testicular germinal epithelium. |
| *LOAEL* | 100 ppm |
| *NOAEL* | 30 ppm |
| *Exposure continuity* | 6 hours/day, 5 days/week |
| *Average experimental exposure* | 5.4 ppm for NOAEL group |
| *Human equivalent concentration* | 5.4 ppm for NOAEL group (gas with systemic effects, based on RGDR = 1.0 using default assumption that lambda (a) = lambda (h)) |
| *Exposure duration* | 13 weeks |
| *LOAEL uncertainty factor* | 1 |
| *Subchronic uncertainty factor* | 10 |
| *Interspecies uncertainty factor* | 3 |
| *Intraspecies uncertainty factor* | 10 |
| *Cumulative uncertainty factors* | 300 |
| *Inhalation reference exposure level* | 0.02 ppm (20 ppb; 0.06 mg/m$^3$; 60µg/m$^3$) |

The REL is based on the same study on which U.S. EPA based its RfC. However, OEHHA declined to use a modifying factor because the criteria for use of such factors are not well described by U.S. EPA. However, since rabbits were the more sensitive species and live 6 years (312 weeks), a 13 week study in rabbits merits a subchronic UF of 10.

A comparison with the proposed REL for EGME of 20 ppb (60 µg/m$^3$) can be made using the occupational study of Welch *et al*. (1988) of the semen of shipyard painters exposed to both EGEE and EGME. The men supplied demographic characteristics, medical conditions, personal habits, and reproductive history; underwent a physical examination; and provided a semen sample. The painters were exposed to EGEE at a TWA concentration of 0 to 80.5 mg/m$^3$ (mean = 9.9 mg/m$^3$, and to EGME at a TWA concentration of 0 to 17.7 mg/m$^3$ (mean = 2.6 mg/m$^3$). The painters had an increased prevalence of oligospermia and azoospermia and an increased odds ratio for a lower sperm count per ejaculate compared to shipyard employees who were not painters. (The results were controlled for smoking.) Adding the mean exposure levels together results in a total glycol ether concentration (EGME + EGEE) of 12.5 mg/m$^3$, equivalent to a continuous exposure of 4.5 mg/m$^3$. Division by a UF of 10 for a LOAEL and by another of 10 for human intraspecies variability results in a REL of 40 µg/m$^3$ (10 ppb), similar to the REL based on rabbits. Since exposure was primarily to EGEE with co-exposure to EGME, and exposure assessment ws difficult to quantify, this study was not deemed suitable for developing a REL. Nonetheless, the REL developed using this study is close in value to the proposed REL of 20 ppb.

## VII. Data Strengths and Limitations for Development of the REL

The strengths of the inhalation REL for EGME include the availability of subchronic inhalation exposure data from a well-conducted study with histopathological analysis and the observation of a NOAEL. In addition, there are a number of human studies showing similar toxicological endpoints to those demonstrated in animal studies. Major areas of uncertainty are the lack of adequate human exposure data, and the lack of chronic inhalation exposure studies.

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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

| | |
|---|---|
| *Inhalation reference exposure level* | **90 µg/m$^3$ ( 20 ppb)** |
| *Critical effect(s)* | Reproductive (testicular) toxicity in rabbits (EGME) |
| *Hazard index target(s)* | Reproductive system |

## II. Chemical Property Summary (HSDB, 1995)

| | |
|---|---|
| *Description* | Colorless liquid |
| *Molecular formula* | $C_5H_{10}O_3$ |
| *Molecular weight* | 118.3 g/mol |
| *Boiling point* | 144-145°C |
| *Vapor pressure* | 2 torr @ 20°C |
| *Solubility* | Miscible with water, organic solvents, oils |
| *Conversion factor* | 4.83 µg/m$^3$ per ppb at 25ºC |

## III. Major Uses and Sources

Ethylene glycol monomethyl ether acetate (EGMEA) is used as a solvent for nitrocellulose, cellulose acetate, and various other gums, resins, waxes, and oils (HSDB, 1995). It is also used in the semiconductor industry and in textile printing, photographic films, lacquers, and silk-screening inks. The annual specific statewide industrial emissions of EGMEA from facilities reporting under the Air Toxics Hot Spots Act in California based on the most recent inventory were estimated to be 3,060 pounds (CARB, 1999).

## IV. Effects of Human Exposure

Developmental defects have been described in the offspring of a mother who was occupationally exposed to EGMEA during pregnancy (Bolt and Golka, 1990). The mother was exposed during pregnancy by skin absorption and inhalation for approximately 1-4 hours/day to 1-2 liters of EGMEA. Her first child was born with congenital hypospadia, chordee, micropenis, and

scrotum bifida and her second child (3 years later) was born with chordee, cryptorchidism, penile hypospadia and scrotum bifida. Both children had normal karyotypes. No estimates of exposure were made.

A single case report described allergic dermatitis which may have developed from contact with EGMEA (Jordan and Dahl, 1971). A 58-year-old woman developed dermatitis on the nose possibly from contact with EGMEA on her eyeglasses. Ethylene glycol monoethyl ether acetate (EGEEA) was also present.

## V. Effects of Animal Exposure

Cats, rabbits, guinea pigs, and mice were repeatedly exposed by inhalation for 8 hours daily to 500 and 1000 ppm EGMEA (Gross, 1943; as described by Gingell *et al.*, 1982). This exposure regimen was fatal to cats at 500 ppm EGMEA. Death occurred after the animals showed slight narcosis. Similarly, exposure to 1000 ppm EGMEA produced deaths among rabbits, guinea pigs, and mice within a few days. Kidney toxicity was observed in animals in both dose groups. Repeated 4- and 6-hour exposure of cats to 200 ppm EGMEA resulted in decreased “blood pigments” and red blood cell counts.

The toxic effects of EGMEA were examined in male mice treated by gastric intubation 5 days/week for 5 weeks with 0, 62.5, 125, 250, 500, 1000, or 2000 mg EGMEA/kg/day (Nagano *et al.*, 1984). Dose-related testicular atrophy was observed at doses above 250 mg EGMEA/kg/day. Decreased white blood cell counts were observed in all EGMEA-exposed groups.

EGMEA was readily converted in vitro to ethylene glycol monomethyl ether (EGME) by the nasal mucosal carboxylesterases of mice and rabbits (Stott and McKenna, 1985). The enzyme activity in the nasal mucosa was equal to that of the liver and greater than that of the kidney and lung.

A concentration dependent decrease in testes weight was observed in male rabbits exposed to 30, 100, or 300 ppm ethylene glycol monomethyl ether (EGME) 6 hours/day, 5 days/week for 13 weeks (Miller *et al.*, 1983). Degenerative changes in the germinal epithelium were observed in male rabbits of all exposed groups, but the changes were not statistically significant at 30 ppm. Two of five male rabbits exposed to 300 ppm EGME died during the course of the study. Female rabbits were also exposed; two of five female rabbits exposed to 100 or 300 ppm EGME died during the course of the study. Reduced body weight gain, pancytopenia (abnormal depression of all the cellular elements of the blood), and thymic atrophy were observed in rabbits of both sexes exposed to 300 ppm EGME. No effects on the reproductive organs of the female rabbits were observed.

In the same study male and female rats were exposed to 30, 100, or 300 ppm EGME 6 hrs/day, 5 days/week for 13 weeks. Moderate to severe degeneration of the germinal epithelium and seminiferous tubules was observed in male rats exposed to 300 ppm EGME. A significant decrease in body weight was observed in male rats exposed to 300 ppm and in female rats

exposed to concentrations of EGME of 100 ppm or greater. Pancytopenia, lymphoid tissue atrophy, and decreased liver weights were observed in animals of both sexes exposed to the highest concentration. Also in the highest exposure group, mean values for total serum protein, albumin and globulins were lower than control values.

## VI. Derivation of Chronic Reference Exposure Level (REL)

| | |
|---|---|
| *Study* | Miller *et al.*, 1983 (see below) |
| *Study population* | Rabbits |
| *Exposure method* | Discontinuous inhalation exposure (0, 30, 100, or 300 ppm EGME) |
| *Critical effects* | Testicular effects |
| *LOAEL* | 100 ppm EGME |
| *NOAEL* | 30 ppm EGME |
| *Exposure continuity* | 6 hr/day, 5 days/week |
| *Exposure duration* | 13 weeks |
| *Average experimental exposure* | 5.4 ppm EGME for NOAEL group (30 x 6/24 x 5/7) |
| *Human equivalent concentration* | 5.4 ppm EGME for NOAEL group (gas with systemic effects, based on RGDR = 1.0 using default assumption that lambda (a) = lambda (h)) |
| *LOAEL uncertainty factor* | 1 |
| *Subchronic uncertainty factor* | 10 |
| *Interspecies factor* | 3 |
| *Intraspecies factor* | 10 |
| *Cumulative uncertainty factor* | 300 |
| *Inhalation reference exposure level* | 0.02 ppm (20 ppb, 0.06 mg/m$^3$, 60 µg/m$^3$) EGME<br>90 µg/m$^3$ EGMEA (20 ppb)<br>(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

| | |
|---|---|
| *Inhalation reference exposure level* | **30 μg/m$^3$** (18 ppb) |
| *Critical effect(s)* | Neurotoxicity in rats |
| *Hazard index target(s)* | Nervous system |

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

| | |
|---|---|
| *Description* | Colorless gas |
| *Molecular formula* | $C_2H_4O$ |
| *Molecular weight* | 44.06 g/mol |
| *Density* | 1.80 g/L @ 25ºC |
| *Boiling point* | 10.6ºC |
| *Melting point* | –111.6ºC |
| *Vapor pressure* | 1095 torr @ 20ºC |
| *Conversion factor* | 1 ppm = 1.80 mg/m$^3$ |

## III. Major Uses or Sources

The majority of all ethylene oxide (EtO) produced is used as a chemical intermediate in the production of various compounds including ethylene glycol, glycol ethers, and non-ionic surfactants (ATSDR, 1990). EtO is also used as a fumigant for food and cosmetics, and in hospital sterilization of surgical equipment and heat sensitive materials such as plastics. The annual statewide industrial emissions from facilities reporting under the Air Toxics Hot Spots Act in California based on the most recent inventory were estimated to be 43,972 pounds of ethylene oxide (CARB, 2000).

## IV. Effects of Human Exposure

Ten hospital sterilizer workers were matched with controls and examined for physical and neuropsychological health (Estrin *et al.*, 1990). The workers had operated sterilizers using 12% EtO and 88% Freon for an average of 5 years (range 0.5-10 years). Regular monitoring of workroom air had not been done. Measurements at the time of the study indicated concentrations of 15 ppm EtO or less. However, a second measurement showed an air concentration of 250 ppm EtO. A significantly greater percent of exposed workers exhibited a

bilateral reflex reduction in the ankle compared to controls. Nerve conduction tests did not identify significant differences between control and exposed workers, but a highly significant reduction ($p = 0.009$) in finger tapping speed was observed in exposed workers. The exposed group also performed more poorly on tests of spatial and visual abilities, and on tests of visual motor function. The results extended previous work by the same group (Estrin *et al*., 1987).

Cognitive impairment and personality dysfunction were observed more frequently in hospital workers chronically exposed to EtO, compared to a control group (Klees *et al.*, 1990). A group of 22 hospital workers, who had been exposed to an 8-hour TWA of 4.7 ppm EtO for a mean of 6.13 years (range 1-11 years), were matched with 24 control subjects. Neuropsychological function in the workers was classified as either normal or impaired on the basis of the questionnaires and of neuropsychological tests by 2 clinical psychologists (who were unaware of exposure status). (If the classification of the two clinicians did not agree, the subject was classified as "disagreement." Disagreement occurred in 7/23 (30%) of the controls and 10/22 (45%) of the exposed.) Exposed subjects were significantly more frequently classified as impaired (5/12) compared to controls (1/16) ($\chi^2 = 6.0861$; $p<0.05$). The Klees *et al*. (1990) study cites several earlier case reports of EtO neurotoxicity.

Recent studies have identified hemoglobin adducts, sister chromatid exchanges, and other hematological effects as indicators of ethylene oxide exposure (Ribeiro *et al.*, 1994; Sarto *et al.*, 1991). However, a recent study of 68 female workers from 9 hospitals in the U.S. and one in Mexico not only reports biological indicators of ethylene oxide exposure, but also provides a complete blood count with differential (Schulte *et al.*, 1995). The workers were classified as low- or high-exposure based on a mean 8-hour time weighted average of 0.08 or 0.17 ppm EtO. The mean length of employment for workers from U.S. hospitals was 5.5 and 10 years for low- and high-exposure workers, respectively. The mean length of employment in low- and high-exposure workers from the hospital in Mexico was 5.9 and 4.2 years, respectively. In workers from U.S. hospitals only, statistically significant decreases in hematocrit and hemoglobin were observed in high-exposure workers compared to low-exposure workers. Also, a statistically significant increase in lymphocytes and a significant decrease in neutrophils were observed in high-exposure workers compared to controls. In the workers from the hospital in Mexico, a significant relationship of EtO exposure and elevated neutrophil count was observed using regression.

At least 2 epidemiological reports indicate a possible association of EtO exposure and spontaneous abortion. Hemminki *et al.* (1982) analyzed spontaneous abortions in Finnish hospital sterilizing staff using data from a postal questionnaire and from a hospital discharge register. The study included all sterilizing staff employed in Finnish hospitals in 1980; the controls were nursing auxiliaries. When the women were involved in sterilizing procedures during their pregnancies, the frequency of spontaneous abortion was 16.7% versus 5.6% for the non-exposed pregnancies. The independent analysis of spontaneous abortions using the hospital discharge register confirmed the findings. Thus two analyses suggested that EtO exposure may carry a risk of spontaneous abortion among sterilizing staff.

More recently Rowland *et al*. (1996) sent questionnaires to 7,000 dental assistants (ages 18-39 years) registered in California in 1987. Of these, 4,856 responded (69%). They analyzed 1,320

women whose most recent pregnancy was conceived while working full-time. Thirty-two reported exposure to EtO; unexposed dental assistants comprised the comparison group. Among exposed women, the age-adjusted relative risk (RR) of spontaneous abortion was 2.5 [95% (CI) = 1.0-6.3]. The RR for pre-term birth was 2.7 (95% CI = 0.8-8.8) and the RR for post-term birth was 2.1 (95% CI = 0.7-5.9). The RR of any of these adverse outcomes among exposed women was estimated to be 2.5 (95% CI = 1.0-6.1). These results also indicate a possible relationship of EtO and spontaneous abortion.

## V. Effects of Animal Exposure

A 2 year inhalation bioassay exposed groups of 80 male rats to 0, 50, or 100 ppm EtO 7 hours per day, 5 days per week for 104 weeks (Lynch *et al.*, 1984). Mean body weights were significantly lower and mortality was significantly higher in both exposure groups. Inflammatory lesions of the lung, nasal cavity, trachea, and inner ear were observed more frequently in EtO exposed rats. Skeletal muscle myopathy, consisting of atrophy and degeneration of skeletal muscle fibers, was observed more frequently in rats exposed to 100 ppm EtO compared to controls. Neoplastic changes were also observed in EtO exposed rats.

Mice (30 per sex) were exposed to 0, 10, 50, 100, or 250 ppm EtO for 6 hours per day, 5 days per week, for 10 weeks (males) or 11 weeks (females) (Snellings *et al.*, 1984). Neuromuscular screening was conducted, and samples of urine and blood were collected. A significantly greater percent of exposed mice exhibited abnormal posture during gait and reduced locomotor activity. A dose-response was observed for these effects, with significant changes at 50 ppm and greater. An abnormal righting reflex was observed in a significantly greater percent of mice exposed to 100 ppm and above. Reduced or absent toe and tail pinch reflexes were observed in a significantly greater percent of mice exposed to 250 ppm EtO. Hematological changes observed in mice exposed to 250 ppm include slight, yet significant, decreases in red blood cell count, packed cell volume, and hemoglobin concentration. Absolute and relative spleen weights were significantly decreased in female mice exposed to 100 and 250 ppm and in male mice exposed to 250 ppm EtO. A significant increase in relative liver weight was observed in female mice exposed to 250 ppm EtO. Male mice exhibited a significant decrease in body weight at 10, 50, and 250 ppm and a significant decrease in absolute testes weights at 50, 100, or 250 ppm EtO. This study indicates a subchronic NOAEL for neurological effects of 10 ppm EtO.

In a study of the testicular effects of EtO, male rats were exposed to 500 ppm EtO 6 hours per day, 3 days per week for 2, 4, 6, or 13 weeks (Kaido *et al.*, 1992). An awkward gait was observed in rats after 6-9 weeks of exposure. Although no significant changes in body weight were observed, a statistically significant dose-related decrease in testes weight was observed at 4, 6, and 13 weeks of exposure. Progressive degeneration and loss of germ cells were also observed during the 13 week exposure. While severe loss of germ cells and marked morphological changes in remaining germ cells were observed at 6 weeks of exposure, some intact spermatids were observed at 13 weeks of exposure. This suggests that recovery of spermatogenesis occurred.

Saillenfait *et al*. (1996) studied the developmental toxicity of EtO in pregnant Sprague-Dawley rats using inhalation exposure during gestation days 6 to 15. Two protocols were used: (1)

exposure for 0.5 hr once a day to 0, 400, 800, or 1200 ppm EtO; or (2) exposure for 0.5 hr three times a day to 0, 200, or 400 ppm EtO or to 0, 800, or 1200 ppm EtO. The second protocol caused fetal toxicity as indicated by reduced fetal weight at 800 ppm (the LOAEL for this endpoint) and at 1200 ppm, and overt maternal toxicity manifested as reduced body weight gain at 1200 ppm. No embryolethality or teratogenicity occurred in either exposure protocol.

## VI. Derivation of Chronic Reference Exposure Level (REL)

| | |
|---|---|
| *Study* | Snellings *et al*., 1984 |
| *Study population* | Male and female B6C3F1 mice |
| *Exposure method* | Inhalation chamber exposure to 0, 10, 50, 100, or 250 ppm ethylene oxide |
| *Critical effects* | Impaired neurological function |
| *LOAEL* | 50 ppm |
| *NOAEL* | 10 ppm |
| *Exposure continuity* | 6-hours/day, 5 days/week |
| *Exposure duration* | 10 weeks (males), or 11 weeks (females) |
| *Average experimental exposure* | 1.79 ppm (10 x 8/24 x 5/7) |
| *Human equivalent concentration* | 1.79 ppm ((gas with systemic effects, based on RGDR = 1.0 using default assumption that lambda (a) = lambda (h)) ) |
| *LOAEL uncertainty factor* | 1 |
| *Subchronic uncertainty factor* | 3 |
| *Interspecies uncertainty factor* | 3 |
| *Intraspecies uncertainty factor* | 10 |
| *Cumulative uncertainty factor* | 100 |
| *Inhalation reference exposure level* | 18 ppb (30 $\mu g/m^3$) |

Snellings *et al*. (1984) found a subchronic NOAEL of 10 ppm for neurological effects in mice. A neuromuscular screening test indicated that certain reflex responses and locomotor activities were altered in EtO-exposed animals. Human studies have also indicated neurological impairment in ethylene oxide exposed workers.

## VII. Data Strengths and Limitations for Development of the REL

The strengths of the inhalation REL for ethylene oxide include the use of an animal study with both a LOAEL and a NOAEL and the use of an endpoint seen in both animals and humans.

Major areas of uncertainty are the short time-frame of the key study, the lack of an appropriate human study, and the limited number of developmental toxicity studies.

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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

| | |
|---|---|
| *Inhalation reference exposure level* | **14 μg HF/m$^3$** (17 ppb); **13 μg F/m$^3$** |
| *Oral reference exposure level* | **0.04 mg/kg-day** |
| *Critical effect(s)* | Skeletal fluorosis |
| *Hazard index target(s)* | Bone and teeth; respiratory system |

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

| | |
|---|---|
| *Description* | Colorless gas (HF), or as particulates |
| *Molecular formula* | HF |
| *Molecular weight* | 20.0 g/mol |
| *Density* | 0.83 g/L @ 25°C |
| *Boiling point* | 19.54°C |
| *Melting point* | –83.1°C |
| *Vapor pressure* | 400 torr @ 2.5°C |
| *Solubility* | Soluble in water and alcohol |
| *Conversion factor* | 1 ppm = 0.83 mg/m$^3$ @ 25°C |

## III. Major Uses or Sources

Hydrofluoric acid (HF) is a colorless, fuming liquid with a sharp, penetrating odor (Fairhall, 1949). This acid is used in the glass etching, electronic, microelectronic, and petroleum refining and chemical industries (Bertolini, 1992). These industries use HF in the manufacture of such things as computer chips (an important industry in California), phosphate fertilizer, metal cans, plastics, refrigerant chemicals (fluorocarbons), inorganic chemicals, soaps and detergents, high-octane gasoline, and aircraft parts (Wohlslagel *et al.*, 1976; Wing *et al.*, 1991). HF is also used in commercial rust removal products. Another high profile use of HF in California has been as a catalyst in petroleum alkylation to make high-octane gasoline. HF is also a product of combustion of any F containing materials; as such, it is produced during structural fires.

Sodium fluoride has been used as a topical and ingested anticaries agent due to its ability to harden tooth enamel during development. The optimal doses are not well established, but have been suggested to be approximately 0.080 mg/kg/day for 7 to 9 month old infants decreasing to 0.034 mg/kg/day at 13 years of age (Shulman *et al*., 1995). A dose of 1.0 mg F ingested per day was reported to reduce dental caries 43%, and to be associated with a greatly increased rate of minor tooth mottling which caused no esthetic damage (Van Nieuwenhuysen and D'Hoore, 1992). Many communities in California routinely add fluoride to the drinking water. The California Department of Health Services has adopted regulations that establish standards for the addition of F (CDHS, 2002). Any public water system using fluoridation must maintain F levels within the range established for its climate. The ranges vary according to average air temperatures, since people in cooler climates typically drink less water per day than people in warmer climates. Thus, in cooler areas, more F is required to provide the same dental benefit. For 2001-2002, F levels in San Francisco municipal water ranged from 0.65 to 1.1 ppm, while in Los Angeles the range was 0.44 to 0.83 ppm (CDHS, 2002).

The annual statewide industrial emissions from facilities reporting under the Air Toxics Hot Spots Act in California based on the most recent inventory were estimated to be 48,221 pounds of fluorides and compounds, and 62,670 pounds of hydrogen fluoride (CARB, 2000).

## IV. Effects of Human Exposure

The chronic exposure to fluorides, including HF, and the incidence of minimal osseous changes were studied in the workplace by Derryberry *et al.* (1963). In this study, the 8-hour time-weighted average fluoride exposure was calculated for the employment period of each of 74 male workers (30 Caucasian, 44 African-American). The overall average fluoride exposure in these workers was measured as a time-weighted average of 2.81 mg F/m$^3$. In comparison, the 17 workers within this group who had evidence of minimally increased bone density had an average fluoride exposure of 3.38 mg F/m$^3$. The other workers were exposed to an average measured concentration of 2.64 mg F/m$^3$. In addition, urinary fluoride levels were greater in the 17 individuals with greatest exposure compared to the remaining 57 workers (average = 5.18 mg F/L vs. 4.53 mg F/L). No differences between exposed and unexposed individuals were observed for gastrointestinal, cardiovascular, or hematologic systems, or in a physical exam. A statistically significant ($p < 0.05$) increase in the incidence of acute respiratory disease as determined from past medical histories was observed in fluoride-exposed individuals (19/74 vs. 8/67 in controls); radiographic examination revealed a difference of lesser significance ($p < 0.10$) for pulmonary changes (11/74 vs. 4/67). No pulmonary function tests were reported.

An analysis of these data by OEHHA (see derivation section below) showed a statistically significant relationship between air fluoride and the minimal bone density increases. The raw data from the Derryberry *et al.* (1963) study are shown in Table 1. A Pearson correlation matrix of the variables measured in the Derryberry *et al.* study indicated that bone density was best correlated with mean air fluoride level, and to a lesser extent with the age of the individual. A log-logistic regression using the log air fluoride concentration as the independent variable showed a significant ($p < 0.033$) relationship between increasing air fluoride concentrations and probability of skeletal fluorosis. The parameters for the regression were $\beta_0 = -2.3468$ (std. error

= 0.6462), and $\beta_1$ = 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/m$^3$. For these reasons, years exposed were not used as the dose-metric for bone-density in this analysis.

Although a threshold was not readily apparent from the logistic regression model, grouping the 74 individuals by air fluoride exposure level into quintiles of 15 each with one group of 14, allowed for a comparison of group mean responses (Table 2). The 14 employees exposed to a time-weighted average concentration of 1.07 mg F/m$^3$ did not exhibit bone density changes. An analysis of the grouped responses using a binomial distribution showed a probability of p = 0.008 for obtaining 4/15 increased bone density observations in the 2.34 mg/m$^3$ group, and a probability of p = 0.047 for obtaining 3/15 positive observations in the 1.89 mg F/m$^3$ group. The 1.89 mg F/m$^3$ group was therefore considered a LOAEL for chronic skeletal fluorosis, and the 1.07 mg/m$^3$ group was considered a NOAEL. The above probabilities assume that a chance occurrence is, at most, 1 in 18 of skeletal fluorosis or other cause leading to an abnormally dense x-ray in the general population. Since osteosclerosis is a rare condition that is associated with several types of hematological malignancies such as myeloid leukemia, the actual incidence of conditions leading to osteosclerosis is far below 1 in 18. This lends strong support to the consideration of 1.89 mg/m$^3$ as a LOAEL for skeletal fluorosis.

**Table 1.** Data on worker exposure to fluoride from Derryberry *et al.* (1963)

| Obser-vation # | ID | Bone density | Years exposed | Urine max F (mg F/L) | Urine min F (mg F/L) | Mean urinary F (mg F/L) | Age (years) | Air fluoride ($mg/m^3$) | *OEHHA exposure grouping* |
|---|---|---|---|---|---|---|---|---|---|
| 1 | 119 | normal | 18.5 | 43.0 | 2.8 | 14.7 | 58 | 8.16 | 5 |
| 2 | 0 | normal | 8.4 | 24.7 | 5.3 | 9.6 | 42 | 3.19 | 4 |
| 3 | 41 | normal | 15.8 | 35.0 | 2.5 | 9.1 | 35 | 3.29 | 4 |
| 4 | 147 | minimally increased | 9.6 | 17.1 | 2.1 | 8.9 | 60 | 5.98 | 5 |
| 5 | 120 | normal | 16.7 | 20.5 | 3.4 | 8.6 | 55 | 3.29 | 4 |
| 6 | 54 | minimally increased | 17.0 | 44.0 | 4.0 | 8.6 | 56 | 7.73 | 5 |
| 7 | 148 | normal | 10.5 | 14.0 | 3.7 | 8.4 | 41 | 8.32 | 5 |
| 8 | 314 | minimally increased | 14.4 | 22.7 | 1.7 | 8.3 | 56 | 3.24 | 4 |
| 9 | 29 | normal | 17.0 | 18.2 | 2.5 | 7.7 | 50 | 2.60 | 3 |
| 10 | 14 | normal | 14.3 | 19.4 | 2.1 | 6.3 | 46 | 2.33 | 3 |
| 11 | 115 | normal | 15.2 | 18.5 | 1.4 | 6.3 | 38 | 2.11 | 3 |
| 12 | 10 | minimally increased | 10.3 | 22.0 | 2.3 | 6.1 | 38 | 2.72 | 4 |
| 13 | 4 | minimally increased | 7.1 | 7.7 | 2.0 | 5.7 | 54 | 3.22 | 4 |
| 14 | 51 | normal | 14.9 | 42.0 | 0.8 | 5.6 | 46 | 3.18 | 4 |
| 15 | 94 | normal | 16.2 | 15.4 | 3.3 | 5.5 | 56 | 5.12 | 5 |
| 16 | 217 | normal | 7.1 | 7.1 | 2.6 | 5.3 | 42 | 2.54 | 3 |
| 17 | 281 | minimally increased | 7.8 | 8.6 | 1.1 | 5.2 | 36 | 3.79 | 4 |
| 18 | 114 | normal | 10.4 | 13.2 | 2.8 | 5.2 | 38 | 7.66 | 5 |
| 19 | 7 | normal | 7.8 | 9.1 | 2.2 | 5.1 | 43 | 2.91 | 4 |
| 20 | 308 | normal | 11.9 | 6.7 | 3.5 | 5.1 | 44 | 1.89 | 2 |
| 21 | 301 | minimally increased | 15.2 | 9.5 | 2.5 | 5 | 36 | 2.56 | 3 |
| 22 | 72 | normal | 25.9 | 13.7 | 2.1 | 4.9 | 55 | 5.55 | 5 |
| 23 | 241 | minimally increased | 17.0 | 10.0 | 1.9 | 4.9 | 46 | 4.48 | 5 |
| 24 | 345 | normal | 10.5 | 7.1 | 2.0 | 4.9 | 47 | 1.49 | 1 |
| 25 | 26 | normal | 16.4 | 12.2 | 0.5 | 4.7 | 39 | 2.41 | 3 |
| 26 | 231 | minimally increased | 16.3 | 8.2 | 2.8 | 4.6 | 62 | 1.88 | 2 |
| 27 | 2 | normal | 24.7 | 8.9 | 2.1 | 4.6 | 46 | 3.53 | 4 |
| 28 | 295 | normal | 14.5 | 10.7 | 0.9 | 4.6 | 44 | 2.07 | 3 |
| 29 | 1 | normal | 8.9 | 5.9 | 2.4 | 4.5 | 30 | 1.92 | 2 |
| 30 | 203 | minimally increased | 18.2 | 6.8 | 1.6 | 4.4 | 43 | 2.66 | 3 |
| 31 | 63 | normal | 16.2 | 7.4 | 2.0 | 4.3 | 55 | 3.90 | 5 |
| 32 | 5 | normal | 4.5 | 11.5 | 1.9 | 4.3 | 43 | 1.12 | 1 |
| 33 | 460 | normal | 12.5 | 6.1 | 1.6 | 4.3 | 60 | 2.13 | 3 |

| Obser-vation # | ID | Bone density | Years exposed | Urine max F (mg F/L) | Urine min F (mg F/L) | Mean urinary F (mg F/L) | Age (years) | Air fluoride (mg F/m$^3$) | *OEHHA exposure grouping* |
|---|---|---|---|---|---|---|---|---|---|
| 34 | 249 | minimally increased | 15.0 | 8.0 | 1.8 | 4.3 | 39 | 2.95 | 4 |
| 35 | 3 | normal | 7.6 | 14.5 | 2.1 | 4.3 | 31 | 3.90 | 5 |
| 36 | 322 | normal | 9.3 | 6.3 | 2.0 | 4.3 | 35 | 4.23 | 5 |
| 37 | 8 | minimally increased | 24.8 | 5.9 | 3.0 | 4.2 | 55 | 2.50 | 3 |
| 38 | 3 | normal | 15.2 | 12.2 | 2.1 | 4.2 | 42 | 1.14 | 1 |
| 39 | 309 | normal | 12.1 | 5.5 | 2.4 | 4.1 | 42 | 1.94 | 2 |
| 40 | 36 | normal | 9.1 | 13.2 | 0.8 | 4.1 | 33 | 1.94 | 2 |
| 41 | 45 | normal | 11.3 | 14.0 | 2.2 | 4.1 | 33 | 3.84 | 4 |
| 42 | 70 | normal | 17.9 | 8.0 | 1.0 | 3.9 | 44 | 4.00 | 5 |
| 43 | 250 | minimally increased | 9.8 | 6.7 | 1.5 | 3.9 | 35 | 1.78 | 2 |
| 44 | 38 | normal | 16.9 | 5.9 | 1.0 | 3.9 | 35 | 2.10 | 3 |
| 45 | 200 | minimally increased | 14.0 | 7.0 | 2.8 | 3.8 | 66 | 3.92 | 5 |
| 46 | 183 | normal | 9.8 | 4.9 | 2.2 | 3.7 | 48 | 1.67 | 2 |
| 47 | 32 | normal | 12.5 | 6.6 | 0.9 | 3.7 | 47 | 2.21 | 3 |
| 48 | 25 | normal | 13.6 | 5.5 | 1.5 | 3.7 | 44 | 1.86 | 2 |
| 49 | 21 | normal | 13.9 | 9.1 | 0.4 | 3.7 | 50 | 1.98 | 2 |
| 50 | 304 | normal | 13.4 | 5.0 | 2.1 | 3.7 | 36 | 2.62 | 3 |
| 51 | 132 | normal | 10.9 | 5.1 | 2.4 | 3.6 | 39 | 1.81 | 2 |
| 52 | 6 | minimally increased | 8.4 | 4.8 | 0.9 | 3.6 | 35 | 3.85 | 5 |
| 53 | 244 | normal | 16.6 | 7.1 | 1.4 | 3.6 | 62 | 2.87 | 4 |
| 54 | 30 | normal | 14.0 | 14.0 | 0.9 | 3.6 | 43 | 1.56 | 1 |
| 55 | 88 | minimally increased | 15.5 | 4.9 | 1.7 | 3.5 | 66 | 2.06 | 2 |
| 56 | 227 | normal | 16.6 | 5.7 | 1.0 | 3.5 | 41 | 1.18 | 1 |
| 57 | 271 | normal | 17.7 | 4.1 | 3.0 | 3.4 | 60 | 1.82 | 2 |
| 58 | 19 | normal | 13.9 | 10.0 | 1.8 | 3.4 | 41 | 1.32 | 1 |
| 59 | 190 | normal | 9.3 | 7.7 | 1.9 | 3.3 | 36 | 1.95 | 2 |
| 60 | 258 | normal | 17.8 | 5.6 | 1.6 | 3.2 | 58 | 0.87 | 1 |
| 61 | 278 | normal | 10.0 | 7.0 | 0.3 | 3.2 | 34 | 1.93 | 2 |
| 62 | 331 | normal | 12.8 | 5.6 | 1.5 | 3.1 | 34 | 1.23 | 1 |
| 63 | 91 | normal | 25.3 | 7.9 | 0.2 | 3.1 | 63 | 3.49 | 4 |
| 64 | 342 | normal | 18.5 | 6.0 | 1.3 | 3 | 40 | 2.73 | 4 |
| 65 | 261 | normal | 18.1 | 5.3 | 0.9 | 2.9 | 52 | 4.41 | 5 |
| 66 | 291 | normal | 13.5 | 4.5 | 1.5 | 2.8 | 34 | 2.14 | 3 |
| 67 | 149 | normal | 11.3 | 4.5 | 2.1 | 2.8 | 34 | 0.76 | 1 |
| 68 | 2 | normal | 24.7 | 4.5 | 1.5 | 2.7 | 51 | 1.15 | 1 |
| 69 | 4 | normal | 16.8 | 5.7 | 1.2 | 2.7 | 56 | 0.71 | 1 |
| 70 | 109 | normal | 8.3 | 5.1 | 0.8 | 2.7 | 36 | 1.89 | 2 |
| 71 | 242 | normal | 18.1 | 4.1 | 1.2 | 2.5 | 49 | 1.26 | 1 |

| Obser-vation # | ID | Bone density | Years exposed | Urine max F (mg F/L) | Urine min F (mg F/L) | Mean urinary F (mg F/L) | Age (years) | Air fluoride (mg F/m$^3$) | *OEHHA exposure grouping* |
|---|---|---|---|---|---|---|---|---|---|
| 72 | 179 | normal | 18.9 | 3.9 | 1.0 | 2.4 | 46 | 0.50 | 1 |
| 73 | 325 | minimally increased | 11.8 | 5.0 | 0.5 | 2.2 | 40 | 2.10 | 3 |
| 74 | 159 | normal | 18.9 | 5.0 | 0.7 | 2.1 | 45 | 0.67 | 1 |

**Table 2.** Grouped mean exposure

| Exposure group | Mean age ± SD | Mean air level mg F/m$^3$ ± SD | Number of responses | Probability of difference from group 1* |
|---|---|---|---|---|
| 1 | 45.0 ± 7.0 | 1.07 ± 0.32 | 0/14** | Not Applicable |
| 2 | 43.9 ± 11.2 | 1.89 ± 0.09 | 3/15*** | 0.047 |
| 3 | 43.0 ± 7.6 | 2.34 ± 0.23 | 4/15 | 0.008 |
| 4 | 45.9 ± 9.8 | 3.22 ± 0.35 | 5/15 | 0.001 |
| 5 | 48.5 ± 10.7 | 5.41 ± 1.72 | 5/15 | 0.001 |

* Probability of obtaining result assuming a chance occurrence of abnormally dense x-ray of, at most, 1 in 18 individuals, using a binomial distribution (Systat for Windows v.5.05, 1994).
** NOAEL
*** LOAEL ($p < 0.05$)

Largent *et al.* (1951) found a significant increase in bone density in the lower thoracic spine, with calcification extending into the lateral ligaments of 3 workers exposed for 17, 14, and 10 years to HF (concentrations not estimated).

A group of 74 men, who were occupationally exposed to unspecified concentrations of HF for an average of 2.7 years, reported occasions of upper respiratory irritation (Evans, 1940). Repeated chest X-rays over a 5-year period did not reveal any visible evidence of lung changes. The death rate of these workers from pneumonia and other pulmonary infections was the same as that of unexposed plant employees.

There are various reports of asthma and related respiratory effects in pot room workers in the primary aluminum smelting industry. Exposure to fluoride (among other materials such as sulfur trioxide and polycyclic aromatic hydrocarbons) was measured as a possible index of exposures related to this condition (Seixas *et al*., 2000). However multiple exposures to respiratory irritants and other compounds which may affect immune response appear to be common in this work environment making it difficult to quantitatively relate the respiratory symptoms to inhaled HF or fluorides.

Workers in a warehouse containing HF retorts experienced transitory hyperemia of the skin on their face and hands (Dale and McCauley, 1948). Twenty four of the 40 workers had definite changes in the thickness and number of trabeculae in the upper and lower jaw.

Examinations of 107 pot room workers in two aluminum plants with airborne fluorides revealed 22 subjects with limited motion of the dorsolumbar spine, compared with none in a control group of 108 workers with no history of exposure to fluorides (Kaltreider *et al.*, 1972). In one plant, 76 of 79 workers had increased bone density as measured by roentgenogram, with diagnosis of slight to moderate fluorosis. Moderate and marked fluorosis was observed after 15 years employment. The 8-hour time-weighted average fluoride content in these workplaces was 2.4 to 6.0 mg/m$^3$. Balazova (1971) measured significant fluoride uptake and distribution in children living near an aluminum smelter but reported no incidence of fluorosis.

No studies regarding the chronic irritant or respiratory effects of pure HF exposure in humans were available.

Fluoride ion produced by various fluorocarbons has been associated with toxicity to human kidney collecting duct cells leading to sodium and water disturbances (Cittanova *et al*., 1996).

Oral supplementation of greater than 0.1 mg F/kg body weight daily has been associated with enamel fluorosis in young children (Forsman, 1977).

The Agency for Toxic Substances and Disease Registry (ATSDR, 2001) recently reviewed fluorides since they are found at hazardous waste sites which are candidates for remediation. The focus of this document was on oral exposure studies as that is the main concern for waste site remediation.

## V. Effects of Chronic Exposures to Animals

Stokinger (1949) studied the subchronic effects of HF inhalation in several animal species. Animals (dogs, rabbits, rats, guinea pigs, and mice; 1 to 6 per group) were exposed to 0, 7.2 mg/m$^3$, or 25.1 mg/m$^3$ 6 hours/day, 6 days/week, for 30 days. Mortality, body weight, blood coagulation mechanisms, and gross pathology were measured. Exposure to 25.1 mg/m$^3$ HF for 30 days resulted in degenerative testicular changes and ulceration of the scrotum in all 4 dogs and hemorrhage and edema in the lungs of 3 dogs. Pulmonary hemorrhage was also seen in 20 of 30 rats, and 4 of 10 rabbits. Renal cortical degeneration was observed in 27 of 30 rats. All of the rats and mice at the 25.1 mg/m$^3$ concentration died. No mortality was observed in the other species tested. Blood fibrinogen levels were significantly increased in dogs, rats, and rabbits exposed to 25.1 mg/m$^3$. Exposure to 7.2 mg/m$^3$ HF resulted in pulmonary hemorrhage in 1 out of 5 dogs. No other significant effects were observed at the lower concentration.

Shusheela and Kumar (1991) administered male rabbits 10 mg NaF/kg-bw per day orally for 18 months (7 rabbits) or 29 months (3 rabbits), then studied the testis, epididymis, and vas deferens microscopically. After 29 months of F administration, the spermatogenic cells in the seminiferous tubules had degenerated and lacked spermatozoa. After both 18 and 29 months, cilia were lost from the epithelial cells lining the ductuli efferentes of the caput epididymidis. Stereocilia on the epithelial cells lining the vas deferens were also lost. In some regions of epithelia, the cell boundaries were not clear, and even appeared to be peeled off. Mucus droplets were abundant in the vas deferens of controls, but none were present in F treated rabbits.

Spermatogenesis ceased sometime between 18 and 29 months. The authors concluded that ingestion of a high concentration of F has adverse effects (including infertility) on the male rabbit reproductive system.

Ghosh *et al*. (2002) investigated the effects of NaF on steroidogenic and gametogenic activities in rat testes. Male Wistar rats were given 20 mg/kg/day NaF by gavage for 29 days. F treatment resulted in significantly lower relative wet weight of the testis, prostate, and seminal vesicle, decreased testicular delta(5),3beta-hydroxysteroid dehydrogenase (HSD) and 17beta-HSD activities, and significant lowering in plasma levels of testosterone. Epididymal sperm count was decreased significantly in F-treated rabbits and there were fewer mature luminal spermatozoa. Indicators of oxidative stress due to F included increased conjugated dienes in the testis, epididymis, and epididymal sperm pellet, and decreases of peroxidase and catalase in the sperm pellet. Thus F, at a dose encountered in drinking water in contaminated areas (at least of India), exerts an adverse effect on the male rat reproductive system. These effects on rats and rabbits (and dogs; see above) may be relevant to anecdotal reports of reproductive system malfunction in human chronic fluorosis.

| Parameter | Control (n=6) | NaF (n=6) | p value |
|---|---|---|---|
| Body weight, final (g) | 127.00±3.75 | 122.00±5.10 | |
| Testis, relative weight (%) | 1.522±0.034 | 1.923±0.081 | < 0.05 |
| Prostate, relative weight | 0.297±0.043 | 0.148±0.014 | < 0.05 |
| Seminal vesicles, rel. weight | 0.448±0.025 | 0.174±0.027 | < 0.05 |
| Testicular delta(5),3beta HSD | ~28[a] | ~24[a] | < 0.05[b] |
| Testicular 17betaHSD | ~29[a] | ~24[a] | < 0.05[b] |
| Plasma testosterone (ng/ml) | ~2[a] | ~1[a] | < 0.05[b] |
| Epididymal sperm count ($10^6$/ml) | 7.02±0.17 | 3.70±0.57 | < 0.05 |

[a] approximate values based on reading Figures 2 and 3 of paper; [b] p values of authors

Long *et al*. (2002) used ligand binding and Western blotting to study neuronal nicotinic acetylcholine receptors (nAChRs) in the brains of male and female Wistar rats ingesting 0.5 ppm (controls), 30 ppm, or 100 ppm F in their drinking water for 7 months. (All received 4 ppm F in their diet.) The brains of rats exposed to 100 ppm had significantly less binding sites for [$^3$H]epibatidine, an analgesic agonist, but no change occurred at 30 ppm. Binding sites for [$^{125}$I]alpha-bungarotoxin, a competitive antagonist, were significantly decreased in the brains of rats exposed to both levels. The brain levels of the nAChR alpha4 subunit protein was significantly lowered by exposure to 100 ppm F. Alpha7 subunit protein was significantly decreased by both levels of F. No significant changes were seen in levels of the beta2 subunit protein. These nicotinic receptors have roles in learning and memory. Some of the effects were also seen in rat PC cells cultured for 48 h in up to 50 ppm F (Chen *et al*., 2003). The results may help to explain anecdotal reports of nervous system symptoms in human chronic fluorosis (Waldbott, 1978).

NTP (1990) exposed F344/N rats and B6C3F1 mice of both sexes for two years to 0, 25, 100, and 175 ppm sodium fluoride (NaF) in their drinking water. NaF caused a dose dependent whitish discoloration of the teeth in both rats and mice. Male rats had an increased incidence of

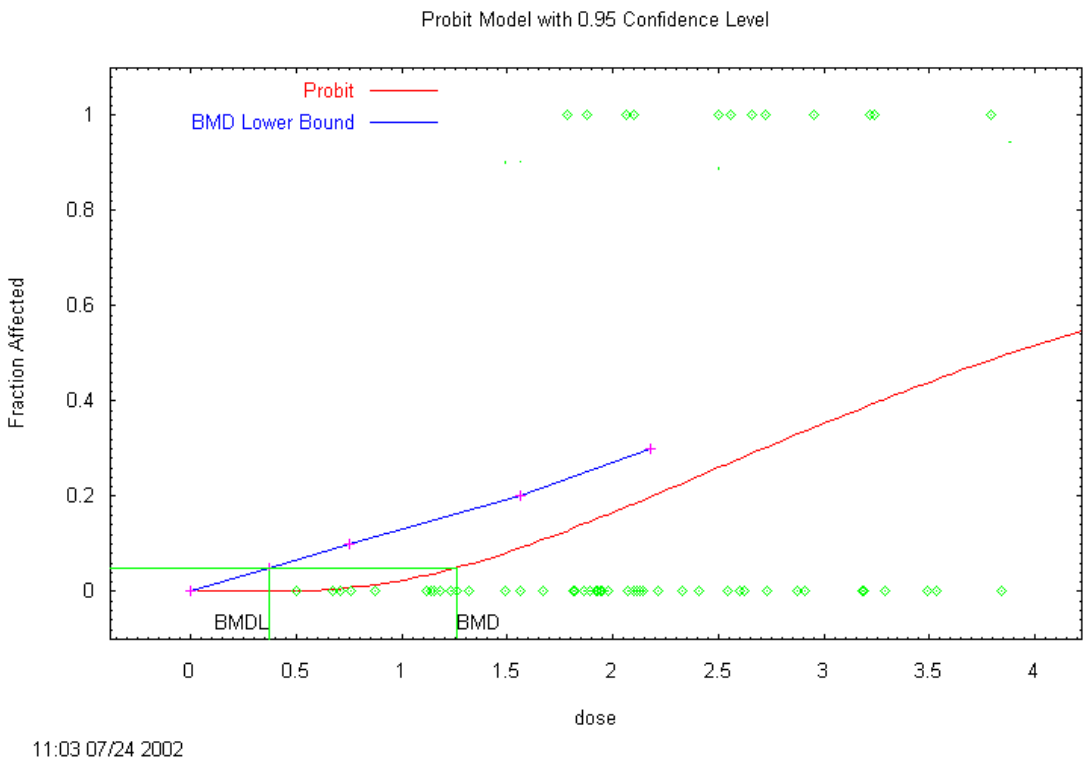
tooth deformities and attrition. NaF increased the dysplasia of dentine in both rats and mice. At the highest dose (175 ppm), osteosclerosis of long bones was increased in female rats. There was also equivocal evidence of carcinogenic activity of NaF in male rats based on four osteosarcomas in dosed animals (Bucher *et al*., 1991). Other organ systems showed no dose-dependent effects.

## VI. Derivation of Chronic Reference Exposure Level (REL)

| | |
|---|---|
| *Study* | Derryberry *et al.* (1963) |
| *Study population* | 74 fertilizer plant workers (67 unexposed control subjects) |
| *Exposure method* | Occupational |
| *Critical effects* | Increased bone density (skeletal fluorosis) |
| *LOAEL* | 1.89 mg F/m$^3$ (1.98 mg HF/m$^3$) |
| *NOAEL* | 1.07 mg F/m$^3$ (1.13 mg HF/m$^3$) |
| *$BMC_{05}$* | 0.37 mg F/m$^3$ (0.39 mg HF/m$^3$) |
| *Exposure continuity* | 8 hours/day, 5 days/week |
| *Exposure duration* | 14.1 years (range = 4.5 to 25.9 years) |
| *Average exposure concentration* | 0.14 mg HF/m$^3$ (0.39 x 10/20 x 5/7) or 0.13 mg F/m$^3$ (0.37 x 10/20 x 5/7) |
| *Human equivalent concentration* | 0.14 mg HF/m$^3$ or 0.13 mg F/m$^3$ |
| *LOAEL uncertainty factor* | 1 |
| *Subchronic uncertainty factor* | 1 |
| *Interspecies uncertainty factor* | 1 |
| *Intraspecies uncertainty factor* | 10 |
| *Cumulative uncertainty factor* | 10 |
| *Inhalation reference exposure level for F or HF* | 0.013 mg F/m$^3$ (13 μg /m$^3$; 0.016 ppm; 16 ppb) or 0.014 mg HF/m$^3$ (14 μg /m$^3$; 0.017 ppm; 17 ppb) |

OEHHA's analysis of the data in Derryberry *et al*. (1963) indicates a LOAEL of 1.89 mg/m$^3$, and a NOAEL of 1.07 mg/m$^3$. A benchmark concentration ($BMC_{05}$) of 0.37 mg/m$^3$ was derived by fitting the probit model to the log dose in the U.S. EPA's BMDS (version 1.3) software, for the individual mean air exposure data and incidence data in Table 1 above. Individuals in the highest dose group (group 5 in Table 2) were not included in the model, since none of the models fit this range of exposures well. Several other models produced reasonable fits to the data, but the probit model with log-transformed dose was selected since it produced a good fit not only by statistical criteria ($p = 0.71$) but also, as determined by inspection, it fit the low dose curve shape better than other models. This model also has the advantage of biological plausibility, in that, since lower doses of fluoride have a beneficial or nutritional effect, a threshold type of response for adverse effects is clearly expected. A graphical representation of the fit is shown in Figure 1. Adjusting for exposure continuity and utilizing an intraspecies uncertainty factor of 10 ($UF_H$) results in a REL for F of 13 μg/m$^3$.

**Figure 1.**




Changes in bone density in association with fluoride exposure have been observed in several studies, and appear to be the most sensitive health effect for chronic exposure. The minimally increased bone density in the Derryberry study was significantly ($p < 0.04$, Fisher's Exact Test) associated with "other osseous changes," which reportedly included disc lesions, arthritis, and calcified ligaments. An increase in pulmonary changes in the workers with high bone density was marginally significant ($p < 0.06$) and included emphysema, fibrosis, and healed tuberculous lesions. Although dental fluorosis is a sensitive endpoint in many fluoride studies, the dental examinations of exposed workers in this study showed healthier teeth than in controls. The increased bone density observed was considered as indicating that adverse effects had occurred, based on the adverse effects associated with the increased density in the study, and on other research showing that increased bone density caused by fluoride exposure (75 mg sodium fluoride per day for four years) also leads to decreased bone strength and increased fragility (Riggs *et al.*, 1990). Symptoms of abdominal pain, backache, restricted joint movement, and respiratory symptoms have been associated with airborne fluoride exposures and bone density increases in industrial settings (Zhiliang *et al.*, 1987).

The absorption of particulate and gaseous fluorides is reported to be similar (Collings *et al.*, 1951). Therefore, it would be expected that the effects on bone density would be similar regardless of the form of fluoride.

As noted in the study description, Derryberry *et al.* (1963) did not find a good correlation between years of exposure to fluoride and bone density change. OEHHA reexamined the original individual data and confirmed that the presence of bone density changes showed a better correlation with mean air fluoride concentration than with years of exposure, or with the product of the individual values of mean air fluoride concentration and years of exposure. However, the product of exposure concentration and time did show a consistent pattern of cumulative incidence suggesting a dose-response relationship for this parameter. An attempt to derive a benchmark value by fitting the probit model to the log of (exposure duration*concentration) and response (presence or absence of bone density change) did not result in an acceptable fit, so a $BMDL_{05}$ could not be reported. However a maximum likelihood estimate of the benchmark ($BMD_{05}$) was found to be 6.04 (mg F*years/$m^3$), with exclusion of the three highest values that appeared to be outliers to the main distribution. If this value is divided by the mean exposure duration for the data set of 14.1 years, a benchmark exposure concentration of 0.43 mg F/$m^3$ is obtained. While this value is evidently less reliable than that obtained by fitting the mean exposure concentration, it is consistent with it, suggesting that, although other confounding factors related to age or duration prevent the demonstration of a relationship between the exposure/time integral and response in this data set, such a relationship probably does exist, as would be expected.

## VII. Data Strengths and Limitations for Development of the REL

The major strengths of the key study for fluoride are the observation of health effects in a large group of workers exposed over many years, the availability of individual exposure estimates for each worker, and the identification of a NOAEL. The primary uncertainty in the study is the lack of a comprehensive health effects examination. Another source for concern is the potentially greater susceptibility of children to the effects of inhaled fluorides, considering the rapid bone growth in early years.

### Derivation of Chronic Oral REL

In addition to being inhaled, airborne fluoride salts in particulate form can settle onto crops and soil and enter the body by ingestion. Thus an oral chronic reference exposure level (REL) for fluoride is also required in order to conduct a health risk assessment under the Air Toxics Hot Spots Act. California has developed a Public Health Goal (PHG) of 1 ppm (1,000 ppb) fluoride in drinking water (OEHHA, 1997). This level is intended to be an approximate year-round average. Thus it has properties similar to a chronic oral REL. (The PHG assumed that drinking water was the only source of fluoride since it was based on comparing communities with and without added fluoridation.)

| | |
|---|---|
| *Study* | Dean, 1942; U.S. Public Health Service, 1991; National Research Council, 1993 |
| *Study population* | Inhabitants of several U.S. cities |
| *Exposure method* | Drinking water |
| *Critical effects* | Dental fluorosis |
| *LOAEL* | 2 ppm |
| *NOAEL* | 1 ppm = 0.04 mg/kg-day* |
| *Exposure continuity* | Continuous |
| *Exposure duration* | Long-term |
| *Average experimental exposure* | 1 ppm = 0.04 mg/kg-day |
| *LOAEL uncertainty factor* | 1 |
| *Subchronic uncertainty factor* | 1 |
| *Interspecies uncertainty factor* | 1 |
| *Intraspecies uncertainty factor* | 1 (studies included children) |
| *Cumulative uncertainty factor* | 1 |
| *Oral reference exposure level* | 0.04 mg/kg-day |

* based on the assumption that an 18 kg child drinks 720 ml of water per day (OEHHA, 2000).

The PHG is based on a no-observed adverse-effect-level (NOAEL) of 1 mg/L for dental fluorosis in children (equivalent to 720 μg/day from drinking water for an 18 kg child drinking 40 ml/kg body weight/day of water). Moderate to severe dental fluorosis is rare when the drinking water fluoride level is near 1 mg/L, but begins to become significant at concentrations close to 2 mg/L. Since the study involved long term exposure to humans including children, a sensitive population, the cumulative uncertainty factor was 1. If one were to do a route-to-route extrapolation from this oral REL using the specific parameters for an 18 kg child breathing 4.2 $m^3$/day, an equivalent inhalation REL would be about 170 μg/$m^3$. Thus, the inhalation REL of 13 μg/$m^3$ based on the adult occupational data is likely to be protective of children.

## VIII. Potential for Differential Impacts on Children's Health

The critical effect for inhalation exposures is skeletal fluorosis. Since infants' and children's skeletons are developing, they may be more sensitive to this effect. This applies with particular importance to the teeth, and it is established that excessive exposure to fluoride during the period of tooth development in infancy and childhood causes dental fluorosis (Dean, 1942; U.S. Public Health Service, 1991; NRC, 1993). The oral REL and the California PHG for fluoride in drinking water are based on dental fluorosis. Although the inhalation chronic REL proposed is based on a study in adults, the inhalation chronic REL (see section VI) is lower than that implied by the oral REL and PHG. Since the oral REL and PHG are based on exposures throughout life, including the pre-natal period, infancy, and childhood, it is reasonable to conclude that the proposed inhalation REL is generally protective of infants and children, barring some unknown difference in toxicity between the two routes of exposure. The ratio of the intake at the PHG level in drinking water is closer to the effect level than the default intraspecies uncertainty factor of 10; this is to be expected since children are a sensitive subpopulation for the dental fluorosis effect.

Extensive interindividual variation in total fluoride intake (930.7 ± 391.5 μg/day) was recently documented for a small group (n = 11) of healthy German children ages 3 to 6 years (Haftenberger *et al*., 2001). Similar interindividual variation has also been reported for slightly younger children in Connersville (n = 14) and Indianapolis, Indiana (n = 29) and in San Juan, Puerto Rico (n = 11) (Rojas-Sanchez *et al*., 1999). Consideration should therefore be given to populations with exceptionally high fluoride intake due to locally elevated concentrations in drinking water, since some of these populations are already close to adverse effect levels of fluoride intake, and certain individuals in California experience dental fluorosis. For these individuals, even exposure to fluorides at the oral and/or inhalation RELs, which are acceptable in isolation, might be deleterious. The table below compares the data of Haftenberger *et al*. (2001) with recent estimates of F intake ranges in California (OEHHA, 1997).

Fluoride Intake (mg/day)

| F in drinking water (mg/L) | F from drinking water | F from food | F from toothpaste | F from mouthwash | F from a supplement | Total F |
|---|---|---|---|---|---|---|
| Children (OEHHA) | | | | | | |
| <0.3 | 0.1 - 0.3 | 0.1 – 0.5 | 0.2 – 1.2 | 0.1 - 0.5 | 0.5 | 1.0 – 3.0 |
| 0.7 - 1.2 | 0.7 - 1.2 | | 0.2 – 1.2 | 0.1 - 0.5 | 0 | 1.1 – 4.6 |
| Haftenberger | | | | | | |
| 0.25 | (see food) | 0.20±0.12 | 0.27±0.18 | No data | 0 - 1.0 | 0.93±0.39 |
| Adults (OEHHA) | | | | | | |
| <0.3 | 0.2 – 0.6 | 0.3 – 1.0 | 0.02 – 0.15 | 0.2 – 1.0 | 0 | 0.7 – 2.8 |
| 0.7 - 1.2 | 1.4 – 2.4 | 0.3 – 3.4 | 0.02 – 0.15 | 0.2 – 1.0 | 0 | 1.9 – 7.0 |

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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

| | |
|---|---|
| *Inhalation reference exposure level* | **0.08 µg/m$^3$** (0.02 ppb) |
| *Critical effect(s)* | Squamous metaplasia of the respiratory epithelium in the nose of male and female mice |
| *Hazard index target(s)* | Respiratory system |

## II. Chemical Property Summary (HSDB, 1996; CRC, 1994; Chemfinder, 2000)

| | |
|---|---|
| *Description* | Colorless liquid/oil |
| *Molecular formula* | $C_5H_8O_2$ |
| *Molecular weight* | 100.12 g/mol |
| *Boiling point* | 188°C (decomposes) (CRC, 1994) |
| *Melting point* | –6°C (Chemfinder, 2000) |
| *Solubility* | Soluble in water, alcohol, benzene |
| *Conversion factor* | 4.1 µg/m$^3$ per ppb at 25ºC |

## III. Major Uses and Sources

Glutaraldehyde is a chemical frequently used as a disinfectant and sterilizing agent against bacteria and viruses (2% solution), an embalming fluid and tissue fixative, a component of leather tanning solutions, and an intermediate in the production of certain sealants, resins, dyes, and electrical products (HSDB, 1996). For commercial purposes, solutions of 99%, 50%, and 20% are available. Glutaraldehyde is also an atmospheric reaction product of cyclohexene. The annual statewide industrial emissions from facilities reporting under the Air Toxics Hot Spots Act in California based on the most recent inventory were estimated to be 29,603 pounds of glutaraldehyde (CARB, 2000).

## IV. Effects of Human Exposure

Evidence of the toxicity of glutaraldehyde to humans is limited to reports of occupational exposure from its use as a disinfectant and sterilizing agent. Frequently observed effects from exposure include skin sensitivity resulting in dermatitis, and irritation of the eyes and nose with accompanying rhinitis (Jordan *et al.*, 1972; Corrado *et al.*, 1986; Hansen, 1983; Wiggins *et al.*,

1989). Occupational asthma has also been reported among workers repeatedly exposed to glutaraldehyde, particularly respiratory technologists who use glutaraldehyde as a sterilizing agent for endoscopes (Chan-Yeung *et al.*, 1993; Stenton *et al.*, 1994; Gannon *et al.*, 1995). Quantitation of the exposure levels that led to glutaraldehyde sensitization was not available from the studies.

## V. Effects of Animal Exposure

The histopathology of the respiratory tract in rats and mice exposed to glutaraldehyde by inhalation was examined (Gross *et al.*, 1994). F344 rats and B6C3F1 mice (20 animals of each sex and of each species at each exposure level for a total of 480 rodents) were continuously exposed to glutaraldehyde in recirculating exposure chambers at concentrations of 0, 62.5, 125, 250, 500, or 1000 ppb glutaraldehyde for one day, 4 days, 6 weeks, or 13 weeks. At termination, respiratory tract tissue as well as duodenum and any gross lesions were collected and formalin fixed. Animals were treated with tritiated thymidine two hours before termination to evaluate cell replication in certain respiratory tract tissues. Respiratory tract tissue sections were made as follows: transverse sections of the nose and trachea, frontal section of the carina, and longitudinal section of the lung. Ten male and 10 female mice exposed to 1000 ppb and one female mouse exposed to 500 ppb group died during the course of the study. Two male and 3 female rats exposed to 1000 ppb died during the course of the study. Histopathological examination of animals surviving to the end of the study entailed scoring the severity of the finding from “no response” to “very severe” response on a 0 to 5 scale. Unit length labeling index, the indicator of cell proliferation, was evaluated by autoradiography at two sites: the nasal vestibule and the dorsal atrioturbinate.

Lesions in animals treated with glutaraldehyde appeared primarily in the anterior third of the nose. Lesions were apparently more increased in mice compared to rats due to some level of “background” non-suppurative lesions in the rats. Mice were considered devoid of background lesions. In the 13-week study, female mice were the most sensitive, with lesions averaging a score of 2 (mild and clear, but of limited extent and/or severity). The lesions were characterized as neutrophilic infiltration primarily in the squamous epithelium of the vestibule, with thickening of the epithelium leading to loss of the characteristic surface grooves. Both cell size and number were reported to be increased. Lesions were generally found to increase in nature and severity with increased time and level of exposure. Obstruction of the nasal vestibule was thought to account for the mortality of animals in the higher dose groups. In female mice at 13 weeks, all glutaraldehyde dose groups showed the accumulation of eosinophilic proteinaceous deposits in the respiratory epithelium of the maxilloturbinate margin. Examination of unit length labeling indices as a measure of growth showed significant increases in all treated groups of female mice. No evidence of exposure related lesions was found in the respiratory tract in the trachea, carina, bronchi, or lungs.

Mean Subjective Pathology Scores for Nasal Lesions in Female Mice at 13 Weeks

| | *Glutaraldehyde* | *Intraepithelial neutrophils* | *Subepithelial neutrophils* | *Squamous metaplasia* |
|---|---|---|---|---|
| | 0 ppb | 0 | 0.4 | 0 |
| | 62.5 ppb | 2.0 | 2.0 | 0 |
| | 125 ppb | 2.4 | 2.8 | 0 |
| | 250 ppb | 3.2 | 3.2 | 0 |
| | 500 ppb | 2.8 | 2.8 | 0.5 |
| | 1000 ppb* | -- | -- | -- |

*Animals exposed to 1000 ppb died early in the experiment.

Greenspan *et al.* (1985) exposed male and female F-344 rats to 0, 0.3, 1.1 and 3.1 ppm glutaraldehyde and 0, 0.2, 0.63, and 2.1 ppm glutaraldehyde, respectively, in a 9-day study, and both sexes to 0, 21, 49, and 194 ppb glutaraldehyde in a 14 week study. Animal numbers were not specified. Exposures were conducted for 6 hours per day, 5 days per week. In the 9-day study, observations in the high and intermediate dose level groups included reduced body weight gain, inflammation of the nasal and olfactory mucosa, and sensory irritation. In the two highest doses of the 14-week study, statistically significant differences in body weight gain were observed as well as perinasal wetness. No histopathological indication of inflammation in olfactory or nasal mucosa was observed.

Mice were exposed to 0, 0.3, 1.0, and 2.6 ppm glutaraldehyde vapors for 6 hours/day for 4, 9, or 14 days (Zissu *et al.*, 1994). These mice were killed immediately after the exposure period. Other groups exposed to 1.0 ppm for 14 days were killed after recovery periods of 1, 2, and 4 weeks. After 4 days of exposure to the lowest dose, mice showed lesions in the respiratory epithelium of the septum, and the naso- and maxilloturbinates. After exposure to 1.0 ppm glutaraldehyde, lesions were still judged as severe after 2 weeks of recovery.

A study comparing the effects of intra-nasally instilled glutaraldehyde and formaldehyde on rat nasal epithelium found inflammation, epithelial degeneration, respiratory epithelial hypertrophy, and squamous metaplasia in treated animals (St. Clair *et al.*, 1990). Acute inhalation exposure to formaldehyde produced identical lesions. Ten-fold higher concentrations of instilled formaldehyde were required to produce the same effect as instilled glutaraldehyde.

In a chronic study, NTP (1998, 1999) exposed groups of 50 male and 50 female F344/N rats to 0, 250, 500, or 750 ppb glutaraldehyde vapor by inhalation for 6 h/day, 5 days/week, for 104 weeks. Survival of 500 and 750 ppb female rats was less than that of the chamber controls. Mean body weights of all exposed groups of male rats and 500 and 750 ppb female rats were generally less than those of the chamber controls. Increased incidences of nonneoplastic nasal lesions occurred primarily within the anterior section of the nose in 500 and 750 ppb rats and to a lesser extent in 250 ppb rats. The more significant lesions included hyperplasia and inflammation of the squamous and respiratory epithelia and squamous metaplasia of the respiratory epithelium. Thus 250 ppb (1000 $\mu g/m^3$) is a chronic LOAEL for rats.

In the same study NTP (1998, 1999) exposed groups of 50 male and 50 female B6C3F1 mice to 0, 62.5, 125, or 250 ppb glutaraldehyde vapor by inhalation for 6 h/day, 5 days/week, for 104

weeks. Survival of exposed mice was similar to that of the chamber controls. Mean body weights of female mice exposed to 250 ppb were generally less than those of the controls. The incidence of inflammation of the nose was marginally increased in 250 ppb females. Incidences of squamous metaplasia of the respiratory epithelium were increased in 250 ppb males and females and 125 ppb females. Incidences of hyaline degeneration of the respiratory epithelium were increased in all exposed groups of females. Thus 62.5 ppb was a chronic LOAEL for female mice.

Incidence of Nasal Lesions in Female Mice exposed for 104 weeks

| | *Glutaraldehyde* | *Inflammation* | *Respiratory epithelium hyaline degeneration* | *Respiratory epithelium squamous metaplasia* |
|---|---|---|---|---|
| | 0 ppb | 6/50 | 16/50 | 7/50 |
| | 62.5 ppb | 7/49 | 35/49 | 11/49 |
| | 125 ppb | 13/50 | 32/50 | 16/50 |
| | 250 ppb | 14/50 | 30/50 | 21/50 |

## VI. Derivation of Chronic Reference Exposure Level (REL)

| | |
|---|---|
| *Study* | NTP 1998, 1999 |
| *Study population* | Male and female F344 rats and B6C3F1 mice (50/sex/group) |
| *Exposure method* | Continuous inhalation exposure (0, 62.5, 125, and 250 ppb in mice; 0, 250, 500, or 750 ppb in rats) |
| *Critical effects* | Respiratory epithelium squamous metaplasia |
| *LOAEL* | 62.5 ppb (female mice) |
| *NOAEL* | Not observed |
| *$BMC_{05}$* | 20.5 ppb |
| *Exposure continuity* | 6 hr/day, 5 days/week |
| *Exposure duration* | 104 weeks |
| *Equivalent continuous exposure* | 3.7 ppb (20.5 x 6/24 x 5/7) |
| *Human equivalent concentration* | 0.62 ppb (gas with extrathoracic respiratory effects, RGDR = 0.17, BW = 28 g, MV = 0.032 L/min, SA = 3 $cm^2$) |
| *LOAEL uncertainty factor* | not needed in BMC approach |
| *Subchronic uncertainty factor* | 1 |
| *Interspecies uncertainty factor* | 3 |
| *Intraspecies uncertainty factor* | 10 |
| *Cumulative uncertainty factor* | 30 |
| *Inhalation reference exposure level* | 0.02 ppb (0.08 $\mu g/m^3$) |

Several studies indicate that the upper respiratory tract is a target for the toxicity of glutaraldehyde from inhalation exposure. Reports of toxicity to humans show that exposure can

lead to occupational asthma as well as cause irritation of the eyes and nose with accompanying rhinitis. Likewise, animals exposed to glutaraldehyde by the inhalation route show evidence of respiratory irritation with the induction of lesions of the anterior nasal cavities upon long-term exposure (Gross *et al.*, 1994; Greenspan *et al.*, 1985; NTP, 1998, 1999). The NTP (1998, 1999) study yielded a chronic LOAEL for female mice of 62.5 ppb. Gross *et al.* (1994) showed neutrophilic infiltration in the olfactory epithelium in the lowest dose exposure group. (Female mice exposed to 62.5 ppb also showed subepithelial neutrophilic infiltration.) This level was taken to be the subchronic LOAEL. This effect on the nasal epithelium was demonstrated to be both concentration- and exposure duration-dependent.

A benchmark concentration was determined using EPA's version 1.20 BMC software and the dose-response data on respiratory epithelium squamous metaplasia in female mice. The quantal-linear model gave an $MLE_{05}$ of 31.24 ppb, a $BMC_{05}$ of 20.51 ppb, and a p value of 0.9471. With the benchmark approach no LOAEL UF is needed. The study was a lifetime study so the subchronic UF is 1. An interspecies UF of 3 rather than 10 was used since an RGDR adjustment had been made. The default intraspecies UF of 10 was used so that the total UF was 30. The resulting chronic REL for glutaraldehyde is 0.02 ppb (0.08 $\mu g/m^3$).

For comparison with the proposed REL, the study of Gross *et al*. (1994) used 62.5 ppb continuous exposure. Multiplying by the RGDR of 0.17 and dividing by a cumulative uncertainty factor of 300 (3 for a LOAEL, 3 for subchronic, 3 for interspecies, and 10 for intraspecies) results in a REL of 0.035 ppb (0.1 $\mu g/m^3$).

## VII. Data Strengths and Limitations for Development of the REL

The major strength of the inhalation REL for glutaraldehyde is the availability of controlled exposure inhalation studies in multiple species at multiple exposure concentrations and with adequate histopathogical analysis. Major areas of uncertainty are the lack of human data, the lack of reproductive and developmental toxicity studies, the lack of dermal sensitization studies, and the lack of observation of a NOAEL.

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*CHRONIC TOXICITY SUMMARY*

# *n*-HEXANE

*(normal hexane)*

**CAS Registry Number: 110-54-3**

## I. Chronic Toxicity Summary

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

## II. Physical and Chemical Properties (HSDB, 1999)

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

## III. Major Uses or Sources

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

## IV. Effects of Human Exposure

In an offset printing factory with 56 workers, symptomatic peripheral neuropathy was noted in 20 of 56 (36%) workers, while another 26 (46%) had evidence of subclinical neuropathy (Chang *et al*., 1993). Reduced sensory action potentials; reduced motor action potentials; decreased motor nerve conduction velocity; and increased distal latency were found in most workers. Giant axonal swellings with accumulation of 10 nm neurofilaments, myelin sheath attenuation, and widening of nodal gaps were noted upon sural nerve biopsy of a severe case. Optic neuropathy and CNS impairment were not usually found. Personal air samples had 80 to 210 ppm hexane (mean = 132 ppm), 20 to 680 ppm isopropanol (mean = 235 ppm), and 20 to 84 ppm (mean = 50 ppm) toluene. The workers worked 12 hours per day for 6 days per week. The mean duration of employment was 2.6 years, with a range of 1 month to 30 years.

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

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

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

Fifteen (25%) of 59 press proofing workers had polyneuropathy (Wang *et al*., 1986). All of the patients with polyneuropathy were regularly exposed to n-hexane, and there was a significant association between n-hexane concentration and prevalence of polyneuropathy. The ambient concentration of n-hexane of 190 ppm was found in one factory in which all six workers

developed polyneuropathy. Workers exposed to less than 100 ppm n-hexane who frequently worked overtime demonstrated significant decreases in motor nerve conduction velocities in median, ulnar, and peroneal nerves. Twelve of 13 workers who regularly slept in the factory had polyneuropathy compared to three (7%) of 46 employees who did not sleep in the factory.

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

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

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

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

## V. Effects of Animal Exposure

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

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

Fischer 344 rats (5/sex/dose) inhaled >99.5% pure n-hexane (0, 3000, 6500, or 10,000 ppm) for 6 hours/day, 5 days/week over 13 weeks (Cavender *et al*., 1984). No statistically significant differences were notes in food consumption, ophthalmologic examination, neurological function, or hematological or serum chemistry parameters in either males or females. Female body weights and clinical observations were unaltered by hexane treatment. The mean body weight gain of male rats in the 10,000-ppm group was significantly decreased compared with controls at 4 weeks of exposure and thereafter. Axonopathy was noted in the tibial nerve of four of five male rats exposed to 10,000 ppm and in one of five male rats exposed to 6500 ppm. Axonopathy in the medulla was noted in one male rat exposed to 10,000-ppm. Males inhaling 10,000 ppm had slightly but significantly lower brain weights. No other adverse histopathological effects were reported. This study identifies a NOAEL for neurotoxicity of 3000 ppm, with an average experimental exposure of 540 ppm.

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

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

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

A dose-dependent decrease in motor nerve conduction velocity and body weight gain was observed in rats exposed to 500, 1200, or 3000 ppm n-hexane for 12 hours per day, 7 days per week for 16 weeks (Huang *et al.*, 1989). The neurotoxicity was significant in the two highest exposure groups; peripheral nerve degeneration, characterized by paranodal swellings and demyelination and remyelination in the myelinated nerve fibers, was observed and was more advanced in the highest exposure group.

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

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

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

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

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

## VI. Derivation of Chronic Reference Exposure Level

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

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

The Sanagi study, which U.S. EPA used as the basis of its RfC, may overestimate hexane effect because of a confounding coexposure to acetone, which is known to potentiate hexane neuropathy. The minimum effective acetone inhalation concentration for potentiating hexane neuropathy is unclear, as studies (Ladefoged et al., 1994; Lam et al., 1991; Larsen et al., 1991) have used orally administered acetone. The minimum effective acetone inhalation dose for potentiation of carbon tetrachloride hepatotoxicity in male Sprague-Dawley rats was 2500 ppm over 4 hours (Charbonneau et al., 1986). A dose of 0.5% acetone in human drinking water is comparable, assuming equal absorption, to an inhalation concentration of approximately 1400 ppm (0.5% w/v x 2 L/day ÷ 2 $m^3$/day = 5 g/$m^3$; 5 g/$m^3$ x 1000 mg/g ÷ 3.52 mg/$m^3$ per ppm = 1400 ppm). As the acetone potentiating effects were all noted at higher exposures than are being considered in occupational studies and are at much higher concentrations than the REL itself, the significance of these findings is uncertain.

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

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

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

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

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

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

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

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

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

## VII. Data Strengths and Limitations for Development of the REL

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

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

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

The major strengths of the REL for hexane include (1) the primary use of an animal study (Miyagaki, 1967) with controlled, nearly continuous chronic hexane exposures not confounded by coexposure to other solvents, which observed both a NOAEL and LOAEL; and (2) the results obtained from two different human studies (Sanagi, 1980; Chang et al., 1993) which were viewed as being generally consistent with the animal study based REL.
There is uncertainty about interspecies as well as intraindividual differences in susceptibility to n-hexane peripheral neuropathy. In one study, controlled TWA exposures of 540 ppm (Cavender

*et al*., 1984) were not found to cause neuropathy in rats. Also human studies (especially that of Chang *et al*., 1993) have shown that some individuals develop peripheral neuropathy within months, whereas others remain symptom-free despite years of employment at the same occupation at the same workplace.

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

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*CHRONIC TOXICITY SUMMARY*

# HYDRAZINE

*(diamine; diamide; nitrogen hydride; levoxine)*

**CAS Registry Number: 302-01-2**

## I. Chronic Toxicity Summary

| | |
|---|---|
| *Inhalation reference exposure level* | **0.2 µg/m$^3$** (0.1 ppb) |
| *Critical effect(s)* | Amyloidosis of the liver and thyroid in hamsters |
| *Hazard index target(s)* | Alimentary system; endocrine system |

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

| | |
|---|---|
| *Description* | Colorless, oily liquid or white crystals |
| *Molecular formula* | $N_2H_4$ |
| *Molecular weight* | 32.05 g/mol |
| *Boiling point* | 113.5°C (Merck, 1983; CRC, 1994) |
| *Melting point* | 2.0°C |
| *Vapor pressure* | 14.4 torr @ 25°C |
| *Solubility* | Miscible with water, methyl-, ethyl-, isobutyl alcohols; slightly miscible with hydrocarbons; insoluble in chloroform, ether |
| *Conversion factor* | 1.31 µg/m$^3$ per ppb at 25ºC |

## III. Major Uses and Sources

Hydrazine is a highly reactive base and reducing agent. Its primary uses are as a high-energy rocket propellant, as a reactant in military fuel cells, in nickel plating, in the polymerization of urethane, for removal of halogens from wastewater, as an oxygen scavenger in boiler feedwater to inhibit corrosion, and in photographic development (Von Burg and Stout, 1991). Hydrazine was historically used experimentally as a therapeutic agent in the treatment of tuberculosis, sickle cell anemia, and non-specific chronic illnesses (Von Burg and Stout, 1991; Gold, 1987). The annual statewide industrial emissions from facilities reporting under the Air Toxics Hot Spots Act in California based on the most recent inventory were estimated to be 1664 pounds of hydrazine (CARB, 2000).

## IV. Effects of Human Exposure

One person was occupationally exposed to hydrazine at unknown levels once per week for a period of 6 months (Sotaniemi *et al.*, 1971). The worker showed symptoms of conjunctivitis, tremors, and lethargy for 1-2 days following each exposure. Vomiting, fever, and diarrhea developed on the last day of exposure and progressed to abdominal pain and incoherence. The previously healthy 59-year old individual died three weeks after the last exposure. Evidence of tracheitis, bronchitis, heart muscle degeneration, and liver and kidney damage was found at autopsy. A single case report can not prove a cause and effect relationship between hydrazine exposures and the noted symptoms and death, but the repeated association between exposures and symptoms is highly suspicious. Liver toxicity is also associated with acute exposure to hydrazine.

The only epidemiological studies of human hydrazine exposures found involve workers in a hydrazine manufacturing plant (Wald *et al*., 1984; Wald, 1985; Morris *et al*., 1995). Workers were exposed to various durations of at least 6 months between 1945 and 1972 and have been followed through 1992. The studies are based on a review of medical records. Only 78 of 427 workers were believed to have had more than incidental exposure to hydrazine. Only cumulative mortality was reviewed. Health effects reported during or after hydrazine exposure were not examined. No increase in mortality was noted for lung cancer, other cancers, or causes other than cancer. However, these small studies have little power to detect increased mortality, and age of death was not examined. The authors reported that relative risks up to 3.5 could have gone undetected.

Dermal sensitization has also been reported from repeated contact with hydrazine (Van Ketal, 1964; Von Keilig and Speer, 1983; Wrangsjo and Martensson, 1986).

## V. Effects of Animal Exposure

An inhalation study of the toxicity and carcinogenicity of hydrazine was conducted in cats, mice, hamsters, and dogs (Vernot *et al.*, 1985). Various animal groups were exposed 6 hours/day, 5 days/weeks for one year to concentrations of 0.05, 0.25, 1.0, and 5.0 ppm anhydrous hydrazine base. Exposed and controls groups were made up of the following animals: 100 Fischer 344 rats/sex at 0.05, 0.25, 1.0, and 5.0 ppm hydrazine plus 150 rats/sex as controls; 400 female C57BL/6 mice at 0.05, 0.25, and 1.0 ppm hydrazine plus 800 female mice as controls; 200 male Golden Syrian hamsters at 0.25, 1.0, and 5.0 ppm hydrazine plus 200 male hamsters as controls; 4 beagle dogs/sex at 0.25 and 1.0 ppm hydrazine plus 4 dogs/sex as controls. Animals were observed post-exposure for the following periods: 18 months for rats, 15 months for mice, 12 months for hamsters, and 38 months for dogs. Animals were observed hourly during the exposure period and daily in the post-exposure period.

No non-cancer toxic effects were observed in mice or dogs, with the exception of a single dog, exposed to 1.0 ppm hydrazine, which showed cyclic elevations in serum glutamic-pyruvic transaminase levels and, upon necropsy at 36 months post-exposure, showed liver effects described as “clusters of swollen hepatocytes that had highly vacuolated cytoplasm.” Of the

other species examined, hamsters showed toxicity at the lowest dose levels, particularly amyloidosis in various organs including liver, spleen, kidney, thyroid, and adrenal glands. An increased incidence of amyloidosis was seen at the lowest exposure level (0.25 ppm hydrazine) in the liver and thyroid (67/160 exposed vs. 42/180 control for the liver and 20/117 exposed vs. 9/155 control in the thyroid; $p \leq 0.01$ by Fisher's exact test). This effect was found to be dose related. The incidence of hemosiderosis of the liver was also significantly increased in all exposed groups. Significantly increased incidences of toxic effects observed in the 1.0 and 5.0 ppm hydrazine groups include amyloidosis of the spleen, kidney glomerulus, and adrenals glands, and lymphadenitis of the lymph nodes. Significantly increased toxic effects observed only in the highest dose group include amyloidosis of the kidney interstitium and thyroid, and senile atrophy of the testis. The authors note these effects appear to reflect accelerated changes commonly associated with aging in hamsters.

Incidence of Nonneoplastic Lesions in Male Hamsters (from Table 3 of Vernot *et al*.)

| *Lesion* | *Control* | *0.25 ppm* | *1.0 ppm* | *5.0 ppm* |
|---|---|---|---|---|
| Liver | | | | |
| Amyloidosis | 42/180 (23)* | 67/160 (42)[a] | 68/148 (46)[a] | 79/159 (50)[a] |
| Hemosiderosis | 42/180 (23) | 63/160 (39)[a] | 77/148 (52)[a] | 94/159 (59)[a] |
| Bile duct hyperplasia | 14/180 (8) | 31/160 (19)[a] | 28/148 (19)[a] | 44/159 (28)[a] |
| Biliary cyst | 45/180 (25) | 45/160 (28) | 42/148 (28) | 55/159 (35)[b] |
| Thyroid | | | | |
| Amyloidosis | 9/155 (6) | 20/117 (17)[a] | 11/127 (9) | 22/137 (16)[a] |
| Adrenal | | | | |
| Amyloidosis | 38/177 (22) | 49/199 (32)[b] | 52/141 (37)[a] | 76/153 (50)[a] |

* Incidence of lesion (% of animals with lesion)
[a] Incidence significantly greater than control, $p \leq 0.01$
[b] Incidence significantly greater than control, $0.01 < p \leq 0.05$

In the hydrazine exposed rats, effects were observed in the respiratory tract of exposed animals. Specifically, squamous metaplasia of the larynx, trachea, and nasal epithelium (males only) was observed in the highest dose group (5.0 ppm hydrazine). Inflammation was also observed in the larynx and trachea of rats exposed to 5.0 ppm hydrazine. Increased incidence of focal cellular change of the liver was observed in female mice at 1.0 and 5.0 ppm hydrazine. Other effects with increased incidence only in the high dose group include hyperplastic lymph nodes in females, endometriosis, and inflammation of the uterine tube.

The toxic effects from inhalation of hydrazine over a six month period from both intermittent and continuous exposure scenarios were examined (Haun and Kinkead, 1973). Groups of 8 male beagle dogs, 4 female rhesus monkeys, 50 male Sprague-Dawley rats, and 40 female ICR rats per dose group were continuously exposed to 0.2 or 1.0 ppm hydrazine or intermittently (6 hours/day, 5 days/week) to 1.0 or 5.0 ppm hydrazine. A control group consisted of equal numbers of animals. The experimental design was such that each intermittent exposure group had a time-weighted-average matching continuous exposure group. Dose-related body weight reductions were observed in all treated groups as well as evidence of hepatic degeneration, fatty

deposition in the liver, central nervous system depression and lethargy, eye irritation, and anemia.

Toxic effects from the exposure of rats, mice, and dogs to airborne hydrazine at levels of 0, 4.6, or 14 ppm intermittently for 6 months were reported (Comstock *et al.*, 1954). Observed adverse effects included anorexia, irregular breathing, vomiting, fatigue, and emphysema in dogs; pulmonary congestion and emphysema in rats and mice; and lung and liver damage in rats.

Lymphoid bronchial hyperplasia was observed in guinea pigs exposed to 2-6 ppm hydrazine for 5 days/week for 19-47 days (Weatherby and Yard, 1955).

## VI. Derivation of Chronic Reference Exposure Level (REL)

| | |
|---|---|
| *Study* | Vernot *et al.*, 1985 |
| *Study population* | Hamster |
| *Exposure method* | Inhalation of 0, 0.25, 1, and 5 ppm |
| *Critical effects* | Amyloidosis and hemosiderosis of the liver; thyroid amyloidosis |
| *LOAEL* | 0.25 ppm |
| *NOAEL* | Not observed |
| *Exposure continuity* | 6 hour/day, 5 days/week |
| *Exposure duration* | 1 year |
| *Average experimental exposure* | 0.045 ppm for LOAEL group (0.25 x 6/24 x 5/7) |
| *Human equivalent concentration* | 0.045 ppm for LOAEL group (gas with systemic effects, based on RGDR = 1.0 using default assumption that lambda (a) = lambda (h)) |
| *LOAEL uncertainty factor* | 10 (low incidence above controls but serious adverse effects) |
| *Subchronic uncertainty factor* | 1 |
| *Interspecies uncertainty factor* | 3 |
| *Intraspecies uncertainty factor* | 10 |
| *Cumulative uncertainty factor* | 300 |
| *Inhalation reference exposure level* | 0.0001 ppm (0.1 ppb, 0.0002 mg/m$^3$, 0.2 µg/m$^3$ ) |

Vernot *et al.*(1985) present a thorough examination of chronic health effects from inhalation exposure to hydrazine. This study was chosen for the development of the chronic reference exposure level because (1) it was conducted with an adequate number of animals, (2) the critical/sensitive adverse effect (degenerative change in the liver in hamsters) showed a dose-response relationship, and (3) the findings of this study support data found in studies by other groups.

This study shows a dose-related increase in the incidence of amyloidosis and hemosiderosis in hamsters intermittently exposed by inhalation to levels of hydrazine greater than 0.25 ppm. Other effects noted at 0.25 ppm included weight depression during exposure, mineralization of the kidney, and amyloidosis of the thyroid. Haun and Kinkead (1973) have also noted lesions of the

liver in dogs, monkeys, and mice exposed continuously to 0.2 ppm hydrazine for 6 months by inhalation. Comstock *et al.* (1954) observed liver damage in groups of rats exposed to hydrazine vapors. The single case report of hydrazine inhalation toxicity in humans showed necrosis and degeneration of the liver (Sotaniemi *et al.*, 1971).

## VII. Data Strengths and Limitations for Development of the REL

The strengths of the inhalation REL for hydrazine include the availability of chronic inhalation exposure data from a well-conducted study with histopathological analysis. Major areas of uncertainty are the lack of adequate human exposure data, the lack of reproductive and developmental toxicity studies, and the lack of observation of a NOAEL in the key study.

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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

| | |
|---|---|
| *Inhalation reference exposure level* | **9 µg/m$^3$ (6 ppb)** |
| *Critical effect(s)* | Hyperplasia of nasal mucosa, larynx, and trachea in rats |
| *Hazard index target(s)* | Respiratory system |

## II. Physical and Chemical Properties (HSDB, 1999)

| | |
|---|---|
| *Description* | Colorless gas |
| *Molecular formula* | HCl |
| *Molecular weight* | 36.46 |
| *Density* | 1.49 g/L @ 25° C |
| *Boiling point* | -84.9° C (HCl gas) |
| *Melting point* | -114.8° C (HCl gas) |
| *Solubility* | Soluble in water, alcohol, benzene, ether; insoluble in hydrocarbons |
| *Conversion factor* | 1 ppm = 1.49 mg/m$^3$ at 25°C |

## III. Major Uses or Sources

Hydrogen chloride (HCl) is used in the manufacture of vinyl chloride, fertilizers, dyes, artificial silk, and pigments for paints. It is also used in electroplating, soap refining, and leather tanning. Other consumers of HCl include the photographic, textile and rubber industries (HSDB, 1999).

Hydrogen chloride is produced in large quantities during combustion of most materials and especially materials with a high chlorine content. Thus, HCl is a major product formed during the thermal decomposition of polyvinyl chloride, a commonly used plastic polymer (Burleigh-Flayer *et al.*, 1985). It is also released in large quantities during the test firing of some rocket and missile engines (Wohlslagel *et al.*, 1976). Since HCl is extremely hygroscopic, it generally exists as an aerosol in the ambient atmosphere. The annual statewide industrial emissions from facilities reporting under the Air Toxics Hot Spots Act in California based on the most recent inventory were estimated to be 2,570,888 pounds of HCl (CARB, 1999b).

## IV. Effects of Human Exposure

Few reports are available on the effects of chronic HCl exposure on humans. Bleeding of the nose and gums and ulceration of the mucous membranes was observed following repeated occupational exposure to HCl mist at high but unquantified concentrations (Stokinger, 1981).

In another report, workers exposed to various mineral acids, including HCl, exhibited etching and erosion of the front teeth (Ten Bruggen Cate, 1968). Dental erosion was noted in 176 of 555 (32%) workers examined between 1962 and 1964, and progressive erosion was reported in 66 of 324 (20%) workers examined repeatedly. Rates of active erosion were highest (50%) in the most highly-exposed category (battery formation workers), intermediate (23%) in an intermediate-exposure category (picklers), and low (7%) in a low-exposure category (other processes). Grade 1 erosion (enamel loss) was noted in workers exposed for greater than 3 months; grade 2 erosion (loss of enamel and dentine) was noted after 2.5 to 5 years exposure; and grade 3 (loss of enamel and dentine with exposure of secondary dentine) was noted after six or more years of exposure.

## V. Effects of Animal Exposure

Male Sprague-Dawley rats were exposed to 10 ppm HCl for 6 hours per day, 5 days per week over their lifetime (Sellakumar *et al.*, 1985). No differences in body weights or survival were observed between 99 exposed and 99 control animals. Increased incidences of hyperplasia of the nasal mucosa (62/99 vs. 51/99), larynx (22/99 vs. 2/99), and trachea (26/99 vs. 2/99) were observed in exposed rats compared to air-exposed controls.

A 90-day inhalation study using B6C3F1 mice and Sprague-Dawley and Fisher 344 rats exposed the animals (groups of 31 males and 31 females for each species and strain) to 10, 20, or 50 ppm HCl for 6 hours per day, 5 days per week over 90 days (Toxigenics, 1984). Several animals died during the study, though the deaths were not considered to be exposure related. A slight but significant decrease in body weight gain was reported in male and female mice and in male Fischer 344 rats in the high-exposure groups. No effect were noted in hematology, clinical chemistry, or urinalysis. Minimal or mild rhinitis was observed in both strains of rats Concentration- and time-related lesions were noted in the anterior portion of the nasal cavity of exposed rats. Cheilitis, eosinophilic globules in the nasal epithelium and accumulation of macrophages in the peripheral tissues were observed in mice of all exposed groups. This study thus observed a LOAEL for both mice and rats of 10 ppm. The U.S. EPA considered this study supportive of the portal-of-entry effects observed at 10 ppm in the lifetime rat study (USEPA, 1999). Female rats (8-15/group) exposed to 302 ppm HCl for 1 hour either 12 days prior to mating or on day 9 of gestation exhibited severe dyspnea and cyanosis; the exposure was lethal to one-third of the exposed animals (Pavlova, 1976). Fetal mortality was significantly higher in rats exposed during pregnancy. Organ functional abnormalities observed in offspring exposed at 2-3 months of age were reported to be similar to those observed in the exposed dams.

Female rats were exposed to 302 ppm HCl for 1 hour prior to mating (GEOMET Technologies, 1981). Exposure killed 20 to 30% of the rats. In rats surviving 6 days after exposure, a decrease in blood oxygen saturation was reported, as were kidney, liver, and spleen effects. Estrus cycles

were also altered. In rats mated 12-16 days postexposure and killed on day 21 of pregnancy, a decrease in fetal weight, an increase in relative fetal lung weights, and reduced numbers of live fetuses were observed.

**Derivation of Chronic Reference Exposure Level**

| | |
|---|---|
| *Study* | Sellakumar *et al.*, 1985 |
| *Study population* | Sprague-Dawley rats (100 males) |
| *Exposure method* | Discontinuous whole-body inhalation (0 or 10 ppm) |
| *Critical effects* | Hyperplasia of the nasal mucosa, larynx and trachea |
| *LOAEL* | 10 ppm |
| *NOAEL* | Not identified |
| *Exposure continuity* | 6 hours per day, 5 days per week |
| *Average experimental exposure* | 1.8 ppm for LOAEL group |
| *Human equivalent concentration* | 0.57 ppm (gas with extrathoracic respiratory effects, RGDR =0.32, based on rat $MV_a$ = 0.33 L/min, $MV_h$ = 13.8 L/min, $SA_a(ET)$ = 15 $cm^2$); $Sa_h$ = 200 $cm^3$) (U.S. EPA, 1994) |
| *Exposure duration* | Lifetime |
| *LOAEL uncertainty factor* | 3 (<30% incidence; mild effect) |
| *Subchronic uncertainty factor* | 1 |
| *Interspecies uncertainty factor* | 3 |
| *Intraspecies uncertainty factor* | 10 |
| *Cumulative uncertainty factor* | 100 |
| *Reference Concentration (RfC)* | 0.006 ppm (6 ppb; 0.009 $mg/m^3$; 9 $\mu g/m^3$) |

Both extrathoracic and tracheobronchial effects have been associated with exposures to hydrogen chloride. The REL was based on extrathoracic effects as humans are predicted to be relatively more susceptible to the effects of hydrogen chloride in that region. An intermediate LOAEL factor was used as the effects were both mild and occurring at a low incidence at the dose tested.

## VII. Data Strengths and Limitations for Development of the REL

The USEPA based its RfC of 7 $\mu g/m^3$ on the same study. U.S. EPA evaluated this RfC as a having a low level of confidence because of (1) the use of only one dose; (2) limited toxicity evaluation; (3) the lack of reproductive toxicity data; and (4) the lack of chronic exposure studies (U.S. EPA, 1994). OEHHA agrees with this assessment. The database for chronic exposure to this common chemical is limited.

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*CHRONIC TOXICITY SUMMARY*

# HYDROGEN CYANIDE

*(Formonitrile; hydrocyanic acid; prussic acid)*

**CAS Registry Number: 74-90-8**

## I. Chronic Toxicity Summary

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

## II. Physical and Chemical Properties (HSDB, 1999 )

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

## III. Major Uses or Sources

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

Another common source of hydrogen cyanide is cigarette smoke. Levels in inhaled mainstream cigarette smoke range from 10 to 400 µg per cigarette (U.S. brands); 0.6% to 27% (w/w) of these mainstream levels are found in secondary or sidestream smoke (Fiskel *et al.*, 1981). The annual statewide industrial emissions from facilities reporting under the Air Toxics Hot Spots Act in

California based on the most recent inventory were estimated to be 188,665 pounds of hydrogen cyanide (CARB, 1999b).

## IV. Effects of Human Exposure

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

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

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

increases in thyroid-stimulating hormone levels, which in combination with the CNS effects, suggest long-term adverse effects associated with cyanide exposure.

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

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

## V. Effects of Animal Exposures

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

demonstrated extensive CNS toxicity, including dyspnea and vomiting, with vascular and cellular CNS lesions identified post-mortem (Valade, 1952).

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

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

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

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

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

## VI. Derivation of Chronic Reference Exposure Level

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

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

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

| | |
|---|---|
| *Study* | Jackson (1988) |
| *Study population* | Miniature swine |
| *Exposure method* | Daily oral administration of aqueous potassium cyanide |
| *Critical effects* | Behavioral effects; decreased blood $T_3$ and $T_4$ |
| *LOAEL* | 0.4 mg/kg-day |
| *NOAEL* | Not observed |
| *Exposure continuity* | Apparently 7 days per week |
| *Average exposure* | 0.4 mg/kg-day (1.4 mg/m$^3$ for LOAEL group assuming 20 m$^3$/day inhalation by a 70 kg person) |
| *Human equivalent concentration* | Not derived due to lack of species-specific data |
| *Exposure duration* | 24 weeks |
| *LOAEL uncertainty factor* | 3 (minimal effects at lowest dose) |
| *Subchronic uncertainty factor* | 10 (based on assumed 27 year lifespan) |
| *Interspecies uncertainty factor* | 10 |
| *Intraspecies uncertainty factor* | 10 |
| *Cumulative uncertainty factor* | 3,000 |
| *Inhalation reference exposure level* | 0.0005 mg/m$^3$ (0.5 µg/m$^3$; 0.0004 ppm; 0.4 ppb) |

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

## VII. Data Strengths and Limitations for Development of the REL

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

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*CHRONIC TOXICITY SUMMARY*

# HYDROGEN SULFIDE

*(hydrogen sulphide; dihydrogen sulfide; dihydrogen monosulfide; sulfur hydride; sulfureted hydrogen; hydrosulfuric acid)*
**CAS registry number: 7783-06-4**

## I. Chronic Toxicity Summary

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

## II. Physical and Chemical Properties (HSDB, 1999)

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

## III. Major Uses or Sources

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

## IV. Effects of Human Exposure

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

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

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

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

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

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

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

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

## V. Effects of Animal Exposure

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

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

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

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

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

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

## VI. Derivation of Chronic REL

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

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

## VII. Data Strengths and Limitations for Development of the REL

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

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

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

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

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

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

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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

| | |
|---|---|
| *Inhalation reference exposure level* | **2,000 μg/m$^3$** (400 ppb) |
| *Critical effect(s)* | Developmental effects (reduced crown-rump length of female rat fetuses); hepatocytomegaly and coagulative necrosis of the liver in mice |
| *Hazard index target(s)* | Development; liver |

## II. Chemical Property Summary (HSDB, 1995; CRC, 1994; CARB, 1997)

| | |
|---|---|
| *Description* | Water-clear liquid with a peppermint-like odor |
| *Molecular formula* | $C_9H_{14}O$ |
| *Molecular weight* | 138.21 g/mol |
| *Boiling point* | 215.2°C |
| *Melting point* | –8.1°C |
| *Vapor pressure* | 0.44 torr at 25°C |
| *Solubility* | Slightly soluble in water (12,000 mg/L water at 25°C); miscible in organic solvents. |
| *Conversion factor* | 5.65 μg/m$^3$ per ppb at 25°C |

## III. Major Uses and Sources

Isophorone is used extensively as a solvent in some printing inks, paints, lacquers, adhesives, vinyl resins, copolymers, coatings, finishes, and pesticides, in addition to being used as a chemical intermediate (HSDB, 1995). Since this compound has many different applications, release to the environment may originate from a wide variety of industrial sources including iron and steel manufacturers, manufacturers of photographic equipment and supplies, automobile tire plants, and printing operations. Coal-fired power plants may also emit isophorone to the air. Although it is mostly a man-made compound, isophorone has been found to occur naturally in cranberries (ATSDR, 1989). Occupational exposure may occur by inhalation or dermal contact. The annual statewide industrial emissions from facilities reporting under the Air Toxics Hot Spots Act in California based on the most recent inventory were estimated to be 2809 pounds of isophorone (CARB, 2000).

## IV. Effects of Human Exposures

No information is available concerning long-term exposure or pharmacokinetics of isophorone in humans. In occupational monitoring studies, the time-weighted average concentration in breathing zones and workplace air of a screening plant ranged from 8.3-23 ppm and from 3.5-14.5 ppm, respectively (Samimi, 1982). Up to 25.7 ppm was detected in air of a silk screening printing plant in Pittsburgh, PA (Kominsky, 1983). The concentration in breathing zone samples from a decal manufacturing plant in Ridgefield, NJ was 0.7-14 ppm (Lee and Frederick, 1982). It was suspected that the reported eye and nose irritation of workers at the silk screening plant and at the decal manufacturing plant was the result of acute and subacute exposure to isophorone vapors.

Workers exposed to 5-8 ppm (28-45 mg/m$^3$) of isophorone for one month complained of fatigue and malaise (NIOSH, 1978). When concentrations were reduced to 1-4 ppm, no adverse effects were reported. Acute exposure studies in humans (up to 400 ppm for 1 to 4 minutes) resulted in eye, nose and throat irritation, nausea, headache, and dizziness or faintness (Union Carbide, 1963). Inhalation exposure for 15 minutes to 10 ppm isophorone produced only mild effects in human subjects while 25 ppm produced irritation to eyes, nose, and throat (Silverman *et al.*, 1946).

## V. Effects of Animal Exposures

Few reports have been published regarding the pharmacokinetics of isophorone in experimental animals. Isophorone was widely distributed in the major organs of the rat following 4 hour inhalation exposure to 400 ppm (ATSDR, 1989). Oral gavage of 4000 mg/kg body wt to rats and a rabbit also resulted in wide distribution of the chemical. The highest blood levels of isophorone were reached by 30 min in rabbits following oral gavage and had decreased dramatically by 21 hours, indicating rapid absorption and elimination of the chemical. Preliminary results of a pharmacokinetic study indicate that rats treated orally with $^{14}$C-isophorone excreted 93% of the radiolabel in the urine, expired air, and feces in 24 hours (ATSDR, 1989). The highest levels of $^{14}$C-isophorone were found in the liver, kidney, preputial gland, testes, brain, and lungs. Several metabolites were identified in the urine of orally dosed rats and rabbits, including 3-carboxy-5,5-dimethyl-2-cyclohexene-1-one, 3,5,5-trimethylcyclohexanol, and some glucuronide conjugates (Dutertre-Catella *et al.*, 1978). A portion of the chemical was excreted unchanged in expired air.

In an early inhalation study, 10 Wistar rats/group and 10 guinea pigs/group, all of mixed sex, were exposed to 0, 25, 50, 100, 200, or 500 ppm isophorone 8 hr/day, 5 days/week for 6 weeks (Smyth *et al.*, 1942). Increased mortality and reduced body weights were observed at 100 ppm and up in both species. However, eye and nose irritation was noted only at the highest dose. Minor changes in blood chemistry and histopathological changes in the kidney and lungs were noted in treated animals. However, later investigations determined that the isophorone used in this study was contaminated with appreciable amounts of compounds (Rowe and Wolf, 1963).

Therefore, some of the adverse effects (i.e., the lung lesions) may have been due to the contaminants. The accuracy of the concentration data in the 1942 study is also questionable.

No treatment-related histopathological lesions were found in lungs, livers, or kidneys of male and female rats exposed intermittently (6 hr/day, 5 days/week) to 37 ppm isophorone for 4 weeks compared to controls (Hazleton Labs, 1968; summarized by ATSDR, 1989). Histological examination was limited to 30% of the control and treated rats. Body weight gain, mean absolute liver weights, and mean liver-to-body weight ratios of treated rats were significantly reduced compared to controls. Slight variations in hematological findings were noted in treated rats (increased lymphocytes and hemoglobin content; decreased neutrophils) but were not considered different from controls.

Rats (10/sex) were exposed to 500 ppm isophorone 6 hr/day, 5 days/week for up to 6 months (Dutertre-Catella, 1976; summarized by ATSDR, 1989). Irritation of eyes and nasal mucosa was observed. One female and three males in the treatment group died during the study, which was considered to be a treatment-related effect. But no exposure-related histopathological lung or liver lesions were observed compared to controls. Dutertre-Catella (1976) also exposed rats and rabbits (number per group per sex not stated) to 250 ppm isophorone 6 hr/day, 5 days/week for 18 months (Dutertre-Catella, 1976). Irritation of eyes and nasal mucosa was observed in both species, but no deaths occurred in the treatment groups. Histopathological examination of the lungs and kidneys, urinalysis, and hematological analysis revealed no exposure-related changes in either species. However, cytoplasmic microvacuolization of hepatocytes was observed in both species (ATSDR, 1989).

In a 90-day feeding study, 20 CFE albino rats/group/sex were given isophorone in their diet at concentrations of 0, 750, 1500, or 3000 ppm. Four beagle dogs/group/sex received isophorone in gelatin capsules at concentrations of 0, 35, 75, or 150 mg/kg body wt-day (AME, 1972a,b). High dose rats exhibited slightly reduced weight gain compared to controls (8-10%) for most of the study. Average weight gain among the exposure groups of beagle dogs remained essentially unchanged during the entire study. Urinalysis, hematology, and clinical chemistry indices found no treatment-related effects in the animals at either the interim or final toxicological examinations. Gross pathology and a limited histopathological examination observed no treatment-related effects in either species. Data on isophorone purity and possible loss of isophorone from rat diet due to vaporization were not presented.

In the most comprehensive isophorone toxicity study to date, 50 F344/N rats/group/sex and 50 B6C3F1 mice/group/sex were administered 0, 250 or 500 mg isophorone/kg body wt 5 days/week by oral gavage (in corn oil) for 103 weeks (Bucher *et al.*, 1986; NTP, 1986). Clinical signs of toxicity were not seen during the length of the study. However, several deaths in male and female rats at the high dose occurred early in the study. A steep decline in survival rate of high dose male rats occurred after week 90. Male and female rats and female mice in the high dose group exhibited only a slight decrease in body weight (<10%) compared to controls. A 13-week range finding study for the 2-year study did not find compound-related lesions in the kidney (or any other organs) of rats and mice exposed up to 1000 mg/kg body wt-day. However, pathological examination of rats exposed to isophorone for 2 years revealed non-neoplastic lesions in the kidney. Increased mineralization of the collecting ducts in isophorone-exposed

male (but not female) rats was observed. This lesion was characterized by basophilic aggregates of mineral most often found in the medullary collecting ducts and occurred coincidentally with lesions of chronic nephropathy. Nephropathy was observed in almost half the female controls and nearly all the male controls. Isophorone exposure appeared to increase both the severity of nephropathy in low dose male rats and the incidence of nephropathy in dosed female rats, but the effects were not pronounced. However, the isophorone potentiation of nephropathy in rats may be due to 'male rat-specific nephropathy' and may not have any relevance to human exposure (Strasser *et al.* 1988). Other adverse effects in kidneys of isophorone-treated male rats include tubular cell hyperplasia (in a dose-related manner) and epithelial hyperplasia of the renal pelvis. In mice, an increased incidence of chronic focal inflammation was observed in the kidneys of males, but was not considered treatment-related. A dose-dependent increase in fatty metamorphosis occurred in the adrenal cortex of male rats, but the biological significance of this change is unknown. All isophorone-exposed male mice had an increased incidence of hepatocytomegaly and coagulative necrosis of the liver. However, treatment-related liver lesions were not observed in female mice. Increased incidence of hyperkeratosis of the forestomach was observed in dosed male and high dose female mice, but was probably not a relevant treatment-related effect.

Published studies on possible reproductive effects of isophorone are lacking. An unpublished inhalation study conducted by a commercial laboratory (Bio/dynamics, 1984b) studied possible teratogenicity due to isophorone in rats or mice at inhaled doses up to 115 ppm. Groups of 22 female rats and 22 female mice were exposed to 0, 25, 50, or 115 ppm isophorone (6 hr/day) on gestational days 6-15. Maternal toxicity in rats included dose-dependent alopecia and cervical/anogenital staining. Low body weights (7-8%) were occasionally observed in the 115 ppm group. In mice, maternal toxicity was confined to slightly decreased weight (7-8%) on one day in the 115 ppm group. No significant differences were found in uterine implantations, fetal toxicity, and external and internal malformations among the animals. However, a slight, but significant, growth retardation in the form of decreased crown-rump length was present among the high dose fetal rats. Also, a slight, but insignificant, increase in extra ribs and/or rudimentary ribs was seen in rat and mouse fetuses at the highest dose. In a pilot study for this developmental toxicity investigation (12 females/species), exencephaly was observed in 1 rat and 1 mouse undergoing late reabsorption and in 2 live rat fetuses from dams exposed to 150 ppm isophorone on gestational days 6-15 (Bio/dynamics, 1984a). Exencephaly was not observed at any dose level in the primary study.

Dutertre-Catella (1976) did not find adverse reproductive or developmental effects in rats exposed to 500 ppm isophorone (6 hr/day, 5 days/week) for 3 months before mating and throughout gestation (females only) as well. The pups were not examined for internal malformations so the study was incomplete for determination of developmental effects.

## VI. Derivation of Chronic Reference Exposure Level (REL)

| | |
|---|---|
| *Study* | Bio/dynamics 1984a,b |
| *Study population* | 22 female mice/group, 22 female rats/group |
| *Exposure method* | Discontinuous whole body inhalation exposure during gestation (0, 25, 50, or 115 ppm) |
| *Critical effects* | Developmental effects (reduced crown-rump length of female rat fetuses); teratogenicity (exencephaly in fetal rats and mice) in range finding study at 150 ppm |
| *LOAEL* | 115 ppm for reduced crown-rump length of female rat fetuses |
| *NOAEL* | 50 ppm |
| *Exposure continuity* | 6 hr/day during gestation |
| *Exposure duration* | Days 6-15 of gestation |
| *Average experimental exposure* | 12.5 ppm (50 x 6/24) |
| *Human equivalent concentration* | 12.5 ppm (gas with systemic effects, based on RGDR = 1.0 using default assumption that lambda (a) = lambda (h)) |
| *LOAEL uncertainty factor* | 1 |
| *Subchronic uncertainty factor* | 1 |
| *Interspecies uncertainty factor* | 3 |
| *Intraspecies uncertainty factor* | 10 |
| *Cumulative uncertainty factor* | 30 |
| *Inhalation reference exposure level* | 0.4 ppm (400 ppb, 2 mg/m$^3$, 2,000 μg/m$^3$) |

The inhalation study by Bio/dynamics (1984a,b) presents data that indicate exposure during gestation may be the most sensitive indicator of non-neoplastic toxicity by isophorone. Exposure of pregnant rats to 115 ppm isophorone during gestation resulted in significant growth retardation of female rat fetuses (reduced crown-rump length). Exposure to 50 ppm isophorone, the NOAEL, produced no developmental effects. The authors had removed the two shortest female fetuses prior to statistical analysis. The result was that there was no significant difference in fetal growth retardation; therefore, this adverse effect is not significant. However, this selective culling before the statistical analysis is not scientifically appropriate in this case. In addition, the authors did not perform some of the scheduled fetal examinations. Otherwise, the growth retardation might have had even greater statistical significance. The pilot study (Bio/dynamics, 1984a) observed exencephaly in a few mouse and rat fetuses at 150 ppm. Exencephaly was not considered significant by the authors because it was not present in any fetuses of the primary study (Bio/dynamics, 1984b). However, exencephaly is included as a critical effect in this summary because it is considered a serious teratogenic effect that was present at a dose (150 ppm) only slightly higher than the LOAEL of the primary study (115 ppm). Alopecia of adult female rats was observed in many of the exposed animals. However, this effect may be considered more of an acute dermal irritation than a chronic effect. In addition, cervical and anogenital staining seen in many exposed rats is not considered a chronic ‘adverse’ effect.

For comparison with the proposed REL of 0.4 ppm, the inhalation LOAEL of 250 ppm for mild liver effects (Dutertre-Catella, 1976) in rats and rabbits intermittently exposed to isophorone for 18 months was used to estimate a REL. Use of a time adjustment (6/24 x 5/7), an RGDR of 1, and a total UF of 100 (LOAEL to NOAEL = 3, interspecies = 3, and intraspecies = 10), also resulted in an estimated REL of 0.4 ppm. These results indicate that the REL will also protect against adverse liver effects.

While the toxicological significance of this liver effect observed by Dutertre-Catella (1976) is unknown, the NTP (1986) study observed an increased incidence of hepatocytomegaly and coagulative necrosis of the liver in treated male mice, but not in female mice and rats, orally gavaged with isophorone. Using 250 mg/kg-day as a LOAEL for mice and dividing by a total UF of 1000 (10 each for LOAEL to NOAEL, 10 for interspecies, and 10 for intraspecies) results in an oral REL of 0.25 mg/kg-day. Multiplying the oral REL by 3,500 $\mu g/m^3$ per mg/kg-day for route-to-route extrapolation results in a chronic inhalation REL estimate of 900 $\mu g/m^3$ (0.16 ppm), which is in good agreement with the REL developed from Dutertre-Catella (1976) and Biodynamics (1984a,b).

## VII. Data Strengths and Limitations for Development of the REL

The strength of the database for isophorone is the consistent lack of relevant severe histopathological effects in the chronic inhalation study (Dutertre-Catella, 1976) and in the oral gavage study (NTP, 1986). Weaknesses of the database for isophorone include the lack of human exposure data, the lack of comprehensive long-term inhalation studies, and the lack of published peer-reviewed reproductive/developmental studies. The lack of human data may be due to isophorone's rather low potency for causing chronic, non-neoplastic, adverse effects. Inhalation of isophorone is a relevant route of exposure under occupational settings, but is most likely a minor route of exposure for the general population. Due to the insufficient characterization of the kidney and liver lesions in the oral gavage NTP study (Bucher *et al*, 1986; NTP, 1986) and the inhalation study (Dutertre-Catella, 1976), a comprehensive chronic study in rodent and non-rodent species would enhance the database for isophorone.

## VIII. Potential for Differential Impacts on Children's Health

Since the REL is based on a developmental study, it is expected to be adequately protective of infants and children. However, there is no direct evidence in the literature to quantify a differential effect of isophorone in children relative to adults. Isophorone occurs in cranberries and thus presumably in cranberry juice, which is often mixed with other fruit juices. Children tend to consume more fruit juice. However, isophorone as a Hot Spot emission is unlikely to be a multimedia chemical, and there is no evidence to suggest that normal dietary levels of isophorone are associated with adverse health effects.

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*CHRONIC TOXICITY SUMMARY*

# ISOPROPANOL

*(2-propanol; dimethylcarbinol; isopropyl alcohol)*

**CAS Registry Number: 67-63-0**

## I. Chronic Toxicity Summary

| | |
|---|---|
| *Inhalation reference exposure level* | **7,000 μg/m$^3$ (3000 ppb)** |
| *Critical effect(s)* | Kidney lesions in mice and rats; fetal growth retardation and developmental anomalies in rats |
| *Hazard index target(s)* | Kidney; development |

## II. Chemical Property Summary (HSDB, 1995)

| | |
|---|---|
| *Description* | Colorless liquid at room temperature (25°C) with a pleasant odor. Slightly bitter taste. |
| *Molecular formula* | $C_3H_8O$ |
| *Molecular Weight* | 60.09 |
| *Boiling point* | 82.5°C |
| *Vapor Pressure* | 44.0 torr at 25°C |
| *Solubility* | Miscible in water and most organic solvents; insoluble in salt solutions. |
| *Conversion factor* | 1 ppb = 2.45 μg/m$^3$ at 25°C |

## III. Major Uses and Sources

Isopropanol is used as a solvent and in making many commercial products (HSDB, 1995). The annual production volume of isopropanol has been in excess of one billion pounds since 1956; it was ranked 50$^{th}$ among chemicals produced in the U.S. in 1994 (C&EN, 1995). Rubbing alcohol is a solution of 70% isopropanol in water. Specific uses and sources include: a component of antifreeze; a solvent for gums, shellac, essential oils, creosote and resins; extraction of alkaloids; component of quick drying oils and inks; component of denaturing alcohol; antiseptic for hand lotions; rubefacient; component of household products (after-shave lotions, cosmetics, etc.); the manufacture of acetone; deicing agent for liquid fuels; dehydrating agent and synthetic flavoring adjuvant. Isopropanol can enter the environment as emissions from its manufacture and use as a solvent. It naturally occurs as a plant volatile and is released during the microbial degradation of animal wastes. Human exposure will be both in occupational atmospheres and from use of consumer products containing isopropanol as a volatile solvent. An odor threshold has been estimated as 22 ppm (Amoore and Hautala, 1983), which is 7-fold higher than the chronic REL.

The annual statewide industrial emissions from facilities reporting under the Air Toxics Hot Spots Act in California based on the most recent inventory were estimated to be 525,826 pounds of isopropanol (CARB, 1999b).

## IV. Effects of Human Exposures

Currently, there are no adequate chronic exposure data for isopropanol in humans. While isopropanol is not considered a dermal irritant, it is a defatting agent and can cause dermatitis with prolonged exposure to skin (IARC, 1977). A subacute study of daily oral intake of isopropanol (2.6 or 6.4 mg/kg body weight) by groups of 8 men for 6 weeks had no effect on blood cells, serum or urine and produced no subjective symptoms (Wills *et al.*, 1969). A pharmacokinetic study of men occupationally exposed to isopropyl alcohol revealed that uptake occurs readily via the inhalation route; acetone is the major metabolite (Brugnone *et al.*, 1983). Acetone was eliminated mainly by the lung but was also eliminated in the urine.

## V. Effects of Animal Exposures

In metabolism studies with rats and mice, up to 92% of the administered dose (via i.v. or inhalation) of isopropanol was exhaled as acetone, $CO_2$ and the unmetabolized alcohol (Slauter *et al.*, 1994). Approximately 3-8% of the administered dose was excreted in urine as isopropanol, acetone, and a metabolite tentatively identified as isopropyl glucuronic acid. Isopropanol is readily absorbed from the GI tract and persists in the circulation longer than ethyl alcohol. Alcohol dehydrogenase oxidizes most isopropanol to acetone. Acetone may be further metabolized to acetate, formate, and finally $CO_2$. In another metabolism study, the amount of acetone in the blood stream was found to be directly related to the air concentration of isopropanol (Laham *et al.*, 1980). This finding indicated that the acetone metabolite could be used as a biochemical indicator of isopropanol exposure.

Subchronic studies by Guseinov and Abasov (1982) and Baikov *et al.* (1974) reported changes in certain hematologic and clinical chemistry parameters, as well as increases in some organ weights. But the Environmental Protection Agency deemed these studies insufficient to reasonably predict subchronic toxicity of isopropanol (Burleigh-Flayer *et al.*, 1994). Three different routes of exposure have been used by researchers for isopropanol toxicity studies: inhalation, oral gavage and presence in drinking water. The following subchronic and chronic studies exposed experimental animals to isopropanol by the inhalation route:

Toxicological and neurobehavioral endpoints were investigated in rats and mice following 13-week inhalation exposure (6 hr/day, 5 days/week) to 0, 100, 500, 1500 or 5000 ppm isopropanol (Burleigh-Flayer *et al.*, 1994). In rats, clinical signs observed following exposures included swollen periocular tissue (females) at the highest dose and perinasal encrustation (males) at 500 ppm and above. Narcosis was observed in a few animals of both species during exposure to 5000 ppm and possibly 1500 ppm as well. However, the animals became tolerant to the narcotic effects of isopropanol after week 2. No neurobehavioral changes were observed in any parameters of the functional observational battery. However, increased motor activity was noted

at week 9 of exposure in female rats of the 5000 ppm group. After an initial drop in body weight gain in the first week of exposure at the high dose (5000 ppm), rats in the 1500 and 5000 ppm groups had significant increases in body weight gain and/or body weight throughout most of the exposure period. But only the 5000 ppm group had greater than 10% body weight gain compared to controls. Increases in body weight and body weight gain greater than 10% were also noted in female mice in the 5000 ppm group. Consistent clinical pathology changes included an increase in mean corpuscular volume (rats; female mice) and mean corpuscular hemoglobin (male rats; female mice) at the 5000 ppm exposure level. Other changes noted include a slight anemia in rats at week 6 only and a slight dehydration in female mice at the end of the study. Relative liver weight in rats was elevated no more than 8% in the 5000 ppm groups. However, a 10 and 21% increase in relative liver weight was observed in female mice at 1500 and 5000 ppm, respectively. No gross lesions were observed in any organs. The only microscopic change observed was hyaline droplets within kidneys of all male rats. This change was not clearly concentration related, although it was most pronounced in the 5000 ppm group.

In a follow-up inhalation study spanning the lifetime of rats and mice, Burleigh-Flayer *et al*. (1997) exposed four groups of animals, each consisting of 75 CD-1 mice/sex and 75 Fischer 344 rats/sex, to 0, 500, 2500, or 5000 ppm isopropanol vapor. Of these, 55 mice/sex/group and 65 rats/sex/group were exposed 6 hr/day, 5 days/week for at least 78 weeks (mice) or 104 weeks (rats). Transient signs of narcosis were observed at the higher doses. Increased mortality and a decreased mean survival time (577 days versus 631 days for controls) were noted for male rats in the 5000 ppm group. Increases in body weight and/or body weight gain were observed for both sexes of mice and rats from the 2500 and 5000 ppm groups throughout the study. Concentration-related increases in absolute and relative liver weight were observed for male and female mice. In addition, increased absolute and/or relative liver and kidney weight were observed for male and/or female rats from the 2500- and 5000-ppm groups. Urinalysis and changes in urine chemistry, indicative of impaired kidney function (i.e. decreased osmolality and increased total protein, volume, and glucose), were noted for male rats in the 2500 ppm group and for male and female rats in the 5000 ppm group. At necropsy, the most significant noncancer lesions in rats were observed in the kidney, and were associated with an exacerbation of spontaneous chronic renal disease. The kidney lesions noted with increased severity and/or frequency included mineralization, tubular dilation, glomerulosclerosis, interstitial nephritis, interstitial fibrosis, hydronephrosis, and transitional cell hyperplasia. The authors considered chronic renal disease to be the main cause of death for male and female rats exposed to 5000 ppm and to account for much of the mortality observed for male rats exposed to 2500 ppm. Unlike the subchronic study, anemia was not observed in rats in the chronic study. In mice, an increased incidence of seminal vesicle enlargement was observed grossly in males in the 2500 and 5000 ppm groups. Microscopically, the lesions in mice included an increased incidence of ectasia (dilation) of the seminal vesicles for male mice in the 2500 and 5000 ppm groups, minimal renal tubular proteinosis for male and female mice from all isopropanol groups, and renal tubular dilation for female mice in the 5000-ppm group. The seminal vesicle effects did not have any associated inflammatory or degenerative changes. The enlargement may have been the result of either increased secretion or decreased evacuation of the secretory product by these glands. Microscopic evaluation of the livers of rats and mice revealed no exposure-related lesions.
In a 13-week behavioral/neurotoxicity study by the same investigators, the reproducibility and reversibility of increased motor activity in isopropanol-exposed female Fischer 344 rats was

investigated (Burleigh-Flayer *et al.* 1998). Rats were exposed to 0 or 5000 ppm isopropanol for 6 hr/day, 5 days/week. Increased motor activity was characterized as the summation of ambulation, rearing and fine movements and was first observed 4 weeks following exposure to 5000 ppm isopropanol. Reversibility of this effect was observed 2 days following cessation of exposure in a subgroup of rats exposed to isopropanol for only 9 weeks. In the subgroup exposed for 13 weeks, reversal of the increased motor activity did not occur until 2 weeks following cessation of exposure. However, complete reversibility of the time versus activity profile, or habituation curve, was not noted until 42 days following exposure to isopropanol for 13 weeks.. Other effects included a significant increase in body weight and an increased incidence of swollen periocular tissue in isopropanol-exposed animals.

In a study conducted to investigate neurochemical and behavioural effects, 20 male Wistar rats/group were exposed to 0 or 300 ppm isopropanol 6 hr/day, 5 days/week for up to 21 weeks (Savolainen *et al.*, 1979). Enzyme activity of superoxide dismutase and azoreductase in cerebellar homogenate was decreased at week 20-21. Acid protease activity in glial cells was increased up to week 10. Open-field tests indicated sporadic changes in urination (10th week) and defecation (15th week). Isopropanol also appeared to depress caffeine stimulation activity at 15 weeks.

In a subchronic neurotoxicity study by Teramoto *et al.* (1993), motor and sensory nerve conduction velocity increased significantly following a 20-week exposure (8 hr/day, 5 days/week) of Jcl-Wistar rats to 8000 ppm isopropanol. Low dose (1000 ppm) exposure had no effect on conduction velocity. Conduction velocities returned to normal following the end of exposure. The sex of the rats in this study was not specified.

A developmental study in rats exposed pregnant dams (15/group) to 0, 3500, 7000 or 10,000 ppm isopropanol 7 hr/day on gestation days 1-19 (Nelson *et al.*, 1988). At the two highest exposure levels, feed intake (weeks 1 and 2 of exposure) and maternal body-weight gain were reduced. Narcosis was evident only at the 10,000 ppm level.Increased fetal resorptions and reduced fetal weights (59% of controls) occurred at the highest exposure level. Fetal weights were also significantly reduced (85% of controls) at 7000 ppm. A slight reduction in fetal weight (96% of controls) occurred at 3500 ppm but was significant in the sense that a dose-dependent relationship in fetal weight reduction was present across all exposed groups. Skeletal malformations (primarily rudimentary cervical ribs) were seen only in the presence of maternal toxicity at the two highest exposure levels. No detectable teratogenic effects were observed in the 3500 ppm group. The authors noted that the developmental effects at 3500 ppm were considered very slight, indicating that this exposure level is close to the LOAEL for isopropanol.

The following studies administered isopropyl alcohol to experimental animals by oral gavage:

In a developmental study, pregnant (VAF)CD(SD) rats (25/group) were gavaged with either 0, 400, 800 or 1200 mg/kg body wt-day of isopropanol daily on gestational days 6 through 15 (Tyl *et al.*, 1994). In the same study, pregnant New Zealand white rabbits (15/group) were dosed orally with either 0, 120, 240 or 480 mg/kg body wt-day of isopropanol daily during gestational days 6 through 18. In rats, fetal body weight exhibited a linear downward trend with increasing dose and was significantly lower at the highest dose compared to controls. However, the fetal

body weight differences at each dose level was less than 10% of controls. Maternal weight gain during gestation was significantly reduced at the highest dose level. In rabbits, maternal weight gain and food consumption was reduced during gestation at 480 mg/kg body wt-day. Four rabbits died after dosing at this level. No differences were observed in reproduction indices or in fetal development. No teratogenic effects were seen in either species.

In another developmental study performed to investigate neurotoxicity in rat pups, 64 time-mated Sprague-Dawley rats/group were administered 0, 200, 700 or 1200 mg/kg body wt-day isopropanol by oral gavage from gestational day 6 through postnatal day 21 (Bates *et al.*, 1994). One high-dose dam died on postnatal day 15, but there were no other clinical observations of effects on maternal weight, food consumption or gestation length. All fetal developmental indices were unaffected at the dose levels used. Developmental neurotoxicity, in the form of motor activity, auditory startle and active avoidance tests, was not found at any dose of isopropanol.

In a multi-generation study carried out to investigate potential reproductive and developmental effects of isopropanol, Sprague-Dawley rats were administered 0, 100, 500 or 1000 mg/kg body wt-day of isopropanol by oral gavage (Bevan *et al*., 1995). P1 and P2 rats were dosed daily for 10 weeks prior to mating, throughout the mating, and during the gestation and lactation period for the F1 and F2 litters, respectively. In adult rats, centrilobular hepatocyte hypertrophy and increased relative liver weight (>10%) was observed in P2 males at 1000 mg/kg. A general increase in absolute and relative liver and kidney weights was observed (less than 10% in most cases) in treated animals in both P1 and P2 generations. However, with the exception of hepatocellular hypertrophy in P2 males, no histopathological effects relevant to human risk were present. Reproductive effects due to isopropanol were not seen at any dose level. Statistically significant reduction of body weights (5-12%) in F1 and F2 offspring and increased mortality (14%) in F1 offspring were observed at the 1000 mg/kg dose level.

The following toxicology studies administered isopropanol to experimental animals in drinking water:

In a study designed to investigate neurotoxicological effects, 22 male SPF rats/group were administered isopropanol in drinking water at concentrations of 0, 1, 2, 3, or 5% (*w*/*v*) for 12 weeks (Pilegaard and Ladefoged, 1993). Average daily intake of isopropanol was 0, 870, 1280, 1680 and 2520 mg/kg body wt, respectively. Water intake and body weights were consistently lower at the two highest doses. Relative weights of liver, kidney and adrenals were increased in a dose-dependent manner. However, histopathology revealed no treatment-related changes in organs other than the male rat-specific kidney lesions. Evidence of astrogliosis, in the form of increased glial fibrillary acidic protein in dorsal hippocampus, was not found in exposed rats.

In a 1-generation study, 10 Wistar-derived rats/sex/group were exposed to 0, 0.5, 1.25, 2.0, and 2.5% isopropanol in water for up to 18 weeks (USEPA/OTS, 1986). The doses are equivalent to 0, 325, 711, 1002, and 1176 mg/kg body wt-day, respectively, for males; to 0, 517, 1131, 1330, and 1335 mg/kg body wt-day, respectively, for females during the pre-mating phase; and to 0, 1167, 2561, 2825, and 2722 mg/kg body wt-day, respectively, in females during the post-partum phase. Exposure periods were: 70 days pre-mating, plus 15 days during mating, plus 42 days for

males; 21 days pre-mating plus 15 days during mating, plus 21 days gestation, plus 21 days rearing in females; and 21 days for the $F_1$ generation. At the highest two levels, body weights of males during the first two weeks were reduced and the body weights of females during the post-partum period were reduced. Water consumption and food ingestion were generally lower at the top three dose levels. The authors concluded that these effects were related to the unpalatability of drinking water containing isopropanol and not due to a toxic effect of the alcohol itself. Anemia was present in post-partum females. Red cell numbers were reduced in a dose-related manner at doses of 1.25% isopropanol or higher. Hematocrit was lower at the two highest doses while hemoglobin was lower at the highest dose. In males, mean cell volume was reduced at 1.25% isopropanol or higher. Absolute and relative liver and kidney weights were higher in most exposure groups at 2.0% or higher in both sexes, but no relevant pathology was seen. Absolute liver weight of females was also higher in the 1.25% group. Fetal weight gain was depressed in a dose-related fashion in the 1.25% and higher groups. Mean pup weights and pup survival were lower than controls at the two highest doses. Fewer pups were born per animal in the 2.5% exposure group. A teratogenic examination was not performed on the pups.

In a similar exposure study investigating the potential teratogenic effects of isopropanol, 20 pregnant Wistar-derived rats/group were exposed to 0, 0.5, 1.25 or 2.5% of the alcohol in drinking water (equivalent to 0, 596, 1242 and 1605 mg/kg body wt-day) during gestational days 6 to 16 (USEPA/OTS, 1992a,b). Water and feed consumption were reduced at the two highest doses while maternal body weight was significantly reduced at the highest dose. Fetal body weights were decreased in the two highest dose groups. Minor abnormalities and variants (reduced ossification of the skeleton) were present in fetuses of exposed groups in a dose-related manner. However, the authors concluded that the reduced fetal weights are probably a consequence of maternal growth retardation during the critical period of organogenesis. Similarly, the fetal abnormalities are probably due to small fetal size, related to slightly retarded development. Therefore, the study found no indication of teratogenesis.

A multi-generation study performed in ‘white’ rats also observed reduced body weights in F1 offspring (Lehman *et al.*, 1945). Body weights of F2 offspring were the same as controls. The adult rats had imbibed an average of 1.9 ml/kg (1470 mg/kg body wt) of isopropanol per day in drinking water 80 days prior to mating. No other developmental or reproductive effects were seen. In the same study, several dogs were given 4% isopropanol in drinking water for approximately 7 months. Histopathology at the end of exposure revealed a decrease in the number of nephrons with hydropic changes and necrosis of some of the tubular epithelium. Some capillary hemorrhages were also noted in the brains of two of the dogs. Average daily dose of isopropanol imbibed by the dogs could not be determined from data provided in the report.

## VI. Derivation of Chronic Reference Exposure Level (REL)

| | |
|---|---|
| *Study* | Burleigh-Flayer *et al.* (1997) |
| *Study population* | Rats and mice |
| *Exposure method* | Discontinuous whole-body inhalation (0, 504, 2,509 or 5,037 ppm) |
| *Critical effects* | Kidney lesions in mice and rats |
| *LOAEL* | 2,509 ppm |
| *NOAEL* | 504 ppm |
| *Exposure continuity* | 6 hours/day, 5 days/week |
| *Exposure duration* | 78 weeks in mice; 104 weeks in rats |
| *Average experimental exposure* | 90 ppm for NOAEL group (500 x 6/24 x 5/7) |
| *Human equivalent concentration* | 90 ppm for NOAEL group (gas with systemic effects, based on RGDR = 1.0 using default assumption that lambda (a) = lambda (h)) |
| *LOAEL uncertainty factor* | 1 |
| *Subchronic uncertainty factor* | 1 |
| *Interspecies uncertainty factor* | 3 |
| *Intraspecies factor* | 10 |
| *Cumulative uncertainty factor* | 30 |
| *Inhalation reference exposure level* | 3 ppm (3000 ppb, 7 mg/m$^3$, 7000 μg/m$^3$) |

The Burleigh-Flayer et al. (1997) study was selected because it was a chronic study, was recent, and was published in a respected, peer-reviewed journal. While numerous subchronic studies have been performed, this was the only study that conducted lifetime animal exposures. In addition, the chronic kidney effects observed in rats and mice were not seen in the subchronic studies, indicating that chronic exposure is necessary for development of these lesions.

The lesions observed in the kidneys of male rats in some of the studies described above is typical of a male rat-specific chronic renal disease and is not considered to be relevant to human risk assessment (Phillips and Cockrell, 1984; Beyer, 1992). However, the exacerbation of chronic renal disease in male and female rats, and the slight kidney damage observed in mice of both sexes following chronic isopropanol exposure indicates that the kidney is a sensitive indicator for nonneoplastic effects (Burleigh-Flayer *et al*., 1997). Suggestive evidence also exists for kidney damage in dogs following subchronic exposure to isopropanol in drinking water (Lehman *et al*., 1945).

Some isopropanol exposure studies noted increased liver and kidney weights in exposed animals but no observable relevant pathology. With particular relevance to the liver, this weight change may be considered to be more of a metabolic response rather than a toxic effect of the alcohol. The changes noted in the neurochemical and behavioural study by Savolainen *et al.* (1979) may have also been more of a metabolic response to the increased load of isopropanol. It is also possible that these changes reflected the development of tolerance. The changes in behavior

were small and unconvincing. This study would have benefited from additional dose levels to analyze for dose-response trends.

Other possible sensitive indicators of isopropanol toxicity include blood chemistry changes and reduced fetal body weights. However, the blood chemistry findings were conflicting among the various studies that investigated this endpoint. Reduced fetal weights at doses below maternal body weight reductions were minor (<10% compared to controls), but consistent, suggesting that reduced fetal weights are a manifestation of isopropanol developmental toxicity.

A comparative REL was calculated from the only reproduction/developmental study that utilized inhalation as the route of exposure (Nelson *et al*., 1988). Exposure of pregnant rats to isopropanol during gestation caused dose-dependent reduction in fetal body weights across all treatment groups, resulting in a LOAEL of 3500 ppm (average measured concentration = 3510 ppm). A NOAEL was not observed for this effect. Skeletal malformations probably related to reduced fetal weight was observed at 7000 ppm and 10,000 ppm. The average exposure duration at the LOAEL for this study is 1024 ppm (7hr/24hr x 3510 ppm). Use of an RGDR of 1 and a cumulative uncertainty factor of 100 (3 for LOAEL to NOAEL, 3 for interspecies, and 10 for intraspecies) resulted in a REL of 10 ppm (25 $mg/m^3$). Since the endpoint is a function of exposure only during gestation, no subchronic to chronic UF was used. This developmental REL is within an order of magnitude of the chronic REL for kidney lesions, and therefore, is also considered to be a critical effect.

The oral dose developmental studies by Tyl *et al*. (1994), Bevan *et al*. (1995), USEPA/OTS (1986), and USEPA/OTS (1992 a, b) provide supportive evidence that reduced fetal weights is a sensitive developmental endpoint. The USEPA/OTS (1992a,b) study provides supportive evidence for skeletal malformations in exposed rat fetuses.

## VII. Data Strengths and Limitations for Development of the REL

Strengths of the database for isopropanol include availability of a well-conducted chronic study in two species, similar toxicological endpoints among different studies, and pharmacokinetic similarities between humans and experimental animals. Isopropanol is metabolized through a similar pathway to acetone and $CO_2$.

Weaknesses of the database for isopropanol include a lack of literature regarding chronic toxicity endpoints in humans. The deficiency of chronic toxicity cases in humans may be related to the relatively low chronic toxicity of isopropanol. Another weakness is that, while most developmental studies observed maternal and fetal effects, only one study was performed via the inhalation route.

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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

| | |
|---|---|
| *Inhalation reference exposure level* | **0.7 µg/m$^3$** (2.5 ppb**)** |
| *Critical effect(s)* | Neutrophilic infiltration of the nasal epithelium; irritation of the respiratory system in rats, hamsters and monkeys |
| *Hazard index target(s)* | Respiratory system |

## II. Chemical Property Summary (HSDB, 1995)

| | |
|---|---|
| *Description* | Colorless or white solid |
| *Molecular formula* | $C_4H_2O_3$ |
| *Molecular weight* | 98.06 g/mol |
| *Boiling point* | 202°C |
| *Melting point* | 52.8°C |
| *Vapor pressure* | 0.1 torr @ 25°C (AIHA, 1970) |
| *Solubility* | Soluble in water, ether, acetate, chloroform, dioxane; @ 25°C, 227 g/100 g acetone, 112 g/100 g ethyl acetate, 52.5 g/100 g chloroform, 50 g/100 g benzene, 23.4 g/100 g toluene, 19.4 g/100 g o-xylene, 0.6 g/100 g $CCl_4$, 0.25 g/100 g ligroin |
| *Conversion factor* | 4.0 µg/m$^3$ per ppb at 25ºC |

## III. Major Uses and Sources

Maleic anhydride is used as a chemical intermediate in the synthesis of fumaric and tartaric acid, certain agricultural chemicals, resins in numerous products, dye intermediates, and pharmaceuticals (HSDB, 1995). It is also used as a co-monomer for unsaturated polyester resins, an ingredient in bonding agents used to manufacture plywood, a corrosion inhibitor, and a preservative in oils and fats. The annual statewide industrial emissions from facilities reporting under the Air Toxics Hot Spots Act in California based on the most recent inventory were estimated to be 7366 pounds of maleic anhydride (CARB, 2000).

## IV. Effects of Human Exposure

In many occupational situations workers are exposed to mixtures of acid anhydrides, including maleic anhydride, phthalic anhydride, and trimellitic anhydride. For example, Barker *et al*. (1998) studied a cohort of 506 workers exposed to these anhydrides. In one factory, workers were exposed only to trimellitic anhydride, which has the lowest acceptable occupational exposure limit (40 $\mu g/m^3$) of the three anhydrides. In that factory there was an increased prevalence of sensitization to acid anhydride and work related respiratory symptoms with increasing full shift exposure even extending down to levels below the current occupational standard. However, none of the workplaces had exposure only to maleic anhydride and a dose-response relationship was not seen with mixed exposures.

The following reports involve exposure only to maleic anhydride.

There are several case reports describing asthmatic responses possibly resulting from exposure to maleic anhydride. An individual showed an acute asthmatic reaction after exposure to dust containing maleic anhydride (Lee *et al.*, 1991). Workplace concentrations of maleic anhydride were 0.83 $mg/m^3$ in the inspirable particulate mass and 0.17 $mg/m^3$ in the respirable particulate mass. Bronchial provocation testing was performed with phthalic anhydride, lactose, and maleic anhydride. Exposure of this individual to maleic anhydride (by bronchial provocation testing) at 0.83 $mg/m^3$ and 0.09 $mg/m^3$ in inspirable and respirable particulate mass, respectively, showed a response of cough, rhinitis, and tearing within two minutes. Within 30 minutes, rales developed in both lungs and peak flow rate decreased 55%.

An individual occupationally exposed to maleic anhydride developed wheezing and dyspnea upon exposure (Gannon *et al.*, 1992). After a period without exposure, two re-exposures both resulted in episodes of severe hemolytic anemia. There was no evidence of pulmonary hemorrhage. Radioallergosorbent testing showed specific IgE antibodies against human serum albumin conjugates with maleic anhydride, phthalic anhydride, and trimellitic anhydride, but not with tetrachlorphthalic anhydride. A critique of the Gannon *et al.* (1992) study by Jackson and Jones (1993) questions the relationship of maleic anhydride exposure to the onset of the anemia, since there were extended periods of exposure to maleic anhydride before symptoms appeared.

Another case report described occupational asthma due to exposure to maleic anhydride (Guerin *et al.*, 1980).

Humans exposed to maleic anhydride showed respiratory tract and eye irritation at concentrations of 0.25 to 0.38 ppm (1 to 1.6 $mg/m^3$) maleic anhydride (Grigor'eva, 1964). No irritation was reported at 0.22 ppm maleic anhydride.

## V. Effects of Animal Exposure

Short *et al.* (1988) chronically exposed CD rats (15/sex/group), Engle hamsters (15/sex/group), and rhesus monkeys (3/sex/group) to maleic anhydride by inhalation. Four groups of each species were exposed to concentrations of 0, 1.1, 3.3, or 9.8 $mg/m^3$ maleic anhydride for 6

hours/day, 5 days/week, for 6 months in stainless steel and glass inhalation chambers. Solid maleic anhydride was heated to 53°C to generate vapors, which were then mixed with a stream of nitrogen. Chamber target levels were monitored by gas chromatography as total maleic (maleic anhydride plus maleic acid). No exposure-related increase in mortality occurred. Of the species examined, only rats showed significant changes in body weight during the course of the experiment, with reductions among males in the high-dose groups after exposure day 40 and a transient weight reduction from days 78-127 in the mid-dose group. All species exposed to any level of maleic anhydride showed signs of irritation of the nose and eyes, with nasal discharge, dyspnea, and sneezing reported frequently. No exposure-related eye abnormalities were reported. The severity of symptoms was reported to increase with increased dose. No dose-related effects were observed in hematological parameters, clinical chemistry, or urinalysis. No effects on pulmonary function in monkeys were observed. Dose-related increases in the incidence of hyperplastic change in the nasal epithelium occurred in rats in all exposed groups, and in hamsters in the mid- and high-dose groups. Neutrophilic infiltration of the epithelium of the nasal tissue was observed in all species examined at all exposure levels. All changes in the nasal tissues were judged to be reversible. The only other significant histopathological observation was slight hemosiderin pigmentation in the spleens of female rats in the high-dose group.

Incidence of epithelial hyperplasia of the nasal mucosa in animals from Short *et al*. (1988)

| Maleic anhydride (mg/m$^3$) | 0 | 0 | 1.1 | 1.1 | 3.3 | 3.3 | 9.8 | 9.8 |
|---|---|---|---|---|---|---|---|---|
| Pathology grade | Trace | Mild | Trace | Mild | Trace | Mild | Trace | Mild |
| Rat | | | | | | | | |
| Male | 0/15 | 0/15 | 2/15 | 6/15 | 1/15 | 14/15 | 0/15 | 12/15 |
| Female | 0/15 | 0/15 | 6/15 | 5/15 | 4/15 | 10/15 | 0/15 | 14/15 |
| Combined | | 0/30 | | 11/30 | | 24/30 | | 26/30 |
| Hamster | | | | | | | | |
| Male | 0/15 | 0/15 | 0/15 | 0/15 | 0/15 | 5/15 | 0/15 | 8/15 |
| Female | 0/15 | 0/15 | 0/15 | 0/15 | 4/15 | 4/15 | 1/15 | 4/15 |
| Combined | | 0/30 | | 0/30 | | 9/30 | | 12/30 |
| Monkey | | | | | | | | |
| Male | 0/3 | 0/3 | 0/3 | 0/3 | 0/3 | 0/3 | 0/3 | 0/3 |
| Female | 0/3 | 0/3 | 0/3 | 0/3 | 0/3 | 0/3 | 0/3 | 0/3 |
| Combined | | 0/6 | | 0/6 | | 0/6 | | 0/6 |

The teratogenicity and multigeneration reproductive toxicity of maleic anhydride were also investigated (Short *et al.*, 1986). To evaluate teratogenicity, pregnant CD rats were treated orally with maleic anhydride in corn oil at concentrations of 0, 30, 90, or 140 mg/kg-day from gestational days 6-15. Animals were necropsied on gestational day 20. No statistically significant dose-related effects were observed in maternal weight gain, implantation, fetal viability, post-implantation loss, fetal weight, or malformations. Groups of 10 male rats and 20 female rats/group ($F_0$ animals) were orally treated with 0, 20, 55, or 150 mg/kg-day maleic anhydride in corn oil to study multigeneration reproductive toxicity. Animals within the same dose group were bred together after 80 days of treatment to produce two $F_1$ generation animals ($F_{1a}$ and $F_{1b}$) and animals from the $F_1$ generation were interbred to produce two $F_2$ generation animals ($F_{2a}$ and $F_{2b}$). A significant increase in mortality was observed among both $F_0$ and $F_1$

generation animals in the high-dose group. Total body weight was significantly reduced in animals in the high-dose group at Week 11 of exposure for the $F_0$ generation males and females and at Week 30 of exposure in the $F_1$ generation males. No consistent pattern of dose- or treatment-related effect on fertility, litter size, or pup survival was observed. Examination of $F_0$ animals showed necrosis of the renal cortex in the high-dose group (60% of males and 15% of females). Absolute kidney weights were significantly increased in $F_1$ females in the low- and mid-dose groups, although there was no histological correlate. No changes in organ weight or histology were observed in the $F_2$ generation animals.

## VI. Derivation of Chronic Reference Exposure Level (REL)

| | |
|---|---|
| *Study* | Short *et al.*, 1988 |
| *Study population* | Rats (15/sex/group), hamsters (15/sex/group), monkeys (3/sex/group) |
| *Exposure method* | Discontinuous inhalation exposure (0, 1.1, 3.3, or 9.8 mg/m$^3$) |
| *Critical effects* | Neutrophilic infiltration of the nasal epithelium; epithelial hyperplasia; respiratory irritation |
| *LOAEL* | 1.1 mg/m$^3$ |
| *NOAEL* | Not observed in rats |
| *$BMC_{05}$* | 0.12 mg/m$^3$ for mild epithelial hyperplasia in rats (males and females combined) |
| *Exposure continuity* | 6 hr/day, 5 days/week |
| *Exposure duration* | 6 months |
| *Average experimental exposure* | 21 μg/m$^3$ for the $BMC_{05}$ (0.12 x 6/24 x 5/7 x 1000) |
| *Human equivalent concentration* | 21 μg/m$^3$ for the $BMC_{05}$ (Due to the lack of aerosol particle size data for the critical study, a human equivalent concentration could not be developed using recommended methods of inhalation dosimetry.) |
| *LOAEL uncertainty factor* | not needed in benchmark approach |
| *Subchronic uncertainty factor* | 1 |
| *Interspecies uncertainty factor* | 3 (see below) |
| *Intraspecies uncertainty factor* | 10 |
| *Cumulative uncertainty factor* | 30 |
| *Inhalation reference exposure level* | 0.7 μg/m$^3$ 0.2 ppb) |

Short *et al.* (1988) examined the toxicity of maleic anhydride to rats, hamsters, and monkeys by the inhalation route of exposure. Dose- and exposure related effects, although mild and reversible, were observed at all exposure levels. Specifically, exposure to maleic anhydride vapors resulted in hyperplastic change in the nasal epithelium of rats and hamsters (obligate nose breathers). Neutrophilic infiltration of the nasal epithelium was observed in all three species at all levels of exposure. All species also showed signs of irritation at all exposure levels. The observation that acute maleic anhydride is a strong respiratory irritant to humans (ACGIH, 1992)

suggests that this is a valid endpoint of toxicity to humans as well. Human exposure at levels as low as ~1 mg/m$^3$ appears to trigger acute asthmatic reactions in sensitive individuals (Lee *et al.*, 1991). The histological changes observed by Short *et al*. occurring as a result of inhalation exposure to a known strong irritant such as maleic anhydride are considered to be the adverse effect of repetitive acute exposures, rather than a chronic response, in the development of the REL.

The chronic REL was developed using the benchmark approach. The gamma model in the U.S. EPA's BMDS software yielded a $BMC_{05}$ of 0.12 mg/m3 for mild epithelial hyperplasia in male and female rats combined. Because of the similarities among species and the inclusion of monkeys in the study, an interspecies uncertainty factor of 3, rather than 10, was used. Although there is no evidence of a toxic response similar to the development of asthma in animals, the 1.1 mg/m$^3$ LOAEL from the animal studies of Short *et al.* (1988) results in a REL of 0.7 μg/m$^3$ which should protect asthmatics from maleic and other anhydrides.

## VII. Data Strengths and Limitations for Development of the REL

The major strengths of the REL for maleic anhydride are the availability of multiple-species, multiple-dose subchronic inhalation studies, and the observation of a mild effect LOAEL. The major uncertainties are the lack of human data and the lack of a NOAEL observation.

## VIII. Potential for Differential Impacts on Children's Health

Minimal teratogenic and reproductive adverse effects were seen at the lowest oral dose of maleic anhydride (20 mg/kg-day), given to rats during gestation (Short et al., 1986). This dose is equivalent to a person inhaling 70 mg/m$^3$. Thus the chronic REL of 0.7 μg/m$^3$ should protect children. Maleic anhydride is a respiratory irritant and an inducer of asthma. Exacerbation of asthma has a more severe impact on children than on adults. However, there is no direct evidence in the literature to quantify a differential effect of maleic anhydride in children.

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*CHRONIC TOXICITY SUMMARY*

# METHANOL

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

**CAS Registry Number: 67-56-1**

## I. Chronic Toxicity Exposure Level

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

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

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

## III. Major Uses and Sources

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

## IV. Effects of Human Exposure

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

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

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

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

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

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

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

## V. Effects of Animal Exposure

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

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

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

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

Ubaydullayev (1968) exposed rats (15 per group) to 0, 0.57, or 5.31 mg/m$^3$ methanol continuously for 90 days. Chronaxy ratios of flexor and extensor muscles were measured in addition to hematologic parameters and acetyl cholinesterase activity. No changes were apparent in the 0.57 mg/m$^3$ group. Effects observed in the 5.31 mg/m$^3$ group included decreased blood albumin content beginning 7 weeks after exposure, slightly decreased acetylcholinesterase activity, decreased coproporphyrin levels in the urine after 7 weeks, and changes in muscle chronaxy. (Chronaxy is the minimum time an electric current must flow at a voltage twice the rheobase to cause a muscle to contract. The rheobase is the minimal electric current necessary to produce stimulation (Dorland, 1981).
Pregnant rats were exposed by inhalation to methanol at concentrations ranging from 5000 to 20,000 ppm for 7 hours per day on days 1-19 gestation, and days 7-15 for the highest dose group (Nelson *et al.*, 1985). A dose-related decrease in fetal weight, an increase in extra or rudimentary cervical ribs, and urinary or cardiovascular defects were observed. Exencephaly and encephalocoele were observed in the 20,000 ppm dose group. The no-observed-adverse-effect level (NOAEL) was 5000 ppm.

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

## VI. Derivation of Chronic Reference Exposure Level (REL)

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

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

## VII. Data Strengths and Limitations for Development of the REL

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

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*CHRONIC TOXICITY SUMMARY*

# METHYL BROMIDE

*(bromomethane; monobromomethane)*

**CAS Registry Number: 74-83-9**

## I. Chronic Toxicity Summary

| | |
|---|---|
| *Inhalation reference exposure level* | **5 μg/m$^3$ (1 ppb)** |
| *Critical effect(s)* | Histological lesions of the olfactory epithelium of the nasal cavity in rats |
| *Hazard index target(s)* | Respiratory system; nervous system; development |

## II. Physical and Chemical Properties (HSDB, 1994)

| | |
|---|---|
| *Description* | Colorless gas |
| *Molecular formula* | $CH_3Br$ |
| *Molecular weight* | 94.95 g/mol |
| *Density* | 3.89 g/L @ 25°C |
| *Boiling point* | 3.6°C |
| *Vapor pressure* | 1420 torr @ 20°C |
| *Solubility* | Soluble in ethanol, benzene, carbon disulfide, and 1.75% (w/w) in water |
| *Odor threshold* | 20.6 ppm |
| *Odor description* | Sweetish odor |
| *Metabolites* | Methanol, bromide, 5-methylcysteine |
| *Conversion factor* | 1 ppm = 3.89 mg/m$^3$ @ 25° C |

## III. Major Uses and Sources

Methyl bromide (MeBr) was used historically as an industrial fire extinguishing agent and was introduced in the U.S. from Europe in the 1920s. Current uses of MeBr include the fumigation of homes and other structures for termites and other pests. Methyl bromide is also used to fumigate soil before planting and fruits and vegetables after harvest. In 1981, 6.3 million pounds of MeBr were reportedly used in California (Alexeeff and Kilgore, 1983). By 1991, its use had grown to 18.7 million pounds in the state (Cal/EPA, 1993). The annual statewide industrial emissions from facilities reporting under the Air Toxics Hot Spots Act in California, based on the most recent inventory, were estimated to be 75,575 pounds of methyl bromide (CARB, 1999). This does not include emissions of methyl bromide during its use as a pesticide.

## IV. Effects of Human Exposure

Workers (n = 32) exposed to MeBr during fumigation of soil or structures were compared to a referent group of 29 workers not exposed to MeBr, but exposed to other fumigants (Anger *et al.*, 1986). Exposures to MeBr were not quantified. It was found that workers exposed to MeBr had a higher rate of neurological symptoms and performed less well on several behavioral tests. Several confounding factors were present in this study, including lack of adjustments for age, alcohol consumption, prescription medication, illegal drugs, education, or ethnic group between the exposed and the referent groups.

## V. Effects of Animal Exposure

The first experimental animal study on repeated MeBr exposures was carried out and reported by Irish and associates (1940). In this study, rats (135 per group), rabbits (104 per group), or female rhesus monkeys (13 per group) were exposed to 0, 17, 33, 66, 100, or 220 ppm (0, 66, 128, 256, 388, or 853 mg/m$^3$) 7-8 hours/day, 5 days/week for 6 months or until the majority of the animals exhibited severe signs of toxicity. Mortality was seen in rats, guinea pigs, and monkeys at 100 ppm. Rabbits began to die at 33 ppm. Severe effects, including paralysis, were seen after exposure to 66 ppm in rabbits and monkeys. None of the species exhibited adverse effects after exposure to 17 ppm.

Kato and associates (1986) observed focal lesions in the brain and heart in rats (10-12 per group) after inhalation of 150 ppm (585 mg/m$^3$) MeBr 4 hours/day, 5 days/week for 11 weeks. In another experiment, rats were exposed to 0, 200, 300, or 400 ppm (0, 777, 1160, or 1550 mg/m$^3$) MeBr 4 hours/day, 5 days/week for 6 weeks. In this experiment, rats exposed to any concentration of MeBr exhibited coronary lesions, and exposures of 300 ppm or greater resulted in neurological dysfunction, including ataxia and paralysis. Testicular atrophy was noted in 6 of the 8 animals exposed to 400 ppm.

Anger *et al.* (1981) determined that rabbits are more sensitive than rats to neurotoxicity of MeBr. In this study, rats or rabbits were exposed to 0 or 65 ppm (0 or 254 mg/m$^3$) MeBr for 7.5 hours/day, 4 days/week, for 4 weeks. Nerve conduction velocity and eyeblink reflex were impaired in the rabbits but not rats exposed to 65 ppm MeBr. Similarly, rats did not exhibit neurological signs after exposure to 55 ppm (215 mg/m$^3$) MeBr for 36 weeks. Rabbits exposed to 26.6 ppm (104 mg/m$^3$) did not display any neurological effects after 8 months exposure (Russo *et al.*, 1984).

In the studies of Reuzel and associates (1987, 1991), groups of 50 male and 60 female Wistar rats were exposed to 0, 3, 30, or 90 ppm methyl bromide (98.8%) for 6 hours per day, 5 days per week. Three groups of animals (10/sex/exposure level) were killed for observations at 14, 53, and 105 weeks of exposure. Body weight, hematology, clinical chemistry, and urinalyses were examined throughout the experiment in addition to histopathology and organ weights at time of necropsy. Exposures of males and females to 90 ppm resulted in reduced body weight. Exposure to 90 ppm also resulted in significant lesions in the heart in the form of cartilaginous metaplasia and thrombus in the males, and myocardial degeneration and thrombus in the

females. Exposure of males to 30 or 90 ppm resulted in a decrease in relative kidney weight. Histological changes in the nose, heart, esophagus, and forestomach were the principal effects of methyl bromide toxicity. At the lowest concentration (3 ppm), very slight degenerative changes in the nasal epithelium, and olfactory basal cell hyperplasia were noted in both sexes at 29 months. Based on this study, a LOAEL of 3 ppm (11.7 mg/m$^3$) was determined.

The National Toxicology Program (NTP) conducted a 13-week and a chronic study on the toxicology and carcinogenesis of methyl bromide in rats and mice (NTP, 1990). In the 13-week study, 18 rats/sex/group were exposed to 0, 30, 60, or 120 ppm (0, 117, 233, or 466 mg/m$^3$) MeBr 6 hours/day, 5 days/week. The mice were exposed to 0, 10, 20, 40, 80, or 120 ppm (0, 39, 78, 155, 311, or 466 mg/m$^3$) 6 hours/day, 5 days/week. Hematological parameters and selected organ weights were measured in both species, in addition to histopathological changes. Pseudocholinesterase activity and neurobehavioral tests were conducted in the mice. Serious effects, including 58% body weight loss, 17% mortality and severe curling and crossing of the hindlimbs were observed in mice exposed to 120 ppm MeBr. Exposure of males to 40 ppm or higher resulted in significant effects on several hematological parameters, including decreased mean cell hemoglobin and increased red blood cell count. The only exposure-related histological effect was olfactory epithelial dysplasia and cysts in the rats of both sexes exposed to 120 ppm.

A 6-week study in rats and mice (5 animals/sex/group) exposed to 0 or 160 ppm (0 or 624 mg/m$^3$) showed high mortality rates, loss in body weight and histological changes in multiple organ systems including brain, kidney, nasal cavity, heart, adrenal gland, liver, and testes (NTP, 1990).

An exposure of mice (86 animals/group) to 0, 10, 33, or 100 ppm (0, 38.8, 128, or 388 mg/m$^3$) MeBr for 6 hours/day, 5 days/week, for 103 weeks was also conducted by NTP (1990). In this study, high mortality rates in both males and females in the 100 ppm group resulted in a discontinuation of exposure after 20 weeks. A low incidence of sternal dysplasia and a significant decrease in locomotor activity were noted in the 10 ppm group.

A 5-day exposure of rats (10 animals/group) to 0, 90, 175, 250, or 325 ppm (0, 350, 680, 971, or 1260 mg/m$^3$) resulted in lesions in the nasal olfactory sensory cells, the cerebellum and adrenal gland beginning at 175 ppm (Hurtt *et al.*, 1987). Hurtt and Working (1988) later observed severe histological damage to the nasal epithelium following a single exposure to 90 or 200 ppm (351 or 780 mg/m$^3$) MeBr. Olfactory function, measured by the ability to locate buried food, was impaired at the 200 ppm exposure. In this study, reduced testosterone and testicular glutathione levels were observed in the male rats exposed to 200 ppm, but no effects on spermatogenesis, sperm quality, or testes histopathology were noted.

Sikov *et al.* (1981) examined the teratogenic potential of MeBr in rats and rabbits exposed to 0, 20, or 70 ppm (0, 78, or 272 mg/m$^3$) 7 hours/day, 5 days/week for 3 weeks during days 1-19 (rats) or 1-24 (rabbits) of gestation. No maternal or fetal effects were observed in the rats, however, severe maternal neurotoxic effects were observed in the rabbits that resulted in 24/25 deaths. In this study, no significant maternal or fetal effects were observed at a concentration of 20 ppm.

Another developmental toxicity study was conducted in rabbits by Breslin *et al.* (1990). In this study, rabbits were exposed to 0, 20, 40, or 80 ppm (0, 78, 156, or 312 mg/m$^3$) MeBr for 6 hours/day on gestation days 6-19. Maternal toxicity was observed at 80 ppm and included reduced body weight gain and signs of neurotoxicity. In addition to the maternal effects observed, a significant increase in incidence of gall bladder agenesis and fused sternebrae were observed in the offspring exposed to 80 ppm. No adverse effects were observed at 40 ppm or lower concentrations.

A 2-generation reproduction and developmental toxicity study on MeBr in rats was conducted by American Biogenics Corporation (1986). Groups of rats (25/sex/concentration) were exposed to 0, 3, 30, or 90 ppm (0, 12, 117, or 350 mg/m$^3$) MeBr 6 hours/day, 5 days/week during premating, gestation, and lactation through 2 generations. Significant decreases in body weight during the pre-mating period and at the end of the study were observed in the males exposed to 90 ppm. Although some adult organ weights were affected in the 90-ppm group, there was no evidence of histopathology in these organs. Neonatal body weights were decreased by exposure to 30 ppm. There was a decreased cerebral cortex width in the 90 ppm $F_1$ group, reduced brain weight in 30 ppm $F_1$ females, and reduced fertility in the 30 and 90 ppm $F_{2b}$ groups.

## VI. Derivation of Chronic Reference Exposure Level

| | |
|---|---|
| *Study* | Reuzel *et al.*, 1987; 1991 |
| *Study population* | Male and female Wistar rats (50 and 60 per group, respectively) |
| *Exposure method* | Discontinuous inhalation exposures (0, 3, 30, or 90 ppm) over 29 months |
| *Critical effects* | Basal cell hyperplasia of the olfactory epithelium of the nasal cavity |
| *LOAEL* | 3 ppm |
| *NOAEL* | Not observed |
| *Exposure continuity* | 6 hr/day, 5 days/week |
| *Exposure duration* | 29 months |
| *Average experimental exposure* | 0.54 ppm for the LOAEL group |
| *Human equivalent concentration* | 0.12 ppm for the LOAEL group (gas with extrathoracic respiratory effects, RGDR = 0.23, based on MV = 0.03 m$^3$/min, SA = 11.6 cm$^2$) |
| *LOAEL uncertainty factor* | 3 (20% extra risk of a mild effect) |
| *Subchronic uncertainty factor* | 1 |
| *Interspecies uncertainty factor* | 3 |
| *Intraspecies uncertainty factor* | 10 |
| *Cumulative uncertainty factor* | 100 |
| *Inhalation reference exposure level* | 0.001 ppm (1 ppb, 0.005 mg/m$^3$, 5 μg/m$^3$) |

The chronic REL for methyl bromide is also the U.S EPA RfC.

## VII. Data Strengths and Limitations for Development of the REL

The major strengths of the REL for methyl bromide are the use of a comprehensive, long-term, multiple dose study with large sample sizes, and the availability of supporting data including long-term studies in other species and reproductive and developmental studies. The major uncertainties are the lack of human data and the lack of a NOAEL observation for the critical effect.

The California Department of Pesticide Regulation used a different approach that adjusts for respiration rate differences between humans and animals and which uses 10-fold uncertainty factors for interspecies differences, for intraspecies variability, and for a LOAEL to NOAEL extrapolation. Applying these factors to the same 3 ppm LOAEL results in a level for children and adults of 1 and 2 ppb (4 and 8 $\mu g/m^3$), respectively.

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*CHRONIC TOXICITY SUMMARY*

# METHYL CHLOROFORM

*(1,1,1-trichloroethane, methyltrichloromethane)*

**CAS Registry Number: 71-55-6**

## I. Chronic Toxicity Summary

| | |
|---|---|
| *Inhalation reference exposure level* | **1,000 µg/m$^3$ (200 ppb)** |
| *Critical effect(s)* | Astrogliosis in the sensorimotor cortex (brain) of gerbils |
| *Hazard index target(s)* | Nervous system |

## II. Chemical Property Summary (HSDB, 1999)

| | |
|---|---|
| *Description* | Colorless liquid |
| *Molecular formula* | $C_2H_3Cl_3$ |
| *Molecular weight* | 133.42 g/mol |
| *Density* | 1.3376 g/cm$^3$ @ 20° C |
| *Boiling point* | 74.1° C |
| *Melting point* | -30.4° C |
| *Vapor pressure* | 127 torr @ 25° C |
| *Solubility* | Soluble in acetone, benzene, methanol, carbon tetrachloride |
| *Conversion factor* | 5.47 µg/m$^3$ per ppb at 25ºC |

## III. Major Uses and Sources

Methyl chloroform is used as a solvent for adhesives and for metal degreasing (ACGIH, 1992). It is also used in the manufacture of vinylidene chloride and in textile processing and dry cleaning. The annual statewide industrial emissions from facilities reporting under the Air Toxics Hot Spots Act in California, based on the most recent inventory, were estimated to be 25,316,458 pounds of methyl chloroform (CARB, 1999a). Statewide monitored median and mean concentrations of methyl chloroform have been generally declining; decreasing from 0.8 or 1.71 ppb in 1990 to 0.12 or 0.30 ppb in 1996 (CARB, 1999b).

## IV. Effects of Human Exposure

A 44-year old woman was diagnosed with peripheral neuropathy following 18 months of occupational exposure to methyl chloroform in a solvent bath (House *et al.*, 1994). There was no identified exposure to agents known to cause peripheral neuropathy, such as n-hexane or

trichloroethylene. The worker reported that she wore protective gloves and a respirator, both of which frequently leaked. Seven months following removal from exposure, the worker showed improved nerve conduction.

Other case reports have identified the nervous system as a target of methyl chloroform toxicity in similar exposure scenarios. Three workers developed distal sensory neuropathy after working with methyl chloroform in a degreasing operation with repeated dermal exposure (Liss, 1988; Howse *et al.*, 1989). Changes were observed in nerve conduction in the upper extremities accompanied by both axonopathy and myelonopathy.

Twenty-eight workers with chronic exposure to high (but unquantified) concentrations of 1,1,1-trichloroethane had significant deficits in memory, intermediate memory, rhythm, and speed based on the Luria-Nebraska Neuropsychological Battery (Kelafant *et al*., 1994). Deficits in vestibular, somatosensory, and ocular components of balance were noted.

A 13-year-old male died after intentional inhalation of 1,1,1-trichloroethane (Winek *et al*., 1997). Autopsy findings included tissue congestion of lung, liver and kidney.

Cardiac arrhythmia resulting from heightened cardiac sensitivity to epinephrine has been reported in several case reports of high acute inhalation exposures to methyl chloroform (ATSDR, 1990). There are case reports of arrhythmias persisting for two weeks or more after cessation of exposure to methyl chloroform (McLeod *et al.*, 1987).

An epidemiological study of workers chronically exposed to low levels of methyl chloroform (<250 ppm) found no changes in blood pressure, heart rate, or electrocardiogram (Kramer *et al.*, 1978). This study consisted of 151 workers who had been exposed for more than one year. No neurophysiological testing was done.

Another study of 22 female workers exposed to methyl chloroform (plus 7 unexposed control workers) at concentrations ranging from 110-345 ppm in air for a mean of 6.7 years failed to identify neurotoxicity resulting from methyl chloroform exposure (Maroni *et al.*, 1977). The examination included evaluation for neurologic symptoms, changes in nerve conduction, and psychomotor tests.

Liver disease was observed in a worker exposed to methyl chloroform in a clothing factory screen printing room (Cohen and Frank, 1994). The worker was exposed for a total of 4 years before occupational exposure was identified as the cause of the liver disease. The worker sprayed an adhesive (containing 65% methyl chloroform, 25% propane and dimethyl ether, and 10% inert ingredients) during which the worker reported often feeling dizzy or intoxicated. Three months following removal of the worker from exposure, liver function tests, although still abnormal, were significantly improved. Other case reports support these findings (Hodgson *et al.*, 1989; Halevy *et al.*, 1980).

Six male volunteers were exposed to 35 and 350 ppm methyl chloroform for 6-hours on two separate occasions (Nolan *et al.*, 1984). Absorption was determined to be 25% of the inhaled dose. Of the absorbed dose, 91% was excreted unchanged in the expired air. Although the odor

was perceptible for the duration of the exposure, no subjective symptoms were reported by the volunteers.

## V. Effects of Animal Exposure

Gerbils (4/sex/dose plus 24 sex-matched control animals) were continuously exposed to 70, 210, or 1000 ppm methyl chloroform for 3 months (Rosengren *et al.*, 1985). A 4-month (solvent-free) recovery period following exposure was included to evaluate "lasting or permanent changes." Body weights were not changed significantly as a result of exposure. Brain weights in the animals in the 1000 ppm dose group were significantly decreased. Fibrillary astrocytes are formed in the brain in response to injury. Brain injury in methyl chloroform exposed gerbils was evaluated by detection of glial fibrillary acidic (GFA) protein, the main protein subunit of astroglial filaments. Increased levels of GFA protein were detected in the sensorimotor cerebral cortex of animals exposed to 210 or 1000 ppm methyl chloroform.

A later study in gerbils examined the effects of a 3-month continuous exposure to 70 ppm methyl chloroform followed by a 4-month recovery period (Karlsson *et al.*, 1987). DNA content was significantly decreased in three areas of the brain: posterior cerebellar hemisphere, anterior cerebellar vermis, and hippocampus. The authors contended that depressions in DNA content reflect decreased cell density.

No evidence of peripheral neuropathy or other neurotoxicity was detected in rats exposed to 200, 620, or 2000 ppm methyl chloroform 6 hours per day, 5 days per week for 13 weeks (Mattson *et al.*, 1993). The study included a functional observational test battery and measured visual, somatosensory, auditory and caudal nerve-evoked potentials. Histopathology of the brain, spinal cord, peripheral nerves and limb muscles was also examined at the end of the 13-week exposure.

Forty percent of all mice continuously exposed to 1000 ppm methyl chloroform for 14 weeks exhibited evidence of hepatocellular necrosis (McNutt *et al.*, 1975). A statistically significant increase in liver weight per body mass was observed throughout the study. Electron microscopy revealed accumulation of triglyceride droplets in the centrilobular hepatocytes following one week of exposure to 1000 ppm methyl chloroform. After 4 weeks of exposure, cytoplasmic alterations in centrilobular hepatocytes included a loss of polyribosomes and increased smooth endoplasmic reticulum. Similar changes observed occasionally in hepatocytes from mice exposed to 250 ppm were not as dramatic.

Mild hepatocellular changes were observed in rats exposed to 1500 ppm methyl chloroform 6 hours per day, 5 days per week for 6, 12, and 18 months (Quast *et al.*, 1988). At 24 months, these slight effects were no longer discernible due to confounding geriatric changes. No hepatocellular changes or other adverse effects were observed in rats exposed to 150 or 500 ppm methyl chloroform for up to 24 months.

The developmental toxicity of inhaled methyl chloroform was studied in CD-1 mice. Mice were exposed on gestation days 12 through 17 to either 2000 ppm methyl chloroform for 17 hours per day or 8000 ppm methyl chloroform for 1 hour three times per day (Jones *et al*., 1996). There

were no effects on pregnancy outcome, but exposed pups has reduced weight gain, had poorer results on motor coordination tests and showed delays in negative geotaxis (orienting towards the top of a sloped screen).

## VI. Derivation of Chronic Reference Exposure Level (REL)

| | |
|---|---|
| *Study* | Rosengren *et al.* (1985) |
| *Study population* | Mongolian gerbils (4/sex/dose) |
| *Exposure method* | Whole-body inhalation exposure |
| *Critical effects* | Astrogliosis in the sensorimotor cerebral cortex |
| *LOAEL* | 210 ppm |
| *NOAEL* | 70 ppm |
| *Exposure continuity* | Continuous |
| *Average experimental exposure* | 70 ppm for NOAEL group |
| *Human equivalent concentration* | Not derived (species-specific data for gerbils unavailable to validate assumption of RGDR=1) |
| *Exposure duration* | 3 months |
| *LOAEL uncertainty factor* | 1 |
| *Subchronic uncertainty factor* | 3 |
| *Interspecies uncertainty factor* | 10 |
| *Intraspecies uncertainty factor* | 10 |
| *Cumulative uncertainty factor* | 300 |
| *Inhalation reference exposure level* | 0.2 ppm (200 ppb; 1 mg/m$^3$; 1,000 µg/m$^3$) |

## VII. Data Strengths and Limitations for Development of the REL

Case reports indicate that the nervous system and the liver are targets of the toxicity of methyl chloroform (House *et al.*, 1994; Liss, 1988; Howse *et al.*, 1989; Cohen and Frank, 1994). The largest of the epidemiological studies (Kramer *et al.*, 1978; Maroni *et al.*, 1977), however, did not identify adverse effects as a result of chronic methyl chloroform exposure. The Kramer *et al.* (1978) study limited its evaluation to changes in blood pressure, heart rate, or electrocardiogram and exposure levels were only characterized as less than 250 ppm. Maroni *et al.* (1977) conducted their study among 22 women exposed occupationally to methyl chloroform levels as low as 110 ppm. Although the subjects were evaluated specifically for signs of neurotoxicity, the small sample size limits conclusions that can be drawn from their failure to identify adverse effects in this population. If no effects are associated with the exposures in the 2 studies (Kramer *et al.*, 1978; Maroni *et al.*, 1977), the REL predicted would be approximately 3 ppm.

Data from animal studies generally support the findings of the case reports from human exposures. Both neurotoxicity and hepatotoxicity have been identified among animals exposed by inhalation to methyl chloroform. The adverse effect observed at the lowest level in these studies was the development of astrogliosis in the brains of gerbils exposed for 3 months to 210 ppm methyl chloroform (Rosengren *et al.*, 1985). A no-observed-adverse-effect-level (NOAEL)

in this study was 70 ppm methyl chloroform. A subsequent study identified a more subtle change in the brains of gerbils exposed similarly to 70 ppm methyl chloroform, with slightly decreased DNA content found in several discrete brain regions of exposed animals. However, the relationship between tissue DNA content and cell density as an indication of adverse effect in the brain was considered too tenuous for the development of a guidance value for chronic exposure to methyl chloroform.

The major strengths of the REL for methyl chloroform are the observation of the NOAEL and the continuous subchronic exposure regimen. The major uncertainties are the lack of human exposure data, the lack of dose-response information, and the lack of comprehensive multi-organ effects data.

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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

| | |
|---|---|
| *Inhalation reference exposure level* | **1 µg/m$^3$** (0.5 ppb) |
| *Critical effects(s)* | Decreased weight gain and lung pathology at cessation of exposure in rats |
| *Hazard index target(s)* | Respiratory system; reproductive system |

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

| | |
|---|---|
| *Description* | Colorless liquid |
| *Molecular formula* | $C_2H_3NO$ |
| *Molecular weight* | 57.06 g/mol |
| *Boiling point* | 39.5$^o$C |
| *Melting point* | –45$^o$C |
| *Vapor pressure* | 348 torr @ 20$^o$C, 600 torr @ 30$^o$C (Varma and Guest, 1993) |
| *Solubility* | 10 percent in water @ 15$^o$C |
| *Conversion factor* | 2.3 µg/m$^3$ per ppb at 25$^o$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$^o$C), or heating metal methylisocyanates. Because of its high reactivity, MIC is used as an intermediate in organic synthesis, most notably in the production of carbamate based pesticides. Tobacco smoke from some brands of cigarettes also contains MIC (about 4 µg per cigarette). Workers exposed to the MIC 8-hour threshold limit value of 0.02 ppm (46 µg/m$^3$) are exposed to approximately 460 µg MIC in a workday. Based on the most recent inventory, the annual statewide industrial emissions from facilities reporting under the Air Toxics Hot Spots Act in California were negligible (CARB, 2000). This does not include estimates of emissions of breakdown products from the use of metam sodium in agricultural applications. Use of metam sodium averaged 15,400,000 pounds/year from 1995 to 1999.

## IV. Effects of Human Exposure

Although occupational exposures to MIC have been documented (Varma, 1986), few known exposures to the general public have occurred. A major exposure occurred in Bhopal, India in December 1984. Because of the sudden, short-term release (30-45 minutes), no measurements occurred, but the air concentration was estimated as 13 ppm (Dave, 1985) to 100 ppm (Varma, 1986).

The chemical identity of the ultimate toxicant has not been unequivocally determined and may consist of more than one chemical species. Although the chemistry of MIC suggests that hydrolysis to methylamine and dimethylurea is rapid, such hydrolysis in moist air is probably slow, and the reaction with photochemically produced hydroxyl radical is also slow (chemical $T_{1/2}$ about 3 months) (U.S. EPA, 1986). Brown *et al.* (1987) have shown that the alkylisocyanates (e.g., MIC) are relatively resistant (compared to the arylisocyanates) to hydrolysis in water. Hence, despite the high water reactivity of MIC, this compound could possibly persist in the environment for many days after an initial release.

Within 5 days of the initial exposure to MIC at Bhopal, more than 2,000 deaths occurred (Dave, 1985), while 4,000 more deaths were documented during the following decade (Lepkowski, 1994). The initial symptoms among the population living near the MIC plant were irritation and difficulty in breathing (Varma, 1986). Blindness occurred in more than 10,000 exposed persons but later resolved in most cases (Andersson *et al.*, 1990). The acute damage that led to death was mainly to the respiratory system, most likely pulmonary edema, bronchospasm, and electrolyte imbalance (Varma, 1986). However extrapulmonary damage, including tissue anoxia, gastrointestinal symptoms, and muscular weakness, were also observed (Dave, 1985). Within a year of the exposure, survivors continued to exhibit damage to the lung and eyes. Fibrosis of the lungs was seen in 30 percent of this group (Dave, 1985).

Reproductive toxicity was observed among women exposed to MIC in Bhopal. Varma (1987) reported 43 percent unsuccessful pregnancies among 865 women who were pregnant at the time of the MIC release. Among the live births, 14 percent of the infants died within 30 days, whereas a death rate of only 3 percent for the same interval was recorded 2 years prior to the release. Bhandari *et al.* (1990) reported increased spontaneous abortions and neonatal deaths among exposed women who were pregnant at the time of exposure compared to a control group in another city. In the latter study, stillbirths and congenital malformations were similar in the exposed and non-exposed groups.

Non-reproductive, non-pulmonary responses were evident in a group of exposed Bhopal residents, 3-years following exposure to the MIC vapors. Loss of vision and loss of visual acuity were more prominent among exposed residents than among unexposed people, and the losses appeared to be dose-dependent (Andersson *et al.*, 1990). In this study, the surrogate for dose was extent of early deaths in a housing cluster. Similarly, cataracts were reported more often among the exposed than among the unexposed group.

The lesions associated with lung damage may be expressed as pulmonary edema for immediate effects (Varma, 1986), and lesions associated with the bronchoalveolar area for long-term effects

(Dave, 1985, Varma, 1986). Vijayan *et al.* (1995) studied cellular components of bronchoalveolar lavage (BAL) and pulmonary function in Bhopal patients 1.3, 2.7, and 5.1 years after exposure to MIC. All had lived within 3-miles of the factory and all experienced acute respiratory and ophthalmic symptoms on the day of exposure. All were experiencing continued respiratory symptoms. Among the exposed people, decrements in forced vital capacity and forced expiratory volume (at 1-minute) were observed. In general, the decrements ranged from 12 - 21 percent of predicted values, whereas the control group exhibited decrements of 2 - 4 percent of the expected values. Analysis of the BAL revealed increases in total cells (all exposed groups), increased absolute numbers of macrophages (all exposure groups), decreased percentage of lymphocytes (2.7 and 5.1 year groups), and increased numbers and percentage of neutrophils (5.1 year group). These cell types are involved, through the secretion of various factors, in inflammatory and immunologic processes in the lung (Reiser and Last, 1986). The Vijayan *et al.* (1995) study thus suggests long term damage to lung parenchyma among people who survived the initial acute effects of MIC exposure.

In summary, humans exposed acutely by inhalation to MIC may experience long-term (as well as immediate) damage to pulmonary and extrapulmonary systems. The lung is probably the critical target organ for long-term effects from acute exposure, although adverse effects on other organs (e.g., eye, reproductive, and gastrointestinal) also exist. The late responses to the acute exposure suggest an immunological component, which could involve several systems including lung, eye, liver, and kidney. The chemical identity of the ultimate toxicant is unknown and may be more than one compound.

Avashia *et al*. (1996) assessed pulmonary effects from long-term, low-level MIC for more than 400 workers at a large chemical facility. Serial pulmonary function data, cigarette smoking histories, and industrial-hygiene measurements were available. Jobs were classified according to level of MIC exposure as none, low, moderate or high. Where work records were incomplete, exposures were based on the ratings of supervisors and coworkers. The frequency of pulmonary impairment was evaluated for the assumed four levels of exposure. No specific or consistent pulmonary impairment was evident. Unfortunately the report gave no quantitative classification of low, moderate or high MIC levels.

## V. Effects of Animal Exposure

Experimental animal studies have been designed to address the experiences of the victims of the Bhopal disaster, in which the exposure has been described as acute because of the short duration (30-45 min). No studies were found that described exposure duration greater than 10 days. However, a chronic component to MIC exposure may exist as a result of slower rates of hydrolysis in air (compared to water), the presence of carbamylated hemoglobin in MIC-exposed people, and the change from edematous to inflammatory and/or fibrotic lesions with time. Further, a glutathione-dependent reversible MIC transport system has been suggested in experimental animals (see below).

MIC is absorbed through the respiratory tract and distributed to non-respiratory organs in experimental animals. In an acute (30 min) inhalation exposure to a dose of $^{14}$C-MIC (labeled in

the isocyanate moiety) equivalent to one-$LC_{50}$ (23 mg/L), rats accumulated protein-bound radioactivity (including carbamylated proteins) in brain, liver, kidney, and lung, but not in blood (Bhattacharya *et al.*, 1988). Ferguson *et al.* (1988) exposed guinea pigs by inhalation to 0.47 ppm $^{14}C$-MIC (methyl group) for 6-hours. At the end of exposure, the label was found in arterial and venous blood, bile, and urine. At 2.7 days post-exposure, the label decreased to 2-7 percent. MIC was retained in the nasal-laryngeal area of the guinea pigs.

MIC, like reactive isocyanates in general, can react with biological molecules containing amino, alcohol, or sulfhydryl groups, as well as with water. While hydrolysis in an aqueous environment, such as the lung, is theoretically possible, measurements show that alkyl isocyanates are relatively resistant (compared to arylisocyanates) to such hydrolysis (Brown *et al.* 1987). The absence of a role for MIC hydrolytic products, methylamine (MA) or dimethylurea (DMU), is also suggested by the work of Jeevaratnam and Sriramachari (1994) and Sriramachari *et al*. (1994). Inhalation (30 min) or subcutaneous exposure of rats to either hydrolytic product at levels equivalent to the $LC_{50}$ or $LD_{50}$ did not result in death. Similarly, neither methylamine nor dimethylurea duplicated the acute effects of respiratory necrosis and congestion. However, exposure to these hydrolytic products did lead to interstitial pneumonitis, an observation that suggests MA and/or DMU could lead to subsequent inflammatory responses if sufficient amounts are present.

A role for methylamine in reproductive/developmental toxicity was investigated by Guest and Varma (1991). In a mouse study, pregnant dams were exposed to varying doses (intraperitoneal) of methylamine (as well as the di- and trimethyl compounds). Reproductive toxicity was not observed for methylamines. However, in cultured embryo experiments, decrements in crown-rump length, yolk-sac diameter, head length, and embryo survival were observed. The concentrations were high (>0.75 mM) and the interpretation of the biological activity of methylamine in terms of inhalation exposure is difficult.

MIC is a carbamylating intermediate; this is the basis for its use in the manufacture of carbamate based pesticides. In the same way, MIC should react with the appropriate functional groups of proteins, peptides, and nucleic acids. However, i*n vitro* studies with cholinesterases show that such a reaction is not efficient (Brown *et al.*, 1987), an observation which may be explained by the presence of protonated amino groups at physiological pH (Baillie and Slatter, 1991).

A transport system for MIC via reduced glutathione (GSH) has been suggested by the discovery of the MIC-adduct, S-(N-methylcarbamoyl)glutathione (SMG), in the bile and the MIC-adduct of N-acetylcysteine (mercapturic acid, AMCC) in the urine of rats exposed to MIC by non-inhalation routes (Pearson *et al.*, 1990; Slatter *et al.*, 1991). The reaction of MIC with GSH and with cysteine is reversible, and can provide a source of free MIC in the tissues (Baille and Slatter, 1991). Similar studies in experimental animals exposed to MIC by inhalation have not been reported. However, humans exposed by inhalation to N,N-dimethylformamide (H-C-(=O)-$N(CH_3)_2$) excrete AMCC in urine (Mraz and Nohova, 1992). Hence a reversible MIC-transport system in animals, including humans, is possible, and the presence of high levels of GSH in human lavage fluid (Cantin *et al.*, 1987) would permit the initiation of this mechanism.

The toxicity of the adduct SMG was tested in mouse embryo culture (Guest *et al*., 1992). Mouse embryos, at day 8 of gestation in vivo, were removed from their dams and cultured in the presence (and absence) of SMG. Dose-dependent (0.25 - 2 mM) decrements were observed for yolk sac diameter, crown-rump length, somite number, and protein content. Delayed DNA synthesis in the embryos and in yolk-sacs occurred in the presence of 0.25 mM SMG. Similar to the results obtained with methylamine, the SMG concentrations were high and the exposures were not by inhalation. However, the data show that a MIC metabolite, SMG, has toxic properties. In the presence of GSH (1 or 3 mM), the extent of the SMG-dependent toxicities was decreased. Such data demonstrate the reversibility of the binding between MIC and GSH.

Three inhalation studies were identified in which experimental animals were exposed to more than one dose of MIC. Among these studies, two used exposure durations for more than one day (Dodd and Fowler, 1986; Mitsumori *et al.*, 1987). Rats and mice were exposed by inhalation to 0, 1.1, and 2.8 (female) or 3.0 (male) ppm MIC for 6 hr/day for a total of 4 days, and then followed during a 91-day post-exposure interval (Mitsumori *et al.*, 1987). Among the rats, post-exposure deaths occurred by 49 days (male) and 14 days (female) at the high dose. Among the mice, only 1 male mouse died at 16 days post-exposure. Reduced weight gain was observed among the female and male rats in the high dose group, prior to death, although the absolute weights were not different from the unexposed rats one day before the end of exposure. Among the mice, a slowed weight gain was observed at 3- and 6-days post exposure (male) and 1 day post exposure (female) at the high dose, but normal weight gain returned by 1 week following cessation of exposure. At 7 days post-exposure, microscopic changes were observed in the respiratory system among the high dose rats of both sexes. Between 8- and 27 days post-exposure, increased lesions in the respiratory tract and also in liver, thymus, spleen, heart, and brain were observed at the high dose. Similar lesions were not observed in rats exposed to 1.1 ppm MIC and followed to the 8-27 day post-exposure. Among survivors, the incidence of lesions decreased to control values by 91 days. Among the mice, treatment related changes in the respiratory tract were observed at the high dose at 7 days post-exposure. Between 28 and 91 days, the lesions associated with the upper respiratory tract disappeared, whereas those associated with the major bronchi remained, although somewhat attenuated. These data suggest that the rat is more sensitive than the mouse to the effects of MIC. A LOAEL of 2.9 ppm is indicated, based on post-exposure decreased weight gain and respiratory tract changes in rats.

Dodd and Fowler (1986) exposed rats to 0, 0.15, 0.6, and 3.1 ppm MIC for two 4-day sessions at 6-hours/day and examined the animals within 1-day following exposure. The 2-cycle exposure included a 2-day recess from exposure. No deaths occurred at any MIC concentration during the exposure. Lesser weight gain occurred for rats in the 3.1 ppm groups, whereas weight among the rats in the 0.15 and 0.6 ppm MIC groups was indistinguishable from the air-exposed control animals. On exposure days 3 and 8, mean food consumption values in the high dose group were below those for the non-exposed group. At the time of termination, male rats exposed to 3.1 ppm MIC exhibited a 38 percent increase in hemoglobin concentration and a 26 percent decrease ($p<0.001$) in oxygen saturation, compared to the unexposed rats ($p<0.001$). Such changes were not observed for the female rats exposed to 3.1 ppm or for rats of either sex exposed to 0.15 or 0.6 ppm MIC. Absolute lung weights increased ($p<0.001$) in both sexes after exposure to 3.1 ppm, compared to the control rats. Decreases in liver, kidney and testes absolute weights were observed in this exposure group, but the authors interpreted these data as a reflection of the body

weight losses. No weight changes were observed in rats exposed to 0.15 or 0.60 ppm MIC. Gross and microscopic lesions were observed in rats (female and male) exposed to 3.1 ppm, but not in rats exposed to 0, 0.15, or 0.6 ppm MIC. The microscopic lesions occurred in the respiratory tract and consisted of inflammation, epithelial necrosis, squamous metaplasia, and epithelial hyperplasia. These lesions extended into the bronchioles. These data suggest a NOAEL of 0.6 ppm MIC, based on weight gain loss, absolute lung weight, and lung histopathology in rats, immediately following cessation of exposure.

Post-exposure changes in lung pathology also occurred in the rats surviving 3.1 ppm in the Dodd and Fowler (1986) study. The early lesions associated with inflammation, epithelial necrosis, squamous metaplasia, and epithelial hyperplasia extending to the bronchioles either decreased in severity or receded toward the upper respiratory tract by 85-days post-exposure. In males, the intraluminal and submucosal fibroplasia changed in appearance during this interval, due in part to the maturation of fibrous tissue. Mucous plugs were also seen in the terminal bronchioles and alveoli in some rats. The importance of this observation is the progressive character of MIC induced lung disease. Such progression may be difficult to follow at lower doses, if the times involved are of insufficient duration.

Sethi *et al.* (1989) exposed rats by inhalation to 0, 0.21, 0.26, and 0.35 ppm MIC for 6 days at 0.5 hr/day. Statistical evaluation was not presented. No post-exposure deaths were reported, although lethality was recorded for rats exposed to 3.5 and 35 ppm for only 10 minutes. Following the 0.5 hr × 6-day exposure, the weight gain declined in proportion to the exposure dose. At the lowest dose (0.21 ppm) the weight gain was 111 g after 91 days post-exposure, compared to a weight gain of 218 g during the same interval among the non-exposed rats. The absolute weights of the rats at the end of the exposure were not given. According to the narrative, inflammatory lesions of bronchopulmonary tissue were present; their extent increased with dose. A dose-response increase in markers of lung infection was present and suggests that the MIC exposed rats were more prone to infectious agents than were the unexposed animals. Non-specific lesions in liver and kidneys were also observed and appeared to be dose dependent, but the authors suggested that these effects could be a result of the lung infections.

Fetotoxicity was observed in two experimental animal studies (Schwetz *et al.*, 1987; Varma, 1987). Among female mice exposed to 0, 1, or 3 ppm MIC during gestation days 14 - 17 for 6 hr/day, an increased incidence of fetal deaths was observed at 1 ppm (Schwetz *et al.*, 1987). At 3 ppm, the average number of pups/litter decreased relative to the air-exposed controls. The dams were unaffected in terms of survival, body weight, or length of gestation. Non-gestational exposure (6 hr/day, 4 days) did not affect the number of pregnancies or the live litter sizes, suggesting that the fetotoxic effect may be specific to the female reproductive tract rather than a general attribute of systemic toxicity. Similarly, female mice exposed for 3 hours on gestation day 8 to 0, 2, 6, 9 or 15 ppm MIC gave birth to pups with decreased body weights at the lowest dose, although a good dose-response was not observed (Varma, 1987). At 9 or 15 ppm MIC, the surviving dams lost 75 - 80 percent of their fetuses. Maternal mortality and decreased skeletal lengths were also observed at 9 and 15 ppm. A distinction between maternally induced fetotoxicity and a direct effect on fetal health could not be made. Because the inhalation exposure to the dams occurred for only 3 hrs on one day, a chronic LOAEL is not suggested. Exposure of male rats to one dose of 3.2 mg/L for 8 minutes resulted in a 21 percent fertility rate

among the cohabited female rats within the day 8-14 period post-exposure compared to a fertility rate of 40% for controls; however, the rates increased after 15 days post-exposure (Agarwal and Bose, 1992). There was no evidence of fetotoxicity among the dams impregnated by the MIC-exposed male rats. Exposure of male and female mice to 0, 1, or 3 ppm MIC did not result in altered body weights, fertility, or litter size (Schwetz *et al*, 1987). The results suggest that exposures to MIC at doses that are not toxic to adult male or female (pregestational) mice or rats do not result in adverse reproductive outcomes.

## VI. Derivation of Chronic Reference Exposure Level (REL)

| | |
|---|---|
| *Study* | Dodd and Fowler (1986) |
| *Study populations* | F344 rats |
| *Exposure method* | Inhalation (0, 0.15, 0.6, or 3.1 ppm) |
| *Critical effects* | Decreased weight gain and lung pathology immediately after cessation of exposure |
| *LOAEL* | 3.1 ppm |
| *NOAEL* | 0.6 ppm |
| *Exposure continuity* | 6 hours/day, 8 days/10 day experiment (2-cycles, with one 2-day recess from exposure) |
| *Exposure duration* | 10 days |
| *Average experimental exposure* | 0.12 ppm for the NOAEL group (0.6 x 8/10 x 6/24) |
| *Human equivalent factor* | 0.15 ppm for the NOAEL group (gas with pulmonary respiratory effects, RGDR = 1.23, based on BW = 152 g, MV = 0.12 L/min, SA = 225 $cm^2$) |
| *LOAEL uncertainty factor* | 1 |
| *Subchronic uncertainty factor* | 10 |
| *Interspecies uncertainty factor* | 3 |
| *Intraspecies uncertainty factor* | 10 |
| *Cumulative uncertainty factor* | 300 |
| *Inhalation reference exposure level* | 0.5 ppb (1 $\mu g/m^3$) |

Although the exposure was for only 10 days, the Dodd and Fowler (1986) study includes the longest exposure duration of the available investigations and also uses some of the lower exposure levels (down to 0.15 ppm). The microscopic findings of the respiratory tract were statistically analyzed, although an observation of the tabulated data at the four doses (0, 0.15, 0.6, and 3.1 ppm) clearly shows a NOAEL of 0.6 ppm. Other endpoints with the same NOAEL were increased hemoglobin and increased absolute lung weights. The symptomatic ramifications of the increased hemoglobin are unknown, although similar increases were reported for humans exposed to MIC in Bhopal (Srivastava *et al.*, 1988). The lung weight gain may be a reflection of the pathological changes seen in the microscopic studies.

Decreased body weight gain was also seen in the experimental 4 day rat inhalation study of Mitsumori *et al.* (1987) (NOAEL = 1.1 ppm), except that the decrease in the latter study did not

occur until 1 and 3 days (female and male, respectively) post-exposure. The apparent discrepancy could be explained, in part, on the basis of the length of exposure, which was twice as long in the Dodd and Fowler (1986) study. However, the weight gain loss in the Dodd and Fowler (1986) study was initiated within one day of the start of exposure.

The MIC chronic REL of 0.5 ppb is based on endpoints observed within 1 day of cessation of exposure. Post-exposure evaluation showed that, at a higher exposure level (3.1 ppm), progressive changes, including death, occurred. Post-exposure observations, however, were not reported at the 0.15 and 0.6 ppm MIC levels. The attribute of delayed MIC inhalation toxicity has also been observed in other experimental animals studies (Dodd and Fowler, 1986, Mitsumori *et al.*, 1987). In the case of the human MIC exposure in Bhopal, India, death did not occur during the immediate 30 - 45 minute exposure, but exhibited a lag phase. A few deaths occurred during the first few hours, the maximum occurred at 2 - 3 days, and by the end of a week about 2500 deaths were documented (Dave, 1985; Varma, 1986; Varma and Guest , 1993), although Varma (1986) suggests that the immediate number may be closer to 5,000. One report suggests that during the intervening decade as many as 6,000 deaths may be attributed to the initial exposure in Bhopal (Lepkowski, 1994). Such information suggests that the presence of an adverse effect at the NOAEL of 0.6 ppm (Dodd and Fowler, 1986) might be possible if the rats were observed during an extended post-exposure interval. Experimental evidence is needed to test this hypothesis.

Only one study was identified in which post-exposure observations were made on experimental animals exposed subchronically by inhalation to multiple doses of MIC. Mitsumori *et al.* (1987) exposed rats to 0, 1.1, and 2.8 (females) or 3.0 (males) ppm MIC for 6 hr/day for 4 days and observed the rats for 91 days. No deaths and no weight gain loss (in contrast to Dodd and Fowler, 1986) were present until the post-exposure period and were mainly observed in animals exposed at the high dose. Using a NOAEL of 1.1 ppm MIC, a chronic REL of 1.1 ppb (2.6 $\mu g/m^3$) was derived. The REL based on the Mitsumori *et al.* (1987) study is similar to the REL based on immediate effects (Dodd and Fowler, 1986), and may indicate that the time of occurrence of exposure related effects may not be as important as the MIC air concentration.

## VII. Data Strengths and Limitations for Development of the REL

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

## VIII. Potential for Differential Impacts on Children's Health

Since exposures to MIC at levels that are not toxic to adult male or female (pregestational) mice or rats do not result in adverse reproductive outcomes, the chronic REL of 1 $\mu g/m^3$ should adequately protect infants and children. MIC is a respiratory irritant and the developing respiratory system is more sensitive than that of adults. However, there is no direct evidence in the literature to quantify a differential effect of MIC on the respiratory system of infants and children.

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*CHRONIC TOXICITY SUMMARY*

# METHYL *t*-BUTYL ETHER

*(MTBE; 2-methoxy-2-methylpropane; tert-butyl methyl ether; methyl 1,1dimethyl ether)*

**CAS Registry Number: 1634-04-4**

## I. Chronic Toxicity Summary

| | |
|---|---|
| *Inhalation reference exposure level* | **8000 μg/m$^3$ (2000 ppb)** |
| *Critical effect(s)* | Nephrotoxicity, prostration, periocular swelling in Fischer 344 rats |
| *Hazard index target(s)* | Kidney; eyes; alimentary system |

## II. Physical and Chemical Properties (HSDB, 1994)

| | |
|---|---|
| *Description* | Colorless liquid |
| *Molecular formula* | $C_5H_{l2}0$ |
| *Molecular weight* | 88.15 g/mol |
| *Density* | 0.7405 g/cm$^3$ @ 20°C |
| *Boiling point* | 55.2°C @ 760 mm Hg |
| *Vapor pressure* | 245 torr @ 20°C |
| *Solubility* | Soluble in alcohol, ether, and 5% soluble in water |
| *Conversion factor* | 1 ppm = 3.61 mg/m$^3$ @ 25° C; 3.67 mg/m$^3$ @ 20° C |

## III. Major Uses or Sources

Methyl t-butyl ether (MTBE) is used as a gasoline additive to improve octane ratings and reduce emissions of some pollutants, in industry to improve miscibility of solvents, and in clinical medicine to dissolve cholesterol gall stones (Yoshikawa *et al.*, 1994). The annual statewide industrial emissions from facilities reporting under the Air Toxics Hot Spots Act in California,based on the most recent inventory, were estimated to be 215,182 pounds of MTBE (CARB, 1999).

## IV. Effects of Human Exposure

Gasoline (with 10% MTBE) tanker drivers reported significantly higher fatigue at the end of the work week than before the work week, and those with longer exposure to gasoline with MTBE during the work week reported significantly higher fatigue than drivers with shorter exposure (Hakkola *et al*., 1997). 20% of drivers reported symptoms such as headache, dizziness, nausea,

and dyspnoea at the end of work week. No human chronic toxicity or chronic epidemiology information for MTBE without coexposure to gasoline was found.

Ten healthy male volunteers undergoing light physical work were exposed to 5, 25, and 50 ppm MTBE vapor for 2 hours (Nihlen *et al*., 1998). While a solvent smell was noted at these concentrations, there were no consistent concentration-related effects on reported ocular or nasal irritation. The blockage index (a measure of nasal airway resistance) increased significantly after exposure but was not correlated with exposure concentration.

## V. Effects of Animal Exposure

Male and female rats (50/sex/group) were exposed by inhalation for 6 hours/day, 5 days/week to mean concentrations of 0, 403, 3023, or 7977 ppm (0, 1453, 10,900, or 28,760 mg/m$^3$) MTBE for 24 months (Chun *et al.*, 1992). Clinical signs, hematology, body weights and food consumption were monitored. Necropsy included measurements of organ weights and histopathology. Corticosterone levels were measured on 10 animals prior to sacrifice. Serum enzymes were not monitored. The NOAEL for several endpoints, including non-alpha-2μ-globulin induced nephrotoxicity, increased relative liver and kidney weights and prostration in females, and periocular swelling in both sexes was 403 ppm (1453 mg/m$^3$).

Mice were exposed for 6 hours/day, 5 days/week for 18 months to MTBE concentrations of 0, 402, 3014, or 7973 ppm (0, 111, 835, or 2208 mg/m$^3$) (Burleigh-Flayer *et al.*, 1992). The mice exposed to the highest concentration (7973 ppm) all exhibited ataxia. Prostration was also noted in 8 of 50 animals in this group. Liver weights were elevated in a concentration-dependent manner in the female mice but this change was not significant at the lowest concentration (402 ppm). Kidney weights were elevated in the female mice exposed to 7973 ppm. At the highest concentration, a significant increase in hepatocellular hypertrophy and adrenal gland weight was detected in the male mice. Spleen weights were increased in the females exposed to the highest concentration.

Moser *et al*. (1998) exposed female B6C3F$_1$ mice to 7924 ppm (2195 mg/m$^3$) MTBE for 4 months, or 7919 ppm (2194 mg/m$^3$) MTBE for 8 months; controls received plain air. Body weight increases for control and MTBE-exposed mice, respectively, were 57% and 37% at 4 months and 79% and 45% at 8 months: the reduced weight gain in MTBE-exposed mice was significantly different from the controls at both time points. In MTBE-exposed mice, mean uterine weight was 83% reduced relative to controls at 4 and 8 months. Ovary weight was also reduced in exposed mice, the mean weight being 55% of control at 4 months and 51% of control at 8 months. Pituitary weights were decreased by 44% and 31% at 4 and 8 months, relative to controls. Disturbances of the estrus cycle and histological changes in the reproductive organs were also noted. Although the changes in organ weights and histology were suggestive of an anti-estrogenic effect of MTBE, serum estrogen levels were unaffected. No changes in estrogen receptor (ER) immunoreactivity in reproductive system tissues were observed. Experiments *in vitro* failed to demonstrate any inhibition of estradiol binding to ER by MTBE or its metabolites. No inhibition of ER by MTBE was detected, nor was there any inhibition of the induction of ER by estradiol. The authors concluded that the apparent anti-estrogenic effects of MTBE were not

mediated via the ER, and drew a parallel with the anti-estrogenic effects of dioxins and chlorinated biphenyls.

Tests for histopathology in the respiratory tract, plasma corticosterone levels, motor activity and neurobehavioral endpoints were performed in rats exposed to MTBE at concentrations of 0, 797, 3920, or 8043 ppm (0, 2877, 14151, or 29035 mg/m$^3$), 6 hours/day, 5 days/week for 13 weeks (Dodd and Kintigh, 1989). Of these endpoints, the most significant finding was an elevation in plasma corticosterone in the high dose group. This finding was consistent with the elevated adrenal weights reported by Burleigh-Flayer *et al.* (1992). A clear dose-response for neurotoxic effects in these rats was not established.Biles *et al.* (1987) reported a NOAEL of 300 ppm (1083 mg/m$^3$) MTBE for decreased pup viability in rats exposed for 6 hours/day, 5 days/week for a total of 16 weeks. Animals exposed to 1240 ppm (4470 mg/m$^3$) or 2860 ppm (10,311 mg/m$^3$) MTBE exhibited slightly decreased pup survival.

Neeper-Bradley (1991) exposed rats to 0, 402, 3019, or 8007 ppm (0, 111, 836, and 2218 mg/m$^3$) MTBE over 2 generations. Exposures were for 6 hours/day, 5 days/week during the prebreeding period, and for 7 hours/day, 5 days/week during gestation and lactation. Parental effects of MTBE exposure were observed, including ataxia, blepharospasm, lack of startle reflex, and increased relative liver weights (F1 generation only). There were no histological changes in the organs from either parental generation. Reduced body weights were observed in the F1 and F2 pups at the 3019 and 8007 ppm concentrations. Reduced survivability to postnatal day 4 was observed in the 8007 ppm group. No adverse effects were noted at the 403 ppm (111 mg/m$^3$) concentration.

In a developmental and reproductive toxicity study, Conaway and associates (1985) found no significant increases in maternal or fetal toxicity, nor in pregnancy rates or in any gross toxicologic parameter tested with pregnant rats or mice exposed during gestation to concentrations of MTBE up to 3300 ppm (11,897 mg/m$^3$).

Maternal toxicity, in the form of hypoactivity and ataxia, was observed in pregnant mice exposed during gestation to 4076 ppm (14,690 mg/m$^3$) MTBE (Bushy Run Research Center, 1989a). Significant reductions in food intake and body weight gain were observed in dams exposed to 8153 ppm (29,390 mg/m$^3$). Fetal body weight was significantly reduced in the 4076 ppm group, and there were significant increases in the incidences of skeletal variations and unossified phalanges in the 4076 and 8153 ppm groups. Pregnant rabbits exposed to similar concentrations during gestation showed no significant maternal or fetal toxicity or developmental toxicity up to a concentration of 8021 ppm (28,918 mg/m$^3$) (Bushy Run Research Center, 1989b).

## VI. Derivation of Chronic Reference Exposure Level

| | |
|---|---|
| *Study* | Chun *et al.*, 1992; Bird 1997 |
| *Study population* | Male and female rats (50 per sex/group) |
| *Exposure method* | Discontinuous whole-body inhalation exposures (0, 403, 3023, or 7977 ppm) |
| *Critical effects* | Nephrotoxicity, increased liver and kidney weight, prostration and periocular swelling |
| *LOAEL* | 3023 ppm |
| *NOAEL* | 403 ppm |
| *Exposure continuity* | 6 hours per day, 5 days per week |
| *Exposure duration* | 24 months |
| *Average experimental exposure* | 72 ppm for the NOAEL group |
| *Human equivalent concentration* | 72 ppm for the NOAEL group (gas with systemic effects, based on RGDR = 1.0 using default assumption that lambda (a) = lambda (h)) |
| *LOAEL uncertainty factor* | 1 |
| *Subchronic uncertainty factor* | 1 |
| *Interspecies uncertainty factor* | 3 |
| *Intraspecies uncertainty factor* | 10 |
| *Cumulative uncertainty factor* | 30 |
| *Inhalation reference exposure level* | 2 ppm (2000 ppb, 8 mg/m$^3$, 8000 μg/m$^3$) |

The USEPA (1995) based its RfC of 3000 μg/m$^3$ on the same study but included a Modifying Factor (MF) of 3 for database deficiencies. The criteria for use of modifying factors are not well specified by U.S. EPA. Such modifying factors were not used by OEHHA.

## VII. Data Strengths and Limitations for Development of the REL

The major strengths of the REL for MTBE are the use of a comprehensive, long-term multiple dose study with large sample sizes and the observation of a NOAEL. The major uncertainty is the lack of human data.

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*CHRONIC TOXICITY SUMMARY*

# METHYLENE CHLORIDE

*(dichloromethane, methylene dichloride)*

**CAS Registry Number: 75-09-2**

## I. Chronic Toxicity Summary

| | |
|---|---|
| *Inhalation reference exposure level* | **400 µg/m$^3$ (100 ppb)** |
| *Critical effect(s)* | Carboxyhemoglobin formation above 2% in human workers |
| *Hazard index target(s)* | Cardiovascular system; nervous system |

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

| | |
|---|---|
| *Description* | Colorless liquid |
| *Molecular formula* | $CH_2Cl_2$ |
| *Molecular weight* | 84.93 |
| *Density* | 1.32 g/cm$^3$ @ 20° C (ACGIH, 1991) |
| *Boiling point* | 39.75° C |
| *Vapor pressure* | 400 torr @ 24.1° C |
| *Solubility* | Miscible with most organic solvents, slightly soluble in water (ACGIH, 1991) |
| *Conversion factor* | 1 ppm = 3.47 mg/m$^3$ @ 25° C |

## III. Major Uses and Sources

Methylene chloride (MC) is used in paint and varnish remover, in aerosols as a cosolvent or vapor pressure depressant, and in solvent degreasing and metal cleaning. It is also used in plastics processing and in extraction of fats and oils from food products (HSDB, 1999). The annual statewide industrial emissions from facilities reporting under the Air Toxics Hot Spots Act in California, based on the most recent inventory, were estimated to be 3,504,271 pounds of methylene chloride (CARB, 1999a). Both mean and maximum monitored ambient methylene chloride concentrations have decreased slightly between 1990 and 1996 (CARB, 1999b). Median and maximum concentrations were 1.09 and 11 ppb in 1990 and 0.66 and 5.6 ppb in 1996.

## IV. Effects of Human Exposure

Effects of a controlled 2-hour inhalation exposure to MC included CNS depression at concentrations of 1000 ppm (3500 mg/m$^3$) or more and increased blood carboxyhemoglobin

(COHb) content at lower concentrations (500 ppm) due to metabolism of MC to carbon monoxide (Stewart *et al.*, 1972). High levels of COHb can be found in the blood hours after exposure to methylene chloride, due to its partitioning into fat and its slow release into circulation with subsequent metabolism, leading to formation of carbon monoxide (Engstrom and Bjurstrom, 1977). In situations of chronic exposure, carbon monoxide toxicity is also of concern. Barrowcliff (1978) documented the case of an adult male who developed an unsteady gait, a peculiar dysarthria and a loss of memory. The man had worked with 15-50 liters of methylene chloride daily for 3 years in a poorly ventilated room while cleansing road materials. No natural disease could be found to explain his conditions and the effects were attributed to chronic carbon monoxide poisoning.

Twelve women volunteer subjects were exposed to 0, 300, or 800 ppm methylene chloride for 4 hours (Fodor and Winneke, 1971). Neurobehavioral vigilance was measured by auditory discrimination of intensity of certain sound pulses against a background of continuous white noise. A significant interactive effect between methylene chloride concentration and duration of exposure using 2-way ANOVA ($p < 0.01$) was found.

Human erythrocytes enzymatically convert methylene chloride to formaldehyde in cell-culture experiments (Hallier *et al.*, 1994).

A subacute controlled exposure of eleven resting non-smokers to methylene chloride was conducted by DiVincenzo and Kaplan (1981a). The eleven subjects were exposed to 50, 100, 150, or 200 ppm methylene chloride for 7.5 hours on 5 consecutive days. Exposure to all concentrations led to dose-dependent elevation in COHb concentrations in the blood and elevated exhaled CO. The peak blood COHb saturations were 1.9, 3.4, 5.3, and 6.8%, respectively, for the 50, 100, 150, and 200 ppm groups.

Divencenzo and Kaplan (1981a) also measured COHb percentage in the blood of workers occupationally exposed to methylene chloride and a group of workers not exposed to methylene chloride. The 19 workers exposed to methylene chloride had mean blood COHb concentrations of 2.3% in the morning and 3.9% at the end of the work-shift. Ambient concentrations in the workplace were estimated from 57 samples, which ranged from 0 to 250 ppm, with a mean concentration of 40 ppm. Three exposed workers also wore monitors to estimate personal exposures. The time-weighted average exposure for these workers was 33 ppm. Controls (8 subjects) had significantly lower mean blood COHb concentrations of 0.8% in the AM and 1.3% in the PM compared with the exposed workers. The length of employment of the exposed workers was not given.

A companion study by DiVincenzo and Kaplan (1981b) showed that smoking and methylene chloride exposure result in an additive effect on COHb levels compared with levels in non-smokers. Similarly, light, moderate or heavy exercise workloads resulted in higher COHb levels.

Soden *et al.* (1996) showed a dose-response increase in carboxyhemoglobin levels in non-smokers with increasing methylene chloride exposure in workers involved in triacetate fiber production. Carboxyhemoglobin levels ranged from 1.77% to 4% from exposures ranging from 6.5 to 89.7 ppm, respectively. The number of employees in the study was not reported.

Although animal studies have shown COHb-induced cardiovascular effects following MC exposure (Aviado *et al.*, 1977), no data exist on this outcome in humans. However, studies of men with coronary artery disease and exercise-induced angina report a decrease in time to onset of exercise-induced angina following exposure to carbon monoxide (CO) at concentrations sufficient to result in blood COHb levels of about 2% (Kleinman *et al.*, 1989; Allred *et al.*, 1989). A physiologically based pharmacokinetic model of MC and CO estimated that a 1-hour exposure to 340 ppm (1200 mg/m$^3$) MC at a ventilation rate of 9 liters/min would result in a peak blood COHb level of 2% (Andersen *et al.*, 1991; Reitz, 1994). The California Ambient Air Quality Standard for CO is based on a blood COHb level of 2% (CARB, 1982).

An epidemiological study of 751 male workers in the Eastman Kodak Company exposed to daily 8-hour time-weighted average concentrations of 30-125 ppm methylene chloride for up to 30 years was conducted by Friedlander and associates (1978). A control group of workers in production but not exposed to methylene chloride was used together with New York state cause and age-specific mortality rates. The follow-up period for these workers was 13 years, with 97% success. The studies did not indicate any increase in risk of death from circulatory disease, cancer, or other causes due to methylene chloride exposure.

A study of female pharmaceutical workers in eight different factories exposed to a variety of organic solvents indicated that solvent exposure, and particularly methylene chloride exposure, resulted in an increase in spontaneous abortions (Taskinen *et al.*, 1986). In all, 1795 pregnancies were followed, with 142 spontaneous abortions occurring. The odds ratio for methylene chloride exposure was 1.0 to 5.7 (average = 2.3; $p < 0.06$). There was a significant effect of exposure to 4 or more solvents, compared with age-matched controls ($p < 0.05$). The concentrations of MC were not reported in the study.

The U.S. Occupational Safety and Health Administration reduced its permissible exposure limits (PEL) for MC from 500 ppm to 25 ppm in 1997 (U.S. CFR, 1997).

## V. Effects of Animal Exposure

Nitschke *et al.* (1988) found that a 2-year exposure to 0, 50, 200, or 500 ppm MC for 6 hours/day, 5 days/week resulted in significant histopathologic lesions in the livers of rats exposed to 500 ppm. No significant adverse effects were observed at 200 ppm or lower. The predominant hepatocellular lesion was fatty vacuolization of hepatocytes.

Female B6C3F1 mice inhaling 2000 ppm MC for 1 to 26 weeks had 40 to 60% lower cell turnover rates of bronchiolar cells compared with controls (Kanno *et al*., 1993). At this concentration no observable pathological changes were found in the lungs of MC exposed animals.

A continuous exposure of mice (16 per group) to 100 ppm MC for 1, 2, 3, 4 or 10 weeks resulted in signifcant elevation in liver triglycerides beginning at 2 weeks and lasting throughout the 10-week period (Weinstein and Diamond, 1972). Liver/body weight ratios were unaffected at any

time point. After 1 week, small fat droplets were apparent in centrilobular hepatocytes and a decrease in hepatic glycogen was also noted. Necrosis was not observed during the 10-week period, but fat droplet size increased and glycogen depletion persisted.

Male and female Sprague-Dawley rats and Golden Syrian hamsters inhaled methylene chloride (0, 500, 1500, or 3500 ppm) for 6 hr per day, 5 days a week over 2 years (Burek *et al.*, 1984). The groups consisted of 129 rats per sex per concentration, and 107 to 109 hamsters per sex per concentration. Females rats inhaling 3500 ppm had an increased mortality rate while female hamsters inhaling 1500 or 3500 ppm had decreased mortality rates. Slight histopathological findings were noted in livers of rats exposed to 500, 1500, or 3500 ppm MC. Decreased amyloidosis was also found in livers and other organs of hamsters at each of the three MC concentrations. Overall, effects were more potent in rats compared with hamsters, which had fewer spontaneous age-related changes, decreased mortality (at least for females), and evidence of specific target organ toxicity was weak. Carboxyhemoglobin values were elevated in both rats and hamsters exposed to 500 ppm or more of MC, with the percentage increase greater in hamsters than in rats.
Monkeys were observed to be more susceptible subjects for methylene chloride induced COHb than dogs upon 14-week subchronic continuous exposure to 25 or 100 ppm (Haun *et al.*, 1972). At 25 ppm, approximately 1.5% COHb was reached in the 4 monkeys, compared to approximately 0.5% in 16 dogs. Monkeys exposed to 100 ppm MC had COHb levels of approximately 4% compared with 2% in the dogs.

Oral ethanol pretreatment in rats has been shown to suppress the COHb formation characteristic of methylene chloride exposure through inhibition of biotransformation of methylene chloride (Glatzel *et al.*, 1987).

Gerbils (10/sex per group; 60 controls) exposed continuously to MC concentrations of 210, 350, or 700 ppm for a period of 3 months, with a 4-month follow-up period, showed irreversible cellular and biochemical changes in brain (Rosengren *et al.*, 1986). A high mortality rate (19/20) was observed in the 700 ppm group, and this exposure was terminated after 7 weeks. The gerbils exposed to 350 ppm also had a high mortality rate (9/20) and this exposure was terminated after 10 weeks. The gerbils exposed to 210 ppm had no premature mortality and the exposure continued for the full 3 months. Four months after termination of exposure, the animals in the 350 and 210 ppm groups had significantly decreased brain DNA content in the hippocampus. The 350 ppm group exhibited elevated astroglial proteins in the frontal and sensory motor cerebral cortex, consistent with astrogliosis in these regions. In addition, the gerbils exposed to 350 ppm MC had significantly decreased DNA in the cerebellar hemispheres. Complimentary studies by these investigators showed that the formation of carboxyhemoglobin did not increase in gerbils between the 210 and 350 ppm exposures, indicating that the metabolism of MC to CO is saturable at concentrations below those in the study. On the other hand, the neurotoxic brain biochemical alterations were significantly greater in gerbils exposed to 350 ppm as compared with the 210 ppm group, implying that carboxyhemoglobin induced cerebral hypoxia is not the major cause of MC-induced neurotoxicity in the brain.

Rats (50 per sex per group) were exposed to 0, 1000, 2000, or 4000 ppm methylene chloride 6 hours/day, 5 days/week for 102 weeks (NTP, 1986). Both sexes exhibited hemosiderin

pigmentation in the liver in a dose-dependent fashion, beginning with the 1000 ppm concentration. Squamous metaplasia of the nasal cavity was observed in female rats, and thyroid C-cell hyperplasia was observed in males exposed to 2000 ppm or greater. Kidney tubule degeneration (not otherwise specified) was increased at all exposure levels.

Mice (50 per sex per group) exposed to 0, 2000, or 4000 ppm methylene chloride 6 hours/day, 5 days/week for 102 weeks showed increased incidence of liver cytologic degeneration and splenic atrophy at 4000 ppm (males) (NTP, 1986). Male and female mice also had an increased incidence of kidney tubule casts (not otherwise specified) at 2000 ppm or greater, and significant testicular atrophy was observed in males at 4000 ppm. Female mice showed cytologic degeneration in the liver at 2000 ppm or greater, and ovarian atrophy at 2000 ppm or greater.

A six month exposure to 5000 ppm MC of 8 guinea pigs for 7 hours/day, 5 days/week resulted in 3 deaths; 2 showed moderate centrilobular fatty degeneration of the liver and extensive pneumonia at necropsy (Heppel *et al.*, 1944). None of the 14 control animals died. Food consumption and body weight were lower in the exposed guinea pigs, compared with control pigs. One out of 12 rats died at this concentration, and the liver histology in this animal revealed multiple thrombi in renal vessels, associated with marked cortical infarction. By comparison, dogs and rabbits showed no signs of illness, nor were blood pressure or hematological values altered at the 5000 ppm concentration. At 10,000 ppm, 2 of 4 dogs showed moderate centrilobular congestion, narrowing of liver cell cords, and slight to moderate fatty degeneration. One of 2 monkeys revealed disseminated tuberculosis lesions, but no other histological alterations. Four out of 6 guinea pigs had moderate fatty degeneration of the liver at this concentration.

The offspring of rats (10 dams per group) exposed during gestation to 0 or 4500 ppm methylene chloride exhibited altered rates of behavioral habituation to novel environments (Bornschein *et al.*, 1980). This effect was observed beginning at 10 days of age but was still demonstrable in rats 150 days old. The authors concluded that elevated maternal COHb could have been a contributing factor in the developmental impairment.

In a study of the effects of methylene chloride on estrous cycle and serum prolactin, groups of 15 female rats were exposed to 0 or 3500 ppm for 6 hours/day for 15 to 19 consecutive days (Breslin and Landry, 1986). Males (15 per group) were exposed for 5 hours/day for 5 consecutive days. Female rats exhibited decreased body weight and increases in the estrous cycle duration and in serum prolactin. Males did not show any significant effects on serum prolactin from methylene chloride exposure.

Pregnant mice and rats were exposed to 0 or 1250 ppm MC 7 hours/day, on days 6 through 15 of gestation (Schwetz *et al.*, 1975). Significantly elevated absolute liver weights were seen in maternal animals from both species. In addition, significantly increased incidences of delayed ossification of the sternebrae were seen in both species, compared to controls.

Methylene chloride exposure of female rats before or during gestation to 4500 ppm resulted in elevated maternal liver weights and decreased birth weights of the offspring, but no terata or skeletal/soft tissue anomalies (Hardin and Manson, 1980).

A 2-generation reproduction test was conducted by Dow Chemical Company (Nitschke *et al.*, 1985) which showed no significant reproductive or developmental effects in rats exposed to 0, 100, 500, or 1500 ppm MC 6 hours/day, 5 days/week, for 14 weeks. The exposure conditions were identical for the $F_0$ and $F_1$ generations.

## VI. Derivation of Chronic Reference Exposure Level (REL)

| | |
|---|---|
| *Study* | DiVincenzo and Kaplan (1981a) |
| *Study population* | 19 workers, 8 controls |
| *Exposure method* | Occupational inhalation exposure |
| *Critical effects* | Significantly elevated carboxyhemoglobin levels (> 2%) |
| *LOAEL* | 40 ppm (ambient workplace exposures averaged 40 ppm with a range of 0 to 250 ppm); controls = 0 ppm |
| *NOAEL* | Not observed |
| *Exposure continuity* | 8 hours/day, 5 days/week |
| *Exposure duration* | Length of employment unspecified |
| **Average occcupational exposure** | 14 ppm for LOAEL group (40 x 10/20 x 5/7) |
| *LOAEL uncertainty factor* | 10 |
| *Subchronic uncertainty factor* | 1 (see following text for explanation) |
| *Interspecies uncertainty factor* | 1 |
| *Intraspecies uncertainty factor* | 10 |
| *Cumulative uncertainty factor* | 100 |
| *Inhalation reference exposure level* | 0.1 ppm (100 pbb; 0.4 mg/m$^3$; 400 µg/m$^3$) |

Workers were exposed to average measured concentrations of 40 ppm during the workday, and the personal monitors on 3 of the subjects indicated a 8-hour time-weighted average of 33 ppm over a 2-week period. The average COHb levels were 3.9% at the end of the work-shift. Elevated carboxyhemoglobin concentrations of above 2% are considered to aggravate angina in some individuals (CARB, 1982). In effect, 2% COHb can be considered a NOAEL for aggravation of angina. Therefore, the 33 ppm concentration was considered a LOAEL for the formation of greater than 2% COHb. The duration of the employment period was not specified. However, in the DiVincenzo and Kaplan (1981a) study, the levels of COHb did not appear to increase over a period of 5 days in experimental exposures using volunteers, therefore an uncertainty factor for subchronic exposure was not necessary. A number of factors contribute to the uncertainty in determining the degree of sensitivity to methylene chloride, including activity level, metabolic enzyme activity, age, and background COHb status (e.g., from smoking, etc.).

The subchronic study by Haun *et al.* (1972) with monkeys reported a NOAEL of 25 ppm and a LOAEL of 100 ppm for 2% COHb formation following a 14-week exposure. These results are consistent with the LOAEL reported in the DiVincenzo and Kaplan study. However, the human occupational study likely contains less uncertainty, since the toxicokinetics of the effect,

including rate of formation of CO and thus COHb is metabolism-dependent, resulting in considerable potential interspecies differences.

The study in hamsters by Burek *et al.*(1984) showed a LOAEL for elevated carboxyhemoglobin of 500 ppm. A time-weight average exposure and HEC of 89 ppm was calculated. Using a 10-fold LOAEL uncertainty factor, a 3-fold interspecies uncertainty factor for residual uncertainty not accounted for in the HEC calculation, and a 10-fold intraspecies uncertainty factor, a REL of 300 ppb or 1000 µg/m$^3$ was derived. Thus, the REL derived from the best available animal study is comparable to the 400 µg/m$^3$ REL derived from the best-available human study.

## VII. Data Strengths and Limitations for Development of the REL

The major strength of the key study (DiVincenzo and Kaplan, 1981a) used to derive the REL for methylene chloride is that human health effects were observed. The major uncertainties from the key study itself are the lack of a NOAEL observation, the difficulty in estimating exposures, and the discontinuous and variable nature of the exposures.

The health effects database for methylene chloride includes, in addition to an adequate study of human occupational exposures (DiVincenzo and Kaplan, 1981a), an adequate lifetime inhalation exposure study in 2 species of laboratory animals (Burek *et al*., 1984). The REL values derived from these studies (400 µg/m$^3$ vs. 1,000 µg/m$^3$) are comparable. That both the human and animal studies measured the same endpoint and arrived at similar conclusions is a circumstance that is rarely found but one that considerably increases the weight of evidence from which the REL was derived. The two studies complement each other, as the animal study involved controlled, measured exposures over a lifetime but introduces the uncertainty of predicting human health effects from animal observations, and the human study involved poorly characterized human exposures but lacks the uncertainty inherent in interspecies extrapolation.

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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

| | |
|---|---|
| *Inhalation reference exposure level* | **20 µg/m$^3$** (2 ppb) |
| *Critical effect(s)* | Ocular toxicity to the retinas of guinea pigs |
| *Hazard index target(s)* | Eyes; alimentary system (hepatotoxicity) |

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

| | |
|---|---|
| *Description* | Colorless to pale yellow flakes; tan |
| *Molecular formula* | $C_{13}H_{14}N_2$ |
| *Molecular weight* | 198.3 g/mol |
| *Boiling point* | 398-399°C |
| *Melting point* | 92.5°C |
| *Vapor pressure* | 1 torr @ 197°C |
| *Solubility* | Soluble in alcohol, benzene, ether; 273 g/100 g acetone; 0.1 g/100 g water @ 25°C |
| *Conversion factor* | 8.1 µg/m$^3$ per ppb at 25ºC |

## III. Major Uses and Sources

4,4'-Methylene dianiline (MDA) is synthesized by the reaction of aniline with formaldehyde. MDA's major uses are as a chemical intermediate in the synthesis of certain isocyanates and polyurethane polymers, as a corrosion inhibitor, in the preparation of azo dyes, as a rubber preservative, and in the curing of epoxy resins and neoprene (HSDB, 1995; ACGIH, 1992). The annual statewide industrial emissions from facilities reporting under the Air Toxics Hot Spots Act in California based on the most recent inventory were estimated to be 1133 pounds of MDA (and its dichloride) (CARB, 2000).

## IV. Effects of Human Exposure

Several cases of human exposure to MDA have identified the compound as a hepatotoxicant which produces cholestatic jaundice (Kopelman *et al.*, 1966; McGill and Motto, 1974; Williams *et al.*, 1974; Bastian, 1984). Bastian (1984) described cases of acute hepatic illness in four workers exposed from laying floors using an epoxy resin base, which contained MDA as a curing agent. The workers were exposed via fumes and dusts in the air as well from hand

contact with powder and had worked with epoxy resins for periods ranging from one to 12 years. The level of exposure was not quantified. The workers initially reported to the hospital with symptoms of abdominal pain three days after the most recent exposure and all were discharged within four days. Two workers continued to show severe symptoms five days after the onset, with abdominal pain, jaundice, a tender liver, nausea, dyspnea, and muscular pain. Plasma bilirubin, alkaline phosphatase, and aspartate aminotransferase levels were elevated. Some symptoms did not subside until two months after the onset. One worker, after another exposure, experienced nausea, abdominal pain, and muscular pain. A second worker reported further symptoms of headache, tiredness, and decreased libido.

Williams *et al.* (1974) reported symptoms in 6 of approximately 300 workers exposed to MDA by surface coating concrete walls with epoxy resins. Exposure probably occurred by inhalation, ingestion, and skin contact as a result of mixing powder containing MDA. Symptoms of clinical hepatitis in the 6 workers appeared two days to two weeks after beginning work; five of the six had elevated bilirubin levels, and one liver biopsy showed bile stasis. All the workers recovered completely after an unspecified time.

McGill and Motto (1974) described hepatitis among 13 men who, over the course of 6 years, were occupationally exposed to MDA in the blending of epoxy resins used in the manufacture of insulating material. Among the 13 patients showing symptoms, all reported weakness, jaundice, and dark urine; 11 reported abdominal pain, nausea or vomiting, and anorexia; and over half reported fever, chills and/or headache. All the workers recovered within a 10 week period. After the first cases of hepatitis occurred, air sampling showed initial levels of MDA to be 0.1 ppm in the work area. After additional cases of hepatitis occurred, measures were taken to reduce worker exposure, and air levels were reduced to as low as 0.0064 ppm. The authors concluded that percutaneous absorption was the likely major route of exposure in light of the fact that cases occurred in spite of measures taken to reduce air levels and there was evidence that significant hand contact with the compound occurred during the workday. Since the symptoms appeared within one to 18 days after “working intensively” with the compound and exposure routes were not clearly established, quantitation of exposure levels was considered difficult.

The most well-known incident of MDA toxicity to humans resulted from ingestion of bread made with flour contaminated with MDA during transport (Kopelman *et al.*, 1966a). Eighty-four persons showed symptoms of abdominal pain and some degree of jaundice. All patients had elevated serum alkaline phosphatase and glutamic oxaloacetic transaminase levels. Seventeen had serum bilirubin levels over 5 mg/100 ml. Liver biopsy was performed on 8 persons and evaluated in a separate study (Kopelman *et al.*, 1966b). The primary finding was an unusual lesion described during the early course of the disease as portal zone cholangitis and later as centrilobular cholestasis with necrosis. The initial study reported that all but 2 patients had complete recovery within several weeks. However, a two year follow-up study of 14 individuals showed that 10 still had symptoms of some severity 7 to 23 months after initial onset including food intolerance, gastrointestinal disturbances, fatigue, and visual disturbances (Kopelman, 1968).

Human effects other than hepatotoxicity have been described including several cases of contact dermatitis and skin sensitization (LeVine, 1983; Van Joost *et al.*, 1987; de Pablo *et al.*, 1992;

Bruynzeel and van der Wegen-Keijser, 1993). A case report of a man exposed to MDA with potassium carbonate and γ-butyrolactone by accidental ingestion has been described (Roy *et al.*, 1985). In addition to hepatitis and abnormal liver function, which persisted over 18 months, the patient developed a progressively worsening retinopathy described as a "malfunction of the retinal pigment epithelium" accompanied by diminished visual acuity. The patient improved after approximately 3 months, but after examination at 18 months had not completely recovered.

Another report described the development of acute cardiomyopathy in addition to hepatitis in a worker exposed to a large quantity of MDA dust as the result of air filtration malfunction (Brooks *et al.*, 1979). The patient showed an abnormal ECG and an elevated cardiac LDH isoenzyme profile, which returned to normal within one month of onset.

## V. Effects of Animal Exposure

The carcinogenicity of MDA was investigated in F344/N rats and $B6C3F_1$ mice (50/sex/dose group) administered in the drinking water at concentrations of 0, 150, and 300 ppm MDA (dihydrochloride) for 103 weeks (Lamb *et al.*, 1986). A 14-day range finding study was also conducted with 5 animal/sex/species/dose group, with exposure levels of 0, 200, 400, 800, 1600, and 3200 ppm MDA. A 13-week subchronic study was conducted with 10 animals/sex/species/dose group and exposure levels of 0, 25 (mice), 50, 100, 200, 400, and 800 (rats) ppm MDA. Using body weight and drinking water values from the study, low and high daily doses in the chronic study were calculated to be 9 and 16 mg/kg-day for male rats, 10 and 19 for female rats, 25 and 57 for male mice, and 19 and 43 for female mice. In the chronic study, survival was reduced among male mice treated with 300 ppm MDA. Final mean body weights were reduced in the 300 ppm dose group of female rats (-9%), male mice (-13%), and female mice (-16%). Among rats, non-cancer effects included follicular cysts and follicular-cell hyperplasia of the thyroid (significantly increased incidence in high-dose females; $p<0.05$ by Fisher's exact test). In the liver, the incidence of fatty and focal cellular change was elevated in low-dose male and female rats and also in high dose male rats. Incidence of unspecified dilatation of the liver was also elevated in high-dose male rats. Increased incidence of kidney mineralization was found in male rats treated with 300 ppm MDA. Among mice, incidence of liver degeneration was elevated in males in both treatment groups and females in the high-dose group ($p<0.01$ by Fisher's exact test). Incidence of kidney nephropathy was increased in male and female mice in both treatment groups and mineralization of the renal papilla was increased in both sexes in the high-dose group ($p<0.01$). From the 13-week study, the authors noted thyroid and bile duct effects in rats at 800 ppm MDA in water and in mice at 400 ppm MDA in water.

Albino and pigmented guinea pigs were exposed to aerosols of methylene dianiline in polyethylene glycol 200 (PEG) in nose-only exposure chambers (Leong *et al.*, 1987). Animals (8 of each strain) were exposed to a time-weighted average aerosol concentration of 0.44 g MDA/$m^3$ in air for 4 hours/day, 5 days/week for 2 weeks. Eight control animals were neither exposed to aerosol nor placed in the exposure chamber. Two weeks after the exposure period, animals were evaluated for dermal sensitization and irritation by challenge with 0.05 ml of 0, 2, 20, and 200 mg MDA/ml in PEG for up to 24 hours. No evidence of dermal irritation or

sensitivity was found. Subsequently, the animals were also examined for pulmonary sensitization by challenge with aerosols containing 0.01 and 0.05 ml of 200 mg MDA/ml PEG. Lung insufflation pressures were measured as an indication of changes in lung distensibility. No evidence of pulmonary sensitization was found. After the pulmonary challenge, the animals were examined histopathologically, with emphasis on eye, lung, liver, and kidney toxicity. Ocular toxicity ranging from mild to more severe was observed in all MDA-treated animals, but in none of the control animals. Pigmented animals did not differ in sensitivity or effect compared to albino animals. Mild lesions were described as "retraction and thickening of the outer segments of the photoreceptor cells" while more severe effects included swelling "through the inner segments of the photoreceptor cells to the outer nuclear layer." Some evidence of inflammatory cell infiltration was also noted and the pigmented epithelial layer was also degenerated. The authors conclude that the effects were attributable to MDA because no retinal lesions have been associated with exposure to the PEG vehicle. Furthermore, the inhalation exposures to MDA are the likely cause rather than the dermal and lung sensitization study exposures because these subsequent studies were conducted on control as well as treated animals. Pulmonary granulomas consisting of "an aggregate of macrophages surrounded by a thin mantle of lymphocytes" were found in 7 of the 16 MDA-exposed animals and one of the 8 control animals (level of significance was not stated). Treated and control animals had a high background incidence of pulmonary lesions including slight to mild bronchitis. No liver or kidney effects were detected in treated animals.

Nine purebred beagle dogs were treated orally (by capsule) with 70 mg "crude" (4 dogs) or "purified" (5 dogs) MDA in corn oil three days per week for a period ranging from approximately 3 to 7 years (Deichmann *et al.*, 1978). No concurrent controls were included since untreated animals were regularly maintained in the laboratory. After 2 years, cystoscopic examination was performed at 15-month intervals. After 4½ years, clinical chemistry tests were performed at 4 month intervals on 3 dogs from each group. Microscopic examination of urinary bladder, liver, heart, ovaries, uterus, and lymph nodes was performed on moribund animals or at the end of the experimental period (7 years, 2 months). Liver toxicity was noted in all the treated animals. Effects were described as fatty change, cell degeneration and necrosis, and lymphoid cell infiltration. One dog from each treatment group died from the toxic effect on the liver. The kidneys of four treated animals (two from each group) showed toxic effects including granuloma, glomerular nephritis, and congestion with cloudy swelling. Two dogs treated with "purified" and one dog treated with "crude" MDA showed toxicity to the spleen described as hemosiderosis and swelling with lymphocyte infiltration.

Wistar rats (5/sex/dose) were treated orally with 0, 0.0083, and 0.083 g MDA/kg body weight in propylene glycol daily for 12 weeks (Pludro *et al.*, 1969). Doses were 1% and 10% of the experimentally determined median lethal dose. No significant changes in body weight or hematological parameters were found, although serum albumin, β-globulin, and γ-globulin were elevated in animals in the 0.083 mg/kg dose group. The livers of all the animals in the high dose group showed signs of degeneration, including atrophy of the parenchyma and stromal hyperplasia in the portal areas. Also in this dose group, all animals showed hypertrophy of the lymphatic nodules of the spleen. In the low dose group, one animal showed a liver lesion and one a lesion in the spleen.

Schoental (1968) treated rats (8/sex) with MDA in 25% aqueous ethanol by stomach tube. Rats were given 20 mg doses a total of 2-5 times over several weeks up to 7½ months (frequency not specified). Animals showed necrosis of the liver and kidney and congestion and edema of the lungs.

Visual toxicity was reported in 15 cats treated perorally with 25-100 mg MDA/kg body weight in a 1% aqueous suspension (Schilling von Canstatt *et al.*, 1966). In four animals treated once with 100 mg/kg, no blindness was reported. In all the other treated animals (four with one dose of 100 mg/kg, two with one dose of 150 mg/kg, and two with three doses of 25 mg/kg and 3 doses of 50 mg/kg), blindness occurred within 8 days. Three of the eight recovered sight within 4 days. Two other treated animals were examined microscopically, one treated with 25 and then 50 mg/kg and one treated once with 200 mg/kg. The first was examined after 7 days and showed signs of granular degeneration of the rods and cones with some proliferation of the pigmented epithelium. The second was examined after 4¼ years and showed atrophy of the retinal neuroepithelium. The authors noted that no visual disturbances were found in other MDA treated experimental animals, including dog, rabbit, guinea pig, and rat.

## VI. Derivation of Chronic Reference Exposure Level (REL)

| | |
|---|---|
| *Study* | Leong *et al.*, 1987 |
| *Study population* | Guinea pigs |
| *Exposure method* | Discontinuous inhalation exposure (nose only) of aerosols |
| *Critical effects* | Degeneration of retinal epithelium |
| *LOAEL* | 440 mg/m$^3$ (54 ppm) |
| *NOAEL* | Not observed |
| *Exposure continuity* | 4 hours/day, 5 days/week |
| *Exposure duration* | 2 weeks |
| *Average experimental exposure* | 52 mg/m$^3$ for LOAEL group (440 x 4/24 x 5/7) (6.4 ppm) |
| *Human equivalent concentration* | 52 mg/m$^3$ using the default assumption of RGDR = 1 for a gas with systemic effects |
| *LOAEL uncertainty factor* | 10 (incidence = 100%) |
| *Subchronic uncertainty factor* | 10 |
| *Interspecies uncertainty factor* | 3 |
| *Intraspecies uncertainty factor* | 10 |
| *Cumulative uncertainty factor* | 3,000 |
| *Inhalation reference exposure level* | 0.02 mg/m$^3$ (20 µg/m$^3$; 0.002 ppm; 2 ppb) |

Two specific types of toxicity have been associated with exposure to MDA: hepatotoxicity and ocular toxicity. Several studies have demonstrated hepatotoxicity in experimental animals. The best study of long term toxicity of MDA was the report by Lamb *et al.* (1986). In addition to addressing the carcinogenicity of MDA, Lamb described non-cancer health effects, which resulted from lifetime exposure of two species, rats and mice, to MDA at two concentrations in the drinking water. The 150 ppm dose level was a LOAEL for fatty change and focal cellular

change to the livers of male and female rats as well as for liver degeneration in male mice. The corresponding effects were also observed in high-dose male rats and male mice. Nephropathy was observed in mice of both sexes at the 150 and 300 ppm. There is abundant evidence from both human and animal studies that MDA is hepatotoxic. Bastian (1984), Williams *et al.* (1974), and McGill and Motto (1974) reported hepatitis in people exposed by inhalation and dermal absorption routes. Kopelman *et al.* (1966a,b) demonstrated human hepatotoxicity from exposure by the oral route. However, limited data detailing exposure levels associated with adverse health effects in humans preclude the development of a chronic REL from studies in humans.

The other toxic effect of potential concern from MDA exposure is ocular toxicity. Leong *et al.* (1987) reported damage to the retinas of guinea pigs exposed for 2 weeks to MDA aerosols (0.44 $g/m^3$ for 4 hr/day, 5 days/week; average experimental exposure = 52 $mg/m^3$) by inhalation. Schilling von Canstatt *et al.* (1966) also reported blindness in cats treated orally with MDA. A single case of retinopathy and visual toxicity in humans was reported in a man who accidentally ingested MDA with potassium carbonate and γ-butyrolactone. The Leong *et al.* (1986) study was selected for the development of the chronic REL because, although conducted for a relatively short period of time, the study appears to address the most sensitive endpoint of toxicity by the most appropriate route of exposure (inhalation). The studies, which established the hepatotoxicity of MDA, were conducted by the oral route of exposure.

As a comparison with the proposed REL, the study by Lamb *et al*. (1986) found a LOAEL of 9 mg/kg-day for liver changes in male rats. Use of a LOAEL UF of 3, an interspecies UF of 10, and an intraspecies UF of 10 results in an oral chronic REL of 0.03 mg/kg-day. Use of route-to-route extrapolation with the assumption that a 70 kg person breathes 20 $m^3$ of air per day leads to an inhalation chronic REL estimate of 100 $\mu g/m^3$. The proposed chronic REL based on Leong *et al*. (1987) is lower by a factor of 5 than that obtaimed by using Lamb *et al*. (1986) and should be protective of hepatotoxicity.

## VII. Data Strengths and Limitations for Development of the REL

The strengths of the inhalation REL for 4,4′-methylene dianiline include the availability of a controlled exposure inhalation study. Major areas of uncertainty are the lack of adequate human exposure data, the lack of chronic inhalation exposure studies, the lack of reproductive and developmental toxicity studies, and the lack of observation of a NOAEL. In addition the test animals were under additional stress due to the restraint used to obtain nose-only exposure, while the control animals were not restrained. Liver toxicity has been included as a potential critical effect because of uncertainty regarding the relative potency of this compound in causing liver toxicity in different species by different routes of exposure.

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

## VIII. Potential for Differential Impacts on Children's Health

No evidence to support a differential effect of methylene dianiline on infants and children was found in the literature.

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*CHRONIC TOXICITY SUMMARY*

# METHYLENE DIPHENYL ISOCYANATE

*(diphenylmethane diisocyanate)*

**CAS Registry Number: 101-68-8**

## I. Chronic Reference Exposure Level

| | |
|---|---|
| *Inhalation reference exposure level* | **0.7 µg/m$^3$** |
| *Critical effect(s)* | Hyperplasia of the olfactory epithelium in rats |
| *Hazard index target(s)* | Respiratory system |

## II. Physical and Chemical Properties (HSDB, 1995)

| | |
|---|---|
| *Description* | Light yellow solid |
| *Molecular formula* | $C_{15}H_{10}N_2O_2$ (monomer) |
| *Molecular weight* | Variable (monomer = 250.27 g/mol) |
| *Density* | 1.197 g/cm$^3$ @ 70°C (monomer) |
| *Boiling point* | 196°C (monomer) |
| *Melting point* | 37°C (monomer) |
| *Vapor pressure* | 0.001 torr @ 40°C (monomer) |
| *Solubility* | Soluble in acetone, benzene, kerosene, and nitrobenzene (monomer) |
| *Conversion factor* | Monomer: 1 ppm = 10.2 mg/m$^3$ at 25°C; Not applicable for polymer |

## III. Major Uses or Sources

Methylene diphenyl isocyanate (MDI) is used for bonding rubber to nylon. MDI is also used in the manufacture of lacquer coatings and in the production of polyurethane resins and spandex fibers (HSDB, 1995). It is often handled in a partially polymerized form ("MDI polymer"), which has a much lower vapor pressure than the monomer. The annual statewide industrial emissions from facilities reporting under the Air Toxics Hot Spots Act in California based on the most recent inventory were estimated to be 30,398 pounds of MDI (CARB, 2000).

## IV. Effects of Human Exposure

A 5-year occupational study of 107 workers from a polyurethane plastic manufacturing plant examined pulmonary function, respiratory symptoms, and smoking habits (Musk *et al.*, 1982, 1985). No significant changes in pulmonary function or respiratory symptoms were observed

when controlled for smoking. Mean MDI concentrations measured ranged from 0.0003 to 0.0006 ppm.

Significantly increased prevalence of asthma in female workers and of chronic bronchitis in male and female workers was observed following occupational exposure to low levels of MDI (<0.02 ppm) (Pham *et al.*, 1988). Workers from two plants were grouped by job classification and evaluated in this study conducted in 1976; workers were grouped as unexposed (62 men, 21 women), indirectly exposed (61 men, 56 women), or directly exposed (91 men, 27 women). Further characterization of the exposure groups was not presented. Decrements in pulmonary function (measured by VC, $FEV_1$ and single-breath carbon monoxide diffusion tests) were observed in men in the direct and indirect exposure groups: decrements in men with a history of direct exposure to MDI were statistically significant. Workers were also grouped by duration of occupational exposure (<20 months, 20-60 months, >60 months). Workers with known (direct or indirect) occupational exposure to MDI for greater than 60 months exhibited statistically significant decrements in pulmonary function tests. The follow-up examination of this study describes data from male workers only. At the time of the 5-year follow-up, air levels had been reduced to below the maximum allowed air concentration of 0.005 ppm by a modification of the ventilation system. Statistically significant decrements in pulmonary function were observed again in workers with known direct occupational exposure to MDI. Workers who were exposed at the time of the 1976 study but had since been removed from exposure did not exhibit decrements in pulmonary function, leading the authors to conclude that the effects of low-level exposure to MDI are to some extent reversible. Flaws in study design, including lack of exposure characterization, attrition, and inclusion of asthmatics in cohorts, preclude a quantitative assessment of MDI exposure on lung function.

An epidemiologic study of foundry workers reported more respiratory symptoms and significantly lower mean $FEV_1$ and maximum mid-expiratory flow at 25-75% in exposed workers compared to controls (Johnson *et al.*, 1985). However, MDI-exposed workers also had unquantified exposure to silica, metal dust, phenol formaldehyde, and a pyridine derivative precluding the evaluation of respiratory effects resulting from MDI exposure.

A worker with 5 years occupational exposure and suspected MDI hypersensitivity was exposed continuously in a controlled chamber to 5 ppb for 15 minutes, then 10 ppb for 30 minutes, and 20 ppb for 15 minutes (Marczynski *et al.*, 1992). The worker had not been exposed to MDI in the workplace for 5 days prior to the test challenge. Exposure to MDI resulted in an immediate, moderate, asthmatic reaction associated with significant hypoxemia.

IgG antibodies recognizing MDI-human serum albumin conjugates were detected in 4 of 5 MDI-exposed workers (Aul *et al*., 1999). The levels of specific IgG antibodies were more elevated with polymeric MDI compared with monomeric MDI.

A workplace death of a 39-year-old foundry worker was ascribed to occupational asthma induced by MDI exposure (Carnio *et al*., 1997). Postmortem pulmonary findings included epithelial desquamation, mucosal eosinophilic/neutrophilic infiltration, bronchial vessel dilatation, and edema and hypertrophy of smooth muscle.

## V. Effects of Animal Exposure

Rats were exposed to 0.2, 1.0, and 6.0 mg/m$^3$ aerosolized MDI polymer 6 hours per day, 5 days per week for 24 months (Reuzel *et al.*, 1990; 1994). Statistically significant increased incidences of basal cell hyperplasia, olfactory epithelial degeneration, alveolar duct epithelialization, localized alveolar bronchiolization, and adenomas were observed in male and female rats exposed to 6.0 mg/m$^3$ MDI. An accumulation of macrophages with yellow pigment was also noted in the lungs and mediastinal lymph nodes. Male rats exposed to this concentration also exhibited a statistically significant increase in the incidence of Bowman's gland hyperplasia. Male rats exposed to 1 mg/m$^3$ MDI also exhibited statistically significant increased incidences of basal cell hyperplasia and Bowman's gland hyperplasia. An accumulation of macrophages with yellow pigment was observed in the lungs of female rats and the lungs and mediastinal lymph nodes of male rats exposed to 1 mg/m$^3$. No adverse effects were noted in rats exposed to 0.2 mg/m$^3$ MDI.

Hyperplasia of the olfactory epithelium with MDI exposure (Reuzel *et al*., 1990; 1994)

| Concentration (*mg/m$^3$*) | *Males* | | | *Females* | | | *Combined* | | |
|---|---|---|---|---|---|---|---|---|---|
| | *Responders* | *N* | *Incidence* | *Responders* | *N* | *Incidence* | *Responders* | *N* | *Incidence* |
| 0 | 14 | 60 | 0.23 | 4 | 60 | 0.067 | 18 | 120 | 0.15 |
| 0.2 | 13 | 60 | 0.22 | 8 | 60 | 0.13 | 21 | 120 | 0.18 |
| 1 | 26 | 60 | 0.43 | 8 | 60 | 0.13 | 34 | 120 | 0.28 |
| 6 | 32 | 60 | 0.53 | 49 | 59 | 0.83 | 81 | 119 | 0.68 |

Guinea pigs were exposed to 2 ppm MDI 3 hours per day for 5 days (Aizicovici *et al.*, 1990). Qualitative immunostaining techniques indicated that MDI was localized in the respiratory tract. The spleen, lymph nodes, and thymus had very little staining. However, another study exposed guinea pigs to 4 ppb radiolabelled toluene diisocyanate (TDI) for 1-hour and found measurable radioactivity in extrathoracic tissues and body fluids (Kennedy *et al.*, 1989). Therefore, there is a possibility that MDI may be transported to sites other than the respiratory tract, such as the ovaries and testes, following inhalation exposure.

Gravid Wistar rats, Crl:(WI)BR, were exposed by whole-body inhalation to clean air (control) and to 1, 3, and 9 mg/m$^3$ MDI, respectively, for 6 hr per day from days 6 to 15 post conception (Buschmann *et al*., 1996). Rats were killed on day 20. The lung weights in the high-dose group were significantly increased compared to the sham-treated control animals. Treatment did not influence any other maternal and/or fetal parameters investigated (including maternal weight gain, number of corpora lutea, implantation sites, pre-and postimplantation loss, fetal and placental weights, gross and visceral anomalies, and degree of ossification). A slight but

significant increase in litters with fetuses displaying asymmetric sternebra(e) was observed after treatment with the highest dose. Although the relevance of an increase of this minor anomaly in doses which maternal toxicity is limited and within the limits of biological variability, a substance-induced effect in the high-dose group cannot be excluded with certainty. Thus, the authors reported a NOAEL of 3 mg/m$^3$ for embryotoxic effects.

## VI. Derivation of Chronic Reference Exposure Level (REL)

| | |
|---|---|
| *Study* | Reuzel *et al.*, 1990; 1994 |
| *Study population* | Rats |
| *Exposure method* | Inhalation of polymeric aerosolized MDI (0, 0.2, 1.0, and 6.0 mg/m$^3$) |
| *Critical effects* | Hyperplasia of the olfactory epithelium |
| *LOAEL* | 1 mg/m$^3$ |
| *NOAEL* | 0.2 mg/m$^3$ |
| *Benchmark Concentration ($BMC_{05}$)* | 0.25 mg/m$^3$ (95% lower confidence limit on concentration for a 5% incidence of response based on analysis of the combined male and female data with a linear model, the best-fitting of 6 models examined, p = 0.99) |
| *Study continuity* | 6 hours per day, 5 days per week |
| *Study duration* | 24 months |
| *Average experimental exposure* | 0.046 mg/m$^3$ for $BMC_{05}$ group (0.25 x 6/24 x 5/7) |
| *Human equivalent concentration* | 0.020 mg/m$^3$ for $BMC_{05}$ group (particle with extrathoracic respiratory effects, RDDR = 0.453, based on MMAD = 0.68 µm and sigma g = 2.93) |
| *LOAEL uncertainty factor* | 1 |
| *Subchronic uncertainty factor* | 1 |
| *Interspecies uncertainty factor* | 3 |
| *Intraspecies uncertainty factor* | 10 |
| *Cumulative uncertainty factor* | 30 |
| *Inhalation reference concentration* | 0.7 µg/m$^3$ |

The data of Reuzel *et al*. (1990, 1994) were examined with six quantal dose-response models (linear, log-normal, Weibull, logistic, quadratic, gamma) using USEPA BMDS 1.2. All models except the quadratic gave a good fit to the combined male and female data set. The linear model was selected as the best-fitting model. Possible differences between male and female susceptibility are suggested by the gender-specific data, although the significance of these differences is uncertain.

USEPA used the same two studies and a $BMC_{10}$ approach to develop an RfC of 0.6 µg/m$^3$. Since USEPA used a 3-fold database uncertainty factor, their $BMC_{10}$-based RfC is comparable to the $BMC_{05}$-based OEHHA REL.

## VII. Data Strengths and Limitations for Development of the REL

Strengths of the REL for MDI include the use of a well-conducted, long-term inhalation study, the observation of a NOAEL, and the estimation of a benchmark concentration. A limitation of the REL is that it is based on data on exposures to MDI "polymer" which actually contains nearly 50% monomer. Monomers may in some cases be more toxic than polymers. Thus, effects of pure monomeric MDI may occur at concentrations somewhat lower than observed in the reported study on MDI polymer. However, the capacity of MDI polymer to induce immunologic sensitization is greater than that of MDI monomer (Aul *et al*., 1999). The relative potential of MDI monomer and polymer to induce hyperplasia of the olfactory epithelium is unknown.

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*CHRONIC TOXICITY SUMMARY*

# NAPHTHALENE

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

**CAS Registry Number: 91-20-3**

## I. Chronic Toxicity Summary

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

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

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

## III. Major Uses or Sources

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

## IV. Effects of Human Exposure

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

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

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

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

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

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

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

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

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

## V. Effects of Animal Exposure

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

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

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

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

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

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

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

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

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

## VI. Derivation of Chronic Reference Exposure Level (REL)

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

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

The most important limitation of the study is that the lowest concentration tested caused adverse effects in most (≥96%) of the animals tested. Thus the study amply demonstrates the risk of lifetime exposures to 10 ppm, but is uninformative regarding the concentration-response relationship at lower concentrations. Only a general assumption can be drawn on the magnitude of uncertainty factor needed to predict a concentration at which adverse effects would most likely not be observed. Lacking specific guidance or relevant research for this situation, the default 10-fold factor was applied. U.S. EPA also used the NTP study to develop its RfC of 3 μg/m$^3$ with slightly different assumptions and a cumulative uncertainty factor of 3000 (U.S. EPA, 2000). OEHHA followed the U.S. EPA precedent in using an intraspecies UF of 10 for

naphthalene, rather than using the HEC/RGDR approach. According to U.S. EPA (2000), because of its low water solubility and low reactivity, naphthalene-related effects on the nasal epithelium are expected to result following absorption of naphthalene and its metabolism to reactive oxygenated metabolites, not from direct contact. This is supported by data on naphthalene metabolism indicating that toxic effects on the respiratory tract are due to a naphthalene metabolite that may be formed either in the liver or in the respiratory tract. Necrosis of bronchial epithelial (Clara) cells in mice and necrosis of olfactory epithelium in mice, rats, and hamsters occur following intraperitoneal injection of naphthalene. The nasal effects from inhalation exposure to naphthalene were considered to be extra-respiratory effects of a category 3 gas (U.S. EPA, 1994). The assumption is made that nasal responses in mice to inhaled naphthalene are relevant to humans; however, it is uncertain that the RfC for naphthalene based on nasal effects will be protective for hemolytic anemia and cataracts, the more well-known effects from naphthalene exposure in humans.

## VII. Data Strengths and Limitations for Development of the REL

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

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*CHRONIC TOXICITY SUMMARY*

# NICKEL AND NICKEL COMPOUNDS: NICKEL OXIDE

| *Molecular Formula* | *Molecular Weight* | *Synonyms* | *CAS Registry Number* |
|---|---|---|---|
| Ni | 59 | elemental nickel | 7440-02-0 |
| NiO | 74.69 | nickel oxide | 1313-99-1 |
| $NiCl_2$ | 129.6 | nickel chloride<br>nickel dichloride | 7718-54-9 |
| $NiSO_4$ | 154.75 | nickel sulfate<br>nickelous sulfate | 7786-81-4 |
| $NiCO_3$ | 118.7 | nickel carbonate<br>carbonic acid nickel salt | 3333-67-3 |
| $Ni_3S_2$ | 240.19 | nickel subsulfide<br>trinickel disulfide<br>heazlewoodite | 12035-72-2 |

## I. Chronic Toxicity Summary

### A. Nickel and Nickel Compounds (except nickel oxide)

*Inhalation reference exposure level* **0.05 μg Ni/m$^3$**

*Critical effect(s)* Lung, nasal epithelial and lymphatic pathology in male and female rats

*Hazard index target(s)* Respiratory system; hematopoietic system

## B. Nickel Oxide

*Inhalation reference exposure level* **0.10 μg Ni/m$^3$**

*Critical effect(s)* Lung and lymphatic pathology in male and female rats

*Hazard index target(s)* Respiratory system; hematopoietic system

## II. Physical and Chemical Properties (from HSDB, 1995)

| | |
|---|---|
| *Description* | Ni metal: Silvery metal; $NiCl_2$: deliquescent crystals (U.S.EPA, 1985) |
| *Molecular formula* | See above |
| *Molecular weight* | See above |
| *Density* | 8.9 g/cm$^3$ @ 20°C (Ni) |
| *Boiling point* | 2730°C (Ni) |
| *Vapor pressure* | Not applicable |
| *Solubility* | Elemental nickel, nickel subsulfide, and nickel oxide are insoluble in water, but are soluble in dilute nitric, hydrochloric, and sulfuric acids. The chloride and sulfate forms of nickel are water soluble. |
| *Conversion factor* | Not applicable for fumes and dusts |

## III. Major Uses and Sources

The most common airborne exposures to nickel compounds are to insoluble nickel compounds such as elemental nickel, nickel sulfide, and the nickel oxides from dusts and fumes. Contributions to nickel in the ambient air are made by combustion of fossil fuels, nickel plating, and other metallurgical processes. The most common oxidation state of nickel is the divalent ($Ni^{2+}$) form (U.S.EPA, 1985). Elemental nickel is a malleable, silvery-white metal that is highly resistant to strong alkali. Because of its corrosion resistance, nickel is used in the production of stainless steel, permanent magnets, and other alloys that require resistance to extremes of temperature or stress (U.S.EPA, 1985). Nickel is also used in electroplating baths, batteries, textile dyes, and catalysts (U.S.EPA, 1985). Nickel dust or powder is flammable (CDTSC, 1985). Due to its unique toxicological and physico-chemical properties, nickel carbonyl is not included in this summary. The annual statewide industrial emissions from facilities reporting under the Air Toxics Hot Spots Act in California, based on the most recent inventory, were estimated to be 110,334 pounds of nickel (CARB, 1999).

## IV. Effects of Human Exposure

Several studies have indicated that occupational inhalation exposure to nickel aerosols can result in development of asthma specific to nickel. Davies (1986) found 3 cases of asthma among 53 nickel-plating workers without a history of asthma prior to employment. Novey *et al.* (1983) described biphasic metal-specific bronchial responses in an individual metal-plating worker exposed to nickel and chromium salts. In another case, immunological studies conducted in a 24-year old man showed nickel-specific antibodies in the serum after several weeks of working in a nickel-plating shop using nickel sulfate (McConnell *et al.*, 1973). Dermatitis was observed on exposed areas of his skin, and pulmonary function, measured by $FEV_1$ with and without isoproterenol challenge, was significantly impaired compared with a control subject and normal

values. Dyspnea, non-productive cough, chest-tightness, and wheezing were reported as symptoms by this worker during the work period.

A group of 7 metal plating workers with occupational asthma were evaluated for atopy and pulmonary function challenge in response to inhalational challenge with nickel and other metals (Cirla *et al.*, 1985). Three of the asthmatics tested positive for the presence of nickel-specific IgE antibodies. Positive reactions to skin testing with nickel were found in 3 of the asthmatic workers who also had dermatitis. Six out of the 7 asthmatics exhibited significantly decreased $FEV_1$ (> 15%) when exposed to 0.3 mg/m$^3$ nickel sulfate for 30 minutes. Control challenges with other metal salts did not reveal similar deficits in $FEV_1$.

Although asthma has been described in the above studies, occupational inhalation of nickel dusts has not been found to be associated with pulmonary fibrosis (Muir *et al.*, 1993). An occupational epidemiology report by Broder *et al.* (1989) found no significant effects on pulmonary function in relation to nickel exposure in a nickel smelter, however a healthy worker effect was observed in this study.

## V. Effects of Animal Exposure

Early studies on the chronic non-cancer effects of metallic nickel dust were complicated by early mortality and cancer in guinea pigs and rats (Hueper, 1958).

A 2-year inhalation study of nickel oxide in rats and mice (65 per sex, per group) was conducted by the National Toxicology Program (NTP, 1994a). In the first study, rats were exposed to 0, 0.62, 1.25, or 2.5 mg nickel oxide/m$^3$ (0, 0.5, 1.0, or 2.0 mg Ni/m$^3$) 6 hours/day, 5 days/week for 104 weeks. In addition to the carcinogenic effects of nickel oxide, a number of non-cancerous lesions were observed, particularly in the lungs. The incidence of inflammatory pigmentation in the alveoli was significantly greater in all exposed groups, compared to controls. The severity of the lesions reportedly increased with increasing exposure. Atypical alveolar hyperplasia was also seen in all exposed groups. Lymphoid hyperplasia in the bronchial lymph nodes was observed in males and females exposed to 1 mg Ni/m$^3$ or greater at 7 and 15 months and the incidence generally increased with increasing concentration at the end of the 2-year study. Females had an increased incidence of adrenal medullary hyperplasia at all exposures of nickel oxide. Body weights were significantly lower in the groups exposed to 2.0 mg Ni/m$^3$ for both sexes, and in males exposed to 1.0 mg Ni/m$^3$.

A companion study on nickel oxide in mice conducted by NTP showed similar lung inflammatory changes as seen in the rats, in addition to pigmentation of the alveolar region at all exposure concentrations, compared with controls (NTP, 1994a). The mice were exposed to 0, 1.0, 2.0, or 3.9 mg Ni/m$^3$. Bronchial lymph-node hyperplasia was also evident in all nickel-exposed animals. Body weights were slightly but significantly lower in the 3.9 mg Ni/m$^3$ group, compared with controls.

A continuous exposure of rats (20 - 40 per group) to 0, 60, or 200 μg Ni/m$^3$ as nickel oxide for 2 years resulted in severe pulmonary damage and premature mortality so that carcinogenesis could not be evaluated (Glaser *et al.*, 1986). Pulmonary alveolar proteinosis and septal fibrosis were

observed in the animals exposed to nickel. Only 1 rat per group survived the nickel exposures to the end of the experiment.

A 2-year study on the effects of nickel subsulfide in rats and mice was conducted by NTP (1994b). Rats (52-53 per sex per group) were exposed to 0, 0.15, or 1 mg $Ni_3S_2/m^3$ (0, 0.11, or 0.73 mg $Ni/m^3$) for 6 hours/day, 5 days/week for 104 weeks. Body weights were lowered in rats exposed to 0.73 mg $Ni/m^3$ compared with controls. Lung inflammation, alveolar hyperplasia, macrophage hyperplasia, and pulmonary fibrosis were observed with a significantly increased incidence at both nickel concentrations. Female rats exposed to nickel had significantly increased adrenal medullary hyperplasia. In addition to the pulmonary lesions, nasal inflammation and olfactory epithelial atrophy was observed in both sexes exposed to 0.73 mg $Ni/m^3$.

In the second phase of the NTP study (NTP, 1994b), mice were exposed to 0, 0.6, or 1.2 mg $Ni_3S_2/m^3$ (0, 0.44, or 0.88 mg $Ni/m^3$) for 6 hours/day, 5 days/week for 104 weeks. The same pathological lesions were observed in the lung and nasal passages as in the rats in the above study. These lesions were evident at both the 0.44 mg $Ni/m^3$ and the 0.88 mg $Ni/m^3$ concentrations. The adrenal medullary hyperplasia seen in female rats was not observed in the mice.

An exposure of rats to either 0 or 0.97 mg $Ni_3S_2/m^3$ (0 or 0.71 mg $Ni/m^3$) for 6 hours/day, 5 days/week for 78-80 weeks resulted in decreased body weight, hyperplasia, metaplasia, and neoplasia in the lungs due to Ni (Ottolenghi *et al.*, 1974).

The NTP (1994c) studied the chronic non-cancer and carcinogenic effects of nickel sulfate hexahydrate on rats and mice. Rats were exposed to 0, 0.12, 0.25, or 0.5 mg $NiSO_4/m^3$ (0, 0.03, 0.06, or 0.11 mg $Ni/m^3$) for 6 hours/day, 5 days/week for 104 weeks. Chronic effects of nickel exposure in rats included inflammatory lesions in the lung, lung macrophage hyperplasia, alveolar proteinosis, and fibrosis, in addition to bronchial lymph node hyperplasia and nasal epithelial atrophy. The above effects were seen at exposures of 0.06 mg $Ni/m^3$ or greater.

Mice were exposed to a similar regimen that included 0, 0.06, 0.11, and 0.22 mg $Ni/m^3$ as nickel sulfate hexahydrate (NTP, 1994c). Similar pulmonary, lymphatic and nasal changes were observed in the mice as with the rats. Fibrosis was not reported, but an increased incidence of interstitial infiltration and alveolar proteinosis were observed at exposures of 0.11 mg $Ni/m^3$ or greater. No clinical findings or hematological effects were observed, but body weights were significantly depressed in all groups of nickel-exposed female mice. The body weights of males were reduced only in the group exposed to 0.22 mg $Ni/m^3$.

Rats and mice (10 per group) were exposed to nickel sulfate, nickel subsulfide, or nickel oxide 6 hours/day, 5 days/week, for 13 weeks (Dunnick *et al.*, 1989). Exposure-related increases in lung weight and histological lesions were observed in both species for all nickel exposures. Histological lesions included inflammatory changes, fibrosis, and alveolar macrophage hyperplasia. Nasal lesions were also observed in animals treated with nickel sulfate or nickel subsulfide. Lung weight changes were observed at exposures of 0.05 mg $Ni/m^3$ or greater in female rats. Macrophage hyperplasia in the alveolar region was observed at concentrations as

low as 0.02 mg Ni/m$^3$. Additional inflammatory lesions in the lungs were observed at 0.1 mg Ni/m$^3$.

A similar study by Haley *et al.* (1990) found that exposure of mice to nickel sulfate, nickel subsulfide, or nickel oxide resulted in various immunological effects. Mice were exposed to 0, 0.11, 0.45, or 1.8 mg Ni/m$^3$ as $Ni_3S_2$; 0.47, 2.0, or 7.9 mg Ni/m$^3$ as NiO; and 0.027, 0.11, and 0.45 mg Ni/m$^3$ as $NiSO_4$ for 6 hours/day, 5 days/week for 13 weeks. Nickel exposures consistently decreased splenic antibody-forming cell (AFC) responses, with significant decreases occurring at 1.8 mg Ni/m$^3$ as nickel subsulfide. In contrast, AFC responses in the lung-associated lymph nodes were consistently increased, indicating a possible indirect influence of inflammatory mediators released in the lung on local lymph nodes.

Rabbits (8 nickel exposed and 8 controls) exposed to 0.24 mg Ni/m$^3$ as nickel chloride 6 hours/day, 5 days/week for 4 weeks exhibited significantly decreased macrophage lysozyme activity in pulmonary lavage fluid and in macrophage cultures, compared with control animals (Lundborg and Camner, 1984). Similar exposures of rabbits to chlorides of cadmium, cobalt, or copper did not reduce lysozyme activity.

## VI. Derivation of Chronic Reference Exposure Level (REL)

### A. Nickel and Nickel Compounds (except nickel oxide)

| | |
|---|---|
| *Study* | National Toxicology Program, 1994c |
| *Study population* | Male and female F344/N rats (52-53 per group) |
| *Exposure method* | Discontinuous inhalation |
| *Critical effects* | Pathological changes in lung, lymph nodes, and nasal epithelium: (1) active pulmonary inflammation, (2) macrophage hyperplasia, (3) alveolar proteinosis, (4) fibrosis, (5) lymph node hyperplasia, (6) olfactory epithelial atrophy |
| *LOAEL* | 60 μg Ni/m$^3$ (as nickel sulfate hexahydrate) |
| *NOAEL* | 30 μg Ni/m$^3$ |
| *Exposure continuity* | 6 hours/day, 5 days/week |
| *Exposure duration* | 104 weeks |
| *Average experimental exposure* | 5.4 μg Ni/m$^3$ for NOAEL group (30 x 6/24 x 5/7) |
| *Human equivalent concentration* | 1.6 μg Ni/m$^3$ for NOAEL group males (particulate with respiratory effects, RDDR = 0.29 based on MMAD = 2.5, sigma g = 1.26, male rat body weight = 380 g, SA(PU) = 0.34 m$^2$, DEP(PU) = 0.024) |
| *LOAEL uncertainty factor* | 1 |
| *Subchronic uncertainty factor* | 1 |
| *Interspecies uncertainty factor* | 3 |
| *Intraspecies uncertainty factor* | 10 |
| *Cumulative uncertainty factor* | 30 |
| *Inhalation reference exposure level* | 0.05 μg Ni/m$^3$ |

**Nickel Oxide**

| | |
|---|---|
| *Study* | National Toxicology Program, 1994c |
| *Study population* | Male and female F344/N rats (52-53 per group) |
| *Exposure method* | Discontinuous inhalation |
| *Critical effects* | Pathological changes in lung and lymph nodes: (1) active pulmonary inflammation, (2) lymph node hyperplasia<br>Adrenal medullary hyperplasia (females) |
| *LOAEL* | 500 μg Ni/m$^3$ |
| *NOAEL* | Not observed |
| *Exposure continuity* | 6 hours/day, 5 days/week |
| *Exposure duration* | 104 weeks |
| *Average experimental exposure* | 89.5 μg Ni/m$^3$ for LOAEL group (500 x 6/24 x 5/7) |
| *Human equivalent concentration* | 30 μg Ni/m$^3$ for LOAEL group males (particulate with respiratory effects, RDDR = 0.29 based on MMAD = 2.5, sigma g = 1.26, male rat body weight = 380 g, SA(PU) = 0.34 m$^2$, DEP(PU) = 0.024) |
| *LOAEL uncertainty factor* | 10 |
| *Subchronic uncertainty factor* | 1 |
| *Interspecies uncertainty factor* | 3 |
| *Intraspecies uncertainty factor* | 10 |
| *Cumulative uncertainty factor* | 300 |
| *Inhalation reference exposure level* | 0.10 μg Ni/m$^3$ |

The studies conducted by NTP (1994 a,b, & c) all showed similar non-carcinogenic effects in rats and mice, regardless of the form of nickel administered. It therefore appears that soluble and insoluble forms of nickel cause similar effects in rodents. The human epidemiological literature predominantly describes cancer mortality rates from occupational exposures to nickel compounds, but does not specifically examine non-cancer effects. However, it is clear from many case reports that allergies and dermatitis can occur in exposed workers. Hypersensitive reactions to nickel have not been quantitatively studied in humans or animals, therefore it is not possible to develop an REL based on immunological hypersensitivity at the present time. A host of subacute and subchronic animal studies have shown nickel to affect certain immunological responses unrelated to hypersensitivity, but the applicability of these results to chronic human exposures and responses involves considerable uncertainty. Furthermore, data show that nickel may precipitate onset of asthma in occupational settings.

The results of the NTP studies and these dose response analyses support the speciation of nickel oxide for noncancer effects. The health effects data for nickel oxide indicate that its adverse pulmonary effects were less severe (absence of fibrosis, lower chronic lung inflammation severity scores) at higher doses than the pulmonary effects observed for nickel sulfate and nickel

subsulfide. The higher chronic REL value for nickel oxide of 0.1 $\mu g/m^3$ reflects these dose response differences. Furthermore, while it is based upon a LOAEL, the lower severity of the adverse health effects at the LOAEL mitigates some of the uncertainty associated with use of a LOAEL rather than a NOAEL. OEHHA therefore concludes that 0.1 $\mu g/m^3$ is an appropriate REL for nickel oxide. However, in setting inhalation exposure RELs for groups of compounds, OEHHA uses the most sensitive strain, species, sex, chronic endpoint, and agent for each group of substances. Therefore, as the pulmonary toxicity of the relatively insoluble nickel subsulfide is greater than that of nickel oxide and closer to that of nickel sulfate, OEHHA proposes to use the chronic REL derived from nickel sulfate for all other nickel compounds.

## VII. Data Strengths and Limitations for Development of the REL

The strengths of the inhalation REL include the availability of controlled lifetime exposure inhalation studies in multiple species at multiple exposure concentrations and with adequate histopathogical analysis and the observation of a NOAEL. The major areas of uncertainty are the lack of adequate human exposure data and the lack of lifetime toxicity studies in any non-rodent species.

In addition to being inhaled, airborne nickel can settle onto crops and soil and enter the body by ingestion. Thus an oral chronic refererence exposure level for nickel is also required.

***Derivation of Oral Chronic Reference Exposure Level***

| | |
|---|---|
| *Study* | Ambrose *et al*., 1976 |
| *Study population* | Rats |
| *Exposure method* | Diet |
| *Critical effects* | Decreased body and organ weights |
| *LOAEL* | 1000 ppm (50 mg/kg-day) |
| *NOAEL* | 100 ppm (5 mg/kg-day) |
| *Exposure continuity* | Continuous |
| *Exposure duration* | Lifetime |
| *Average exposure* | 5 mg/kg-day |
| *Human equivalent concentration* | 5 mg/kg-day |
| *LOAEL uncertainty factor* | 1 |
| *Subchronic uncertainty factor* | 1 |
| *Interspecies uncertainty factor* | 10 |
| *Intraspecies uncertainty factor* | 10 |
| *Cumulative uncertainty factor* | 300 |
| *Oral reference exposure level* | 0.05 mg/kg-day |

The oral REL for nickel used the same study used for the U.S. EPA's oral Reference Dose (RfD). U.S.EPA assumed that rat consumption of 1 ppm Ni in the feed resulted in a dose of 0.05 mg/kg/day. An uncertainty factor of 10 was used for interspecies extrapolation and another of 10 to protect sensitive human populations. An additional uncertainty factor of 3 was used by U.S. EPA to account for inadequacies in reproductive studies of nickel. OEHHA has not used such special uncertainty or modifying factor because the criteria for their use are not well presented.

In addition there is an extensive toxicologic database on nickel in general which includes studies on reproductive effects.

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*CHRONIC TOXICITY SUMMARY*

# PHENOL

*(Carbolic acid, phenylic acid, phenyl hydroxide)*

**CAS Registry Number: 108-95-2**

## I. Chronic Toxicity Summary

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

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

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

## III. Major Uses or Sources (HSDB, 1995)

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

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

## IV. Effects of Human Exposures

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

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

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

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

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

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

## V. Effects of Animal Exposures

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

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

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

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

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

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

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

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

## VI. Derivation of Chronic Reference Exposure Level (REL)

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

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

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

sensitive populations and is not for continuous exposure conditions. The workplace standard is consistent with reports indicating that no respiratory irritation occurred among workers exposed regularly to 4 ppm phenol (Connecticut Bureau of Industrial Hygiene, undated) and no adverse effects were mentioned among workers exposed to 3.3 ppm (Ohtsuji and Ikeda, 1972). Neither report was considered appropriate to be the basis of a REL. However, for the sake of comparison adjusting the reported NOAEL of 4 ppm to continuous exposure and dividing by an intraspecies uncertainty factor of 10 results in an estimated chronic REL of 140 ppb, in reasonable agreement with the proposed REL of 50 ppb.

## VII. Data Strengths and Limitations for Development of the REL

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

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*CHRONIC TOXICITY SUMMARY*

# PHOSPHINE

*(hydrogen phosphide; phosphorus trihydride; Celphos; Phostoxin)*

**CAS Registry Number: 7803-51-2**

## I. Chronic Toxicity Summary

| | |
|---|---|
| *Inhalation reference exposure level* | **0.8 μg/m$^3$** (0.6 ppb) |
| *Critical effect(s)* | Decreased body weight gain in mice |
| *Hazard index target(s)* | Respiratory system; alimentary system; nervous system; kidney; hematopoietic system |

## II. Chemical Property Summary (HSDB, 1995, except as noted)

| | |
|---|---|
| *Description* | Colorless gas |
| *Molecular formula* | $PH_3$ |
| *Molecular weight* | 34 g/mol |
| *Vapor density* | 1.17 (air = 1) |
| *Boiling point* | –87.7°C |
| *Vapor pressure* | 20 atm @ –3°C (Weast, 1980) |
| *Solubility* | 0.26 volumes in water @ 20°C; soluble in alcohol, ether (Sax and Lewis, 1989) |
| *Conversion factor* | 1.39 mg/m$^3$ per ppm at 25ºC |

## III. Major Uses and Sources

Phosphine is used as an agricultural fumigant against insects and is among the most acutely toxic of the fumigant gases (HSDB, 1995). In its use as a fumigant, application of aluminum, magnesium, or zinc phosphide pellets generates phosphine gas upon exposure to moisture. Because of high volatility, phosphine residue dissipates from treated material upon ventilation. Inadequate sealing of materials during the course of treatment can result in unplanned environmental exposure.

Phosphine is also used by the semiconductor industry as a chemical doping agent for electronic components (n-type semiconductors) (HSDB, 1995). Other minor sources/uses of phosphine are in chemical syntheses: specifically, in preparations of phosphonium halides, for polymerization initiation, and as condensation catalysts. The annual statewide industrial emissions from facilities reporting under the Air Toxics Hot Spots Act in California based on the most recent inventory were estimated to be 3312 pounds of phosphine (CARB, 1999). In 2000, more than 120,000 pounds of phosphide compounds (including 119,519 pounds of aluminum phosphide and 1607 pounds of zinc phosphide) were applied in California agriculture (CDPR, 2001). In the

presence of water these phosphides break down to phosphine. However, the fraction emitted as phosphine is not known.

## IV. Effects of Exposures to Humans

Toxicity among 22 workers intermittently exposed to phosphine levels of 0.17-2.11 ppm in air from fumigation activity ranging over 0.5 to 29 years (mean = 11.1 years) was evaluated (Misra *et al.*, 1988). The subjects were interviewed within one day of fumigation activity and reported that symptoms subsided when phosphine was not in use. The most frequently reported symptoms include dyspnea (31.8%), headache (31.8%), chest tightness (27.3%), cough (18.2%), anorexia and epigastric pain (18.2%), finger paresthesia and numbness (13.6%), and giddiness, numbness, and lethargy (13.6%). No change in motor or sensory nerve conduction velocity was found.

A similar spectrum of toxic effects among workers involved in grain storage at a seaboard terminal has been reported (Jones *et al.*, 1964). Among 69 men exposed to breathing zone phosphine levels of 0-35 ppm for as long as 16 hours per day, the authors report symptoms of multiple origins. These include gastrointestinal (diarrhea, nausea, epigastric pain, vomiting), cardio-respiratory (chest tightness, dyspnea, pain in chest, palpitations, retrospinal pain), and central nervous (headache, dizziness, staggering gait) systems. Symptoms were reported to appear only at the time of exposure and apparently were reversible.

In another report of chronic occupational exposure, authors cited the appearance of chronic bronchitis, anemia, and digestive disorders (Eichler, 1934).

Most literature reports of human toxic health effects of phosphine, however, come from case reports of acute exposures. Some are suggestive of potential chronic toxicity endpoints because of the irreversible nature of the effect. In a case report of phosphine poisoning of 29 people exposed by inhalation on a grain freighter, pathological findings included evidence of urinary tract injury (occult blood), liver damage (bilirubinuria and increased SGPT, GGPT, and LDH), and myocardial damage (increased MB fraction of CPK, abnormal ECG) (Wilson *et al.*, 1980). A-two-year old child who died as a result of the exposure showed myocardial necrosis with mononuclear infiltrates, pulmonary edema with damaged epithelia, pleural effusion, and an enlarged spleen. In another case report exposure of a 7-months pregnant, 24-year-old woman to aluminum phosphide from a nearby grain storage site was lethal (Garry *et al.*, 1993). There was evidence of severe pulmonary edema, necrosis of individual hepatic cells, and anoxic change in Purkinje cells of the cerebellum. These reported deaths of a small child and a pregnant woman exposed together with individuals who survived exposure to phosphine suggest that there may be sensitive human subpopulations. In another case report of acute phosphine poisoning by inhalation, Schoonbroodt *et al.*, (1992) observed necrosis of the nasal mucosa, delayed onset of pulmonary edema, and myocardial injury. Chopra *et al*. (1986) treated sixteen patients with aluminium phosphide poisoning during 1985. Findings included mucosal necrosis and cardiac abnormalities (due to hypoxemia). Renal failure (1/16), proteinuria (1/16), and increased blood transaminases (2/16) resulted from oral exposure to phosphine. The multi-organ involvement in toxicity suggests that phosphine is a broad-spectrum toxicant.

In a 1994 two deaths and three illnesses were reported due to phosphine fumigation of agricultural products in railroad cars (Perrotta *et al*., 1994). The annual report for the year 2000 of the American Association of Poison Control Centers' Toxic Exposure Surveillance System does not list fumigants as a separate category of pesticides (Litovitz *et al*., 2001). However, two of the case reports of fatalities, that were presented in abstract form, were due to aluminum phosphide.

## V. Effects of Exposures to Animals

A subchronic inhalation toxicity study of phosphine was conducted in Balb-c mice (Barbosa *et al.*, 1994). Twelve animals/sex/dose group were exposed for 6 hours/day, 5 days/week for 13 weeks to 0, 0.3, 1.0, or 4.5 ppm phosphine. Non-cancer toxicity endpoints included reduction in weight gain and changes in relative organ weights of kidneys, lungs, liver, heart, brain and spleen. In the highest dose group, itching and scratching of the eyes, feet and tail, and decreased overall activity were observed. No diarrhea, loss of equilibrium, convulsions, seizures, or other neurological disturbances were noted. A dose-dependent decrease in total body weight gain was observed at all exposure levels with a greater effect observed in females ($p<0.0001$). Statistically significant decreases in relative organ weights (kidney, heart, and brain) were observed in males only at the 0.3 ppm exposure level ($p<0.001$). On the other hand, female mice showed increased relative organ weights (lungs, heart, and spleen) predominantly at higher doses (1.0 and 4.5 ppm; $p<0.001$). At 4.5 ppm phosphine absolute kidney and spleen weights were significantly increased in females ($p<0.01$). Increased frequencies of micronuclei in polychromatic erythrocytes from bone marrow and spleen were also seen at 4.5 ppm. This group also conducted a short-term repeated dose experiment. Six mice/sex/group were exposed to 5.5 ppm phosphine for 2 weeks (6 hrs/day, 5 days/wk). No statistically significant changes in weight gain were observed at the end of this exposure period.

In another subchronic inhalation toxicity study, male and female Fischer 344 rats (10/sex/group) were exposed to levels of 0, 0.37, 1.0, and 3.1 ppm phosphine for 6 hours per day, 5 days per week, for 13 weeks (Newton *et al.*, 1993). A higher dose group at 10 ppm was terminated prematurely (at 3 days) because of high mortality. A satellite group exposed to 5.1 ppm for 2 weeks was terminated after 13 days recovery. Observations of overt toxicity and viability were made at the time of each exposure; body weight and food consumption were monitored weekly; ophthalmic examination was done the day before termination; and hematological and clinical chemistry indices were measured after 4 and 13 weeks. Postmortem examination included gross necropsy, with particular attention to orifices, the cranial cavity, surfaces of the brain and spinal cord, nasal cavity and sinuses, the thoracic, abdominal, and pelvic cavities and viscera, and the cervical tissue and organs. Histopathology was performed on 10% buffered formalin-fixed/hematoxylin-eosin-stained tissues. Significant observations after 13 weeks of phosphine exposure included decreased hemoglobin, hematocrit, and erythrocytes in males in the 3.1 ppm dose group. Male rats in the 1 ppm dose group showed decreased weight gain. Increased incidence of small seminal vesicles was noted at 1 and 3.1 ppm, although no histological correlate was observed. Absolute and relative decreases in liver weight were observed in all exposed groups, but there was no evidence that this effect was dose-related. A significant

decrease in serum glutamic pyruvic transaminase (SGPT) was observed at 3.1 ppm, although the authors noted unusually high control levels. None of these effects were observed after the 4 week recovery period. Other effects of a transient nature noted during the exposure include decreased weight gain in female rats at 1 ppm, decreased food consumption at 0.37 ppm in males and females, and increased blood urea nitrogen (BUN) at 3.1 ppm. Observations in the 10 ppm group necropsied after 3 days of exposure included decreased erythrocytes, increased alkaline phosphatase, and increased kidney weight with coagulative necrosis of the tubular epithelium of the outer cortex. In a subchronic study of CD male and female rats under similar conditions (exposure to 0, 0.3, 1, or 3 ppm phosphine 6 h/day, 5 days/week for 13 weeks), no neurotoxicity was observed (Schaefer *et al*., 1998).

Newton *et al.* (1993) also examined developmental toxicity by exposing 24 pregnant female $CD^R$ rats per group to 0, 0.03, 0.33, 2.8, 4.9, and 7.0 ppm phosphine. The highest dose group was terminated prematurely because of high mortality; all other animals were necropsied after 20 days for evaluation of maternal and fetal toxicity. Maternal toxicity endpoints included weight of ovaries and uteri, number of corpora lutea, pregnancy, and implantation rate. Fetal toxicity was evaluated by weight, number, and location of fetuses and resorptions, visceral malformations and variations, and skeletal changes after alizarin staining. No statistically significant differences from control animals were observed for any parameter at any dose, with the exception of a change in mean number of resorption sites ($p \leq 0.01$), mean resorption/implant ratio ($p \leq 0.05$), and incidence of females with resorption ($p \leq 0.05$), all at 0.03 ppm only. In the absence of this effect at higher dose levels, these observations are not considered useful in establishing a low adverse effect level.

A 35-day phosphine inhalation study was conducted exposing rats continuously to 0, 0.05, 0.2, 1.5, and 8.0 $mg/m^3$ phosphine (0, 0.036, 0.14, 1.1, and 5.8 ppm) in which hematological endpoints and histopathological changes of the lungs and kidneys were examined (Pazynich *et al.*, 1984). Observations include a statistically significant change in erythrocytes (increase followed by a decrease at day 35) and decreased hemoglobin at the 0.05 and 0.2 $mg/m^3$ dose levels, although the 1.5 $mg/m^3$ dose group did not show this change. Other significant changes, noted in the lowest dose group, included decreased peroxidase activity after 35 days exposure, decreased sulfhydryl group content in blood after 27 days, and decreased phagocytotic index after 21 days. Some histological changes were noted in the lungs, kidneys, and to a lesser extent, the liver, particularly in the higher dose groups, although the exact nature of the degenerative change is not well described. Unclear dose-response relationships and temporal aspects of the endpoints also make establishment of a low adverse effect level unreliable.

Two rats were exposed to 20 ppm phosphine for 14 days (4 hours/day) (Waritz and Brown, 1975). Animals were monitored for weight gain, and organs/tissues fixed in Bouin's solution and stained with trichrome were examined histopathologically. There were no reported histopathological effects, although there was slightly reduced weight gain in the exposed animals.

In a chronic study of phosphine (Newton *et al*., 1999), 60 male and female F344 rats per group were exposed via whole-body inhalation for 6 h/day, 5 days/wk for up to 104 wk to mean concentrations of 0, 0.3, 1, or 3 ppm phosphine. Three ppm (4.17 $mg/m^3$) was the maximum

exposure level because of lethality seen at the high exposure level (7 ppm = 9.73 mg/m$^3$) in previous repeat dose studies (Newton *et al*., 1993). Ten rats per sex per group were killed after 52 weeks of exposure. Survivors were killed after 104 weeks of exposure. There were no phosphine-related effects seen on clinical observations, body weight, food consumption, hematology, clinical chemistry, urinalysis, or ophthalmology. There were no phosphine-related macroscopic findings or effect on absolute or relative organ weights. No histologic or morphologic alterations attributable to phosphine exposure were seen in the more than 40 organs and tissues examined. Under the conditions of this study, the authors found no treatment-related changes suggestive of a toxic or carcinogenic effect in rats following 52 weeks or 2 years of whole-body inhalation exposure to 0.3, 1, or 3 ppm phosphine. Thus 3 ppm is a chronic NOAEL for rats.

## VI. Derivation of Chronic Reference Exposure Level

| | |
|---|---|
| *Study* | Barbosa *et al*., 1994 |
| *Study population* | Balb-c mice (12 animals/sex/group) |
| *Exposure method* | Discontinuous whole body inhalation exposure (0, 0.3, 1, or 4.5 ppm) |
| *Critical effects* | Decrease in body weight gain, increase in relative organ weights; increase in micronuclei |
| *LOAEL* | 4.5 ppm |
| *NOAEL* | 1 ppm |
| *Exposure continuity* | 6 hr/day, 5 days/ week |
| *Average experimental exposure* | 0.178 ppm for NOAEL group (1 x 6/24 x 5/7) |
| *Human equivalent concentration* | 0.178 ppm for NOAEL group (gas with systemic effects, based on RGDR = 1.0 using default assumption that lambda (a) = lambda (h)) |
| *Exposure duration* | 13 weeks |
| *LOAEL uncertainty factor* | 1 |
| *Subchronic uncertainty factor* | 3 |
| *Interspecies uncertainty factor* | 10 (see below) |
| *Intraspecies uncertainty factor* | 10 |
| *Cumulative uncertainty factor* | 300 |
| *Reference exposure level* | 0.0006 ppm (0.6 ppb; 0.0008 mg/m$^3$; 0.8 μg/m$^3$) |

| | |
|---|---|
| *Supportive study* | Newton *et al*., 1999 |
| *Study population* | Rats (60 animals/sex/exposure level) |
| *Exposure method* | Discontinuous whole body inhalation exposure (0, 0.3, 1, or 3 ppm) |
| *Critical effects* | None |
| *LOAEL* | None detected |
| *NOAEL* | 3 ppm |
| *Exposure continuity* | 6 hours/day, 5 days/week |
| *Average experimental exposure* | 0.53 ppm (3 x 6/24 x 5/7) |
| *Human equivalent concentration* | 0.53 ppm (gas with systemic effects, based on RGDR = 1.0 using default assumption that lambda (a) = lambda (h)) |
| *Exposure duration* | 2 years |
| *LOAEL uncertainty factor* | 1 |
| *Subchronic uncertainty factor* | 1 |
| *Interspecies uncertainty factor* | 10 (see below) |
| *Intraspecies uncertainty factor* | 10 |
| *Cumulative uncertainty factor* | 100 |
| *Reference exposure level* | 0.005 ppm (5 ppb, 0.007 mg/m$^3$, 7 μg/m$^3$) |

Newton *et al*. (1999) found no treatment-related changes suggestive of a toxic effect in F344 rats following 52 weeks or 2 years of whole-body inhalation exposure to 0.3, 1, or 3 ppm phosphine. Thus in this study 3 ppm is a chronic NOAEL for rats. Three ppm was set as the maximum level because in an earlier subchronic study in rats Newton *et al*. (1993) found lethality at 7 ppm. However, the chronic results of Newton *et al*. (1999) differ from the subchronic results of Newton *et al*. (1993), in which at least transient effects were seen in the hematopoietic system after 13 weeks at 3.1 ppm. In a subchronic study in mice (Barbosa *et al*., 1994), 4.5 ppm phosphine was a LOAEL and 1 ppm was a NOAEL for decrease in body weight gain. The results of Barbosa *et al*. (1994) indicated that mice may be more sensitive than rats. Thus, it was selected as the key study, and decrease in body weight gain was selected as the critical effect.

OEHHA has applied a subchronic uncertainty factor of 3 to account for the short duration of the Barbosa *et al*. (1994) study and an intraspecies uncertainty factor of 10 to account for human variability. Due to the general inconsistencies among the various studies in the database on phosphine, and in particular with the observation of mortality at 7 ppm in a short-term developmental study in rats (Newton *et al.*, 1993), it was considered prudent to include the full interspecies uncertainty factor of 10 (even though the HEC adjustment procedure could be applied) to acknowledge the severity of effect in at least one comparison study, and the additional uncertainty associated with the apparent wide and unpredictable variability between species and between different studies in the same species (rats). This results in a cumulative uncertainty factor of 100 to be applied to the NOAEL of 1 ppm in the subchronic study by Barbosa *et al*. (1994) and a chronic REL for phosphine of 0.8 μg/m$^3$ (0.6 ppb).

The U.S. EPA based its RfC of 0.3 μg/m$^3$ on the Barbosa *et al.* (1994) study, an adequate subchronic animal study for the derivation of a REL, and included a Modifying Factor (MF) of 3

for database deficiencies (lack of multigenerational reproduction studies). The criteria for use of modifying factors are not well specified by U.S. EPA. USEPA used its default interspecies uncertainty factor of 10 for a 13 week study.

The lack of adequate data on levels of chronic phosphine exposure to humans precludes development of a REL from human studies. The endpoint used in the determination of the REL (total body weight gain) showed a dose-related decrease with phosphine exposure in Balb-c mice. This endpoint is also consistent with that found by Newton *et al.* (1993), who noted dose-dependent decreases in body weight gain in Fischer 344 rats after a 13 week exposure regimen at 1 ppm, and Waritz and Brown (1975), who reported slightly decreased weight gain in rats exposed for 14 days to 20 ppm. Surprisingly Newton *et al*. (1999) did not find differences in body weight gain at either 1 ppm or 3 ppm and they did not comment on the discrepancy between their 2 reports. Although body weight changes or changes in food consumption were not addressed in human studies, the scant human data do relate phosphine exposure to a broad spectrum of toxic effects (gastrointestinal, cardio-respiratory, CNS). The decrease in weight gain found in the animal studies and reported changes in some relative organ weights (Barbosa *et al.*, 1994) suggest systemic toxicity.

## VII. Data Strengths and Limitations for Development of the REL

The strengths of the inhalation REL for phosphine include the availability of data on multiple inhalation exposure concentrations and the observation of a NOAEL in a lifetime animal study. Major areas of uncertainty are the lack of adequate human exposure data, the lack of reproductive and developmental toxicity studies, and the inconsistency of the dose-response relationship across rodent studies.

## VIII. Potential for Differential Impacts on Children's Health

Based on the lack of a dose-response in developmental toxicity caused by exposing pregnant female rats to 4.9 ppm phosphine (Newton *et al*., 1993) , the proposed REL of 0.8 $\mu g/m^3$ (0.6 ppb) is likely to be protective of developing humans in utero. However, there is no direct evidence in the literature to quantify a differential effect of phosphine on infants and children.

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*CHRONIC TOXICITY SUMMARY*

# PHOSPHORIC ACID

*(Orthophosphoric acid)*

**CAS Registry Number: 7664-38-2**

## I. Chronic Toxicity Summary

| | |
|---|---|
| *Inhalation reference exposure level* | **7 µg/m$^3$** |
| *Critical effect(s)* | Bronchiolar fibrosis of the respiratory tract in rats |
| *Hazard index target(s)* | Respiratory system |

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

| | |
|---|---|
| *Description* | Clear syrupy liquid or unstable crystals; odorless |
| *Molecular formula* | $H_3PO_4$ |
| *Molecular weight* | 98 |
| *Boiling point* | 213°C |
| *Melting point* | 42.35°C |
| *Vapor pressure* | 0.03 torr @ 20°C |
| *Solubility* | Very soluble in hot water; 548 g/100 ml cold water; soluble in alcohol |
| *Conversion factor* | 4.0 µg/m$^3$ per ppb at 25ºC |

## III. Major Uses and Sources

Phosphoric acid has varied uses (HSDB, 1995). In manufacturing, it is a chemical intermediate or reagent in the production of numerous phosphate fertilizers, agricultural feeds, waxes, polishes, soaps, and detergents. It is added to foods as a preservative, acidifying agent, flavor enhancer, and clarifying agent. Phosphoric acid is also used in processes such as the coagulation of rubber latex, electropolishing, soil stabilization, and as a catalyst in the production of propylene and butene polymers, ethylbenzene, and cumene. By far, largest use of phosphoric acid comes in the production of fertilizers (>75%). The annual statewide industrial emissions from facilities reporting under the Air Toxics Hot Spots Act in California, based on the most recent inventory, were estimated to be 81,103 pounds of phosphoric acid (CARB, 1999).

Airborne phosphoric acid can be produced by the hydrolysis of phosphorus oxides generated from either the spontaneous ignition of white phosphorus in air or the combustion of red phosphorus (Burton *et al.*, 1982; US Department of Defense (US DOD), 1981).

## IV. Effects of Human Exposures

The toxic effects to 48 workers exposed (28 unexposed control workers) to oxidation products of phosphorus during the course of phosphorus production were reported (Hughes *et al.*, 1962). Exposure duration ranged from 1 to 17 years. No differences were observed between exposed and control workers with respect to leukocyte count, an effect observed in acute intoxications, or hand bone density, an effect observed in experimentally exposed animals (Inuzuka, 1956).

A prospective study of 131 workers exposed to several compounds including phosphoric acid, phosphorus pentoxide, fluorides and coal tar pitch in the air was conducted at an industrial refinery (Dutton *et al.*, 1993). Mean duration of exposure (employment) was 11.4 years and the maximum exposure level measured was 2.23 mg/m$^3$ (phosphorus pentoxide). Pulmonary function tests were performed annually over a 3 to 7 year period. No significant residual effect was found after adjusting for age and smoking status.

## V. Effects of Animal Exposures

Two 13-week inhalation studies of the effects of exposure to the combustion products of 95% red phosphorus and 5% butyl rubber were conducted in male Sprague-Dawley rats, with the first group exposed to 0, 300, 750, or 1200 mg/m$^3$ combustion products, and the second exposed to 0, 50, 180, or 300 mg/m$^3$ combustion products (Aranyi *et al.*, 1988a; Aranyi *et al.*, 1988b). Group numbers in the first study were 176, 84, 176, and 176, respectively. The second study used 40 animals/group. Animals were exposed for 2¼ hours/day on 4 consecutive days/week. Control animals were exposed to filtered air only. Daily particle measurements showed MMADs of 0.49-0.65 μm and $\sigma_g$s of 1.56-1.83. Fractional content of phosphoric acid in the aerosol was 71-79%. Nineteen of the 176 animals in the 1200 mg/m$^3$ dose group died of treatment related effects. Post-mortem examination of animals that died during the course of the study showed damage to the laryngeal mucosa, which was probably contributory to mortality. The two highest dose groups in the first study also showed decreased weight gain. Twelve animals from each dose group in the first study were examined histologically and neurobehavioral studies were conducted on other animals. Half the animals in the second study were examined strictly for toxic effects on the respiratory tract, with examination of the trachea, 2 sections of the nasal turbinates, and 5 lobes of the lung. Surviving animals in the high-dose study were observed to have moderate to severe fibrosis of the terminal bronchioles, with minimal severity of this effect in the animals in the low-dose study. The reported incidence of this lesion was 9/20 at 300 mg/m$^3$, 4/20 at 180 mg/m$^3$, and 0/20 at 50 mg/m$^3$. Little to no involvement of pulmonary tissue was observed.

The effects of acid aerosols (particularly sulfuric and phosphoric acid) were studied by U.S. EPA (1989). The respiratory tract was the primary target of toxicity resulting from the irritational effect of the acid on the tissues of the larynx and trachea. The nature of the effect was dependent upon the aerosol particle size, duration of exposure, and the hygroscopic character of the acid.

Sprague-Dawley rats were exposed to the smoke and combustion products of white phosphorus in felt pellets at 192.5 (18 animals/sex), 589 (24 animals/sex), or 1161 mg/m$^3$ (34 males, 43

females) phosphoric acid equivalents for 15 minutes/day, 5 days/week, for 13 weeks (US Department of Defense (US DOD), 1981). Control animals numbering half the size of the treated groups were exposed to air only. Groups of animals were sacrificed at 6 and 13 weeks, and 4 weeks post-exposure. Endpoints examined included: hematology, clinical chemistry, gross- and histo-pathology, ECG, pulmonary function, and behavior. Of the animals in the highest dose group, 56% died as a result of exposure, with the only other death occurring in the control group. Findings were restricted to effects on the respiratory system, with tracheitis and laryngitis incidences of 2/35, 32/47, and 28/31among surviving animals in the three dose groups. In the post-exposure examination, bronchiolitis occurred with a frequency of 0/12, 5/24, and 6/16in the three dose groups.

The toxicity of the combustion products of 95% amorphous red phosphorus and 5% polyvinyl butyral BL18 to female Wistar rats, Porton-strain mice, and guinea pigs was reported (Marrs *et al.*, 1989). Rats (50/group), mice (100/group), and guinea pigs (42-48/group) were exposed to concentrations of 0, 16, or 128 mg/m$^3$ for 1 hour/day, 5 days/week for 36 weeks (mice) or 40 weeks (rats and guinea pigs), with an examination conducted at 19 months or when animals appeared unhealthy. All groups, including controls, showed high mortality. Mice showed accumulation of alveolar macrophages with incidences of 2/41, 9/37, and 9/22 in the control, low-, and high-dose groups, respectively. Guinea pigs appeared to be particularly intolerant to the effects of the smoke.

Female rabbits and rats (10/group) were examined for acute toxic effects of smoke generated by the combustion of either 95% red phosphorus / 5% butyl rubber (Smoke I) or 97% red phosphorus / 3% butadiene styrene (Smoke II) (Marrs, 1984). Animals were exposed for 30 minutes and examined one and 14 days later. Smoke I produced inflammation of the larynx and trachea in rats at 1 day with some inflammation still observed at 14 days. Tracheal inflammation was also reported in rabbits exposed to Smoke I. Four of the rats exposed to Smoke II died within the first day, with severe pulmonary congestion observed in the animals.

One hour exposure to the combustion products of 95% red phosphorus / 5% butyl rubber (plus 1% mineral oil) produced epiglottal deformation, laryngeal edema, and laryngeal and tracheal lesions in rats (Burton *et al.*, 1982). A four-hour exposure produced more severe effects of a similar nature plus some hemorrhaging.

Rats (number unspecified) exposed to 150-160 mg/m$^3$ elemental phosphorus for 30 minutes/day for 60 days were examined for toxic effects (Inuzuka, 1956). Limb bone abnormalities were noted and effects included delayed ossification, widening of the epiphysis, and abnormal axial development.

Two studies have addressed the reproductive and developmental toxicity from exposure to the combustion products of white phosphorus and felt for 15 minutes/day during gestational days 6-15 in rats (24/group) (US Department of Defense (US DOD), 1981; US Department of Defense (US DOD), 1982). Fetal effects included increased incidence of some visceral variations and hypoplasia of the xiphoid process although data were incompletely reported. Another study, which exposed dams 3 weeks prior to mating, throughout gestation, and through lactation and males for 10 weeks prior to and during mating, showed decreased pup body weight, 24-hour and

21-day survival, and lactation. An oral study in which elemental phosphorus was administered to male and female rats by gavage in corn oil showed no statistically significant effects (Condray, 1985).

## VI. Derivation of the Chronic Reference Exposure Level

| | |
|---|---|
| *Study* | Aranyi *et al.*, 1988a |
| *Study population* | Male Sprague-Dawley rats (40-176/group) |
| *Exposure method* | Discontinuous whole body inhalation |
| *Critical effects* | Bronchiolar fibrosis of the respiratory tract |
| *LOAEL* | 180 mg/m$^3$ |
| *NOAEL* | 50 mg/m$^3$ |
| *$BMC_{05}$* | 64 mg/m$^3$ |
| *Exposure continuity* | 2¼ hours/day, 4 days/week |
| *Exposure duration* | 13 weeks |
| *Average experimental exposure* | 2.7 mg/m$^3$ for NOAEL group (estimated as 3.5 mg/m$^3$ at $BMC_{05}$) |
| *Human equivalent concentration* | 2.2 mg/m$^3$ at $BMC_{05}$ (particle with respiratory effects, RDDR = 0.63) (3.5 x 0.63) |
| *LOAEL uncertainty factor* | 1 ($BMC_{05}$ assumed to be similar to NOAEL) |
| *Subchronic uncertainty factor* | 10 |
| *Interspecies uncertainty factor* | 3 |
| *Intraspecies uncertainty factor* | 10 |
| *Cumulative uncertainty factor* | 300 |
| *Reference exposure level* | 0.007 mg/m$^3$ (7 µg/m$^3$) |

OEHHA has used the same study which U.S. EPA used in the development of its Reference Concentration (RfC) of 10 µg/m$^3$. The U.S. EPA has used a benchmark dose methodology for the derivation of the RfC for phosphoric acid from the toxicity data in the Aranyi *et al.* (1988) study (U.S. EPA, 1995). The RfC is restricted to "aerosols of phosphoric acid and phosphorus oxidation products and does not apply to elemental phosphorus or other forms of phosphorus, such as phosphorus salts".

The U.S. EPA, using the Weibull model, estimated the lower 95% confidence level bound on the maximum likelihood estimate (MLE = 150 mg/m$^3$) resulting in 10% incidence of lesions in the tracheo-bronchiolar region to be 100 mg/m$^3$ (the $BMC_{10}$). The U.S. EPA considered 10% incidence level to be a correlate to the NOAEL, based on a precedent in the analysis of data with developmental toxicity endpoints (Allen *et al.*, 1994; Faustman *et al.*, 1994). After correction for exposure continuity, a regional deposited dose ratio (RDDR) for the tracheobronchial region of 0.64 was applied due to the availability of data concerning the growth and deposition of phosphoric acid aerosol particles in humans and the similarities in the effects of phosphoric and better-characterized sulfuric acid aerosols. Key assumptions in the generation of this factor include: (1) the lowest $\sigma_g$ of 1.56 µm cited in the study was used in the calculation; (2) geometric rather than aerodynamic diameter approximations were used; (3) particles of this size reach the deposition / lesion site (bronchioles); 4) these hygroscopic particles become more uniform with

growth; and (5) particle growth is similar in humans and rodents. An uncertainty factor of 10 was applied because of the subchronic duration of the study. A factor of 3 was applied for interspecies extrapolation in light of the fact that some correction for human equivalency was made with the RDDR. Finally, a factor of 10 was applied for protection of potentially sensitive human subpopulations. The resulting RfC for phosphoric acid is 0.01 $mg/m^3$.

OEHHA uses a $BMC_{05}$ for development of acute Reference Exposure Levels (OEHHA, 1999; Fowles *et al*., 1999). OEHHA staff believe that the $BMC_{05}$ is more likely to approximate a NOAEL than a $BMC_{10}$ since 5% is closer than 10% to the lower end of average risk levels associated with a NOAEL (Leisenring and Ryan, 1992). A $BMC_{05}$ is more likely to represent a value close to the limit of most studies to detect an effect, and is therefore more like a NOAEL. In contrast, a $BMC_{10}$ is more likely to represent a LOAEL since it is usually in the detectable range of responses. In the specific case of phosphoric acid the $BMC_{10}$ of 100 $mg/m^3$ was twice the NOAEL of 50 $mg/m^3$. The $BMC_{05}$ was calculated to be 64 $mg/m^3$, much closer to the NOAEL. Use of the $BMC_{05}$ results in a chronic REL of 7 $\mu g/m^3$.

## VII. Data Strengths and Limitations for Development of the REL

The strengths of the inhalation REL for phosphoric acid include the availability of subchronic inhalation exposure data from a well-conducted study with histopathological analysis and the observation of a NOAEL. Major areas of uncertainty are the lack of adequate human exposure data, the lack of chronic inhalation exposure studies, and the discontinuous nature of exposures (only 2 1/4 hours per day).

The Aranyi *et al.* (1988a) study represents the most adequate study for the quantitative evaluation of the toxicity of phosphoric acid. It was conducted with a large number of animals with multiple doses, produced good dose-response data, and examined likely targets of toxicity (respiratory system) of smoke generated from the combustion of phosphorus and butyl rubber. Uncertainties associated with these data, however, include that (1) the study used combustion products of phosphorus rather than phosphoric acid itself, (2) the total exposure time was relatively short and discontinuous over the duration of the experiment, and (3) only one species/strain/sex was studied.

## VIII. References

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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

| | |
|---|---|
| *Inhalation reference exposure level* | **20 μg/m$^3$** |
| *Critical effect(s)* | Eye and respiratory irritation, asthma, and bronchitis in occupationally exposed workers |
| *Hazard index target(s)* | Respiratory system |

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

| | |
|---|---|
| *Description* | White or pale yellow crystals |
| *Molecular formula* | $C_8H_4O_3$ |
| *Molecular weight* | 148.11 g/mol |
| *Boiling point* | 295°C |
| *Melting point* | 130.8°C |
| *Vapor pressure* | $5.14 \times 10^{-4}$ torr @ 25°C; 1 torr @ 96.5°C |
| *Solubility* | Soluble in 162 parts water, 125 parts carbon disulfide; soluble in hot benzene |
| *Conversion factor* | 1 μg/m$^3$ per ppb at 25ºC |

## III. Major Uses and Sources

The primary use of phthalic anhydride (PA) is as a chemical intermediate in the production of plastics from vinyl chloride. Phthalate esters, which function as plasticizers, are derived from phthalic anhydride. Phthalic anhydride has another major use in the production of polyester resins and other minor uses in the production of alkyd resins used in paints and lacquers, certain dyes (anthraquinone, phthalein, rhodamine, phthalocyanine, fluorescein, and xanthene dyes), insect repellents, and urethane polyester polyols. It has also been used as a rubber scorch inhibitor and retarder (HSDB, 1995; National Cancer Institute (NCI), 1979). The annual statewide industrial emissions from facilities reporting under the Air Toxics Hot Spots Act in California based on the most recent inventory were estimated to be 11,442 pounds of phthalic anhydride (CARB, 2000).

## IV. Effects of Human Exposure

Symptoms in workers exposed to phthalic anhydride by inhalation in two plants (A and B) manufacturing alkyd and unsaturated polyester resins were studied (Nielsen *et al.*, 1988). Two groups of exposed workers were identified in each plant. One group worked directly loading the reactors from bags of phthalic anhydride ("heavy" exposure – 35 workers) and the other group was involved with "other work" which led to "low" exposure (25 workers). Mean employment times for the "heavy" and "low" exposure groups were 13.3 and 11.9 years, respectively. Time-weighted average air concentrations for workers from the loading of PA was 6.1 (range: 1.8-14.9) and 6.8 mg PA/$m^3$ (range: 1.5-17.4) in plants A and B, respectively. Similar exposure levels in both plants led to pooling of data. The exposure duration of the "heavy" group was estimated at approximately 30 minutes two times a day, corresponding to the time of loading, and resulted in a full-day time weighted exposure estimate of 0.4 mg PA/$m^3$. For those engaged in "other work" exposure levels were estimated at < 0.1 mg PA/$m^3$ (the limit of detection). Other chemicals in use in smaller amounts included maleic anhydride, isophthalic anhydride, and trimellitic anhydride. Comparison of symptom incidence between the "heavy" and "low" exposure groups included conjunctivitis (46% vs. 20%), rhinitis (40% vs. 20%), rhinoconjunctivitis (17% vs. 12%), asthma (17% vs. 0%), and chronic bronchitis (17% vs. 4%). Serum antibodies were measured in both groups of workers and compared to 22 nonexposed workers (employed at a food processing factory). The only significantly changed level was an increase in specific IgG in the "heavy" exposure group. A correlation was also noted between specific IgG level and exposure level, although not all individuals with elevated specific IgG reported symptoms.

In a study conducted at another plant manufacturing alkyd and/or unsaturated polyester resins, serum immunoglobulins and lung function were examined in 23 workers exposed to phthalic anhydride and 18 control subjects (Nielsen *et al.*, 1991). Estimated exposure levels were 6.6 mg PA/$m^3$ (range: 1.5-17) (Nielsen *et al.*, 1988). Workers were examined for sensitization to PA and other allergens and possible development of small airways disease. Among the exposed workers, there was significantly increased reporting of conjunctivitis and rhinoconjunctivitis. One worker showed an asthmatic response to anhydrides. No significant differences in lung function tests were observed between exposed and unexposed groups.

Symptoms in workers occupationally exposed to PA during the course of producing alkyd and/or polyunsaturated polyester resins were described (Wernfors *et al.*, 1986). Exposure estimates of breathing zone PA levels ranged from 3 to 13 mg/$m^3$ for workers engaged directly with the handling of PA. In other areas the estimated level was <0.3 mg/$m^3$. The study examined 48 workers who were employed at the time of the study and 70 former employees who responded to a survey of symptoms related to exposure. No unexposed control group was included in the study. Workers who were employed for at least two months reported symptoms of rhinitis (28%), chronic bronchitis (11%), and asthma (28%). Among a subset of 11 workers with asthma, 3 had positive skin tests for PA sensitivity. Bronchial provocation tests with 6 or 0.5 mg/$m^3$ PA for 5 or 10 minutes were positive in 2 workers.

## V. Effects of Animal Exposure

Male albino rats (6/treatment group) were exposed to phthalic anhydride vapors at 0, 0.02, 0.2, and 1 mg/m$^3$ continuously for 45 days (Protsenko, 1970). After a two week recovery period the testes were examined for spermatozoa motility time as well as for ascorbic acid, dehydroascorbic acid, and nucleic acid content. Motility time was defined as the time it took for spermatozoa to cease motion completely under microscopic examination. Spermatozoa motility time was decreased ~50% in the 1 mg/m$^3$ dose group and ~25% in the 0.2 mg/m$^3$ dose group. Significant decreases in ascorbic acid and dehydroascorbic acid levels were found in animals exposed to 0.2 and 1.0 mg/m$^3$ phthalic anhydride, and dehydroascorbic acid levels were decreased in the 0.02 mg/m$^3$ dose group. At 1 mg/m$^3$, RNA levels and combined RNA and DNA levels were significantly increased over controls. No significant changes were observed in the 0.02 mg/m$^3$ dose group.

Five and six female Hartley guinea pigs were exposed to 0.05-0.2 mg/m$^3$ and 0.6-6 mg/m$^3$ phthalic anhydride dust, respectively, for 3 hours/day for 5 consecutive days (Sarlo and Clark, 1992). Exposures were expressed as ranges due to difficulty in regulating dust levels in the chambers. Sampling of dust showed particles were 65-80% < 10 μm diameter and had a mean mass diameter of 5.8-9.8 μm. Eight control animals were exposed to filtered air only. Two weeks after the last exposure, animals were challenged for 30 minutes with aerosolized PA-guinea pig serum albumin conjugate. All animals in the "high" dose group showed immediate bronchoconstriction and transiently increased respiratory rate. Animals in this dose group also showed elevated IgG antibody titers. No detectable increase in antibody levels was found in the "low" dose group.

Type I hypersensitivity was examined in female Hartley guinea pigs exposed to phthalic anhydride dust (Sarlo *et al.*, 1994). Two groups of 8 animals were exposed to 0.5 or 1.0 mg/m$^3$, and two groups of 16 animals were exposed to 0 (filtered air only) or 5.0 mg/m$^3$ phthalic anhydride dust (respirable size – 5 μm) in stainless steel chambers for 3 hours/day for 5 consecutive days. Groups of 8 animals from the control and 5 mg/m$^3$ groups were challenged after a two week recovery period for 30 minutes with 5.0 mg/m$^3$ phthalic anhydride dust. Respiratory data were collected using a plethysmograph from 30 minutes before the exposure to 60 minutes after the exposure. No significant difference (defined as a change of 3 standard deviations from the same parameter in the control animals) in respiration rate or plethysmograph pressures was found between the exposed and unexposed animals. Eight animals in each of the four exposure groups were also challenged after two weeks of recovery with 2.0 mg/m$^3$ aerosolized PA-guinea pig serum albumin (GPSA) conjugate as described above. Respiratory rate was increased in 4/8 of the high-dose group animals and 1/8 of the low-dose animals. Plethysmograph pressures were increased in 3/8 animals in the high-dose group and one animal each in the low- and mid-dose groups. Serum IgG antibodies to PA-GPSA were elevated in all exposed animal groups and the effect showed a dose-response. Passive cutaneous anaphylaxis testing for anti-phthalic anhydride-GPSA IgG1a immunoglobulins showed positive results for 3/8, 1/8, and 5/8 animals in the 0.5, 1.0, and 5.0 mg/m$^3$ dose groups, respectively. Results in control animals were not described. Three of eight animals in the highest dose group had >189 hemorrhagic foci in their lungs. No control animal had more than 2 such foci. No foci were

observed in animals challenged with albumin conjugate. Serum IgG titer correlated with the presence of these foci.

Slavgorodskiy (1969) studied the toxicity of phthalic anhydride to animals from inhalation exposure. Sixty white male rats (strain not reported; group distribution not stated, but presumed to be 15 animals/treatment group) were exposed in 100 L chambers to 0, 0.18, 0.54, and 1.52 mg PA/$m^3$ aerosol continuously for 70 days. General condition and behavior, body weight, motor chronaxy of flexor and extensor muscles (every 10 days), cholinesterase activity (every two weeks), and hematological parameters were monitored during the course of the study. (Chronaxy is the minimum time for which a current must flow, at a voltage twice the minimal current necessary to produce muscle stimulation, in order to cause a muscle to contract.) No changes in body weight or behavior were observed in the treated animals. In animals in the high-dose group, the chronaxy ratio of flexors and extensors differed from the controls beginning on day 31 of exposure and continued until two weeks after exposure ceased. Significantly decreased whole blood cholinesterase activity occurred in the high- and mid-dose groups, with the change occurring after 42 days of exposure. An increase in thrombocyte count occurred in the high- and mid-dose groups after 70 days of exposure, but returned to normal during the two-week recovery period. Thus, 0.18 mg/$m^3$ PA appears to be a NOAEL in this study.

A chronic feeding study was conducted with phthalic anhydride in rats and mice to evaluate the carcinogenicity of the compound (National Cancer Institute (NCI), 1979). F344 rats (50/sex/dose group plus 20/sex control animals) were treated with diet containing 0, 7500, or 15,000 ppm phthalic anhydride for 105 weeks (which corresponds to approximately 0, 300, and 600 mg/kg-day, assuming that food consumption is 4% body weight/day). Animals were monitored for changes in body weight and for survival, and, upon death or the end of the study, were examined histopathologically. The only group showing significantly lower body weights was male rats in the high-dose group after week 13. No significant change in mortality was observed. Adverse non-cancer effects observed in the dosed groups, but not in the control animals, included “arched back, rough hair coat, ulceration, and corneal opacity”, however, incidences were described as “low”. No significant histopathological effects were found to be associated with exposure to phthalic anhydride. $B6C3F_1$ mice (50/sex/dose group plus 20/sex control animals) were initially treated with diet containing 0, 25,000, or 50,000 ppm phthalic anhydride (approximately 0, 3000, and 6000 mg/kg-day, assuming that food consumption is 12% body weight/day). Because of excessive weight loss after week 32, exposure levels were reduced during the course of the study such that the time-weighted average exposure for males was 16,346 and 32,692 ppm and for females was 12,019 and 24,038 ppm phthalic anhydride. Evaluation of toxicity was conducted at 104 weeks as with the rats. Mean body weight was reduced in male and female mice in a dose-related manner. No other significant treatment-related adverse effects were observed in the mice.

Pregnant female CD-1 mice (10/dose group) were treated intraperitoneally with phthalic anhydride in 0.5%(w/v) carboxymethyl cellulose solution on gestational days 8-10 (Fabro *et al.*, 1982). Dosing was variable, beginning within the 95% confidence limits of the $LD_{01}$ and progressing geometrically downward until no effect was observed. Animals were terminated on Day 18 and examined for teratogenic effects including fetal viability and number, resorption, and gross malformations. The 95% lower confidence limit on the dose producing teratogenicity

(grossly observable malformations and fetal internal malformations) in 5% and 50% of animals were 0.40 and 1.37 mmol/kg-day (59 and 203 mg/kg-day), respectively.

## VI. Derivation of Chronic Reference Exposure Level (REL)

| | |
|---|---|
| *Study* | Neilsen *et al.* (1988; 1991) |
| *Study population* | 23 occupationally-exposed workers |
| *Exposure method* | Discontinuous occupational inhalation exposures |
| *Critical effects* | Increased incidence of conjunctivitis, rhinitis, asthma, and chronic bronchitis |
| *LOAEL* | 6.5 mg/m$^3$ (mean of 6.1 and 6.8) |
| *NOAEL* | Not observed |
| *Exposure continuity* | 8 hours/day, 5 days/week |
| *Exposure duration* | Mean of 13.3 years |
| *Average experimental exposure* | 2.3 mg/m$^3$ for LOAEL group (6.5 mg/m$^3$ × 10/20 × 5/7) |
| *LOAEL uncertainty factor* | 10 |
| *Subchronic uncertainty factor* | 1 |
| *Interspecies uncertainty factor* | 1 |
| *Intraspecies uncertainty factor* | 10 |
| *Cumulative uncertainty factor* | 100 |
| *Inhalation reference exposure level* | 0.02 mg/m$^3$ (20 μg/m$^3$) |

Adverse effects were demonstrated to occur in humans occupationally exposed to phthalic anhydride in the workplace over long periods of time (Nielsen *et al.*, 1988). The symptoms reported primarily affected the respiratory system, with increased incidence of rhinitis, rhinoconjunctivitis, asthma, and chronic bronchitis. Conjunctivitis was also reported in exposed workers. Specific anti-PA IgG was significantly elevated compared to a non-exposed group. Increased incidences of rhinoconjunctivitis, conjunctivitis, or chronic bronchitis have also been reported in workers exposed to similar levels of PA dust (Nielsen *et al.*, 1991; Wernfors *et al.*, 1986). In these reports, adverse effects were clearly observed at the exposure level reported (6.5 mg PA/m$^3$; full-day time weighted exposure of 0.4 mg PA/m$^3$). Although symptoms were reported by Nielsen (1988) in the lower exposure level group, the significance is not clear since a true control group (unexposed workers) was not included in the symptomatology section of the study. The low exposure group's level of exposure was less than the detection limit for phthalic anhydride cited in the study, and this group was considered as a control group.

## VII. Data Strengths and Limitations for Development of the REL

The strengths of the inhalation REL for phthalic anhydride include the use of human exposure data from workers exposed over a period of years. Major areas of uncertainty are (1) the uncertainty in estimating exposure, (2) the potential variability in exposure concentration, (3) the

potential low exposures of the group considered as controls, (4) potential confounding by exposures to other chemicals, (5) the limited nature of the study, (6) the lack of reproductive and developmental toxicity studies, and (6) the lack of observation of a NOAEL in the key study. Another area of uncertainty is the apparent 10-fold greater sensitivity to bronchoconstriction from PA exposure in guinea pigs (a model for human asthmatics) in comparison to occupationally exposed workers.

The study in rats by Protsenko (1970) identified a LOAEL of 0.2 $mg/m^3$ and a NOAEL of 0.02 $mg/m^3$ for decreased sperm motility. However, this result from 1970 has not been verified or further explored in more recent toxicological or epidemiological studies. The small sample size of 6/group further weakens confidence in this result. Therefore, the study in workers by Nielson *et al.* (1988, 1991) was chosen as the basis for the REL for PA.

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*CHRONIC TOXICITY SUMMARY*

# PROPYLENE

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

**CAS Registry Number: 115-07-1**

## I. Chronic Toxicity Summary

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

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

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

## III. Major Uses and Sources (HSDB 1995)

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

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

## IV. Effects of Human Exposures

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

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

## V. Effects of Animal Exposures

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

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

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

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

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

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

## VI. Derivation of Chronic Reference Exposure Level (REL)

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

## VII. Data Strengths and Limitations for Development of the REL

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

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

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

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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

| | |
|---|---|
| *Inhalation reference exposure level* | **7,000 µg/m$^3$** (2000 ppb) |
| | . |
| *Critical effect(s)* | Liver effects in rats |
| *Hazard index target(s)* | Alimentary system (liver) |

## II. Physical and Chemical Properties (HSDB, 1995)

| | |
|---|---|
| *Description* | Colorless liquid |
| *Molecular formula* | $C_4H_{10}O_2$ |
| *Molecular weight* | 90.14 |
| *Density* | 0.962 g/cm$^3$ @ 20° C |
| *Boiling point* | 118-118.5°C |
| *Melting point* | -96.7°C |
| *Vapor pressure* | 11.8 torr @ 25°C |
| *Solubility* | Soluble in water, methanol, ether, and other organic solvents |
| *Conversion factor* | 1 ppm = 3.69 mg/m$^3$ at 25° C |

## III. Major Uses or Sources

Propylene glycol monomethyl ether (PGME) is used as a solvent for cellulose, acrylics, dyes, inks and stains (HSDB, 1995). Thus, the primary use of PGME is in lacquers and paints. Use of PGME is anticipated to increase due to its low systemic toxicity. The annual specific statewide industrial emissions of PGME from facilities reporting under the Air Toxics Hot Spots Act in California, based on the most recent inventory, were estimated to be 205,769 pounds (CARB, 1999). (Many industries did not estimate emissions of specific glycol ethers so that in 1998 there were also emitted 2,922,744 pounds of the general category glycol ethers, some of which may be PGME.)

## IV. Effects of Exposures on Humans

No reports or studies of human toxicity following chronic exposure to PGME were located in the literature. Slight eye irritation was reported by two of six human volunteers exposed to 100 ppm PGME for 2 hours (Stewart *et al.*, 1970). These subjects were exposed for a total of 3 1/2 hours during which no decrement in visual acuity, coordination, neurological responses or reaction time was measured. The same experiment exposed 23 subjects to 250 ppm PGME. After 15 to 30 minutes of exposure, 8/23 reported eye irritation and 3/23 reported throat irritation; lacrimation was observed in 3/23 subjects. Three subjects each reported one of the following symptoms: irritation, headache, and nausea. While the subjects frequently reported the odor to be objectionable upon first entering the chamber, the odor was usually undetectable by the end of the exposure. Clinical chemistry and urinalysis completed following exposure was not altered as compared to pre-exposure measurements.

## V. Effects of Exposures on Animals

Male and female rats (10 per sex per concentration) and rabbits (7 per sex per concentration) were exposed by inhalation to 300, 1000, or 3000 ppm PGME 5 hours per day, 5 days per week for 13 weeks (Landry *et al.*, 1983). Relative liver weights were statistically significantly higher than controls in both male and female rats exposed to 3000 ppm PGME. Hepatocellular hypertrophy was observed upon histopathologic examination of high dose females. The authors conclude that these effects are the result of physiologic adaptation rather than a manifestation of toxicity. The key observation in this study was sedation of rats and rabbits exposed to 3000 ppm PGME. The sedative effects were no longer apparent after 1-2 weeks of exposure.

Similar findings of mild CNS depression were observed by Hanley *et al.* (1984). Pregnant rats and rabbits were exposed to 500, 1500, or 3000 ppm PGME 6 hours per day either days 6-15 or days 6-18 of gestation, respectively. During the first 4-5 days of exposure, rats in the 3000 ppm PGME exposure group were lethargic and moderately ataxic. Statistically significant decreases in food consumption and maternal body weight gain were also observed during this period. A statistically significant increase in the incidence of delayed sternebral ossification was observed in the 3000 ppm exposure group. Rabbits exposed to 3000 ppm exhibited mild lethargy during the first 1-2 days of exposure with rapid post-exposure recovery. Overall maternal weight gain during the exposure (days 6-18 of gestation) was statistically significantly lower than controls.

No significant effect on fetal birth weight or on pup survival indices (e.g., proportion of pups surviving to day 3 post-delivery) was noted following exposure of pregnant rats to 200 or 600 ppm PGME 6 hours per day on days 6-17 of gestation (Doe *et al.*, 1983). Male rats were exposed to 200 or 600 ppm PGME 6 hours per day for 10 consecutive days. No significant effects on testicular weight or pathology were observed.

Increased liver and kidney weights were observed in male and female rats (10 per sex per concentration) following exposure to 6000 ppm for 7 hours per day, for 81 exposures over a 114-day period (Rowe *et al.*, 1954). No histopathological abnormalities were observed at necropsy.

Ciezlak et al. (1998) evaluated the potential chronic toxicity/oncogenicity and the response of liver and kidney tissue of Fischer 344 rats to propylene glycol monomethyl ether (PGME) at targeted vapor concentrations of 0, 300, 1000 or 3000 ppm. Groups of 50 male and female rats per sex were whole-body exposed under dynamic airflow conditions for 6 hours/day, 5 days/week for up to 2 years. Parameters evaluated included the general appearance and demeanor of animals, in-life body weights, survival, hematology, urinalysis and clinical chemistry determinations, survival, selected organ weights, gross and microscopic pathologic changes and tumor incidence. (The metabolic and morphological bases for PGME-induced sedation, hepatic hypertrophy and renal toxicity were characterized in separate groups of male and female rats exposed to PGME for 6, 12 or 18 months. Hepatic enzyme induction and cellular proliferation, as well as renal cellular proliferation and accumulation of $alpha_{2u}$-globulin (males only) in the kidneys, were conducted in these separate groups of animals.)

PGME-induced sedation at 3000 ppm resolved in all animals during the second week of exposure in conjunction with the appearance of adaptive changes in the liver (cytochrome P450 induction and hepatocellular proliferation). Cytochrome P450 (pentoxyresorufin O-demethylase) activities dropped to near control concentrations by week 52, coinciding with a return of sedation at 3000 ppm PGME. In male rats, the loss of metabolic adaptation was followed by a dose-related increase in altered hepatocellular foci after two years of exposure to 1000 or 3000 ppm PGME. The kidney toxicity observed in male rats was confirmed immunohistochemically as an $alpha_{2u}$-globulin nephropathy. No statistically-identified increases in tumors were observed in any tissue. The authors established a NOEL of 300 ppm PGME for the study.

Ethylene glycol methyl ether (EGME), a structurally related compound, exerts considerable toxicity on the blood, thymus, testes, and developing fetus. The toxicity of EGME has been linked to its primary metabolite, methoxyacetic acid. Recent comparative toxicity and metabolism studies (Miller *et al.*, 1983, Miller *et al.*, 1984) indicate that the relatively low systemic toxicity exerted by PGME is due to its different metabolites. Following a single oral dose of PGME, the key urinary metabolites identified in rats were propylene glycol and the sulfate and glucuronide conjugate of PGME (Miller *et al.*, 1983).

## VI. Derivation of Reference Exposure Level

| | |
|---|---|
| *Study* | Ciezlak *et al.*, 1998 |
| *Study population* | Fischer 344 rats (50/sex/concentration) |
| *Exposure method* | Discontinuous whole-body inhalation (0, 300, 1000, or 3000 ppm) |
| *Critical effects* | Increased eosinophilic foci of altered hepatocytes |
| *LOAEL* | 1000 ppm |
| *NOAEL* | 300 ppm |
| *Exposure continuity* | 6 hours per day, 5 days per week |
| *Average experimental exposure* | 54 ppm for NOAEL group (300 x 6/24 x 5/7) |
| *Human equivalent concentration* | 54 ppm for NOAEL group (gas with systemic effects, based on RGDR = 1.0 using default assumption that lambda (a) = lambda (h)) |
| *Exposure duration* | 104 weeks |
| *LOAEL factor* | 1 |
| *Subchronic uncertainty factor* | 1 |
| *Interspecies uncertainty factor* | 3 |
| *Intraspecies uncertainty factor* | 10 |
| *Cumulative uncertainty factor* | 30 |
| *Inhalation reference exposure level* | 2 ppm (2000 ppb, 7 mg/m$^3$, 7000 µg/m$^3$) |

## VII. Data Strengths and Limitations for Development of the REL

Strengths of the PGME RfC include the observation of a NOAEL and a LOAEL in the same study, and the availability of chronic exposure studies involving multiple concentrations. A major area of uncertainty is the lack of human data.

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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

| | |
|---|---|
| *Inhalation reference exposure level* | **30 µg/m$^3$ (9 ppb)** |
| *Critical effect(s)* | Degenerative and hyperplastic changes in the respiratory epithelium of rats |
| *Hazard index target(s)* | Respiratory system |

## II. Physical and Chemical Properties (HSDB, 1994)

| | |
|---|---|
| *Description* | Colorless liquid |
| *Molecular formula* | $C_3H_6O$ |
| *Molecular weight* | 58.08 |
| *Density* | 0.83 g/cm$^3$ @ 20° C |
| *Boiling point* | 34.23° C |
| *Melting point* | -112.13° C |
| *Vapor pressure* | 445 torr @ 20° C |
| *Solubility* | Soluble in water, miscible in acetone, benzene, carbon tetrachloride, methanol, ether |
| *Conversion factor* | 2.38 mg/m$^3$ per ppm at 25° C |

## III. Major Uses or Sources

Propylene oxide is used as a fumigant such as in the sterilization of packaged foods. It is also used as a chemical intermediate in the production of propylene glycol and glycol ethers and as a solvent. Propylene oxide is used in the preparation of surfactants and oil demulsifiers (HSDB, 1994). The annual statewide emissions from facilities reporting under the Air Toxics Hot Spots Act in California, based on the most recent inventory, were estimated to be 619,494 pounds of propylene oxide.

## IV. Effects of Human Exposures

Conclusive data regarding the effects of occupational exposure to propylene oxide were not located.

An epidemiological study examining mortality among workers with exposure to asbestos and several chemicals, including propylene oxide, identified three deaths due to mesothelioma, a rare cancer associated with asbestos exposure, and a statistically significant increase in the number of deaths attributed to forms of heart disease other than ischemia and hypertension (Egedahl *et al.*, 1989). The latter finding was explained by the authors to be the result of differences in diagnostic accuracy between rural and urban, and primary and tertiary medical care settings. A statistically significant decrease in observed deaths was found for all respiratory cancers, cancer of the bronchus and lung, circulatory disease, digestive diseases, cirrhosis and other liver disease, and death due to accidents, poisonings, and violence. These observations may be partially attributed to a "healthy worker effect".

## V. Effects of Animal Exposures

Male and female rats were exposed for 124 or 123 weeks (respectively) to 30, 100 or 300 ppm propylene oxide 6 hours per day, 5 days per week (Kuper *et al.*, 1988). Interim sacrifices were performed at 12, 18, and 24 months. Cumulative mortality was statistically significantly different from controls at 115 weeks in rats of both sexes exposed to 300 ppm propylene oxide. Cumulative mortality was also significantly different from controls at 119 weeks in female rats exposed to 100 ppm. However, a contributing factor to the increased mortality in female rats was the presence of mammary tumors. Atrophy of the olfactory epithelium and degenerative changes in the respiratory epithelium were observed in both male and female rats following 28 months of exposure to 30, 100, or 300 ppm propylene oxide. Severe hyperplastic changes in the olfactory epithelium were observed in male and female rats following 28 months exposure to 300 ppm propylene oxide. Mild hyperplastic changes were observed in the olfactory epithelium of female rats exposed to 100 ppm propylene oxide.

Rats and mice were exposed to 200 and 400 ppm propylene oxide 6 hours per day, 5 days per week for 103 weeks (NTP, 1985). Survival in mice was adversely affected in all groups exposed to propylene oxide; a statistically significant decrease in survival was observed in male and female mice exposed to 400 ppm propylene oxide. Survival in rats was not adversely affected by propylene oxide exposure. Rats exhibited exposure-related increases in suppurative inflammation of the nasal cavity, epithelial hyperplasia and squamous metaplasia.

Rats were exposed to 1500 ppm propylene oxide 6 hours per day, 5 days per week for 7 weeks (Ohnishi *et al.*, 1988). After 3-4 weeks of exposure the rats exhibited an awkward gait; the rats were ataxic by the seventh week. Histopathological examination revealed axonal degeneration of myelinated fibers of the hindleg nerve and fasciculus gracilis indicating central-peripheral distal axonopathy.

Eldridge *et al*. (1995) exposed male F344 rats to 0, 10, 20, 50, 150, or 525 ppm propylene oxide vapor for up to 4 weeks (with up to 4 weeks of recovery). Histopathology showed that the incidence and severity of respiratory epithelial hyperplasia increased with exposure time and regressed after termination of exposure, with complete recovery after 4 weeks. Cell proliferation (determined by bromodeoxyuridine incorporation) was elevated following 1 and 4 weeks of exposure, but decreased to control values after 1 week of recovery. Degeneration of the olfactory epithelium was found after 4 weeks of exposure with a decrease in incidence and severity after termination of exposure. Proliferation of olfactory epithelium was elevated during the 4-week exposure period and 1 week post-exposure and returned to control values after 4 weeks of recovery. The authors report a 4-week NOAEL for propylene oxide effects in nasal epithelium of 50 ppm.

Artificially inseminated rabbits were exposed to 500 ppm propylene oxide on days 1-19 or 7-19 of gestation (Hardin *et al.*, 1983). Maternal toxicity as indicated by a significant reduction in food intake and a significant decrease in maternal body weight gain was observed in both exposed groups. An increased number of resorptions per litter, with no change in total resorptions, was observed in rabbits exposed on days 1-19 of gestation. Sternebral and limb anomalies (considered minor by U.S. EPA and the authors) were significantly increased in the offspring of rabbits exposed on days 1-19 of gestation.

The same study also reported similar findings in sperm-positive rats exposed to 500 ppm propylene oxide on either days 1-16 or 7-16 of gestation or daily for 3 weeks prior to mating and then daily on days 1-16 of gestation. Reproductive capacity was impaired in rats exposed prior to breeding; the number of corpora lutea, implantation sites, and live fetuses were reduced. Those dams exposed pregestationally to propylene oxide also exhibited more resorptions. Maternal toxicity as indicated by decreased food intake and decreased body weight gain was observed in all exposed rats. Significant reductions in fetal body weight and fetal crown-rump length were observed in all exposed groups. An increased incidence of wavy ribs and reduced ossification were observed in the offspring of rats exposed from days 1-16 of gestation.

Harris *et al*. (1989) evaluated the developmental toxicity potential of propylene oxide in Fischer 344 rats. Four groups of 25 mated female rats were exposed to 0, 100, 300, and 500 ppm for 6 hours per day on gestation days 6 through 15. Cesarean sections were performed on all females on gestation day 20 and the fetuses were removed for morphological evaluation. Exposure to propylene oxide did not adversely affect survival, appearance, or behavior at any level. Maternal body weight gain and food consumption were reduced significantly at the 500 ppm level during exposure. Only one exposure-related effect was noted with respect to maternal water consumption, organ weights, cesarean section, or fetal morphological observations: increased frequency of seventh cervical ribs in fetuses at the maternally toxic exposure level of 500 ppm. Thus 300 ppm was considered the NOAEL.

## VI. Derivation of Chronic REL (U.S. EPA Reference Concentration (IRIS, 1995))

| | |
|---|---|
| *Study* | Kuper *et al.*, 1988 |
| *Study population* | Rats (male and female) |
| *Exposure method* | Inhalation (0, 30, 100 or 300 ppm) |
| *LOAEL* | 30 ppm |
| *Critical effects* | Degenerative and hyperplastic changes in the respiratory epithelium |
| *NOAEL* | Not observed |
| *Exposure continuity* | 6 hr/day for 5 days/week |
| *Exposure duration* | 124 weeks |
| *Average experimental exposure* | 5.4 ppm for LOAEL group (30 x 6/24 x 5/7) |
| *Human equivalent concentration* | 1.2 ppm for LOAEL group (gas with extrathoracic respiratory effects, RGDR = 0.23, based on MV = 0.3 $m^3$/day, SA(ET) = 11.6 $cm^2$) |
| *LOAEL uncertainty factor* | 3 (mild effects only observed during last 4 months of exposure) |
| *Subchronic uncertainty factor* | 1 |
| *Interspecies uncertainty factor* | 3 |
| *Intraspecies uncertainty factor* | 10 |
| *Cumulative uncertainty factor* | 100 |
| *Inhalation reference exposure level* | 0.009 ppm (9 ppb, 0.03 mg/$m^3$, 30 µg/$m^3$) |

## VII. Data Strengths and Limitations for Development of the REL

The chronic REL is equivalent to the US EPA RfC. The major strength of the REL for propylene oxide is the use of a well-conducted, long-term, multi-concentration study with adequate histopathological analyses. Weaknesses include the lack of adquate human data and the lack of a chronic NOAEL observation.

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*CHRONIC TOXICITY SUMMARY*

# SELENIUM AND SELENIUM COMPOUNDS

**(**other than Hydrogen Selenide**)**

| *Molecular Formula* | *Synonyms* | *Molecular Weight* (g/mol) | *CAS Reg. No.* |
|---|---|---|---|
| Se | elemental selenium | 78.96 | 7782-49-2 |
| $SeO_2$ | selenium dioxide; selenium oxide; selenious anhydride | 110.96 | 7446-08-4 |
| $H_2SeO_3$ | selenious acid | 128.97 | 7783-00-8 |
| $SeOCl_2$ | seleninyl chloride; selenium oxychloride; selenium oxichloric | 165.86 | 7791-23-3 |
| $Na_2SeO_3$ | disodium selenite | 263.01 | 10102-18-8 |
| $Na_2SeO_4$ | disodium selenate | 188.94 | 13410-01-0 |
| SeS | selenium sulfide; sulfur selenide | 111.02 | 7446-34-6 |

I. Chronic Toxicity Summary

| | |
|---|---|
| *Inhalation reference exposure level* | **20 μg/m$^3$** |
| *Oral reference exposure level* | **0.005 mg/kg/day** (USEPA RfD) |
| *Critical effect(s)* | Clinical selenosis |
| *Hazard index target(s)* | Alimentary system; cardiovascular system; nervous system |

## II. Chemical Property Summary (HSDB, 1995; Weast, 1980; Canady and Hodes, 1994; ACGIH, 1992)

| | |
|---|---|
| *Description* | $Se^0$ crystal: metallic gray<br>$H_2SeO_4$, $Na_2SeO_3$: white crystals<br>$H_2SeO_3$, $Na_2SeO_4$: colorless crystals<br>$SeO_2$: lustrous crystals; yellow vapor<br>SeS: yellow to orange powder |
| *Molecular formula* | see above |
| *Molecular weight* | see above |
| *Vapor pressure* | 0.001 torr @ 20°C |
| *Melting point* | $SeO_2$: 340°C<br>SeS: decomposes at 118-119°C |
| *Solubility* | $Se^0$: insoluble in water, alcohol; slightly soluble in $CS_2$; soluble in ether<br>$H_2SeO_4$: sol. in water; decomposes in alcohol<br>$H_2SeO_3$: sol. in hot water, alcohol<br>$Na_2SeO_3$: sol. in water<br>$Na_2SeO_4$: 84 g/100 ml water at 35°C<br>$SeO_2$: 38.4 g/100 ml water at 14°C<br>SeS: insoluble in water |
| *Conversion factor* | $Se^0$: not applicable (particulate)<br>$SeO_2$: 4.5 μg/m$^3$ per ppb at 20°C |

## III. Major Uses and Sources

Selenium occurs in four valence states: selenates ($Se^{6+}$), selenites ($Se^{4+}$), selenides ($Se^{2-}$), and elemental selenium ($Se^0$) (Goyer, 1991) which include compounds formed with oxygen, sulfur, metals, and/or halogens. Selenium compounds are used in the glass industry as decolorizing agents and in the rubber industry as vulcanizing agents. Selenium compounds are also found in toning baths used in photography and xerography, and in insecticides and photoelectric cells. Selenious acid is a component of gun cleaning chemicals (Quadrani *et al*., 2000). Selenium sulfide is used in shampoos as an anti-dandruff agent. The most widely used selenium compound in industry is selenium dioxide ($SeO_2$) which catalyzes reactions of organic compounds and is produced by the oxidation of selenium with nitric acid followed by evaporation or by burning selenium in oxygen (HSDB, 1995). The largest anthropogenic sources of atmospheric selenium are from the combustion of fossil fuels and the production/refining of copper; particulates are the primary expected form of the compound (National Academy of Sciences (NAS), 1976; U.S. EPA, 1984). The annual statewide industrial emissions from facilities reporting under the Air Toxics Hot Spots Act in California based on the most recent inventory were estimated to be 12,417 pounds of selenium and 4846 pounds of selenium sulfide (CARB, 1999).

Selenium is an essential trace element in humans and other species; selenium deficiency leads to cardiomyopathy in humans (Goyer, 1991). For dietary intake, the National Research Council has

set a U.S. Recommended Daily Allowance (RDA) of 0.87 μg/kg (55-70 μg/person/day) (Subcommittee on the Tenth Edition of the RDAs, 1989). The average daily oral intake of selenium is 125 μg/person (U.S. EPA, 1991). Organic selenium compounds (e.g., dimethyl selenide) are known to occur as metabolites and as microbial degradation products in the environment. These compounds appear to have relatively low toxicity.

## IV. Effects of Human Exposures

Acute occupational exposure to $SeO_2$ resulted in bronchospasm, irritation of the upper respiratory passages, violent coughing, and gagging with nausea and vomiting (Wilson, 1962).

The relationship between inhalation exposure to selenium and the presence of selenium in the urine was investigated in a five year study of workers at a selenium rectifying plant (Glover, 1967). Workers were exposed to fumes and dusts of elemental red selenium, which, the author reported, is converted 80% to $SeO_2$ in the presence of air. Average air concentrations of selenium were reported to be 3.6 mg/m$^3$ in grinding processes, 0.04 mg/m$^3$ in annealing processes, and a range of averages of 0.23-0.87 mg Se/m$^3$ in various "special" processes, e.g., punching, scraping, sorting, refining, and testing. The same author previously reported symptoms among selenium exposed workers including garlic-like odor of the breath, skin rashes, indigestion, and poorly-defined "socio-psychological" effects including lassitude and irritability (Glover, 1954).

Clinical signs of toxicity were observed among a population exposed to high levels of selenium in soils and food supplies in China (Yang *et al.*, 1983). Approximately half of 248 people in this region showed symptoms including hair and nail loss, discoloration and decay of the teeth, and CNS disturbances including pain and anesthesia of the extremities. Animals in the region were also affected, with hoof damage and horn sloughing reported in water buffalo, cattle, and pigs. Populations in low-, medium-, and high-selenium areas of China were later studied to associate the symptoms with selenium intake. Estimated daily intake for adults in these areas were 70, 195, and 1438 μg Se for males and 62, 198, and 1238 μg for females, respectively (Yang *et al.*, 1989). Selenium intake was highly correlated with whole blood, breast milk, and 24-hour urine selenium levels. The authors also suggested the possibility of liver dysfunction as indicated by a delay in prothrombin time among persons with intake of 750-850 μg Se/day. More clearly recognized and characteristic clinical signs, however, were only observed in a group exposed to greater than 1261 μg Se/day and not among those exposed to less than 853 μg Se/day. Assuming a 55 kg body weight, these respective daily dose rates were 0.023 and 0.015 mg/kg-day.

A population of 142 subjects in seleniferous areas of western South Dakota and eastern Wyoming was examined for signs of selenosis over a two-year period with monitoring of selenium levels in diet, whole blood, serum, urine, and toenails (Longnecker *et al.*, 1991). Subjects completed health questionnaires, underwent physical examinations, provided blood samples for clinical assessment, and provided blood, urine, toenails, and duplicate-plate food collections for selenium analysis. About half of the 142 free-living subjects had selenium intakes greater than 2.54 mμmol/day (200 μg/day) (range 0.86-9.20 mμmol/day, or 68-724 μg/day). Average intake among the population was estimated at 239 μg Se/day. No clinical

signs and no changes in hematological function, clinical chemistry, or liver function were observed in the population, even in subjects whose intake was as high as 9.20 mμmol/day (724 μg/day).

## V. Effects of Animal Exposures

Toxic effects from acute inhalation exposure to selenium dust were examined in rats, guinea pigs, and rabbits (Hall *et al.*, 1951). Twenty female rats were exposed once for 8 hours to 33 ± 10 mg Se/$m^3$. Many animals showed signs of pulmonary effects at both one week and 4 weeks after exposure; however, no control group was included in the experiment with which to compare incidence. Similarly, six female rabbits and 10 male guinea pigs were exposed to the same level of selenium dust for four 4-hour periods every 48 hours (8 days total duration). The animals showed signs of interstitial pneumonitis at one week (2 animals of each species) and lung congestion and alveolar infiltration of large macrophages.

Guinea pigs exposed one time to concentrations "less than 0.021 mg $H_2Se$/L" (22 mg Se/$m^3$ as hydrogen selenide) for 2, 4, or 8 hours exhibited difficulty breathing and a red-tinged discharge from the nose (Dudley and Miller, 1941). Mortality studies were conducted with guinea pigs (16/group) using the same exposure duration and selenium concentrations ranging from 1 to 43 mg Se/$m^3$. Fifty percent mortality was observed at 30 days among animals exposed once for 2 hours to 12 mg Se/$m^3$. Mortality after 30 days was 50% among animals exposed once to 1 mg Se/$m^3$ for 8 hours. Histopathological evaluation of guinea pigs exposed once for 4 hours to 8 mg Se/$m^3$ showed fatty change to the liver, pneumonia, lymphoid hyperplasia, and increased reticuloendothelial tissue in the spleen. These effects did not begin to resolve until more than 17 days after the exposure.

Several studies have addressed the toxicity of selenium compounds to animals when administered in either food or drinking water. Mice (50/group) treated with 0, 1, 4, or 8 ppm $Na_2SeO_3$ in drinking water over 50 weeks showed decreased growth rates at 8 ppm (Jacobs and Forst, 1981). The same group reported gross liver pathology in male mice treated by oral gavage for 3 days with 0.5 ml of 64 ppm $Na_2SeO_3$. Hamsters (8/sex/group) treated with 0.1 (unsupplemented), 1, 5, 10, or 20 ppm $Na_2SeO_3$ in the diet for 42 days showed histopathological changes to the liver (Beems and van Beek, 1985). Rats (6-8/group) treated in the diet with $SeS_2$, $Na_2Se$, $Na_2SeO_3$, or $Na_2SeO_4$ showed increased relative liver weights and/or decreased body weight gain at 10 ppm (for each compound) over a 5 week exposure (Dausch and Fullerton, 1993). A 13-week drinking water study of $Na_2SeO_3$, and $Na_2SeO_4$ in rats and mice showed increased mortality, decreased body weights, and histopathological changes to the kidneys in rats and decreased body weight and decreased water consumption in mice (Abdo, 1994). Decreased body weights were observed in rats treated for 6 weeks in drinking water with 2 ppm $Na_2SeO_3$ or $Na_2SeO_4$ (Palmer and Olson, 1974).

Decreased percentage of live spermatozoa, altered sperm morphology, and decreased body weight gain were observed in rats (6/group) treated for 5 weeks with 2 ppm $Na_2SeO_3$ in the diet (Kaur and Parshad, 1994). Rats (7-12/group) exposed to 0, 4, 8, or 16 ppm $Na_2SeO_3$ in drinking

water for 240 days showed alterations in testicular LDH and β-glucuronidase activity at 4 ppm (Nebbia *et al.*, 1987).

Developmental toxicity endpoints were examined in hamsters (5-10/group) exposed by oral gavage on gestational day 8 to $Na_2SeO_3$ and $Na_2SeO_4$ at concentrations ranging from 0 - 110 μmol/kg body weight (Ferm *et al.*, 1990). Effects observed at 100 μmol $Na_2SeO_3$/kg included decreased fetal crown-rump length and increased percentage of abnormal litters. At 90 μmol $Na_2SeO_4$/kg, an increased percentage of abnormal litters was observed. Mice (10 or 14/group) treated with 0, 3, or 6 ppm $Na_2SeO_3$ in drinking water from 30 days pre-gestation through gestation showed altered estrus cycle length, decreased fetal growth, and a decreased number of ossified vertebrae in offspring (Nobunaga *et al.*, 1979).

## VI. Derivation of Chronic Reference Exposure Level (REL) (for selenium and selenium compounds other than hydrogen selenide)

| | |
|---|---|
| *Study* | Yang *et al*., 1989 |
| *Study population* | 400 people in China |
| *Exposure method* | Low, medium, & high environmental levels of Se |
| *Critical effects* | Clinical selenosis (liver, blood, skin, CNS) |
| *LOAEL* | 0.023 mg/kg-day* (1.261 mg/day / 55 kg) |
| *NOAEL* | 0.015 mg/kg-day* (0.853 mg/day / 55 kg) |
| *Exposure continuity* | Continuous |
| *Exposure duration* | Lifetime |
| *Average experimental exposure* | 70, 195, and 1438 μg/day for adult males;<br>62, 198, and 1238 μg/day for adult females |
| *LOAEL uncertainty factor* | 1 |
| *Subchronic uncertainty factor* | 1 |
| *Interspecies factor* | 1 |
| *Intraspecies factor* | 3 |
| *Cumulative uncertainty factor* | 3 |
| *Oral reference exposure level* | 0.005 mg/kg/day (USEPA RfD) |
| *Inhalation extrapolation factor* | 3,500 $\mu g/m^3$ per mg/kg-day |
| *Inhalation reference exposure level* | 20 $\mu g/m^3$ |

*Factors: NOAEL (0.853 mg/day) and LOAEL (1.261 mg/day) calculated from regression analysis (log Y = 0.767 log X - 2.248, where Y = blood selenium and X = selenium intake) based upon the correlation (r = 0.962) between dietary selenium intake and blood selenium level for data showing incidence of clinical selenosis in adults based on an average adult body weight of 55 kg.

The inhalation chronic REL is based on the oral chronic REL, which is the same as the USEPA's oral reference dose (RfD) (U.S. EPA, 1996). In addition to being inhaled, airborne selenium can settle onto crops and soil and enter the body by ingestion. Thus an oral chronic reference exposure level for selenium is also required for Air Toxics Hot Spots health risk assessments. The chronic inhalation REL was derived by route-to-route extrapolation of the RfD. The

principal study used for the REL/RfD was that of Yang *et al*. (1989). Yang *et al*. (1989), in a follow-up to an earlier study (Yang *et al*., 1983), studied a population of approximately 400 individuals living in an area of China with unusually high environmental concentrations of selenium (Se). The subjects were evaluated for clinical and biochemical signs of Se intoxication. Three geographical areas with low, medium, and high selenium levels in the soil and food supply were chosen for comparison in the studies. The earlier study was conducted in response to endemic selenium intoxication in two separate areas with sample sizes of only 6 and 3. Comparisons were then made to a selenium-adequate area (n=8) and low-selenium area (n=13). The Yang *et al*. (1989) studies provide a much larger sample size and include additional analysis of tissue selenium levels. This allows a more accurate estimation of the dose-response relationship observed for selenium toxicity. Selenium levels in soil and approximately 30 typical food types commonly eaten by the exposed population showed a positive correlation with blood and tissue Se levels. The daily average Se intakes, based on lifetime exposure, were 70, 195, and 1438 μg for adult males and 62, 198, and 1238 μg for adult females in the low-, medium- and high-selenium areas, respectively. Significant correlations, demonstrated between Se concentrations of various tissues, were used to estimate the minimal daily Se intake values that elicited various alterations in biochemical parameters indicative of possible Se-induced liver dysfunction (i.e., prolongation of clotting time and serum glutathione titer) and clinical signs of selenosis (i.e., hair or nail loss, morphological changes of the nails, etc.). In this manner, a marginal safe level of daily Se intake was estimated. Analysis of the results indicated that persistent clinical signs of selenosis were observed only in 5/349 adults, a potentially sensitive subpopulation. The blood selenium concentration in this group ranged from 1.054 to 1.854 mg/L with a mean of 1.346 mg/L. Clinical signs observed included the characteristic "garlic odor" of excess selenium excretion in the breath and urine, thickened and brittle nails, hair and nail loss, lowered hemoglobin levels, mottled teeth, skin lesions, and CNS abnormalities (peripheral anesthesia, acroparesthesia, and pain in the extremities). Alterations in the measured biochemical parameters occurred at dietary intake levels of 750-850 μg/day. These alterations were described as a delay in prothrombin time, i.e., increase in blood coagulation time and reduction in blood glutathione concentration. However, these indicators were poorly characterized and are not typically used as an index for clinical selenosis resulting from chronic exposure to selenium (NAS, 1989). Based upon the blood selenium levels shown to reflect clinical signs of selenium intoxication, a whole blood selenium concentration of 1.35 mg/L corresponding to 1.261 mg of daily selenium intake is indicative of the lowest correlative selenium intake causing overt signs of selenosis. The next lowest whole blood selenium concentration of 1.0 mg/L, corresponding to 0.853 mg selenium/day, produces no clinical signs of selenosis. The NOAEL for this study is 0.85 mg Se/day and the LOAEL is 1.26 mg Se/day.

An intraspecies uncertainty factor of 3 was applied to the NOAEL to account for sensitive individuals. A full factor of 10 was not deemed necessary since similar NOAELs were identified in two moderately-sized human populations exposed to selenium levels in excess of the RDA throughout a lifetime without apparent clinical signs of selenosis. No modifying factor was applied by USEPA. OEHHA accepted the USEPA analysis.

Route-to-route extrapolation assumes by default that a chemical is equally absorbed by the inhalation and the oral routes and that the first pass effect due to metabolism by the liver is not important for the chemical. The latter assumption is applicable to most metals. There are

limited data to evaluate the assumption of equal absorption across the gastrointestinal tract and the lungs. Limited data indicate that 60% (range = 44-100%) of ingested Se is absorbed by the gastrointestinal tract, while in one study 30% (single estimate) of inhaled selenium was deposited in the respiratory tract (Owen, 1990). Deposition is dependent on particle size. The available data are not adequate to depart from the default assumption.

The USEPA stated its confidence in the RfD as: Study - Medium; Data Base - High; and RfD - High. Confidence in the chosen principal study is medium. Although this is a human epidemiological study in which a sizable population with sensitive subpopulations was studied, there are still several possible interactions that were not fully accounted for, e.g., fluoride intake and protein status. Also, except for clinical signs of selenosis there are no other reliable indicators, biochemical or clinical, of selenium toxicity. Confidence in the database is high because many animal studies and epidemiologic studies support the principal study. An additional human study with a freestanding NOAEL (Longnecker et al., 1991) provides support for the NOAEL identified in the principal study. Longnecker *et al*. (1991) found no effects at 238 μg Se per day, which would equate to 0.004 mg/kg-day for a 55 kg person. Therefore, high confidence in the RfD is selected based upon support of the critical study and the high level of confidence in the database.

There are insufficient data relating human inhalation exposure to selenium compounds to adverse health effects to use for the development of a chronic REL although toxicity has been reported from occupational exposure to gases of both $H_2Se$ and $SeO_2$ (Buchan, 1947; Wilson, 1962). Experiments in animals have shown that $H_2Se$ is toxic following inhalation exposure, with 8-hour exposures to concentrations as low as 1 mg $H_2Se/m^3$ causing "irritation sufficiently damaging to cause pneumonitis" and subsequently increasing 30-day mortality (Dudley, 1937; Dudley and Miller, 1941). Thus the selenium chronic REL is not meant to be applied to $H_2Se$, which may be considerably more toxic than other selenium compounds. At this time there are inadequate data to develop a REL for $H_2Se$. It is also not intended to be applied to organic metabolites of selenium.

## VII. Data Strengths and Limitations for Development of the REL

The strengths of the REL for selenium include its basis on a study with a large number of human subjects in a non-occupational setting that determined both a NOAEL and a LOAEL. The weaknesses include its basis on a route of exposure other than inhalation and its lack of applicability to hydrogen selenide, the most toxic selenium compound.

## VIII. Potential for Differential Impacts on Children's Health

The key study (Yang *et al*., 1989) included evaluation of children as young as one year old. Thus the chronic REL should be protective of infants and children. No adverse reproductive outcomes were reported, although only 400 people were studied. However, the inhalation REL is based on an oral REL of 0.005 mg/kg-day (0.06 μmol/kg-day). Ferm *et al*. (1990) did not find adverse effects on hamster development with Se doses below 34 μmol/kg. Thus the chronic REL should also be protective of infants and children.

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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

| | |
|---|---|
| *Inhalation Reference Exposure Level* | **3 µg/m$^3$** [respirable, as defined occupationally by ACGIH (2004)/ISO (1995) |
| *Critical effect(s)* | Silicosis in miners and other workers |
| *Hazard index target(s)* | Respiratory system |

## II. Physical and Chemical Properties (HSDB, 2001)

| | |
|---|---|
| *Description* | Transparent crystals |
| *Molecular formula* | $SiO_2$ |
| *Molecular weight* | 60.09 g/mol |
| *Density* | 2.65 g/cm$^3$ @ 0 °C (quartz) |
| *Melting point* | 1610 °C |
| *Boiling point* | 2230 °C (2503.20 °K) |
| *Vapor pressure* | 10 torr @ 1732 °C |
| *Solubility* | Practically insoluble in water or acids, except hydrofluoric acid; very slightly sol. in alkali. |
| *Conversion factor* | Not applicable |

In crystalline silica, the silicon and oxygen atoms are arranged in a definite regular pattern throughout the crystal. The characteristic crystal faces of a crystalline form of silica are the outward expression of this regular arrangement of the atoms (HSDB, 2001). This REL is meant to be applied only to particles of crystalline silica (quartz, cristobalite, tridymite), of respirable size, as defined by the occupational hygiene methods described by ACGIH (2004)/ISO (1995) which has a 50% cut-point at 4 µm particle aerodynamic diameter. This occupational definition of respirable differs from the environmental definition of respirable, which is $PM_{10}$. (The occupational particle category "thoracic" has a 50% cut-point at 10 µm particle diameter (ACGIH, 2004) and the category "inhalable" has a 50% cut-point at 100 µm particle diameter (ACGIH, 2004).)

## III. Major Uses and Sources

At least 11 chemically identical forms (polymorphs) have been described for crystalline silica. Alpha-quartz is the most abundant polymorph and constitutes 12% of the earth's crust (Elzea, 1997). Silica is also found in the amorphous (non-crystalline) state. The amorphous silica in diatomaceous earth (composed mainly of the cell walls of diatoms) can be converted to the crystalline form cristobalite by heating to 1000-1100 ºC (calcining). Silica is often associated

with silicates, which, in addition to silicon and oxygen, contain other metals such as iron, magnesium, aluminum, calcium, potassium, and sodium.

The major uses of silica are in the manufacture of glass, abrasives, ceramics, and enamels, in scouring and grinding compounds, and in molds for castings. Silica is also used in decolorizing and purifying oils and petroleum products; as a clarifying agent; in filtering liquids; and in the manufacture of heat insulators, firebrick, and fire- and acid-proof packing materials. As diatomite (naturally occurring diatomaceous earth), silica is used as a filtration agent, as an abrasive, and as an industrial filler. Sources of ambient respirable crystalline silica in California include mines, quarries, diatomaceous earth calcining plants, sand blasting, and entrained fines (e.g., PM10) from surface soil. The annual statewide industrial emissions from facilities reporting under the Air Toxics Hot Spots Act in California based on the most recent inventory were estimated to be 2,514,981 pounds of crystalline silica (CARB, 2001. The fraction, which is respirable as defined either occupationally or environmentally, is not known.

Measurement of crystalline silica has evolved. Instrumentation has varied by country. In South Africa since the 1930s, dust was collected with a konimeter (Le Roux, 1970; Cherrie and Aitken, 1999). A small volume of air (e.g., 5 cm3 captured in less than a second) was collected (impacted) onto a small area of a glass slide coated with adhesive. Total dust particles were counted and expressed as dust particles per cubic centimeter. Later, slides were heated to 500-550 ºC (ignition) to remove carbonaceous materials and immersed in hot 50% hydrochloric acid followed by a second ignition to remove acid-soluble materials. The remainder was mostly silica particles, which could be counted. The konimeter was superseded by the thermal precipitator, which also deposited particles onto glass but could sample larger air volumes at high flow rates (> 1 L/minute) for several hours. With time, particle counting was replaced by estimation of a particle's surface area, initially by examining slides but more recently by an automated method (Kitto, 1960; 1970).

In the United States the impinger method was used from 1922 until 1984 (Lippmann, 2001). Air was drawn into a trap containing fluid, particles in an aliquot of the fluid were counted under magnification, and concentrations were expressed as million particles per cubic foot of air sampled. Later, gravimetric analysis was introduced. Gravimetric analysis is dominated by the larger particles in any given size range.

When it was realized that only a fraction of the dust was responsible for silicosis, respirable dust was collected onto filters using size-specific dust collectors, such as horizontal plate elutriators in South Africa and cyclones in the United States. The sizes of particles collected on the filter were a function of the apparatus used and the rate of airflow through the apparatus. Quartz dust was quantified by examining filters in an electron microscope with a specific X-ray diffraction beam absorbed by crystalline silica. The National Institute of Occupational Sciences and Health (NIOSH, 2003) has approved Method 7500, which uses one of three approved cyclones and a 5 µm PVC membrane filter to sample, and X-ray diffraction to measure crystalline silica. The ARB has used Method 7500 in research projects.

In order to harmonize respirable particulate sampling methodology in workers, an international agreement has been reached to use dust samplers that have a 50% cut point for particles of 4 µm aerodynamic diameter (ISO, 1995; ACGIH, 2004).

Various attempts have been made to estimate the changes in silica levels in workplaces over time (e.g., Seixas et al., 1997 for diatomaceous earth facilities in California; Verma et al., 1989 for Ontario hard rock miners). However, although some conversion factors have been proposed, correlation between dust particle number in earlier studies, when dust concentrations were higher, and dust particle weight in the later studies, when the dust concentrations have been lowered, is imprecise so it is difficult to compare the earlier silica measurements with the more recent ones.

## IV. Effects of Human Exposures

Inhalation of crystalline silica initially causes respiratory irritation and an inflammatory reaction in the lungs (e.g., Vallyathan et al., 1995). Acute exposures to high concentrations cause cough, shortness of breath, and pulmonary alveolar lipoproteinosis (acute silicosis). After chronic but lower workplace exposures to silica for six to sixteen years, the small airways become obstructed as measured by pulmonary function tests (e.g., decreased FEV1) in granite quarry workers (no measurement of silica levels reported; Chia et al., 1992). In a report on the hazards of exposure to crystalline silica, the American Thoracic Society (1997) stated: "Studies from many different work environments suggest that exposure to working environments contaminated by silica at dust levels that appear not to cause roentgenographically visible simple silicosis can cause chronic airflow limitation and/or mucus hypersecretion and/or pathologic emphysema." Hnizdo and Vallyathan (2003) also concluded that "chronic levels of silica dust that do not cause disabling silicosis may cause the development of chronic bronchitis, emphysema, and/or small airways disease that can lead to airflow obstruction, even in the absence of radiological silicosis." Fibrotic lesions associated with crystalline silica have also been found at autopsy in the lungs of granite workers who lacked radiological evidence of silicosis (Craighead and Vallyathan, 1980).

Silicosis results from chronic exposure; it is characterized by the presence of histologically unique silicotic nodules and by fibrotic scarring of the lung. The histological progression of silicosis has been described as: (1) granuloma composed of histiocytic cells, collagen, and lymphocytes; (2) cellular fibrotic nodule with irregular collagen at the center and circular collagen at the periphery; (3) more mature nodule with acellular and avascular center; and (4) late mature nodule composed of dust and collagen including a calcified center (Green and Vallyathan, 1996). Lung diseases other than cancer associated with silica exposure include silicosis, tuberculosis/silicotuberculosis, chronic bronchitis, small airways disease, and emphysema (Oxman et al., 1993; Park et al., 2002; Hnizdo and Vallyathan, 2003; Balmes et al., 2003). Silica exposure has been implicated in autoimmune diseases (rheumatoid arthritis, scleroderma, systemic lupus erythematosus) in gold miners and granite workers (Steenland and Goldsmith, 1995; Parks et al., 1999) and in the causation of kidney disease in some occupations (Goldsmith and Goldsmith, 1993; Stratta et al., 2001), possibly by an immune mechanism.

At the cellular level, silica particles are engulfed in the lung by alveolar macrophages (AM). According to the generally assumed pathological model, the AM subsequently release various growth factors and reactive oxygen species (ROS; superoxide anion, hydrogen peroxide, hydroxyl radical) (Lapp and Castranova, 1993; Mossman and Churg, 1998; Ding et al., 2002). ROS and some growth factors (e.g., activator protein-1, platelet activating factor) are inflammatory and attract neutrophils to the site of inflammation, while other factors (fibronectin,

alveolar macrophage-derived growth factor) stimulate fibroblasts to proliferate and to make collagen. Since silica particles cannot be digested by the macrophage, the inflammatory process becomes chronic (frustrated phagocytosis). An increased silica burden leads to more foci of inflammation, nodule formation, and fibrosis. The internal process can continue after external exposure ends. Silica particles also enter into alveolar Type I epithelial cells (Churg, 1996), which can lead to cell death of Type I cells and to hypertrophy and proliferation of Type II epithelial cells to replace the Type I cells. The epithelial repair process is associated with a subsequent increase in collagen formation.

The initial diagnosis of silicosis is often based on chest radiographs. Recent papers have used the 1980 classification by the International Labor Organization (ILO, 1980) to identify and classify silicosis into categories and subcategories of seriousness by comparison of patient radiographs with ILO-supplied reference radiographs taken at various stages of silicosis (Table 1):

**Table 1. International Labor Organization categorization of silicosis (ILO, 1980).**

| ***ILO Category*** | ***Qualitative Description*** |
|---|---|
| 0/0 | No small (up to 1 cm) silicotic opacities (nodules) are present |
| 0/1 | Probably no nodules, but some areas of radiograph are suspect [possible silicosis] |
| 1/0 | Small silicotic nodules are most likely present, but not certainly [probable silicosis] |
| 1/1 | Small silicotic nodules are definitely present |
| 1/2 | Small silicotic nodules are definitely present; other areas of the radiograph may indicate more advanced lesions including large opacities (> 1 cm), pleural thickening, etc. |
| 2/1, 2/2, 2/3, 3/2, 3/3 | More advanced stages of silicosis/increasing certainty of the presence of lung abnormalities |

Some reports (e.g., Kreiss and Zhen, 1996; Hughes et al., 1998) use 1/0 (probable) as the basis of classification of silicosis, since many cases of silicosis are not detected by chest radiographs, yet silicotic nodules and other lesions are found at autopsy (Craighead and Vallayathan, 1980; Hnizdo et al., 1993). Other reports (e.g., Hnizdo and Sluis-Cremer, 1993) use the definite 1/1 as the lowest category indicating silicosis. Some disease is missed by radiography and is determined only by autopsy (Hnizdo et al., 1993). The ILO criteria are intended as an epidemiologic classification and comparison tool, not as a diagnostic classification on an individual basis. In occupational medicine practice, a group of tests is used to clinically diagnose silica-related lung disease including physical examination, X-rays, and high resolution computed tomography (CT) scans of the lung (e.g., Begin et al., 1991; Olivetti et al., 1993).

### A. Environmental silicosis

Several studies have reported "environmental silicosis", cases where the silicosis occurs in the absence of an industry usually associated with the disease (reviewed by USEPA, 1996). In one of the stronger examples, Saiyed et al. (1991) investigated non-occupational pneumoconiosis in Ladakh, India, high in the western Himalayas where there are no mines or industries. Among 449 randomly selected inhabitants of three villages, there were many cases of pneumoconiosis

associated with progressive massive fibrosis (nodules > 1 cm) and "egg shell" calcification of hilar glands. The prevalence of pneumoconiosis was 2.0% (3/150) in the village of Saboo, 20.1% (31/149) in Shey, and 45.3% (68/150) in Chushot, and corresponded with the severity of dust storms and the presence or absence of chimneys in the kitchens (i.e., ventilated cooking). Without chimneys (Chushot), dust concentrations in kitchens averaged 7.5 mg/m3 during cooking periods. The free silica content of the dust storms was 60-70%. The authors suggested that exposure to free silica from dust storms and to soot from cooking with domestic fuels caused the pneumoconiosis. Perhaps the interaction of silica and soot led to the disease. Such exposures in this and other studies, such as Bar-Ziv and Goldberg (1974), might be considered to be non-industrial but occupational, since the subjects studied by Saiyed et al. (1991) were involved in the domestic work of cleaning and cooking (USEPA, 1996). In any case, the exposures were very high and thus similar to some occupational exposures.

**B. Occupational silicosis**

Several relatively recent reports have presented data that allow a quantitative relationship between occupational dust exposure and the development of silicosis in workers to be calculated.

Hard rock miners in Ontario, Canada (Muir *et al*., 1989)

Muir *et al.* (1989) examined the relationship between cumulative exposure to silica (free crystalline silica, specifically alpha-quartz) and the development of silicosis in 2109 male hard rock (uranium, gold, mixed metals) miners in Ontario, Canada. The miners began work between 1940 and 1959 and were followed either until they ended their dust exposure or until December 31, 1982 (whichever came first). Five X-ray readers examined chest radiographs; one or more readers identified 32 cases of silicosis, defined as ILO category 1/1 or greater with round opacities. All five readers agreed on only six cases, while 12 cases were identified by only one reader (Table 2). A Weibull model of the form

$$R(x) = 1 - \exp[-(\alpha x)^{\beta}] \quad (x \geq 0, \beta > 0)$$

gave the best fit to the data for cumulative risk R of silicosis as a function of cumulative exposure in units of $(mg/m^3)$-yr. In this model x is the cumulative exposure (lagged five years), $\alpha$ is the Weibull scale parameter, and $\beta$ is the Weibull shape parameter (Table 2). Estimates of $\alpha$ and $\beta$ for each reader are given in Table II of Muir *et al*. (1989).

**Table 2. Silicosis Risk *vs*. Cumulative Respirable Silica in $(mg/m^3)$-y (Table IV of Muir *et al.*)**

| ***Reader*** | ***Cases (n)*** | ***1% risk*** [a] | ***2% risk*** | ***5% risk*** | ***10% risk*** |
|---|---|---|---|---|---|
| 1 | 14 | 3.5 (2.4-5.1) | 5.7 (3.9-8.4) | 11.2 (6.8-18.2) | 18.6 (9.9-35.0) |
| 2 | 24 | 2.7 (2.0-3.6) | 4.1 (3.2-5.3) | 7.1 (5.5-9.1) | 10.9 (8.1-14.8) |
| 3 | 24 | 3.0 (2.3-3.9) | 4.3 (3.4-5.3) | 6.9 (5.6-8.5) | 9.9 (7.8-12.7) |
| 4 | 14 | 3.7 (2.6-5.2) | 5.6 (4.1-7.7) | 9.8 (6.7-14.3) | 15.1 (9.3-24.4) |
| 5 | 7 | 5.7 (4.0-8.0) | 7.8 (5.5-11.0) | 11.9 (7.8-18.3) | 16.5 (9.7-28.2) |
| Any reader | 32 | 2.1 (1.6-2.9) | 3.3 (2.6-4.2) | 6.0 (4.8-7.5) | 9.6 (7.3-12.5) |
| At least 3 | 15 | 3.5 (2.5-4.9) | 5.4 (4.0-7.3) | 9.5 (6.6-13.6) | 14.6 (9.3-23.2) |
| All readers | 6 | 6.1 (4.1-8.9) | 8.5 (5.6-12.8) | 13.2 (7.8-22.5) | 18.7 (9.7-36.1) |

[a] In parentheses is the 95% confidence interval (CI) for each risk estimate.

The Ontario cohort gives the shallowest dose-response relationship for silicosis of the several cohorts examined (see Summary Table 15 below) due in part to the lack of follow-up of members who left the mines (either for another type of work or for retirement). Silicosis often develops after leaving employment (Hnizdo and Sluis-Cremer, 1993; Chen *et al.*, 2001). In Hnizdo and Sluis-Cremer (1993), for more than half the cases of silicosis radiographic signs developed at an average of 7.4 years after mining exposure ended. In addition, some of the Ontario miners in the Muir et al. study may have changed to a less dusty job if their physician told them that their (annual) radiograph showed abnormalities. The lack of follow-up, leading to under-ascertainment of silicosis, is a serious limitation of this study.

Gray iron foundry workers (Rosenman et al., 1996)

Rosenman et al. (1996) evaluated 1,072 (96.8% males) current and retired workers in a Mid-western gray iron foundry, which produces engine blocks for the automotive industry. Medical records and silica exposure data were analyzed for those with at least 5 years of employment as of June 1991. Nearly half had worked at the foundry for 20 years. Sixty had radiographic evidence of pneumoconiosis (ILO categories 1/0 and greater). Twenty-eight workers had radiographs consistent with silicosis; of these 25 had simple silicosis and three had progressive massive fibrosis. The prevalence of radiographic changes consistent with silicosis increased with years at the foundry, work area, quantitative silica exposure, and cigarette smoking. In regard to quantitative silica exposure, the authors stated that 0.3-2.7% of workers at the OSHA standard (90-100 $\mu g/m^3$) were silicotic, as were 4.9-9.9% of workers above 100 $\mu g/m^3$. After controlling for confounders, Rosenman et al. (1996) used a logistic regression analysis based on cumulative silica exposure to determine an odds ratio of 1.45 for developing a radiograph consistent with silicosis after 20 years of work at 100 $\mu g/m^3$ and an odds ratio of 2.10 after 40 years of work at 100 $\mu g/m^3$ (Tables 3 and 4). This study probably underestimates risk due to lack of follow-up of the current workers. Although silica is not the only toxic chemical in a foundry, the unique nature of the silicotic nodule diminishes the likelihood of confounding by other exposures.

**Table 3. Silicosis risk based on Rosenman *et al*. data (Finkelstein, 2000)**

| ***Cumulative silica exposure*** | ***Prevalence of silicosis*** |
|---|---|
| < 2 ($mg/m^3$)-y | 0.4% |
| 2-6 ($mg/m^3$)-y | 2.7% |
| > 6 ($mg/m^3$)-y | 10% |

**Table 4. Odds ratios for silicosis (from Table 8 of Rosenman *et al.*)[a]**

| *Time-weighted average silica exposure (mg/m$^3$)* | *20-year cumulative exposure [(mg/m$^3$)-y]* | *Odds ratio (95% C.I.)* | *40-year cumulative exposure [(mg/m$^3$)-y]* | *Odds ratio (95% C.I.)* |
|---|---|---|---|---|
| 0.010 | 0.2 | 1.04 (1.02-1.15) | 0.4 | 1.08 (1.05-1.11) |
| 0.025 | 0.5 | 1.10 (1.06-1.14) | 1.0 | 1.20 (1.12-1.30) |
| 0.050 | 1.0 | 1.20 (1.12-1.30) | 2.0 | 1.45 (1.25-1.68) |
| 0.075 | 1.5 | 1.32 (1.18-1.47) | 3.0 | 1.74 (1.40-2.17) |
| 0.100 | 2.0 | 1.45 (1.25-1.68) | 4.0 | 2.10 (1.15-2.82) |
| 0.150 | 3.0 | 1.74 (1.40-2.17) | 6.0 | 3.04 (1.96-4.72) |
| 0.200 | 4.0 | 2.10 (1.56-2.82) | 8.0 | 4.40 (2.45-7.93) |
| 0.300 | 6.0 | 3.04 (1.96-4.72) | 12.0 | 9.24 (3.83-22.3) |

[a] Additional mean silica exposures, their calculated odds ratios, and 95% confidence intervals (C.I.) are given in the paper.

Diatomaceous earth workers in California (Hughes *et al.*, 1998; Park *et al.*, 2002)

Hughes *et al.* (1998) investigated 1,809 Caucasian male diatomaceous earth workers in Lompoc, California, who had at least one year of exposure to cristobalite between 1942 and 1987. The crystalline silica isomorph cristobalite is formed when the amorphous silica in diatomaceous earth is calcined at 1000-1100 ºC. Quantitative estimates of dust exposure were made and published in the peer-reviewed literature by Seixas *et al*. (1997) based on 6395 air sampling records taken from 1948-1988. The average estimated respirable dust concentrations for 135 jobs were 3.55 ± 1.25 mg/m$^3$ prior to 1949, 1.37 ± 0.48 mg/m$^3$ from 1949-1953, 0.47 ± 0.16 mg/m$^3$ from 1954-1973, and 0.29 ± 0.10 mg/m$^3$ from 1974-1988. The workers had periodic chest radiographs. Based on the median of radiographic readings by three independent readers, 81 workers (4.5%) were judged to have opacities on chest radiographs (small opacities, ILO profusion ≥ 1/0, and/or large opacities). Age-adjusted relative risk of opacities increased significantly with cumulative exposure to crystalline silica. The concentration of respirable crystalline silica was an important determinant of risk after accounting for cumulative exposure. The workers were split into two categories: those exposed to < 0.50 mg/m$^3$ (or hired after 1950) and those exposed to > 0.50 mg/m$^3$ (or hired before 1950). The risk of opacities for a cumulative exposure to crystalline silica of 2.0 mg/m$^3$-yr is shown in Table 5.

**Table 5. Silica exposure and silicosis based on data of Hughes *et al*. (1998)**

| *Average crystalline silica exposure* | *Cumulative risk of silicotic opacities* |
|---|---|
| < 0.50 mg/m$^3$ (or hired after 1950) | 1.1% |
| > 0.50 mg/m$^3$ (or hired before 1950) | 3.7% |

The findings of Hughes *et al.* (1998) indicate an exposure-response relationship between cumulative exposure to crystalline silica as cristobalite and radiographic opacities. The relationship was substantially steeper among those exposed at the highest average concentrations of crystalline silica. The authors believe that the data do not support the regulatory assumption that cristobalite is more fibrogenic than quartz (i.e., prior to 2000 the occupational limit for cristobalite was half that for quartz), since at average silica levels comparable to other

epidemiologic studies quartz gave a higher incidence of silicosis than did cristobalite in this study. However, since radiography can under-diagnose silicosis, complete accounting for silicosis will require evaluation at autopsy. The ACGIH recently lowered the TLV for alpha-quartz from 100 to 50 μg/m$^3$, so that it has the same TLV as cristobalite (ACGIH, 2000).

Park *et al*. (2002) carried out a quantitative risk assessment, by Poisson regression methods, of the onset of silicosis among the diatomaceous earth workers in Lompoc. A linear relative risk model gave the best fit to the data. They estimated an excess lifetime risk for radiographic silicosis of 68-75 cases per thousand workers exposed to 50 μg/m$^3$ silica (cristobalite) for a 45 year work-life, then living to age 85. At 1 μg/m$^3$ silica the excess lifetime risk was estimated to be 1.6 cases of lung disease other than cancer per thousand workers exposed (Table 6).

**Table 6. Excess lifetime risk of silicosis predicted by Park *et al*. (2002)**

| ***Silica concentration (mg/m$^3$)*** | ***45 year cumulative exposure in mg/m$^3$-y*** | ***Radiographic silicosis - all workers*** | ***Radiographic silicosis in workers with < 10 mg/m$^3$-y*** |
|---|---|---|---|
| 0.001 | 0.045 | 6.2/1000* | 1.6/1000 |
| 0.005 | 0.225 | 17/1000 | 7.8/1000 |
| 0.01 | 0.45 | 26/1000 | 16/1000 |
| 0.02 | 1.8 | 39/1000 | 31/1000 |
| 0.05 | 2.25 | 68/1000 | 75/1000 |
| 0.1 | 4.5 | 100/1000 | 140/1000 |
| 0.2 | 9 | 150/1000 | 260/1000 |

* Excess risk estimates assume that workers were exposed to a constant silica concentration for up to 45 years (ages 20-65). Annual risks are accumulated up to age 85.

White South African gold miners (Hnizdo and Sluis-Cremer, 1993)

Hnizdo and Sluis-Cremer (1993) investigated silicosis risk retrospectively in a cohort of 2,235 white male South African gold miners. Exposure estimates were made for nine separate occupational categories based on a special study of dust levels in these mines done by Beadle in the 1960s (Beadle, 1971). To compensate for the fact that the average hours working in dust ranged among the 9 categories from 4 hours for “other officials” to 8 hours for “shaft sinkers and developers,” exposure was “normalized” to 8-hour shifts. The workers had a minimum of 10 years and an average of 24 years service from 1940 until the early 1970s. Dust levels were fairly constant during this period (see, e.g., Table 2 in Gibbs and DuToit (2002)). The miners had an annual chest radiograph while mining; they were followed until 1991 for radiographic signs of the onset of silicosis. An ILO category 1/1 (definite silicosis) or greater was selected to designate silicosis. Two independent readers initially read the chest films, but only the reader whose interpretations correlated better with autopsy results was used for additional analysis; the use of one reader is a limitation of the study. There were 313 miners (14% of the cohort) who developed radiographic signs of silicosis at an average age of 55.9 years. The latency period was largely independent of the cumulative dust exposure (CDE). In 57% of the silicotics, the radiographic signs developed at an average of 7.4 years after mining exposure ceased. The risk of silicosis determined by chest radiographs increased exponentially with cumulative dust dose. At the highest level of 15-(mg/m$^3$)-years CDE (approximately 37 years of gold mining at an

average respirable dust concentration of 0.4 mg/m$^3$), the cumulative risk for silicosis reached 77% as estimated by the accelerated failure time model using the log-logistic distribution (SAS Proc LIFEREG):

$$CR(t) = 1 - \{1/[1 + \exp(-\mu/\sigma) \times t^{(1/\sigma)}]\}$$

where CR(t) = cumulative risk at time t, and μ (2.439) is the intercept and σ (0.2199) is the scale parameter estimated by SAS's LIFEREG procedure. The authors concluded that the risk of silicosis was strongly dose-dependent, but that the latency period was largely independent of dose. The life table analysis (SAS Proc LIFETEST) below (Table 7) shows the number of miners who developed silicosis ("cases"), the number of miners considered by the authors to be at risk, and the risk per unit of CDE (also as calculated by the authors). In the table in column 1 (in parentheses) are OEHHA's determination of the mg/m$^3$–yr respirable silica exposure, based on Hnizdo and Sluis-Cremer's estimate of 30% silica in the dust, and in column 4 is the total number of miners actually at each midpoint level of CDE or silica. The values in column 4 of Table 7 are the number of workers in the group with the temporally integrated dust exposure in column 1.

**Table 7. Life table results - Risk of silicosis per unit Cumulative Dust Exposure (CDE)** (from Table IV of Hnizdo and Sluis-Cremer, 1993)

| ***Midpoint in (mg/m$^3$)-y of CDE (silica)*** | ***Cases of silicosis*** | ***Number of workers at risk based on life table*** | ***Number of workers remaining at this CDE midpoint*** | ***"Risk/ unit CDE"*** | ***Mean years in dust*** | ***Mean dust conc. (mg/m$^3$)*** |
|---|---|---|---|---|---|---|
| 1 (0.3) | 0 | 2218 | 204 | | | |
| 3 (0.9) | 9 | 2014 | 474 | 0.002 | 20.5 | 0.17 |
| 5 (1.5) | 48 | 1540 | 556 | 0.016 | 23.5 | 0.24 |
| 7 (2.1) | 85 | 984 | 469 | 0.045 | 27.2 | 0.30 |
| 9 (2.7) | 93 | 515 | 318 | 0.099 | 28.0 | 0.33 |
| 11 (3.3) | 53 | 197 | 142 | 0.156 | 29.4 | 0.38 |
| 13 (3.9) | 20 | 55 | 44 | 0.222 | 31.5 | 0.41 |
| 15 (4.5) | 5 | 11 | 11 | 0.227 | 37.0 | 0.42 |

[a] CDE = Σ number of dusty shifts x mean mass respirable dust conc. x average number of hours spent underground / (270 shifts/year x 8 h/shift)

A plot of risk of silicosis per unit of Cumulative Dust Exposure (CDE) versus the mid-point unit CDE, as given in Figure 1 of the Hnizdo and Sluis-Cremer report, and a plot of % silicosis among the workers actually exposed to a given level of silica (Figure 2), as determined by OEHHA staff, respectively, are given below.

**Figure 1. Risk of silicosis per unit CDE vs. CDE mid-point**

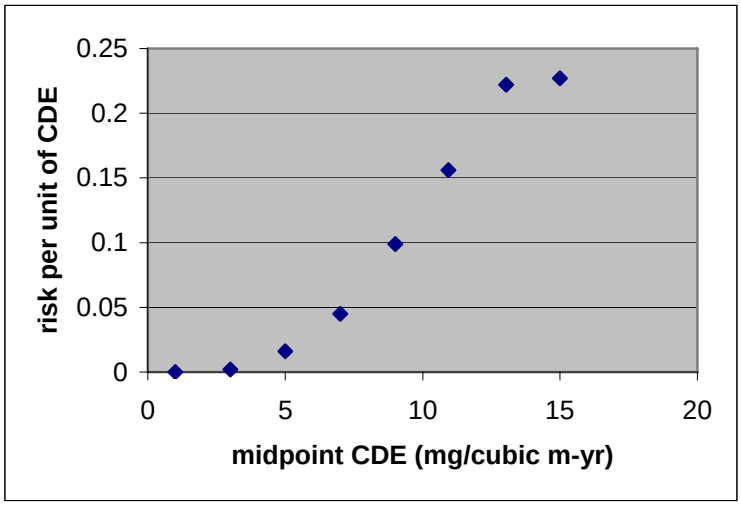
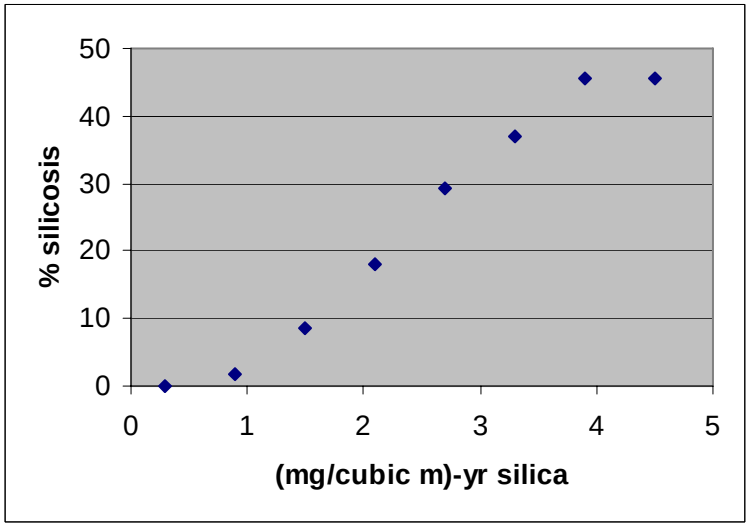



**Figure 2. Percent silicosis among workers at each silica level**




Black South African gold miners (Churchyard *et al*., 2004; Murray *et al*., 1996)

Black migrant contract workers constitute a large majority (85 - 90%) of South African gold miners. In a cross sectional study, Churchyard *et al*. (2004) interviewed and took chest radiographs of 520 black gold miners (mean age = 46.7 years, range = 37.1 – 59.9) who were still mining (average service = 21.8 years, range 6.3-34.5). Two readers examined the radiographs. As in the Hnizdo and Sluis-Cremer study, silicosis was defined as an ILO (1980) profusion of ≥ 1/1. The mean respirable dust concentration was 0.37 mg/m$^3$ (0 - 0.70); the mean quartz concentration was 0.053 mg/m$^3$ (0 - 0.095). The prevalence of silicosis was determined to be 18.3% by one reader and 19.9% by the other (mean 19.1%) (Table 8). This included several workers with more serious silicosis as indicated by ILO profusions ≥ 2/1 (see Table 1).

Significant trends were found between the prevalence of silicosis and: (1) length of service (OR = 1.69 per 5 years), (2) mean intensity of exposure (OR = 1.18 per 0.01 mg/m$^3$), and (3) cumulative exposure to quartz (OR = 3.2). The study confirms the large burden of silicosis among older black workers in this industry (see next paragraph). The burden is likely to worsen with continuous employment in dusty jobs. For this cohort the prevalence of silicosis will increase even if the miners stop mining immediately. If, as assumed by the authors, the dust levels during the working life of these black miners were constant, silicosis developed while they were exposed to a quartz level below the workplace limit of 0.100 mg/m$^3$.

**Table 8. Silicosis in black gold miners (Churchyard *et al*., 2003; 2004)**

| ***Cumulative quartz exposure in mg/m$^3$-yr*** | ***Mid-point of cumulative quartz exposure*** | ***Number in quintile**** | ***Cases of silicosis*** | ***Percent silicosis*** |
|---|---|---|---|---|
| 0 – 0.80 | 0.4 | 103 | 11 | 10.7 |
| 0.80 – 0.99 | 0.9 | 97 | 8 | 8.2 |
| 0.99 – 1.24 | 1.12 | 103 | 18 | 17.5 |
| 1.24 – 1.48 | 1.36 | 104 | 23 | 22.1 |
| 1.48 –3.08 | 2.28 | 103 | 33 | 32.0 |
| (Total) | | (510)** | (93) | (18.2) |

* Personal communication from Dr. J. teWaterNaude, December 2, 2004.
** Ten of the 520 films were unreadable.

Murray *et al*. (1996) analyzed data from 16,454 black South African gold miners dying from unnatural causes between 1975 and 1991 in order to study change in prevalence in silicosis and pulmonary tuberculosis (TB). TB prevalence increased from 0.9% in 1975 to 3.9% in 1991, while that for silicosis increased from 9.3% to 12.8%. The prevalence of both increased with age and duration of service. Silicosis was the most significant predictor of TB (OR = 1.78, CI = 1.27 - 2.30, p = 0.0001). A highly significant trend for TB, for year of autopsy, remained after adjustment for other variables, such as age and duration of service (OR = 1.04, CI = 1.01 – 1.06, p = 0.0046). (Another 21,202 black gold miners died of natural causes during the study period.)

<u>Hong Kong granite workers (Ng and Chan, 1994)</u>

Ng and Chan (1994) investigated silicosis among 338 male workers, who had worked at least one year between 1967 and 1985 in two granite quarries in Hong Kong. Three readers examined the chest radiographs. Silicosis was defined as an ILO classification of at least 1/1 (for small rounded opacities) or greater, assigned by at least two of the three readers. Exposure was estimated for each worker based on job category and particle counts. Thirty-six workers (10.6%) were designated silicotic. Both a logistic and a linear model fit the data well. The study suffered because only about half of the previously employed granite workers were studied, which probably led to an underestimate of silicosis risk in at least the highest exposure category and maybe in others. The data are summarized in Table 9.

**Table 9. Silica exposure and silicosis in Ng and Chan (Finkelstein, 2000)**

| *Mean cumulative exposure (mg/m$^3$)-y* | *Prevalence of silicosis* [a] |
|---|---|
| < 1 | 0% |
| 3.1 | 13% |
| 7.1 | 25% |
| 22 | 22% |

[a] rounded opacities determined by at least 2 of 3 readers (Table 3 of Ng and Chan)

Gold miners in South Dakota (Steenland and Brown, 1995)

Steenland and Brown (1995) studied a very large cohort (3330) of white male gold miners in South Dakota, who had worked at least 1 year underground between 1940 and 1965 (average = 9 years underground). The mine dust contained on average 13% silica (range = 1-48%). A job-exposure matrix was created for full-time underground workers grouped into five categories. The authors estimated that most miners were exposed to a median silica level of 0.05 mg/m$^3$, but that those hired before 1930 were exposed to a median level of 0.15 mg/m$^3$. A total of 170 cases of silicosis (5.1% of the cohort) was determined from death certificates only (n = 128 cases), from two cross-sectional radiographic surveys in 1960 and 1976 (n = 29 cases; ILO category 1/1 or greater), or from both (n = 13 cases). Unfortunately, only 25% of living cohort members were surveyed radiographically. The life-time risk of silicosis was less than 1% with a cumulative exposure under 0.5 mg/m$^3$-years and increased to 68% to 84% for the highest cumulative exposure category (more than 4 (mg/m$^3$)-years) (Table 10).

**Table 10. Risk of silicosis for cohort by cumulative exposure (Table 3, Steenland and Brown)**

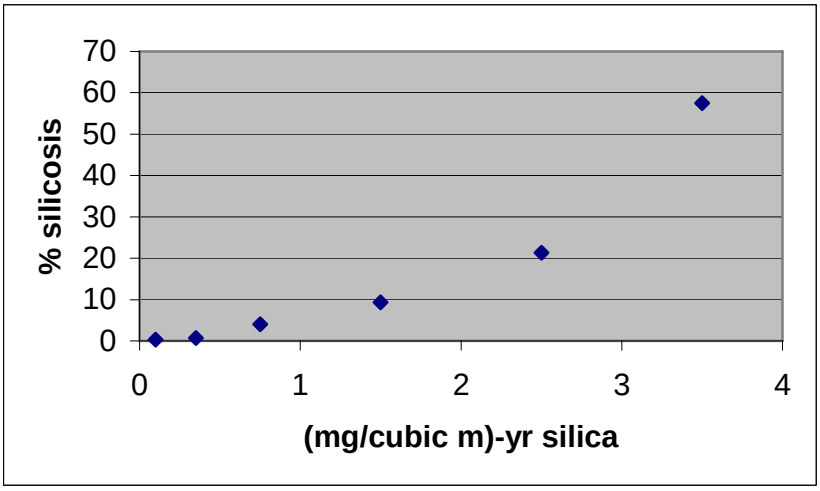
| *Silica exposure in (mg/m$^3$)-yrs: range (midpoint)* | *Miners with silicosis* | *Number entering exposure category (from life table)* | *Number remaining at this exposure level* | *Cumulative* [a] *Risk* | *Mean years of exposure* | *Mean year first exposed* |
|---|---|---|---|---|---|---|
| 0-0.2 (0.10) | 5 | 3330 | 1530 | 0.002 | 2.9 | 1953 |
| 0.2-0.5 (0.35) | 5 | 1800 | 740 | 0.005 | 9.7 | 1948 |
| 0.5-1.0 (0.75) | 15 | 1060 | 376 | 0.017-0.022[b] | 15.4 | 1942 |
| 1.0-2.0 (1.50) | 33 | 684 | 353 | 0.060-0.084[b] | 13.2 | 1931 |
| 2.0-3.0 (2.50) | 44 | 331 | 206 | 0.167-0.245[b] | 18.8 | 1926 |
| 3.0-4.0 (3.50) | 42 | 125 | 73 | 0.403-0.534[b] | 25.5 | 1921 |
| >4.0 | 26 | 52 | 52 | 0.678-0.844[b] | 30.6 | 1914 |

[a] Cumulative risk = 1−exp[−sum of (hazards * interval width)], where the hazards for each category of cumulative exposure are:
no. cases/(width*(no. entering category – 0.5*no. cases – 0.5*no. withdrawals))
[b] Cumulative risk adjusted for age and calendar time (Steenland and Brown, 1995)

The best predictor of disease was cumulative exposure ((mg/m$^3$) – years), followed by duration of exposure (years), and then by average exposure (mg/m$^3$). Figure 1 of Steenland and Brown indicates that a plot of their data for silicosis risk versus cumulative silica exposure was similar

to a plot of the data of Hnizdo and Sluis-Cremer (1993). After adjustment for competing risks of death, Steenland and Brown estimate that a 45-year exposure to 90 - 100 μg/m$^3$ silica would lead to a lifetime risk of silicosis for gold miners of 35% to 47%. A limitation of this study is the reliance on death certificates rather than on ILO interpretation of radiographs. In addition, no mention was made of validating the data on the death certificates. It was also not clear what, if any, autopsy data were available. A plot of silicosis incidence among the workers (as determined by OEHHA staff) actually exposed to the estimated level of silica is given in Figure 3 below. An accompanying editorial (Wagner, 1995) commended the article for estimating both the risk of silicosis while working and the lifetime risk of silicosis resulting from exposure during work.

**Figure 3. % Silicosis vs. silica exposure in Steenland and Brown (see Table 10)**




Miners in Leadville, Colorado (Kreiss and Zhen, 1996)

Kreiss and Zhen (1996) investigated the exposure-response relationships for silicosis among 134 male miners over 40 years old in Leadville, Colorado. The men had been studied three years earlier in a random sample of respiratory disease in their community (Kreiss *et al*., 1989). Of 100 dust-exposed miners, 32 had radiological profusions of small opacities of ILO category 1/0 or greater at a mean of 36.1 years since their first silica exposure. Of miners with cumulative silica, exposures of 2 (mg/m$^3$)-years or less, 20% had silicosis while 63% of miners accumulating greater than 2 (mg/m$^3$)-years had silicosis. Average silica exposure was also strongly associated with silicosis prevalence rates (Table 11).

**Table 11. Miners studied by Kreiss and Zhen (1996)**

| ***Average silica exposure*** | ***% silicotics*** |
|---|---|
| 0.025-0.05 mg/m$^3$ | 13% (5/38) |
| > 0.05-0.1 mg/m$^3$ | 34% (15/44) |
| > 0.1 mg/m$^3$ | 75% (9/12) |
| *Cumulative silica exposure* | *% silicotics* |
| ≤ 2 (mg/m$^3$)-y | 20% (14/70) |
| 2 – 4 (mg/m$^3$)-y | 63% (15/24) |

Based on logistic regression models of the form $R(x) = [1 + \exp(-\alpha - B'x]^{-1}$, Kreiss and Zhen concluded that the risk of silicosis was best predicted by elapsed time since last silica exposure together with either (1) cumulative silica exposure or (2) a combination of average silica exposure and duration of exposure. Exposure-response relationships were substantially higher using measured silica exposures (compared to using estimated silica exposures based on measured total dust exposures and assuming a constant silica proportion of dust). The risk of silicosis in this study is higher than in workforce studies having no follow-up of those leaving the mining industry (e.g., Muir *et al*., 1989) and in studies without job title-specific silica measurements (e.g., Hnizdo and Sluis-Cremer, 1993). However, the risk is comparable to several recent studies of exposure-response relationships for mining dust (e.g., Ng and Chan, 1994; Steenland and Brown, 1995) (see Summary Table 15 below). A limitation relative to other studies is the small number of subjects (100) in the group.

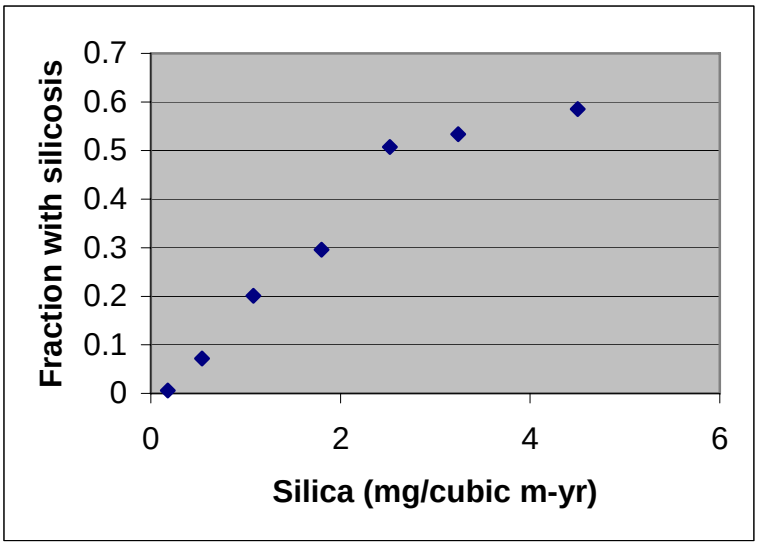
Chinese tin miners (Chen *et al.*, 2001)

Chen *et al.* (2001) found a clear exposure-response relationship between silica dust exposure and silicosis in a cohort of 3010 (2795 male and 215 female) miners employed for at least 1 year during the period 1960-1965 in any of four Chinese tin mines. No other diseases due to silica or tin were mentioned. Each cohort member was followed through 1994. Historical Chinese total dust (CTD) data were used to create a job exposure matrix for each facility, job title, and calendar year. The CTD data were converted to estimates of respirable crystalline silica for comparison with findings from other epidemiological studies of silicosis (including some of those above). Each miner's work history was abstracted from employment records. The diagnosis of silicosis was based on 1986 Chinese Roentgen diagnostic criteria for pneumoconiosis. The criteria classified silicosis as stages I-III, similar to an ILO classification of 1/1 or greater. Of the 3010 miners, 1015 (33.7%) were identified as silicotic (mean age = 48.3 years, with a mean of 21.3 years after first exposure) (Table 12). Among the silicotics, 684 (67.4%) developed silicosis after their tin mine exposure had ended (mean = 3.7 years after). The risk of silicosis was strongly related to cumulative exposure to silica. The Weibull distribution gave a very good fit to the data. The risk of silicosis was less than 0.1% when CTD was less than 10 (mg/m$^3$)-yr (= 0.36 (mg/m$^3$)-yr of respirable crystalline silica). The risk of silicosis increased to 68.7% when CTD exposure was equal to 150 (mg/m$^3$)-yr (= 5.4 (mg/m$^3$)-yr of respirable crystalline silica). Latency period was not correlated to the risk of silicosis or to cumulative dose. From their data, the authors predicted a 55% risk of silicosis for 45 years exposure to 0.1 mg/m$^3$ respirable crystalline silica, the workplace exposure limit (4.5-(mg/m$^3$)-years silica). Figure 4 plots the fraction of the workers in Chen et al. with silicosis (column 2 in Table 12 divided by column 4) exposed to a given level of silica (mid-point – in parentheses in column 1 of Table 12), as calculated by OEHHA staff.

**Table 12. Cumulative silicosis risk based on cumulative total dust (CTD) (Table 5, Chen *et al*., 2001)**

| *Range of CTD exposure in (mg/m$^3$)-y/ (silica mid-point)* | *Cases of silicosis (n)* | *Workers entering category* | *Workers at this level of CTD/silica* | *Cumulative risk based on Weibull model* | *Mean net exposure (years)* | *Mean latency (years)* |
|---|---|---|---|---|---|---|
| <10 (0.18) | 2 | 3010 | 333 | 0.001 | 2.2 | 14.7 |
| 10-19.99 (0.54) | 24 | 2677 | 334 | 0.010 | 5.3 | 21.3 |
| 20-39.99 (1.08) | 126 | 2343 | 626 | 0.070 | 9.3 | 22.0 |
| 40-59.99 (1.80) | 127 | 1717 | 429 | 0.145 | 11.9 | 21.5 |
| 60-79.99 (2.52) | 196 | 1288 | 386 | 0.285 | 9.9 | 20.3 |
| 80-99.99 (3.24) | 141 | 902 | 264 | 0.405 | 10.8 | 19.0 |
| 100-149.99 (4.50) | 244 | 638 | 417 | 0.663 | 13.1 | 20.4 |
| ≥ 150 (≥ 5.4) | 155 | 221 | 221 | 0.917 | 15.7 | 25.4 |

**Figure 4. Percent silicosis vs. silica level from Chen *et al*.**




<u>Industrial sand workers (McDonald et al., 2001; Hughes et al., 2001; Rando et al., 2001)</u>

McDonald *et al*. (2001) studied a cohort of 2670 men employed before 1980 for 3 years or more and followed through 1994 in one of nine North American sand-producing plants and in a large associated office complex (since most of the office employees had previously worked in the mines). They found 37 deaths due to silicosis and silicotuberculosis. The mean exposure of the cohort was 42 µg/m$^3$ silica (Rando *et al.*, 2001). Odds ratios for silicosis mortality, determined using conditional multiple logistic regression (SAS software), were significantly related to cumulative silica exposure (Hughes *et al.*, 2001) (Table 13). The odds ratios are in general agreement with those in the gray foundry workers of Rosenman *et al.* (1996) (Table 4).

**Table 13. Median cumulative silica exposure and odds ratio (Table 3 in Hughes *et al*., 2001)**

| *No lagging* | | | *Lagged 15 yr* | | |
|---|---|---|---|---|---|
| ***Median exposure in (mg/m$^3$)-y*** | ***Silicotics (n)*** | ***Odds ratio [a] for mortality*** | ***Median exposure in (mg/m$^3$)-y*** | ***Silicotics (n)*** | ***Odds ratio [a,b] for mortality*** |
| 0.832 | 7 | 1.00 | 0.142 | 7 | 1.00 |
| 2.744 | 7 | 1.27 | 1.229 | 7 | 2.54 |
| 6.916 | 8 | 2.62 | 2.583 | 7 | 4.55 |
| 12.084 | 7 | 2.13 | 7.990 | 8 | 5.16 |

[a] Matched odds ratio relative to lowest cumulative exposure category. Although labeled a cohort study, the data analysis compared cases of silicosis with non-silicotic controls.
[b] Significant increasing trend across exposure categories (see Hughes *et al*. for more details)

Ceramic workers (Cavariani *et al*., 1995; Legrand-Cattan *et al*. (1998)

Cavariani *et al*. (1995) investigated the incidence of silicosis among 2,480 men in the ceramics industry in central Italy. The workers were surveyed during the period 1974-1987 and followed through 1991 with annual chest radiographs. The cumulative risk of silicosis (ILO category 1/1 or greater) was 48% after 30 years of employment. A multivariate Cox's proportional hazards model indicated that silicosis increased linearly up to the period of 25-29 years employment. A hazard risk of 14.6 was found comparing those with ≥ 30 years exposure to those employed 10 years. Smoking significantly contributed to the model, but its role was unclear.

Legrand-Cattan *et al*. (1998) examined the dose-response relationship in two French ceramic plants. A 1992 cross-sectional study included more than 200 silica-exposed workers. Three ILO certified B readers read chest radiographs. Silica was sampled in the airborne dust. The results are tabulated below (Table 14).

**Table 14. Silicosis in two French ceramic plants (Legrand-Cattan *et al*., 1998)**

| ***Cumulative exposure to silica in (mg/m$^3$ – years)*** | ***Number of workers at this level*** | ***Number with small opacities with ILO profusion ≥ 1/0*** | ***Percent*** |
|---|---|---|---|
| < 0.35 | 50 | 2 | 4 |
| 0.35 – 1.08 | 57 | 8 | 14 |
| 1.09 – 1.77 | 55 | 11 | 20 |
| > 1.77 | 55 | 17 | 31 |
| Total | 217 | 38 | (18) |

A dose response relationship is clear; the authors reported a p value of 0.002. However, the study is limited by the lack of follow-up of the workers.

Slate workers (Glover *et al*., 1980; Saiyed *et al*., 1985; Saiyed and Bannerjee, 1985)

Slate contains calcium carbonate, iron oxides, silicates, amorphous silica, and crystalline silica. Glover *et al*. (1980) studied slate workers in North Wales. The respirable slate dust contained 13-32% crystalline silica. In the study group were 725 current and former workers exposed only to slate dust, while the controls were 530 men from the same area who had never been exposed to dust. Pneumoconiosis was found in 239 slate workers (33 %), and 10% had degrees of pneumoconiosis (category 2 or higher using the 1971 ILO scheme) that would bring worker's compensation. The prevalence of respiratory symptoms (cough, phlegm, dyspnea) was high. There was evidence of an effect of both simple and complicated pneumoconiosis on lung function (declines in FVC and $FEV_1$) additional to the effect of age. The high prevalence (40-50%) of radiological lesions suggested the presence of healed tubercular lesions in men over 55. Either pneumoconiosis or old tubercular lesions (or both) could account for the symptomatology and disability of the men.

Saiyed *et al*. (1985) surveyed the slate-pencil industry in India. An industrial hygiene survey revealed very high levels of free silica (2-10 $mg/m^3$), while a medical survey showed that 324 of 593 workers (54.6%) had silicosis. Of these, 105 had "conglomerate" silicosis (progressive massive fibrosis, PMF). Some lung lesions were detectable after less than five years of exposure to slate dust. Saiyed and Bannerjee (1985) conducted a follow-up examination 16 months later. The progression of silicosis was very rapid, and a total of 23 workers had died during this period (mean age = 34.7 years; mean exposure = 11.9 years). The authors attributed the high mortality to high levels of silica leading to early onset of PMF. The progression of silicosis was related to the intensity and duration of dust exposure, and to the severity of silicosis found initially.

Silicosis has been reported in other groups of slate workers in Norway (Bang and Suhr, 1998; Suhr *et al*., 2003) and in Germany (Mehnert *et al*., 1990).

Silica particle size

Data on silica particle size in the various workplaces are limited. According to Witschi and Last (2001), silica particles with a diameter of 1 μm (range = 0.5 - 3 μm) appear to be the most fibrotic in humans. NIOSH (1974) reviewed the existing literature and found that in five diatomite plants the mean silica diameter was 1.1 μm (range = 0.5 - 2 μm). For nine potteries, the particle size was 1.2 μm. For 18 foundries, more than 90% of the particles were less than 3 μm. The majority of particles to which shipyard sandblasters were exposed was also less than 3 μm. In the Vermont granite sheds, 10 mppcf (million particles per cubic foot) granite dust were initially estimated to be equal to 0.1 $mg/m^3$ respirable quartz. Steenland and Brown (1995) used this estimate for silica in South Dakota gold mines. Assuming that the density of quartz is 2.65 $g/cm^3$ and that the quartz particles are spherical, the data indicate that the particles have a diameter of 0.59 μm. NIOSH (1974) listed 0.94 μm as the median particle size in metal mines. No indication was given of the dispersion of the particle sizes around the average value. Davis *et al*. (1983) used the value of 10 mppcf in granite sheds as equal to 0.075 $mg/m^3$ silica. For that estimation, OEHHA staff calculated the particle diameter to be 0.53 μm. Thus, existing data indicate that the majority of silica in the workplace is respirable. In most of the occupational studies examined, the exposures were measured using a calibrated cyclone sampler similar to that recommended in the current NIOSH (2003) method. This allows collection of particles

primarily in the 0.5 – 5 μm range, with a collection efficiency profile intended to match the penetration of particles into the alveolar region of the human lung. In the case of the South African gold mine studies (Beadle, 1971; Page-Shipp and Harris, 1972; Hnizdo and Sluis-Cremer, 1993), particle number was determined by an optical method selecting respirable particles (range of 0.5 to 5 μm). Thus, the risk estimates obtained from these studies refer to particles in the size range where penetration occurs into the respiratory region of the lung. This corresponds to the size range of particles thought to be responsible for silicosis. It differs from the definition of "respirable" particles (*i.e.* $PM_{10}$) commonly used in environmental measurements, which refers to particles capable of penetrating anywhere in the lower respiratory tract (described as "thoracic" particles in occupational studies).

Risk estimation for silicosis from epidemiologic studies

The data from the above studies have been used by a number of investigators (Finkelstein, 2000; Chen *et al*., 2001; Hughes, 1995) and by OEHHA staff to estimate percent silicosis based on cumulative silica exposure in units of ($mg/m^3$)-yr. The results are summarized in Table 15.

In Table 15, more than 14,000 workers were studied, of whom approximately 12% were classified as silicotic. The 12% is likely an underestimate of the incidence of silicosis due to lack of follow-up by chest radiographs during life in some cohorts and to the lack of an autopsy after death.

**Table 15. Summary - Estimates of % silicosis based on cumulative silica exposure in (mg/m$^3$)-y**

| ***Study*** | ***Population (number with silicosis)*** | ***Exposure of 2 (mg/m$^3$)-y*** | ***Exposure of 4 (mg/m$^3$)-y*** | ***Exposure of 4.5 (mg/m$^3$)-y*** |
|---|---|---|---|---|
| Muir *et al*., 1989 | 2109 male Ontario hard rock miners (“15”) | 0.4[a,c] | 1.2[a,c] | 2[b] |
| Rosenman *et al*., 1996 | 1072 Midwestern foundry workers (28) | 2[a] | 10[a] | 3[b] |
| Graham *et al*., 1991 | 408 Vermont granite workers (35) | ~3[c] | — | — |
| Hughes *et al*., 1998 | 1809 white male diatomaceous earth workers (81) | 1.1 (low intensity)<br>3.7 (high intens.)[a] | 4 (low)<br>12 (high)[a] | — |
| Park *et al*., 2002 | 2342 white male diatomaceous earth workers (80) | ~7[e] | 13[e] | 14[e] |
| Hnizdo & Sluis-Cremer 1993 | 2235 white male South African gold miners (313) | 5[a]<br>10[c] | 52[a]<br>60[c] | 77[b] |
| Ng & Chan, 1994 | 338 male Hong Kong granite workers (36) | 6[a] | 15[a] | 15-20[b] |
| Steenland & Brown, 1995 | 3330 male S. Dakota gold miners (170) | 8[a] | 53[a] | 70[b] |
| Kreiss & Zhen, 1996 | 100 miners in Leadville, CO (32) | 11[a] | 53[a] | 92[b] |
| Chen *et al*., 2001 | 3010 Chinese tin miners (1015) | 14[d] | 47[d] | 55[b] |
| Churchyard *et al*., 2004 | 510 black gold miners (93) | ~28 | - | - |

[a] From Table II of Finkelstein (2000)
[b] From Table 6 of Chen *et al*. (2001)
[c] From Tables 3 and 4 of Hughes (1995)
[d] Interpolated by OEHHA staff from Fig. 2 of Chen *et al*. (2001).
[e] Estimated by OEHHA staff from Table 4 of Park *et al*. (2002)
[f] 158 had an ILO reading ≥ 1/0, while 103 had an ILO reading ≥ 1/1.

Determination of LOAEL and NOAEL for silicosis (Rice and Stayner, 1995)

In another approach to the data, Rice and Stayner (1995) identified the NOAEL and LOAEL for silicosis in several studies (Table 16). The study of Hnizdo and Sluis-Cremer (1993) yielded both a LOAEL and a NOAEL.

**Table 16. Estimates of NOAELs and LOAELs for silicosis (Rice and Stayner, 1995)**

| ***Study*** | ***Subjects*** | ***NOAEL in μg/m³*** | ***LOAEL in μg/m³*** |
|---|---|---|---|
| Davis *et al*., 1983 | 969 granite workers | 67.5 | |
| Hnizdo and Sluis-Cremer, 1993 | 2235 gold miners | 7 | 20 |
| McDonald and Oakes, 1984 | 1321 gold miners | - | 8[a] |
| | 64 gypsum miners | 35 | 49 |
| Muir *et al*., 1989 | 2109 gold miners | Could not determine | Could not determine |
| Rice *et al*., 1986 | 888 dusty trade workers | 80-100 | 200-252 |

[a] McDonald and Oakes (1984) considered this value to be only an approximation.

Proposals to change the occupational exposure limit

Silicosis is still being diagnosed at death in workers who were supposed to be exposed to occupational levels of 50-100 μg/m$^3$. Thus, there have been recommendations that the occupational exposure limit for respirable, crystalline silica (specifically alpha-quartz) be lowered from the current level of 100 μg/m$^3$ to 50 μg/m$^3$ (NIOSH, 1974; Rosenman *et al*., 1996; ACGIH, 1999; Finkelstein, 2000). In 2000, the ACGIH lowered its TLV for quartz from 100 to 50 μg/m$^3$. In 1986, WHO recommended that the occupational level be set at 40 μg/m$^3$ (WHO, 1986). Greaves (2000) recommended that the TLV be lowered to 10 μg/m$^3$. Based on existing data Greaves (2000) estimated that at 10 μg/m$^3$ the incidence rate for ILO grade 1/0 silicosis would be less than 5%, while for grade 1/1 it would be less than 2%. Chen *et al*. (2001) recommended that the TLV be lowered to 5 μg/m$^3$. "If the lifetime risk of silicosis is to be under 1 in 1000 (a criterion used by OSHA) for a lifetime exposure of 45 years, then the mean Chinese total dust concentration must be lower than 0.14 mg/m$^3$ (or lower than 0.005 mg/m$^3$ respirable crystalline silica)" (Chen *et al*., 2001). Mannetje *et al*. (2002) pooled data from six occupational cohorts. These included four groups discussed above: diatomaceous earth workers, Vermont granite workers, U.S. industrial sand workers, and South Dakota gold miners. Among them 170 deaths from silicosis were reported. The estimated mortality risk from silicosis to age 65 after 45 years of exposure at 100 μg/m$^3$ silica was 13 per 1000, while the risk of death at 50 μg/m$^3$ was estimated at 6 per 1000. Both estimates are above the 1 per 1000 risk acceptable to OSHA. Mannetje *et al*. also concluded that the occupational standards for silica should be lowered, but they did not specify a level. They further state that their estimates of silicosis mortality are probably underestimates due to exposure misclassification and to outcome misclassification, since deaths due to silicosis might have been coded to tuberculosis or chronic obstructive pulmonary disease.

### C. Silica exposure and lung cancer in workers

In 1997, IARC classified respirable crystalline silica in Class 1, a Known Human Carcinogen, based on occupational epidemiologic studies. However, chronic RELs are not based on cancer endpoints. Further, there is no approved cancer potency factor for silica.

## V. Effects of Animal Exposures

Several papers have reported that freshly fractured quartz, which has increased surface activity, causes greater inflammation than "aged" quartz. Vallyathan *et al*. (1991) reported that “fresh” silica was 4.2-fold more potent than silica aged for 1-2 days in decreasing the membrane integrity of male rat macrophages; 50% more potent in activating hydrogen peroxide secretion by macrophages; and 4.6-fold more potent in stimulating cellular chemiluminescence. Vallyathan *et al*. (1995) reported that inhalation of 19.3 mg/m$^3$ aged (for 2 months) quartz for five hours/day for 10 days by male Fischer 344 rats increased the number of cells recoverable by bronchoalveolar lavage (BAL) (Table 17). Aged quartz also gave histopathological evidence of increased pulmonary infiltrates, showed higher levels of biochemical markers of lung injury, increased lipid peroxidation, and increased the ability of pulmonary phagocytes to produce more oxygen radicals than air-exposed controls. These pulmonary responses were significantly more pronounced after inhalation of 22.4 mg/m$^3$ freshly fractured quartz.

**Table 17. Cells recovered in bronchoalveolar lavage from rats (Vallyathan *et al*., 1995)**

| *Cell type* | *Room air* | *Aged quartz* | *Freshly fractured* | *Fresh/aged* |
|---|---|---|---|---|
| Total cells | 7.1±0.78* | 9.3±1.2 | 20.4±2.2 | 2.2 |
| Macrophages | 6.7±0.69 | 4.7±0.79 | 5.4±0.78 | 1.1 |
| Neutrophils | ≥ 0.038 | 5.3±0.66 | 10.4±1.44 | 2.0 |
| Lymphocytes | ≥ 0.038 | 1.7±0.25 | 3.6±0.27 | 2.1 |
| Red blood cells | ≥ 0.038 | 1.7±0.26 | 6.0±0.57 | 3.5 |

* Cell counts are in millions. Each value is the mean ± standard error of 5 rats.

Burns *et al.* (1980) exposed female Balb/c mice for up to 39 weeks to 4.9 mg/m$^3$ Min-U-Sil brand crystalline silica. By 24 weeks, silica-laden macrophages were present in the lungs. After 39 weeks of exposure, silicotic lesions were seen in the lungs and adjacent lymph nodes (Table 18).

Davis *et al*. (1998) exposed mice to an aerosol of cristobalite silica (mass median aerodynamic diameter (MMAD) = 1.7 μm) for five hours/day in order to examine (1) the effects of exposure dose, (2) the evolution of disease over time, and (3) the variation in responses among strains. In C3H/HeN mice, incremental, cumulative exposure doses of cristobalite (10 mg/m$^3$ for 8 days, 43 mg/m$^3$ for 9 days, and 70 mg/m$^3$ for 12 days) caused (1) increased initial lung dust burden at 12 to 16 weeks post-exposure, (2) progressively intense pathological responses, and (3) increased total lung collagen (as measured by hydroxyproline).

The histopathological changes and total lung collagen increased with time after exposure. Silicosis was compared in four inbred strains of mice (BALB/c, C3H/HeN, MRL/MpJ, New Zealand Black) 16 weeks after aerosol inhalation exposure to cristobalite (70 mg/m$^3$, 5 hours/day, 12 days). C3H/HeN mice had histopathological silicotic lesions, enlarged

intrapulmonary lymphoid tissue, and increased lung wet weight, increased bronchoalveolar lavage (BAL) recoverable macrophages, lymphocytes, and neutrophils, and increased total lung collagen (hydroxyproline analyses). BALB/c mice developed slight pulmonary lesions. MRL/MpJ mice showed prominent pulmonary infiltrates with lymphocytes. New Zealand Black (NZB) mice developed extensive alveolar proteinaceous deposits, inflammation, and fibrosis. The authors found both dose-time-response relationships and a substantial variation of responses among mouse strains to the high level, short duration exposure.

At Brookhaven National Laboratory, groups of Fischer 344 rats were exposed to 0, 2, 10, and 20 mg/m$^3$ Min-U-Sil brand silica (alpha-quartz) for six months (Kutzman, 1984a; as summarized by USEPA, 1996). Other groups of rats had the same exposure, but were allowed to "recover" in air for an additional 6 months (Kutzman, 1984b; as summarized by USEPA, 1996). Significant alterations in total lung weight, total lung collagen, total elastin per unit lung dry weight, and total protein per unit lung dry weight at 2 mg/m$^3$ silica and microscopic evidence of silicotic lesions at the higher silica levels indicated that 2 mg/m$^3$ was a LOAEL for silica effects. After six months in clean air, the silica-induced lesions appeared to worsen.

Muhle *et al*. (1989) exposed groups of 50 male and 50 female rats to 1 mg/m$^3$ DQ12 quartz six hours/day, five days/week for 24 months. DQ12 contains 87% crystalline alpha-quartz, has a mass median aerodynamic diameter (MMAD) of 1.3 µm, and is 74% respirable. Moderate fibrosis was seen in 85 animals, slight fibrosis in 13, and very slight fibrosis in 1 (total rats with fibrosis = 99/100). Varying amounts of peribronchial granulomatous foci were noted in 95 rats.

Muhle *et al*. (1998) reported lung fibrosis in hamsters exposed to 3 mg/m$^3$ DQ12 silica. After 18 months of exposure to DQ12 for 6 h/day, 5 days/week, all hamsters in the group of 15-19 animals necropsied had very slight fibrosis. Approximately 100 silica-exposed animals were exposed for five more months to air only. Afterward 22.2% had very slight fibrosis, 68.7 % had slight fibrosis, and 1% had moderate fibrosis (i.e., more than 90/100 hamsters had lung fibrosis). No collagen measurements were reported. Thus, rats, mice, and hamsters show pulmonary fibrosis after crystalline silica exposure at and above 1 mg/m$^3$.

Wagner *et al*. (1968) exposed dogs up to 2.5 years, guinea pigs up to 18 months, and rats up to 2 years for 6 hours/day, 5 days/week to 61% cristobalite (in calcined diatomaceous earth). Dust exposures were 2 and 5 million particles per cubic foot (mppcf), equivalent to 0.2 and 0.5 mg/m$^3$ cristobalite (USEPA, 1996), with occasional excursions to 50 mppcf. No lung fibrosis was detected at these levels but all levels caused accumulation of inflammatory cells in the lung parenchyma. However, in dogs fibrotic nodules developed in the hilar lymph nodes with more nodules at 5 mppcf than at 2 mppcf.

Scheuchenzuber *et al*. (1985) examined immunologic responses in Balb/c mice following inhalation of 1.954 mg/m$^3$ silica for 150, 300, or 570 days. Mice exposed for 570 days were tested immediately post-exposure. Those exposed for 150 or 300 days were tested immediately or were rested for 30 or 150 days to allow for possible recovery from effects of dust inhalation. Silica inhalation suppressed the number of specific plaque-forming cells (PFC) in the spleen produced in response to aerosolized *E. coli*. After 570 days of inhalation, silica also reduced the ability of alveolar macrophages to phagocytize *Staphylococcus aureus in vitro* and impaired the ability to lyse allogeneic tumor cells (from mice other than Balb/c) *in vitro*. Silica inhalation did

not affect antibody-dependent cell-mediated cytotoxic and mitogenic responses by splenic lymphocytes. (Fibrosis was not an endpoint measured, but the effect level is similar to the LOAELs in other animal studies.)

**Table 18. Animal studies of silica inhalation analyzed by USEPA (1996)**

| ***Study*** | ***Species*** | ***Duration*** [a] | ***LOAEL*** |
|---|---|---|---|
| Muhle *et al*., 1989 | Rat | 24 mo | 1.0 mg/m$^3$ |
| Scheuchenzuber *et al*., 1985 | Mice | 150-570 d | 2.0 |
| Burns *et al*., 1980 | Mice | 3-39 wk | 4.9 |
| Kutzman, 1984a | Rat | 6 mo | 2.0 |
| Kutzman, 1984b | Rat | 6 mo + 6 mo recovery | 2.0 |
| Wagner *et al*., 1986 | Dog | Up to 2.5 yr | 0.2 |

[a] Inhalation exposure was generally for 6 h/day, 5 d/wk.

Quartz has the ability to induce the generation of free radicals and to cause oxidative stress in tissues. Many substances that affect the quartz surface can modify this ability. Some of these modifiers could originate from other minerals, which exist together with quartz in nature. Donaldson and Borm (1998) proposed that the hazard posed by quartz may vary widely depending on the origin of the silica sample or on its contact with other chemicals/minerals. Such mechanistic data could assist in the interpretation of epidemiological studies such as those above. Experimentally their group found that DQ12 quartz, a European quartz standard which is often used in experimental studies of silica effects, is much more inflammatory in rat lung than respirable silica collected from two workplaces (Clouter *et al*., 2001).

Humans appear to show adverse effects of silica exposure at lower levels than animals (compare LOAELs in Table 18 to LOAELs/NOAELs in Table 16). Rodents tend to be obligate nose-breathers and to have extensive nasal turbinates, which may result in less silica reaching the lower lung. For silica, results in animals may not be a good predictor of human effect levels.

**VI. Derivation of Chronic Reference Exposure Level (REL)**

| | |
|---|---|
| *Key study* | Hnizdo and Sluis-Cremer, 1993 |
| *Study population* | 2235 white South African gold miners |
| *Exposure method* | Workplace inhalation |
| *Critical effects* | Silicosis (313 miners) (14 %) |
| *LOAEL* | 3 mg/m$^3$-years CDE (9 miners with silicosis) |
| *NOAEL* | 2 mg/m$^3$-years CDE (0 miners with silicosis) or<br>600 μg/m$^3$-years silica (dust = 30% silica) |
| *$BMCL_{01}$* | 2.12 (mg/m$^3$)–yr CDE or 0.636 (mg/m$^3$)–yr silica |
| *Exposure continuity* | 8 h/day, 5 d/wk |
| *Exposure duration* | Average of 24 years dust exposure (10-39 years) |
| *Average experimental exposure* | 235 μg/m$^3$-yr silica at $BMC_{01}$<br>(636 x 10 m$^3$/20 m$^3$ x 270 shifts/365 days)<br>235 μg/m$^3$-yr/24 yr = 9.8 μg/m$^3$ |
| *Human Equivalent Concentration (HEC)* | 9.8 μg/m$^3$ |
| *LOAEL uncertainty factor* | Not needed in BMC approach |
| *Subchronic uncertainty factor* | 1 |
| *Interspecies uncertainty factor* | 1 |
| *Intraspecies uncertainty factor* | 3 |
| *Cumulative uncertainty factor* | 3 |
| *Inhalation Reference Exposure Level* | 3 μg/m$^3$ (based on 30% silica in mine dust)<br>[respirable, as defined occupationally by ACGIH/ISO] |

| | |
|---|---|
| *First supportive study* | Steenland and Brown, 1995 |
| *Study population* | 3330 S. Dakota gold miners |
| *Exposure method* | Workplace inhalation |
| *Critical effects* | Silicosis (170 miners) (5.1%) |
| *LOAEL* | 0-0.2 mg/m$^3$-years (5 miners with silicosis) |
| *NOAEL* | Not found |
| *$BMCL_{01}$* | 0.34 (mg/m$^3$)–yr (see text below) |
| *Exposure continuity* | 8 h/day, 5 d/wk |
| *Exposure duration* | 3-36 years (average 9 years underground) |
| *Average experimental exposure* | 112 μg/m$^3$-y<br>(340 x 10 m$^3$/20 m$^3$ x 5 d/7 d x 48 wk/52 wk)<br>112 μg/m$^3$-y/9 y = 12.4 μg/m$^3$ |
| *Human Equivalent Concentration (HEC)* | 12.4 μg/m$^3$ |
| *LOAEL uncertainty factor* | Not needed in BMC approach |
| *Subchronic uncertainty factor* | 1 |
| *Interspecies uncertainty factor* | 1 |
| *Intraspecies uncertainty factor* | 3 |
| *Cumulative uncertainty factor* | 3 |
| *Inhalation Reference Exposure Level* | 4 μg/m$^3$ [respirable, as defined occupationally] |

| | |
|---|---|
| *Second supportive study* | Hughes *et al*., 1998 |
| *Study population* | 1809 California diatomaceous earth workers |
| *Exposure method* | Workplace inhalation |
| *Critical effects* | Silicosis (81 workers) (4.5%) |
| *LOAEL* | > 1, ≤ 3 mg/m$^3$-years (17 workers with silicosis) |
| *NOAEL* | ≤ 1 mg/m$^3$-years (6 cases). (Six cases were observed, but Hughes *et al*. assigned the group a Relative Risk = 1 for silicosis.) |
| *Exposure continuity* | 8 h/day, 5 d/wk |
| *Exposure duration* | 1-45 years (mean = 11.5 years) |
| *Average experimental exposure* | ≤ 330 μg/m$^3$-y (1000 x 10/20 x 5/7 x 48/52)<br>≤ 330 μg/m$^3$-y/ 11.5years = ≤ 29 μg/m$^3$ |
| *Human Equivalent Concentration (HEC)* | 29 μg/m$^3$ |
| *LOAEL uncertainty factor* | 3 (authors' NOAEL actually is a LOAEL) |
| *Subchronic uncertainty factor* | 1 |
| *Interspecies uncertainty factor* | 1 |
| *Intraspecies uncertainty factor* | 3 |
| *Cumulative uncertainty factor* | 10 |
| *Inhalation Reference Exposure Level* | 3 μg/m$^3$ [respirable, as defined occupationally] |

| | |
|---|---|
| *Third supportive study* | Chen *et al*. (2001) |
| *Study population* | 3010 Chinese tin miners |
| *Exposure method* | Workplace inhalation |
| *Critical effects* | Silicosis (1015 workers) (33.7 %) |
| *LOAEL* | 10-19.99 mg CTD/m$^3$-years (24 cases) |
| *NOAEL* | ≤ 10 mg CTD/m$^3$-years (2 cases)<br>≤ 360 μg silica/m$^3$ - years |
| *BMCL$_{01}$* | 132 μg silica/m$^3$ - years |
| *Exposure continuity* | 8 h/day, 5 d/wk |
| *Exposure duration* | 2.2 years for NOAEL group |
| *Average experimental exposure* | 40 μg/m$^3$-y (132 x 10/20 x 5/7 x 48/52)<br>40 μg/m$^3$-y/2.2 years = 18 μg/m$^3$ |
| *Human Equivalent Concentration (HEC)* | 18 μg/m$^3$ |
| *LOAEL uncertainty factor* | Not needed in BMC approach |
| *Subchronic uncertainty factor* | 1 |
| *Interspecies uncertainty factor* | 1 |
| *Intraspecies uncertainty factor* | 3 |
| *Cumulative uncertainty factor* | 3 |
| *Inhalation Reference Exposure Level* | 6 μg/m$^3$ [respirable, as defined occupationally] |

| | |
|---|---|
| *Fourth supportive study* | Churchyard *et al*., 2004 |
| *Study population* | 510-520 black South African gold miners |
| *Exposure method* | Workplace inhalation |
| *Critical effects* | Silicosis (93 cases) |
| *LOAEL* | 0-0.80 mg/m$^3$-yr (11 cases) |
| *NOAEL* | Not identified |
| *$BMCL_{05}$* | 0.673 (mg/m$^3$)-yr |
| *Exposure continuity* | 270 shifts/year |
| *Exposure duration* | 21.8 yr (6.3-34.5) |
| *Average experimental exposure* | 249 (μg/m$^3$)-yr (673 x 10/20 x 270shifts/365)<br>249 (μg/m$^3$)-yr/21.8 yr= 11.4 μg/m$^3$ |
| *Human equivalent concentration (HEC)* | 11.4 μg/m$^3$ |
| *LOAEL uncertainty factor* | Not needed in BMC approach |
| *Subchronic uncertainty factor* | 1 |
| *Interspecies uncertainty factor* | 1 |
| *Intraspecies uncertainty factor* | 3 |
| *Cumulative uncertainty factor* | 3 |
| *Inhalation Reference Exposure Level* | 4 μg/m$^3$ [respirable, as defined occupationally] |

The study of 2235 white South African gold miners by Hnizdo and Sluis-Cremer (1993) not only determined a NOAEL of 2 (mg/m$^3$)-yr CDE (600 μg/m$^3$-yr silica), but also had sufficient dose-response data for a BMC derivation. This study was powerful enough to detect a 1.9% incidence of silicosis (9 cases out of 474 exposed) at 0.9 mg/m$^3$-yr silica (0/204 vs. 9/474, p = 0.064 by Fisher exact test, two-tailed). Because this incidence represents approximately the sensitivity limit of the data, and silicosis is a severe irreversible endpoint, the $BMCL_{01}$ (*i.e.*, the lower bound estimate of the concentration at which 1% of the population develops silicosis) was selected as the basis of the chronic REL. In benchmark analysis of chronic animal studies, $BMCL_{05}$ is typically regarded by OEHHA as equivalent to a NOAEL. However, the power of this large-scale study is sufficient to demonstrate measurable responses below the 5% incidence level (which cannot then be logically considered a no-effect level). Furthermore, the endpoint measured in this epidemiological study is considered to be severe, since it represents the occurrence of clinically recognizable and irreversible disease, rather than an adverse physiological or biochemical response or a histopathological result seen at autopsy.

Benchmark Concentration (BMC) models, developed by the USEPA (BMDS versions 1.3, 1.3.1, and 1.3.2), were fit to the human data in Hnizdo and Sluis-Cremer (1993) (Table 7 and Figure 2 above). Fitting the probit model to the log dose of the Hnizdo and Sluis-Cremer (1993) data yielded an $MLE_{01}$ of 2.45 (mg/m$^3$)–yr CDE and a $BMCL_{01}$ of 2.12 (mg/m$^3$)–yr CDE ($\chi^2$ = 0.64; p value for fit = 0.9957) (Figure 5, Table 19). (For comparison the $BMCL_{05}$ was 3.73 (mg/m$^3$)–yr CDE.) Fitting the logistic model to the same data yielded a $BMCL_{01}$ of 1.73 (mg/m$^3$)–yr CDE ($\chi^2$ = 2.71; p value for fit = 0.8446) (Table 19). The $BMCL_{01}$ from these data is about the same as the apparent NOAEL. In general, a BMC is preferred to a NOAEL because the BMC takes into account all the dose response data in a study. The apparent NOAEL may be either above or below an actual effect level, depending on the study design and distribution of the data.

**Figure 5. Probit model fit to the log dose of the Hnizdo and Sluis-Cremer data.**

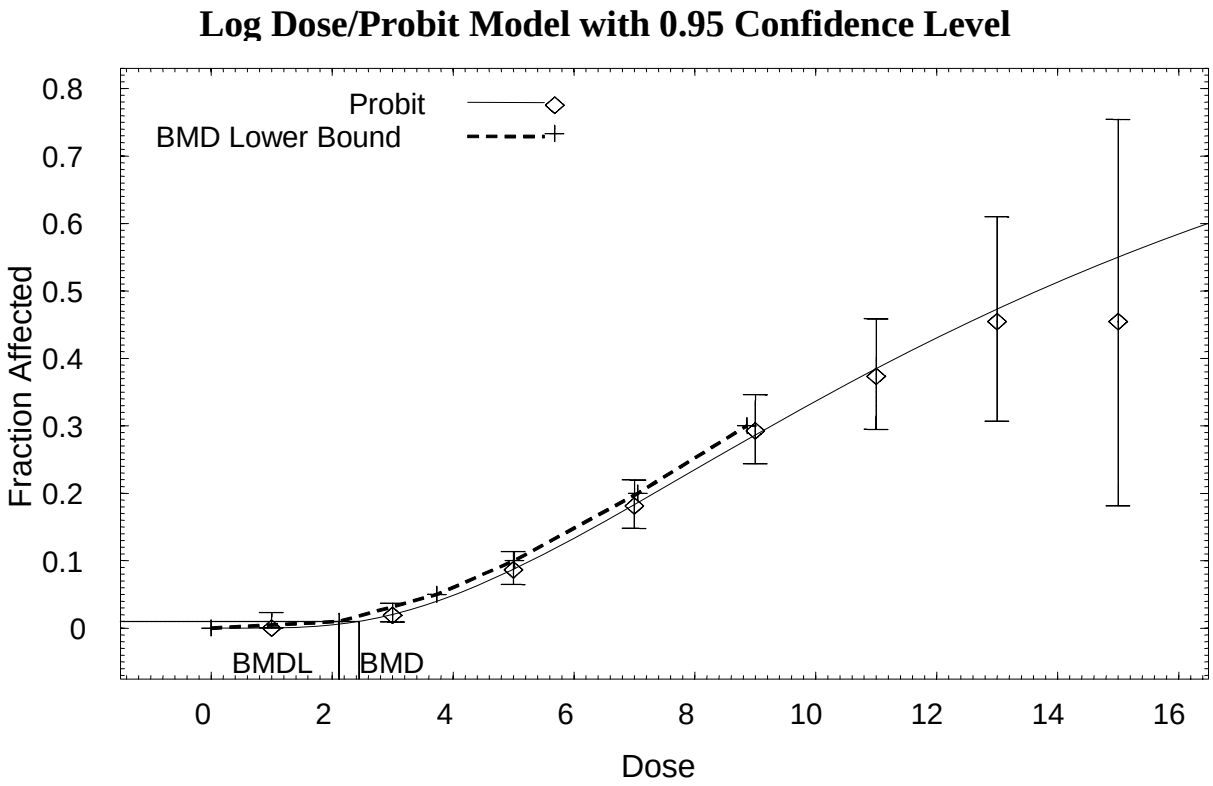



**Table 19. Fits of benchmark models to the Hnizdo and Sluis-Cremer (1993) data**

| *BMDS Model* | *$MLE_{01}$* | *$BMCL_{01}$* | *p value for fit* |
|---|---|---|---|
| Probit-log-dose | 2.45 (mg/m$^3$)–yr CDE | 2.12 (mg/m$^3$)–yr CDE | 0.9957 |
| Logistic-log-dose | 2.07 | 1.73 | 0.8446 |
| Multistage (n=2) | 2.47 | 1.89 | 0.7213 |
| Quantal-quadratic | 1.62 | 1.54 | 0.5017 |
| Probit | 1.56 | 1.32 | 0.0079 |
| Logistic | 1.48 | 1.28 | 0.0003 |
| Quantal-linear | 0.37 | 0.34 | 0.0000 |

For the estimate of 30% silica in the South African gold mine dust, Hnizdo and Sluis-Cremer (1993) relied on estimates for the years 1956-1960 by Beadle (Beadle and Bradley, 1970; Beadle, 1971). The original data, obtained by Corner House Laboratories for the South African Bureau of Mines, are partly presented by Beadle and Bradley (1970), but a more detailed presentation of exposures for various classes of workers is given by Page-Shipp and Harris

(1972). The latter paper also describes in some detail the methodology used to obtain the particle counts, and to convert those data into either respirable surface area or respirable mass values. Gibbs and Du Toit (2002) reviewed the data and methodology used by Hnizdo and Sluis-Cremer (1993) to estimate silica exposures of workers, which apparently depended on an unpublished analysis of the Corner House Laboratories' data done by Du Toit in 1991. Gibbs and Du Toit state that the exact relationship between the observed particle counts and theoretically derived mass concentrations cannot be determined, but that the uncertainties in this conversion do not appear to be severe for the dust characteristics observed in the South African mines. They accept the estimates by Beadle and Bradley (1970) of the quartz percentages in the dust, *i.e.* 54% for incinerated and acid-washed dust and 30% for unmodified dust.

However, Gibbs and Du Toit (2002) assert that Hnizdo and Sluis-Cremer (1993) incorrectly applied the 30% (total dust) silica content to figures for acid-treated dust in calculating the silica exposures of each occupational group. This contention is supported by the footnote to Table II in Hnizdo and Sluis-Cremer (1993) where the respirable dust concentration is described as "After heat and acid treatment". In order to clarify this point, OEHHA reviewed the independent reporting of the underlying data by Page-Shipp and Harris (1972). For most occupational groups, the silica exposures (shown in Table 20) calculated from Appendix I of Page-Shipp and Harris (1972), using the 54% silica content appropriate for acid-washed dust, correspond more closely to those calculated by Hnizdo and Sluis-Cremer (1993) (applying the 30% quartz content to their reported "respirable dust concentrations," i.e., the untreated dust), than to the modified, and higher, quartz exposures proposed by Gibbs and Du Toit (2002). For example, 113 exposure samples were taken for stopers.

**Table 20. Estimates of silica exposures in mg/m$^3$ for different occupational groups in South African gold mines.**

| *Occupation* | *Shaft Sinkers* | *Developers* | *Stopers* | *Assistant miners/ Trammers* | *Shift Bosses* | *Other Officials* | *Banks/ Skips* | *Workers Near shafts* | *Boiler-makers* | *Other Artisans* | *Miscellan-eous* |
|---|---|---|---|---|---|---|---|---|---|---|---|
| **Page-Shipp and Harris (1972) (Table III and Appendix I)** | | | | | | | | | | | |
| Hours/shift (t) | 7.70 | 8.00 | 7.80 | 7.70 | 5.20 | 4.00 | 7.50 | 6.50 | 6.30 | 5.70 | 7.20 |
| Number of samples | 10 | 37 | 113 | 157 | 43 | 106 | 33 | 34 | 41 | 61 | 11 |
| RM x t | 4.44 | 1.96 | 1.57 | 1.20 | 0.87 | 0.77 | 1.31 | 0.56 | 1.00 | 0.64 | 1.01 |
| s.d. | 3.94 | 1.59 | 1.00 | 0.93 | 0.71 | 0.53 | 1.38 | 0.57 | 0.71 | 0.51 | 0.79 |
| Respirable Mass (RM) | 0.58 | 0.25 | 0.20 | 0.16 | 0.17 | 0.19 | 0.17 | 0.09 | 0.16 | 0.11 | 0.14 |
| Silica (54%) | 0.31 | **0.13** | **0.11** | **0.08** | **0.09** | **0.10** | **0.09** | **0.05** | **0.09** | **0.06** | 0.08 |
| (after acid treatment) | | | | | | | | | | | |
| **Hnizdo and Sluis-Cremer (1993) (Table II)** | | | | | | | | | | | |
| RM | | 0.48 | 0.37 | 0.27 | 0.30 | 0.30 | 0.13 | 0.10 | 0.19 | 0.19 | |
| Silica (30%) | | **0.14*** | **0.11*** | **0.08*** | **0.09*** | **0.09*** | 0.04 | 0.03 | 0.06 | **0.06*** | |
| (before acid treatment) | | | | | | | | | | | |
| **Gibbs and Du Toit (2002) (Table 4)** | | | | | | | | | | | |
| RM | | 0.48 | 0.37 | 0.27 | 0.30 | 0.30 | 0.13 | 0.10 | 0.19 | 0.19 | |
| Silica (54%) | | 0.26 | 0.20 | 0.15 | 0.16 | 0.16 | **0.07*** | **0.05*** | **0.10*** | 0.10 | |
| (after acid treatment) | | | | | | | | | | | |

* denotes that value is equal to or closer to the value based on Page-Shipp and Harris

The last line of Appendix I of Page-Shipp and Harris (1972) gives a mean value for stopers of 1.57 (mg/m$^3$)-hours respirable dust mass after acid treatment. Since the average work shift for stopers was 7.8 hours (Page-Shipp and Harris, 1972, Table III, last row), the average exposure level was 0.20 mg/m$^3$. If 54% of this were quartz, the quartz level would be 0.11 mg/m$^3$. Table II of Hnizdo and Sluis-Cremer (1993) lists 0.37 mg/m$^3$ respirable dust for stopers. Thirty % of 0.37 mg/m$^3$ equals 0.11 mg/m$^3$, the same value reported by Page-Shipp and Harris. In Table 4 of Gibbs and Du Toit (2002) stopers are also reported to be exposed to 0.37 mg/m$^3$ respirable dust. If 54% were quartz, as Gibbs and Du Toit contend, the quartz level would be 0.2 mg/m$^3$. For 6 of the 9 categories of workers comprising 83% of the samples taken the silica levels correspond more closely to values used by Hnizdo and Sluis-Cremer than to those suggested by Gibbs and Du Toit.

Several more recent analyses of quartz content of South African mining rock have been reported (Table 21). Kielblock *et al*. (1997) give the overall silica content of the dust as 15% for the late 1980s to early 1990s. Dr. Eva Hnizdo (personal communication, 2003), now with the U.S. National Institute of Occupational Safety and Health (NIOSH), provided a summary of various other estimates that have been made. "Past surveys indicate that the amount of airborne respirable dust in SA gold mines in 1980's and in 1970's was on average around 0.4 mg/m$^3$ with average quartz concentration of 0.08 mg/m$^3$" (about 20%). In a Ph.D. thesis submitted by the late R.E.G. Rendall (1999) on dust in the air of gold mines, the silica percentage averaged 22% during the period from 1964 to 1988. In summary,

(1) Notwithstanding some apparent contradictions in the various accounts, the silica concentrations in air proposed by Hnizdo and Sluis-Cremer, based on the Corner House Laboratory data, are a reasonable contemporary estimate of the exposures experienced by the workers examined in the study by Hnizdo and Sluis-Cremer (1993).

(2) Other, more recent estimates of percent silica in the mine dust were lower than the value of 30% used by Hnizdo and Sluis-Cremer (1993). Newer studies, which using more sophisticated methods to measure silica in the dust, indicate lower silica concentrations in the various occupational settings. Since dust levels in the mines were fairly constant for decades and quantification of silica was improving, 30% is more likely to be an overestimate than an underestimate of silica levels.

(3) Analysis of the data of Page-Shipp and Harris (1972) by OEHHA staff indicated that Hnizdo and Sluis-Cremer (1993) used the correct silica content, despite an erroneous statement in a footnote to Table II of their paper[1].

[1] Dr. Eva Hnizdo reviewed this analysis of the silica content of the dust and agrees with the assessment. ("I am very pleased that you studied carefully all the reports and came to the conclusion that our study was after all reasonably correct. Based on the Churchyard study and the measurements data I have seen in SA during the 1990s, I am also convinced that our results are reasonable estimates of the exposure of the cohort." (Hnizdo, personal communication October 2004)

**Table 21. Estimates of respirable silica fraction of South African gold mine dust**

| *Authors* | *Time frame* | *% silica* | | *Number of samples* | *Methods* |
|---|---|---|---|---|---|
| Beadle and Bradley, 1970 | 1958-1967 | total dust: 25.7%; gravimetric: 28.5%; microscopy. acid-washed: 54% | | 142 grav; 143 elect ppt | gravimetric; precipitator + microscopy |
| Hnizdo and Sluis-Cremer (1993) | 1956-1960 | 30% | | | precipitator + microscopy |
| Rendall (unpublished thesis) | | | | | |
| Survey 1 | 1987-8 | 17% | | 588 | gravimetric |
| Survey 2 | 1977 | 20% | | 166 | gravimetric |
| Survey 3 | 1977 | 17% | | 90 | gravimetric |
| Survey 4a | 1964-7 | 22% | | 112 | gravimetric |
| Hnizdo (personal communication) | 1970-1989 | 20% | | | |
| Kielblock (1997) | ~1990 | 15.08% | | | Not stated |
| Churchyard (2004) | 2000-1 | 14.3% | | | Gravimetric + X-ray diffraction |

In the first supportive study Steenland and Brown (1995) found five cases of silicosis in the lowest dose group of 0 – 0.2 (mg/m$^3$)-yr and considered the group to be a LOAEL (Table 10 above). None of the BMDS models gave an acceptable fit at the $p \geq 0.05$ level using six or seven silica levels. The closest was the quantal quadratic model ($\chi^2 = 9.62$; $p = 0.0473$), which resulted in a $BMC_{01}$ for silica of 0.43 (mg/m$^3$)–yr using the six lowest levels of silica. In risk assessment, the highest dose or doses are often dropped in order to obtain an acceptable fit of the model to the data. This is reasonable with the benchmark approach since the highest doses should be least informative and the doses in the low dose region near the benchmark should be most informative for the benchmark concentration (USEPA, 1995; Filipsson *et al*., 2003). Fitting the probit model to the log dose of the five lowest silica levels from Steenland and Brown yielded a $BMCL_{01}$ of 0.34 (mg/m$^3$)–yr CDE ($\chi^2 = 1.32$; p value for fit = 0.5177). [For comparison, $BMCL_{05} = 0.85$ (mg/m$^3$)–yr CDE.] Fitting the quantal quadratic model gave a $BMCL_{01}$ of 0.45 (mg/m$^3$)–yr ($\chi^2 = 3.36$; $p = 0.3395$). Use of the $BMC_{01}$ value of 0.34 (mg/m$^3$)–yr CDE from the log dose probit model resulted in a chronic REL estimate for crystalline silica of 4 μg/m$^3$. Steenland and Brown stated that "silicosis has no background rate for non-exposed populations that changes with age or calendar time" and thus they assumed that the five silicotics in the 0 – 0.2 (mg/m$^3$)-yr were exposed to silica in the mines.

In a second supportive study, Hughes *et al*. (1998) found six cases of silicosis in the lowest exposure group of ≤ 1 mg/m$^3$-yr but considered that group to be a NOAEL, not a LOAEL. If the lowest exposure group is used as a NOAEL, a chronic REL of 10 μg/m$^3$ is calculated from the

data. Hughes *et al*. (1998) cite examples of possible non-occupational chest radiograph opacities (due, for example, to age or smoking) to explain the six cases in the lowest exposure group. However, due to the rarity of silicosis the six cases are biologically significant. OEHHA considers that the six cases may be work related, not cases of environmental or background silicosis. When a LOAEL to NOAEL UF of 3 is applied to the data of Hughes *et al*. (1998), the estimated REL is 3 $\mu g/m^3$.

In a third supportive study, Chen *et al*. (2001) found two cases of silicosis in the lowest exposure group of ≤ 10 mg CTD/$m^3$-years and considered that exposure level to be a NOAEL. One of the advantages of the benchmark dose analysis is that a NOAEL/LOAEL controversy, such as the one above with the Hughes *et al*. (1998) data, does not impact the procedure. The chart of the Chen *et al*. data above (Figure 4) indicates that the dose response is linear at low doses. Fitting the probit model to the log dose of the four lowest data points yielded a $BMC_{01}$ of 0.132 (mg/$m^3$) - yr CDE ($\chi^2$ = 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 (mg/$m^3$) - yr CDE ($\chi^2$ = 4.86; p value for fit = 0.0879). An inhalation chronic Reference Exposure Level for crystalline silica of 6 $\mu g/m^3$ was estimated from the Chen *et al*. data.

The fourth supportive study is that of black South African gold miners by Churchyard *et al*. (2003, 2004). A problem with this data set is the statistical "noise" in the lower exposure groups; e.g., the lowest exposure group has a higher incidence of silicosis (11/103) than the next group (8/97). This noise causes problems in estimating a low benchmark such as the $BMCL_{01}$ used with the Hnizdo and Sluis-Cremer (1993) data and with data from Steenland and Brown (1995) and Chen *et al*. (2001). The calculation therefore uses a 5% BMCL of 0.673 (mg/$m^3$)-yr from the probit log dose model as the benchmark, which is reasonably well within the range of the reliably observed data and which does not differ too widely from the $MLE_{05}$ estimate of 0.955 (mg/$m^3$)-yr for that parameter. This $BMCL_{05}$ is not strictly comparable to the $BMCL_{01}$ calculated from the data of Hnizdo and Sluis-Cremer, but the concerns about the severity of the effect noted in the discussion of that derivation apply with equal or greater force here.

On the other hand, the variability in the low-dose data in this study implies that an unobserved factor is affecting the data. All the models predict that the "background" is substantially (as much as 5 – 10%) above zero, which is intrinsically implausible for silicosis unless there is an unrecorded additional source of silica exposure. Possibly, there were occasional excursions in the exposure of the workers in less exposed jobs, which were not captured by the systematic assessments for these job classifications. Alternatively, perhaps the assignment of an assumed zero exposure value to "non-dusty" job classifications noted in the paper was in fact inaccurate for some individuals. There may also be some distortion of the curve resulting from the "binning" of the exposure categories; certainly, the bin widths hinder the attempt to calculate a $BMDL_{01}$ in this case. In relation to the model fit, other models (including the quantal linear model, which emphasizes the likely more reliable incidences at higher dose levels) are consistent with the $BMCL_{05}$ results from the log probit model used here. Finally, the total number of cases and controls examined is fewer than in Hnizdo and Sluis-Cremer's study, which reduces the precision.

None of these issues can be resolved without recourse to the individual data, which were not available for this analysis, and may not be resolved even then. However, the derivation of an HEC of 11.2 $\mu g/m^3$ (and thus a comparison REL of 4 $\mu g/m^3$) indicates at least that the results of the earlier analysis are unlikely to have underestimated the proper value of the REL. Although the uncertainties in the Churchyard *et al*. data prevent us from being more precise, we cannot eliminate the possibility that the REL should be set lower. However, the California ambient monitoring data, although subject to considerable uncertainty as to the relevant particle size distributions, suggest a plausible lower bound on the REL, which is consistent with our analysis of Hnizdo and Sluis-Cremer's data.

Other investigators have approached the possibility that some opacities on radiographs may be due to background influences such as age and smoking. In regard to smoking, Blanc and Gamsu (1988) reviewed the literature and concluded that smoking would not interfere with the determination of silicosis by the ILO system. Based on reading 1422 films of unexposed blue-collar workers, Castellan *et al*. (1985) stated that the use of the median result of 3 readers (the same number used by Hughes *et al*.) rarely results in interpreting a chest radiograph as ILO category $\geq$ 1/0 in workers who were not exposed to dust (and regardless of smoking status).

The USEPA (1996) did a benchmark analysis with the Hnizdo and Sluis-Cremer (1993) data. They estimated that the lower bound for a 1% risk for silicosis ($BMCL_{01}$) was 1.31 ($mg/m^3$)-yr, which by their methods is equivalent to a continuous, 70-year exposure to 6.7 $\mu g/m^3$ silica. However, USEPA did not do a formal Reference Concentration (RfC) derivation for silica by either the BMC/UF or NOAEL/UF approach.

The key (Hnizdo and Sluis-Cremer, 1993) and supporting (Steenland and Brown, 1995; Hughes *et al*., 1998; Chen *et al*., 2001) studies were of human adults, nearly all males, who were presumably healthy, at least initially, since they were able to work. Thus there is need to protect the sensitive members of the population, especially children, in whose airways penetration of silica particles will be greater (Phalen *et al*., 1985; Schiller-Scotland *et al*., 1994; Oldham *et al*., 1997; Bennett and Zeman, 1998). In addition, women may be more sensitive than men to the development of silicosis (Gerhardsson and Ahlmark, 1985; Katsnelson *et al*., 1986). The selection of three as the intraspecies uncertainty factor ($UF_H$) was based on several considerations.

(1) The workers who developed silicosis at low silica concentrations are by definition the most sensitive workers to silica-induced silicosis. Because of the large population of workers examined in these studies (more than 14,000), the sensitive individuals represent at least part of the range of sensitivity to be expected in the general population. This may justify reducing the $UF_H$ from the default value of 10. Since these workers did not include children, the elderly, or females (except for the 215 females in Chen *et al*.), some uncertainty related to inter-individual variability remains. Therefore, a $UF_H$ of 3 rather than 1 is chosen.

(2) Mukherji *et al*. (1993) reported mean ambient silica levels (in $PM_{10}$) at three locations in the northern part of Santa Barbara County, California (see the Appendix to this report). At Santa Maria (an urban site) the level was 2.3 $\mu g/m^3$; in Santa Ynez (a rural site) 0.6 $\mu g/m^3$; and in Buellton (a remote background site) 0.2 $\mu g/m^3$ crystalline silica. Thus, use

of a human intraspecies uncertainty factor ($UF_H$) of 10 with the data from the key study would result in an estimated chronic REL of 0.9 μg/m$^3$ (ACGIH method), a level in the range of ambient levels in California. Although the reported levels at the urban site may (according to the authors) have reflected some anthropogenic contributions such as disturbance and tracking of siliceous road dust, the rural and remote site values are apparently (perhaps conservatively) reflective of the natural background to which all California residents are exposed. (U.S. EPA (1996) found slightly higher average ambient levels of silica in $PM_{10}$; this average may include some sites affected by disturbance and emissions.) There is no evidence that these background levels of silica are causing silicosis. On the other hand, silicosis in the general population is not a target for medical attention, and autopsy rates are very low, so the possibility of a low frequency of response at these levels cannot be entirely dismissed. On balance, it appears plausible that a REL of 3 μg/m$^3$ (benchmark + $UF_H$ = 3) would be protective of the general population.

(3) The dose-response curve for silicosis due to inhalation of crystalline silica is steep, and an upward curvature of this dose response was seen in some studies (Figure 7-1 in USEPA, 1996). It is notable that, whereas exposures in the 1-3 μg/m$^3$ range are apparently without effect (based on the benchmark calculations and the California ambient background data), Rice and Stayner (1995) described a LOAEL for silicosis of 8 μg/m$^3$ in gold miners (Table 16; based on data from McDonald and Oakes [1984]). This finding may partly reflect differences in physical state of the silica, and co-exposures, but it might indicate that, although the chronic REL should be protective of public health, chronic exposures only moderately exceeding the REL may lead to clinically observable disease.

The animal studies gave LOAELs for silica of 0.2 mg/m$^3$ in dogs and from 1 to 4.9 mg/m$^3$ in rodents. After extrapolation to equivalent continuous time and application of LOAEL to NOAEL, interspecies, and intraspecies UFs, the estimated chronic RELs from animal data are all less than 1 μg/m$^3$. This reflects in part the greater uncertainty in extrapolating from animal studies to predicted human health effects.

The silica particles of concern in the causation of silicosis are those of respirable size. California EPA defines 'respirable' as particles 10 μm or less MMAD. This reflects one usual type of sampler (for "$PM_{10}$") used for ambient air sampling in the general environment. The other usual type of environmental sampler, $PM_{2.5}$, collects even smaller particles. There are differences in the size range distribution between a typical $PM_{10}$ measuring device and the NIOSH type personal samplers, or other devices with similar size selection properties, used by the investigators in the epidemiological studies. The NIOSH-type samplers capture 50% of particles with a MMAD of 4 μm, and higher percentages of smaller particles. A smaller proportion of larger particles between 4 and 10 μm in aerodynamic diameter will also be collected. Figure 5, from Volume I of U.S. EPA's Third External Review Draft of Air Quality Criteria for Particulate Matter (April 2002), includes particle penetration curves for $PM_{10}$, $PM_{2.5}$, and occupational samplers.

**Figure 5. Size cut curves of particle penetration**

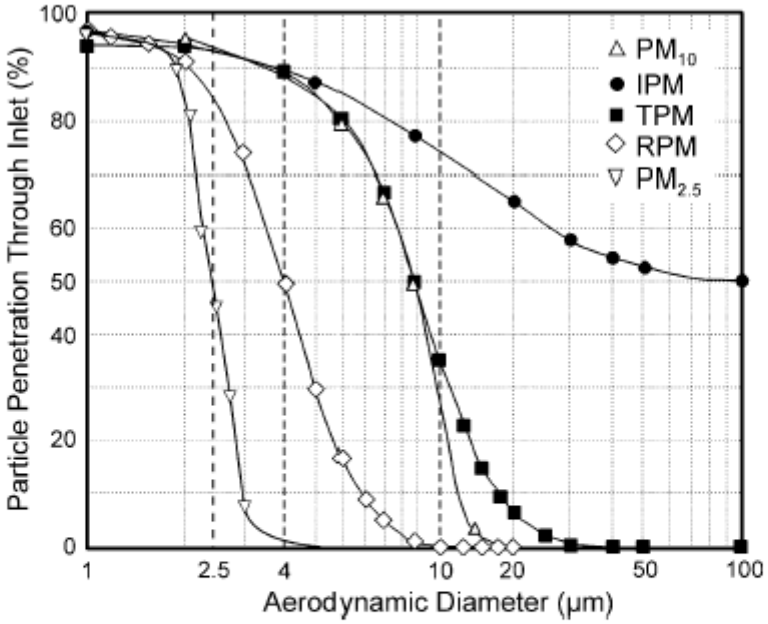



**Figure 2-6. Specified particle penetration (size-cut curves) through an ideal (no-particle-loss) inlet for five different size-selective sampling criteria. Regulatory size cuts are defined in the Code of Federal Regulations; $PM_{2.5}$ (2001c), $PM_{10}$ (2001a). $PM_{2.5}$ is also defined in the Federal Register (1997). Size-cut curves for inhalable particulate matter (IPM), thoracic particulate matter (TPM) and respirable particulate matter (RPM) size cuts are computed from definitions given by American Conference of Governmental and Industrial Hygienists (1994).**

The NIOSH samplers are designed to mimic the size range of particles that reach into the bronchiolar and alveolar spaces (what the occupational community calls respirable). $PM_{10}$ samplers are meant to capture particles that penetrate the entire length of the lower respiratory tree, including those that penetrate to the tracheobronchial and alveolar regions. Penetration (and therefore presumably deposition) by particle size is complex, and is dependent on the aerodynamic diameter, hygroscopicity, and electrostatic charge of the particles, and on a number of host factors including airway structure and geometry, as well as depth, rate, and mode of breathing (nasal vs. oronasal). The fractional penetration in the various regions of the respiratory tract is not linear with respect to size. Generally, though, larger particles impact higher in the respiratory tree (the extrathoracic and tracheobronchial regions), while smaller particles show greater penetration to the lower tracheobronchial and alveolar regions. There are a number of models of regional penetration and deposition in the respiratory tract, as well as some measurements. Chan and Lippmann (1980) showed peak alveolar deposition for particles about 3 μm MMAD with deposition dropping above and below that. Their data and model indicate that tracheobronchial deposition rises rapidly above about 3 μm MMAD. Available data also

indicate significant inter-individual variability in fractional deposition. The ICRP (1994) model used in evaluating risk from radioactive particles indicates that total deposition in the respiratory tract for particles 3 μm in activity median thermodynamic diameter (AMTD) is about 0.78 with a regional deposition fraction of 0.077 for the alveolar region for a reference male worker during nasal breathing. The same model predicts a total deposition in the respiratory tract of 0.77 for 10 μm AMTD particles and a deposition fraction of 0.024 in the alveolar region. Thus, many particles with a 10 μm MMAD get into the alveolar space. A smaller difference in regional penetration and deposition is predicted for mouth breathers. Therefore, if only the size range measured by the samplers used in the studies were considered, the measurement might underestimate the amount of silica that reaches the gas exchange regions of the lung, depending on the actual particle size distributions in the occupational studies and in the environments in which the REL is to be applied. Unfortunately, neither the occupational nor the environmental silica particle size distributions are known in detail; measurements have been reported only in terms of NIOSH sampler results or $PM_{10}$ cutoff values.

It is generally assumed that the silicosis is induced by that fraction of the silica that reaches the alveoli. Nevertheless, no actual data exonerate the coarser particles in the 4 - 10 μm range. A fraction of these particles can enter the bronchioles and alveoli. However, some data from South African gold mines indicate that more than 99% of the crystalline silica dust can be in the $PM_{2.5}$ fraction (Sichel, 1957). Thus, the samplers used in the key study appear to be collecting the biologically relevant range of particles in that situation.

In the absence of comprehensive data on the silicosis-inducing activity of different particle sizes, it is not possible to adjust the REL for different particle size distributions, which might be found in the general environment, or for different measurement methods. The REL is therefore specified as applicable to concentrations of particles having a size range (and reactivity) similar to those measured in the occupational studies [respirable as defined occupationally (ISO, 1995; NIOSH, 2003; ACGIH, 2004)]. Results obtained by other sampling methods would need to be corrected for any difference in size selectivity of the method used. Such a correction factor would be specific to the particle size distribution present at the site studied, so no general correction factors can be proposed. A more inclusive sampling procedure, such as that used for $PM_{10}$, would overestimate the relevant exposure in any situation, and so would be inappropriate for precise risk quantification. However, $PM_{10}$ would be useful as a screening method to establish that a particular situation is unlikely to present a hazard. For example, if the silica concentration in $PM_{10}$ modeled at a receptor is less than the REL (3 $\mu g/m^3$), occupationally respirable silica will also be less than 3 $\mu g/m^3$, so a facility would not pose a risk due to silica at that receptor. If the silica concentration in $PM_{2.5}$ modeled at a receptor is less than 3 $\mu g/m^3$ but $PM_{10}$ is greater than 3 $\mu g/m^3$, further testing would be needed. If both $PM_{2.5}$ and $PM_{10}$ exceeded the REL, the chronic Hazard Index would exceed 1 to an undetermined extent, suggesting a need for risk management. More precise determination of the amount of material in the respirable size fraction for environmental samples may require further work on measurement methodology, since ISO (1995) and similar occupational methods have not been validated for the lower levels encountered in environmental samples.

## VII. Data Strengths and Limitations for Development of the REL

The strengths of the inhalation REL for silica include:

(1) The availability of several long-term studies of inhalation in workers at varying exposure concentrations (see Summary Table 15 above), with adequate histopathological and radiological analysis, and with adequate follow-up.

(2) The finding of a dose-response effect for silicosis in several of the studies (e.g., Hnizdo and Sluis-Cremer, 1993; Steenland and Brown, 1995; Chen *et al*., 2001).

(3) The observation of a NOAEL in some studies including the key study (summarized by Rice and Stayner, 1995).

(4) The power of the Hnizdo and Sluis-Cremer (1993) data to detect a small effect.

Major areas of uncertainty are:

(5) The limited follow-up of the cohort members in some studies (e.g., Muir *et al*., 1989; Rosenman et al., 1996) with consequent under-ascertainment of silicosis (even to the extent that such studies are useless for determining exposure-response).

(6) The general underestimation of silicosis by radiography alone (Hnizdo *et al*., 1993), which results in higher, less health-protective chronic REL estimates.

(7) The possible underreporting of silicosis where complete radiographic data and autopsy data are not available (Steenland and Brown, 1995).

(8) The uncertainties in exposure estimation, especially when reconstructing historical levels of silica exposure (Seixas et al., 1997; Gibbs and Du Toit, 2002) including the variability in the estimates of percent quartz in the South African mine dust (Beadle, 1971; Hnizdo and Sluis-Cremer, 1993; Kielblock *et al*., 1997; Gibbs and Du Toit, 2002; Hnizdo, personal communication) and when converting particle counts to mass.

(9) The differences in percent silicosis in different studies at what were considered similar silica levels and similar exposure duration (see Summary Table 15 above).

(10) The variability in toxicity of various forms of silica (e.g., freshly fractured vs. aged quartz; cristobalite vs. quartz) although all forms have toxicity (Table 17).

(11) The limited information on silica particle size (including its variability) in the epidemiological studies, other than that the silica was respirable, and the variability in particle penetration and deposition as a function of particle size in the respiratory tract in the human population (e.g., Heyder *et al*., 1982; ICRP, 1994; Hattis *et al*., 2001).

(12) The use of area samplers rather than personal samplers to estimate exposure, which usually results in an underestimation of silica exposure (Cherrie, 1999).

## VIII. Potential for Differential Impacts on Children's Health

Silica is a respiratory irritant and a modifier of immune function. Since the key study involved over 2000 men, some were likely to be more sensitive to silica than others. In addition, we used a benchmark of 1% adverse effect, rather than the usual 5 %. Thus, use of the human intraspecies uncertainty factor ($UF_H$) of 3 should result in a REL that adequately protects most members of the general population. Exacerbation of asthma, which has a more severe impact on children than on adults, is a known response to some respiratory irritants. However, there is no data on such a response to silica in infants or children. The epidemiological studies used in the derivation of the REL did not include children. If children's susceptibility were much greater than that of adults, it would be expected that clinical disease would be evident in children following exposures in the upper range of the respirable silica levels measured in ambient air in California. No such reports have been identified in the literature. There are no data on silica's effects on the immune system of children.

OEHHA is currently evaluating its risk assessment methodology, in particular the $UF_H$, for its adequacy in protecting infants and children. Since children have smaller airways than adults and breathe more air on a body weight basis, penetration and deposition of particles in the airways and alveoli in children is likely greater than that in adults exposed to the same concentration (Phalen *et al*., 1985; Schiller-Scotland *et al*., 1994; Oldham *et al*., 1997; Bennett and Zeman, 1998).

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## X. Appendix

*Particulate Levels of Interest for Exposure to Respirable Crystalline Silica Isomorphs*

| | |
|---|---|
| 150 μg/m$^3$ | Federal 24 hour $PM_{10}$ standard |
| 65 μg/m$^3$ | Federal 24 hour $PM_{2.5}$ standard |
| 50 μg/m$^3$ | California 24 hour $PM_{10}$ standard |
| 50 μg/m$^3$ | Federal $PM_{10}$ annual standard (chronic exposure) |
| 50 μg/m$^3$ | 8 hour TLV for quartz, cristobalite, and tridymite for workers (ACGIH Method) |
| 50 μg/m$^3$ | estimated workplace LOAEL for silicosis from studies by Theriault *et al*. (“) |
| 20 μg/m$^3$ | CA annual $PM_{10}$ standard (chronic exposure) (arithmetic mean) |
| 15 μg/m$^3$ | Federal annual $PM_{2.5}$ standard (chronic exposure) |
| 12 μg/m$^3$ | CA annual $PM_{2.5}$ standard (chronic exposure) (arithmetic mean) |
| 12 μg/m$^3$ | current silica TLV adjusted to equivalent continuous exposure (50 μg/m$^3$ x 8 h/24 h x 5 d/7d) (ACGIH) |
| 10 μg/m$^3$ | TLV for silica proposed by Greaves (2000) (ACGIH) |
| 8 μg/m$^3$ | current silica TLV further adjusted by 46/70 years occupational exposure (ACGIH) |
| 8 μg/m$^3$ | estimated high-end ambient crystalline silica level in US (USEPA, 1996) ($PM_{10}$) |
| 6.7 μg/m$^3$ | lower bound on 1% risk of silicosis estimated by USEPA (1996) ($PM_{10}$) |
| 5 μg/m$^3$ | TLV for silica proposed by Chen *et al*. (2001) (ACGIH) |
| 5 μg/m$^3$ | “acceptable” ambient level for silica (10% of $PM_{10}$) (USEPA, 1996) |
| 5 μg/m$^3$ | RfC for diesel exhaust particulate, a respirable PM ($PM_{2.5}$) |
| 3 μg/m$^3$ | estimated average ambient exposure to crystalline silica (USEPA, 1996) ($PM_{10}$) |
| 3 μg/m$^3$ | draft silica chronic REL proposed by OEHHA (ACGIH) |
| 2.3 μg/m$^3$ (1.17-3.46; n=12)* | silica level during 1989 in Santa Maria, CA (urban site) ($PM_{10}$) |
| 0.6 μg/m$^3$ (0-1.44; n=16)* | silica level during 1989 in Santa Ynez, CA (rural site) (“) |
| 0.2 μg/m$^3$ (0-1.15; n=18)* | silica level during 1989 in Buellton, CA (remote background)(“) |

* mean, range, and number of crystalline silica measurements (Mukherji *et al*., 1993)

*CHRONIC TOXICITY SUMMARY*

# STYRENE

*(ethenylbenzene, phenylethylene, vinylbenzene)*

**CAS Registry Number: 100-42-5**

## I. Chronic Toxicity Summary

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

## II. Chemical Property Summary

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

## III. Major Uses and Sources

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

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

## IV. Effects of Human Exposure

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

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

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

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

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

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

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

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

Female workers employed in the reinforced plastics industry (RPI) were studied for levels of substances associated with neuroendocrine function (Mutti *et al.*, 1984a**).** Serum prolactin, thyroid stimulating hormone, human growth hormone, follicle stimulating hormone, and luteinizing hormone were measured in 30 women who were between the 5th and 15th day of the menstrual cycle. Exposure was based on the next-morning MA+PGA, and levels of the neuroendocrine substances were measured in venous blood samples taken the next morning before the start of work. On the basis of a relationship (not detailed in the report) between urinary metabolites and styrene air concentration, the authors estimated that the average styrene TWA/8 hr was about 130 ppm. Controls consisted of women factory workers living in the same area as the styrene-exposed women, but not knowingly exposed to styrene. After controlling for age and exposure time, the increased prolactin and thyroid stimulating hormone levels were correlated with the concentration of next-morning urinary MA+PGA, although only the increased prolactin levels were statistically significant.Numerous occupational studies have noted CNS disturbances in styrene-exposed workers. Decreased manual dexterity, increased reaction times, and/or abnormal vestibuloocular reflex (ability to track moving objects) were observed by Gotell *et al*. (1972), Gamberale *et al*. (1975), Lindstrom *et al*. (1976), Mackay and Kelman (1986), Flodin *et al.* (1989), Moller *et al*. (1990), and Cherry and Gautrin (1990) for air

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

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

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

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

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

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

[a] Data from Table IV in Mutti *et al.* (1984b).
[b] "Next-morning" styrene urinary metabolites.
[c] The quantal grouping of the number of subjects that performed abnormally in ≥3 tests based on their styrene urinary metabolite concentrations, both shown in bold, were used in a benchmark concentration (BMC) analysis for the derivation of the REL (see Section VI below).

[d] Based on Guillemin *et al.* (1982), a linear relationship exists for converting the urinary metabolite concentrations to ppm air styrene levels (4.97 mmoles MA+PGA/mole creatinine is equivalent to 1 ppm styrene). Thus, the mean styrene concentrations per group are 0, 15, 44, 74,

and 115 ppm.In addition to dyschromatopsia observed by Chia *et al*. (1994), Gobba and Cavalleri (1993) and Campagna *et al*. (1995) also reported this visual dysfunction among styrene workers in the reinforced plastics industry. Workers (n=36) exposed to an average of 16 ppm styrene exhibited significantly greater dyschromatopsia than controls, matched for age, ethanol consumption and tobacco smoking (Gobba and Cavalleri, 1993). Among the study population, only 1/36 styrene-exposed workers (compared to 16/36 controls) performed the test with 100 percent accuracy. When a different group of styrene-exposed workers was tested, those exposed to > 50 ppm styrene exhibited greater dyschromatopsia than those exposed to ≤ 50 ppm, and within this group, a subset exhibited a similar decrement after returning from a one month vacation. In the Campagna *et al.* (1995) study, the test for dyschromatopsia was given to 81 reinforced plastics industry workers (79 male and 2 female) exposed to 4.6, 10.1, and 88.8 ppm styrene (first quartile, median, and third quartile, respectively). No control group was used in this study. Statistical analysis revealed a correlation of color vision loss with exposure to styrene (defined as next-morning urinary mandelic acid), age, and ethanol consumption.

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

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

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

Immune system alterations were reported in a study conducted by Bergamaschi *et al.* (1995). Reinforced plastics industry workers (n=32 female/39 male, average age = 32 years, average exposure duration = 7 years) were compared with non-styrene exposed factory workers and matched for age, sex, tobacco use and ethanol consumption. Air styrene levels, among the different factories, varied between 10 - 50 ppm, and individual worker exposure was measured

by urinary metabolites the morning after the last shift (15 hours post-exposure). Among all workers in the study (median exposure = 16 ppm - according to the data of Guillemin *et al.* (1982)), the proportion of 12/18 lymphocyte subsets and the prevalence of abnormal values of immunologic phenotypes for 11/18 subsets were statistically different from the controls ($p < 0.001$ to $< 0.05$). When the workers were placed into three exposure groups (0, < 25 ppm, and > 25 ppm styrene), dose-response relationships were observed for prevalences of abnormal responses for four lymphocyte subsets and, in the case of two subsets, abnormal responses were observed in the group exposed to < 25 ppm styrene. Natural killer cell activity (a lymphocyte function), measured in a different group of workers in the same study, was decreased compared to unexposed worker controls. The median exposure, given in terms of urinary metabolites, was calculated as 21 ppm based on the data of Guillemin *et al.* (1982). The data show that exposure of these workers to air styrene levels below 50 ppm, and probably at levels near 25 ppm, resulted in alterations of the immune system.

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

## V. Effects of Animal Exposure

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

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

Kishi *et al.* (1995) carried out a developmental study on rat pups born to dams exposed by inhalation to styrene (0, 50, 300 ppm; 6-hr/d; gestation days 7-21). Although the small number of litters (n=2) at the 50 ppm dose prevented detailed statistical analysis, the data suggest that exposure of the dams to 50 ppm styrene resulted in deficits and delays in some motor and coordination abilities among the pups. Pups born to dams exposed to 300 ppm exhibited statistically significant increases in spontaneous activity and in the delay of some neurobehavioral functions. Many of the effects became diminished as the pups aged. Measurements of reproductive toxicity (maternal weight gain, length of gestation, number of live

births) did not change. Postnatal body weights were lower among the styrene-exposed pups, but the differences became less as the pups aged to 125-days.

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

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

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

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

Groups of 70 male and 70 female CD-1 mice were exposed in whole body inhalation chambers to styene vapor concentrations of 0, 20, 40, 80, and 160 ppm 6 hrs/day, 5 days/week, over a period of up to 2 years (Huntingdon Life Sciences, 1998). Ten mice per sex per group were

necropsied after 52 and 78 weeks of exposure, and the remaining survivors necropsied after 104 weeks. Due to increased mortality in female control mice, terminal sacrifice for this group occurred at 98 weeks. Two female mice exposed to 160 ppm styrene died during or immediately following the first week of exposure. Histopathology revealed liver necrosis that was a likely contributor to the deaths. Reduced body weight gain and increased food consumption were observed in male mice at the two highest exposure levels and in female mice at the highest exposure level. Both styrene monomer and styrene oxide in blood increased with exposure concentration. No changes of toxicologic significance in hematology, ophthalmology, clinical chemistry, urinalysis, or organ weights were noted. Styrene-related non-neoplastic histopathologic changes were seen in the lungs (bronchiolar-alveolar hyperplasia) and nasal olfactory epithelium (respiratory metaplasia, degeneration or necrosis, and changes to the underlying Bowman's glands) from all exposure groups. The nasal lesions showed progression with time. Focal loss of bone from the turbinate was also seen more frequently as the study progressed. In addition, atrophy of the olfactory nerve fibers was present in mice at the three highest exposure concentrations.

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

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

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

next-morning urinary MA+PGA is directly related to the air level of styrene. The Guillemin *et al.* (1982) study provides the basis for the conversion of urinary MA+PGA levels to styrene exposure levels used by Mutti *et al.* (1984b).

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

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

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

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

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

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

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

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

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

## VII. Data Strengths and Limitations for Development of the REL

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

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

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

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*CHRONIC TOXICITY SUMMARY*

# SULFURIC ACID

*(dithionic acid; pyrosulphuric acid)*

**CAS Registry Number: 7664-93-9**

## II. I. Chronic Toxicity Summary

| | |
|---|---|
| *Inhalation reference exposure level* | **1 µg/m$^3$** |
| *Critical effect(s)* | Bronchiolar epithelial hyperplasia, and thickening of the bronchial walls in monkeys |
| *Hazard index target(s)* | Respiratory system |

## III. Physical and Chemical Properties (HSDB, 1995; CRC, 1994; CARB, 1997)

| | |
|---|---|
| *Description* | Colorless liquid |
| *Molecular formula* | $H_2SO_4$ |
| **Molecular weight** | 98.1 g/mol |
| *Density* | 1.84 g/cm$^3$ @ 15° C |
| *Boiling point* | 330±0.5°C (100%) |
| *Melting point* | 10.36°C (100%) |
| *Vapor pressure* | <0.001 torr @ 25° C; 1 torr @ 145.8° C |
| *Solubility* | Soluble in water |
| *Conversion factor* | Not applicable |

## III. Major Uses or Sources

Sulfuric acid is a strong acid used as an intermediate in the synthesis of linear alkylbenzene sulfonation surfactants used in dyes, in petroleum refining, for the nitration of explosives, in the manufacture of nitrocellulose, in caprolactam manufacturing, as the electrolyte in lead-acid batteries, and as a drying agent for chlorine and nitric acid. Sulfuric acid is formed in the atmosphere from sulfur dioxide, from sulfur trioxide, and from oleum (a combination of sulfur trioxide and sulfuric acid used industrially). The annual statewide industrial emissions from facilities reporting under the Air Toxics Hot Spots Act in California based on the most recent inventory were estimated to be 4460 pounds of sulfuric acid (CARB, 1999).

## IV. Effects of Human Exposures

Workers in the lead battery industry showed etching and erosion of the teeth after 4 months exposure to an average concentration of 0.23 mg/m$^3$ $H_2SO_4$ (Gamble *et al.*, 1984). Dental erosion increased in a dose-dependent manner with longer duration of exposure.

A study of 33 storage battery plant workers exposed to $H_2SO_4$ concentrations as high as 35 mg/m$^3$ showed a greater group mean decrease in $FEV_1$ across the time of their work shift compared to workers who were not exposed to sulfuric acid (El-Saddik *et al.*, 1972). The salivary pH of the sulfuric acid exposed workers, a qualitative measure of acid exposure, was lower than the controls during the course of the work shift.

OEHHA recently reviewed the California Ambient Air Quality Standard (CAAQS) for sulfates (25 μg/m$^3$ for 24 hours) to see if it adequately protects children (OEHHA, 2000). The report was peer-reviewed by the Air Quality Advisory Committee. The report indicates that H+ itself may play a role in the effects seen in epidemiological studies of sulfate air pollution. Controlled acute inhalation studies in humans and laboratory animals of pH neutral or nearly neutral sulfate salts (e.g., ammonium sulfate) (Utell et al., 1983; Lippman et al., 1987; Schlesinger et al., 1990), even at relatively high concentrations, do not produce the effects reported from epidemiologic studies of sulfates (asthma exacerbation, bronchoconstriction, decrements in lung function) that might be expected from short-term excursions. The controlled exposure studies show that sulfate aerosols containing strong acids, such as sulfuric acid and, to a lesser extent, ammonium bisulfate, produce functional and structural changes in healthy subjects consistent with those observed in epidemiological studies. A working hypothesis is that $H^+$ is a causal factor for adverse human health effects (e.g., see Lippmann and Thurston, 1996) and that, among the commonly measured particulate matter (PM) indices, $SO_4^=$ is the best surrogate metric for $H^+$.

A large number of epidemiologic studies have been conducted showing that elevated levels of several air pollutants, including acid aerosols, sulfur and nitrogen oxides, and particulate sulfates are correlated with an increased prevalence of pulmonary disease (U.S. EPA, 1989; OEHHA 2000). Elevated sulfate levels (1.6 ppb or 6.6 μg/m$^3$) have been associated with statistically significant decrements in FVC and $FEV_1$ in a cohort of Canadian children (Stern *et al.*, 1989). Further analysis of these data led Bates and Sitzo (1989) to conclude that $H_2SO_4$ was the most likely cause for the pulmonary changes observed. Similarly, Ostro *et al.* (1989) reported a statistical association between asthma-related symptoms reported by 209 asthmatics and sulfate and acidity levels in ambient air in Denver. Delfino et al. (1997) found that ambient H+ was associated with emergency room visits by children for respiratory symptoms in a study in Montreal. Additionally, Damokosh et al. (1993) in a follow-up analysis of the 6-City study suggested associations between average H+ concentration and chronic bronchitic symptoms. The relative odds of bronchitic symptoms with the highest acid concentration (58 nmoles/m$^3$ H+)_ versus the lowest concentration (16 nmoles/m$^3$) was 2.4 (95% CI:1.9 to 3.2). Furthermore in a study of children in 24 U.S. and Canadian communities (Dockery et al., 1996) in which the analysis was adjusted for the effects of gender, age, parental asthma, parental education, and parental allergies, bronchitic symptoms were confirmed to be significantly associated with strongly acidic PM (OR= 1.66; 95% CI 1.11-2.48). It was also found that FVC and $FEV_1$ were lower in locales with high particle acidity (Raizenne et al., 1996). Gwynn et al. (2000) reported an association between both H+ and sulfate particles and respiratory hospital admissions and mortality in Buffalo, NY. Acidic sulfates may act to increase the toxicity of particles by enhancing the availability of metals present in the particles to generate reactive oxygen species in the respiratory epithelium. This may account for some of the effects seen in these epidemiological studies and makes it difficult to use these studies as a basis for a Reference Exposure Level for sulfuric acid. The relationship between the effect levels observed in these

studies and the proposed REL is discussed in the section below on the potential for differential impacts on children's health.

The occupational standard for sulfuric acid is based on a study in human subjects by Amdur *et al*. (1952). In their study, 22 healthy male subjects were exposed to 0, 0.35, 0.4, 0.5, 1, 2, or 5 $mg/m^3$ for 5-15 minutes. The odor, taste, and irritation threshold was 1 $mg/m^3$. Since the basis for this standard is an acute exposure, it is not useful in determining a chronic non-cancer REL for sulfuric acid. A review of chronic human exposures to sulfuric acid and resulting carcinogenicity outcomes can be found in IARC (1992). However, none of the studies in that review examined non-cancer endpoints.

Sulfuric acid and oleum (supersaturated anhydrous sulfuric acid with varying concentrations of free sulfur trioxide) are absorbed as salts of sulfate anion ($SO_4^{2-}$), and are excreted as organic sulfates, neutral sulfur, or neutral sulfur compounds such as sulfur-containing amino acids. The low systemic toxicity of these metabolites is likely of secondary importance to the irritation caused by the inhaled acid.

## V. Effects of Animal Exposures

An exposure of 9 cynomolgus monkeys per group to $H_2SO_4$ concentrations of 0, 0.38, 0.48, 2.43, and 4.79 $mg/m^3$ continuously for 78 weeks resulted in dose-dependent adverse histological changes in lung and bronchiolar epithelial and parenchymal tissue in addition to a dose-dependent decrease in blood oxygenation (Alarie *et al.*, 1973). In the animals exposed to 0.38 $mg/m^3$, significant bronchiolar epithelial hyperplasia was observed in 5 of 9 animals; thickening of the bronchiolar walls was observed in 3 of 9 animals. A slight focal bronchial epithelial hyperplasia was present in 4 of the 9 animals. One animal died after 4 weeks exposure to 0.38 $mg/m^3$. Although signs of pulmonary edema and cardiac hypertrophy were found, the cause of death was not determined.

**Respiratory system effects of $H_2SO_4$ exposure in monkeys (Alarie *et al*., 1973)**

| *$H_2SO_4$ ($g/m^3$)* | *Particle size MMD* | *Bronchiolar epithelial hyperplasia Incidence – severity* | *Thickening of walls of respiratory bronchioles Incidence – severity* | *Increase in thickness of alveolar walls Incidence – severity* |
|---|---|---|---|---|
| 0 | | 0/9 | 0/9 | 0/9 |
| 0.38 | 2.15 | 5/8 – slight | 3/8 - slight | 0/8 |
| 0.48 | 0.54 | 0/8 | 0/8 | 0/8 |
| 2.43 | 3.60 | 8/8 – moderate | 8/8 – moderate | 8/8 – moderate |
| 4.79 | 0.73 | 8/8 – moderate to severe | 8/8 – moderate to severe | 0/8 |

Alarie *et al.* (1973) also exposed groups of 50 guinea pigs of each sex to 0, 0.08, or 0.1 $mg/m^3$ $H_2SO_4$ continuously for 52 weeks. The group exposed to 0.1 $mg/m^3$ also received larger sized particulates than the 0.08 $mg/m^3$ group (2.78 μm vs. 0.84 μm, respectively). No exposure related effects were observed in the animals exposed to 0.08 $mg/m^3$, whereas exposure of 0.1 $mg/m^3$ resulted in decreased body weights in the female guinea pigs. No other histological changes in any organs were observed at the end of the 52-week study.

Rabbits (4 per group) were exposed to 250 μg/m$^3$ $H_2SO_4$ 1 hour/day, 5 days/week for 4, 8, or 12 months. They showed significantly increased bronchoconstriction upon acetylcholine challenge after 8 and 12 months exposure, compared with a control group of 4 animals that received no $H_2SO_4$ (Gearhart and Schlesinger, 1986, 1988). Mucociliary clearance was also impaired by $H_2SO_4$ exposure and did not improve 3 months after cessation of exposure. A decline in dynamic lung compliance was observed after 12 months exposure. There was no evidence of inflammatory cell infiltration in the lungs of the exposed animals.

In guinea pigs, significantly slower and irregular breathing patterns were noted when the animals had inhaled albumin followed by 30 minute exposures to $H_2SO_4$ at 1.91 mg/m$^3$ twice per week for 5 weeks (Kitabatake *et al.*, 1979). Similarly, when guinea pigs were exposed to 2.49 mg $H_2SO_4$/m$^3$ for 4 hours/day, 6 days/week for 4 weeks, *in vitro* lung histamine release was significantly enhanced following heterogeneous albumin inhalation, compared to control animals unexposed to albumin (Fujisawa *et al.*, 1986; Iguchi *et al.*, 1986). In guinea pigs, sulfuric acid caused significantly greater lung function changes when adsorbed on the surface of zinc oxide particles as compared with pure sulfuric acid (Amdur and Chen, 1989). An exposure to 24 μg/m$^3$ sulfuric acid, layered on zinc oxide, produced significant reductions in lung function when followed by a brief exposure to 0.15 ppm ozone (Chen *et al.*, 1991).

A chronic exposure of beagle dogs to an average concentration of 889 μg/m$^3$ $H_2SO_4$ for 21 hours/day over a 620 day period resulted in increased expiratory resistance, reduced carbon monoxide diffusing capacity, reduced total and residual lung volume, and decreased lung and heart weights (Lewis *et al.*, 1973).

In apparent contrast to the above studies, rats and guinea pigs exposed to $H_2SO_4$ at 10 mg/m$^3$ for 6 hours/day, 5 days/week for 6 months exhibited no adverse histologic changes in lung tissue. Lung function measurements were not reported in this study (Cavender *et al*., 1978).

Mice inhaled sulfuric acid mist at a concentration of 1.4 mg/m$^3$ in combination with a carbon particle mixture (1.5 mg/m$^3$) for 3 hours/day, 5 days/week for up to 20 weeks. The exposure resulted in significant alterations in specific antibody titer (decreased IgG, $Ig_{2a}$, IgM; increased $IgG_{2b}$), depression of primary splenic antibody response, and decreased resistance to respiratory infection as measured by mortality and survival time compared to controls (Fenters *et al.*, 1979).

There are no reliable studies indicating that sulfuric acid is a developmental or reproductive toxicant. In the absence of massive overexposure leading to maternal acidemia, $H_2SO_4$ will be neutralized in the maternal circulation and is unlikely to reach the fetus.

## VI. Derivation of Chronic Reference Exposure Level

| | |
|---|---|
| *Study* | Alarie *et al.*, 1973 |
| *Study population* | Cynomolgus monkeys (5 males and 4 females per group or vice versa) |
| *Exposure method* | Continuous inhalation exposures (0, 380, 480, 2400, or 4800 μg/m$^3$) for 78 weeks |
| *Critical effects* | Significantly increased bronchial epithelial hyperplasia and bronchial thickening |
| *LOAEL* | 380 μg/m$^3$ |
| *NOAEL* | Not observed |
| *Exposure continuity* | The exposure was continuous during the experiment. |
| *Exposure duration* | 78 weeks |
| *Average experimental exposure* | 380 μg/m$^3$ for the LOAEL group |
| *Human equivalent concentration* | 380 μg/m$^3$ |
| *LOAEL uncertainty factor* | 3 (slight effects) |
| *Subchronic uncertainty factor* | 3 |
| *Interspecies uncertainty factor* | 3 (non-human primate) |
| *Intraspecies uncertainty factor* | 10 |
| *Cumulative uncertainty factor* | 300 |
| *Reference exposure level* | 1 μg/m$^3$ |

The study by Alarie *et al.* (1973) identified a LOAEL for chronic exposure to sulfuric acid of 380 μg/m$^3$. The principal uncertainties of this study are the small sample size of the test groups and the absence of an observed NOAEL. A lower chronic LOAEL for bronchial reactivity is presented by Gearhart and Schlesinger (1986, 1988) for rabbits (250 μg/m$^3$). This study was not selected as the basis of the REL because Gearhart and Schlesinger used only a single concentration of sulfuric acid, exposed the animals only for 1 hour per day for 5 days/week, used only 4 animals per group, and measured effects over the course of up to 12 months. The predominant weakness in the rabbit study, however, was the extreme discontinuity of the exposures (1 hour/day, 5 days/week), which would have necessitated use of a very large continuity adjustment. For these reasons, in addition to obvious physiological and genetic similarity arguments, the study in monkeys by Alarie *et al.* (1973) was felt to be more appropriate as the basis for the chronic REL for sulfuric acid. Alarie *et al*. (1975) determined a NOAEL for sulfuric acid in monkeys of 0.1 mg/m$^3$. However, other particulate matter (fly ash) was also present during the exposure. The Alarie *et al*. (1973) report provides data from exposure to sulfuric acid alone.

A free-standing NOAEL for histological changes in 100 guinea pigs exposed continuously for 1 year to 0.08 mg/m$^3$ was reported by Alarie *et al.* (1973). Guinea pigs respond to high concentrations of sulfuric acid by occasional laryngeal spasms that appear similar to a human asthmatic attack (Silbaugh *et al.*, 1981; Amdur and Chen, 1989). As a result, guinea pigs are thought to be sensitive models for the acute effects of sulfuric acid. For chronic effects of sulfuric acid on the lung, monkeys are likely a suitable model due to their physiological and structural similarites to humans.

For comparison, a chronic REL based on the guinea pig free-standing NOAEL of 0.08 mg/m$^3$ in animals exposed continuously for one year (Alarie *et al*., 1973) would be 0.8 μg/m$^3$.

## VII. Data Strengths and Limitations for Development of the REL

The major strength of the study on sulfuric acid is the use of health effects observations from continuous long-term exposures to a primate. The major weaknesses are the lack of adequate human health effects data and the lack of a NOAEL observation.

## VIII. Potential for Differential Impacts on Children's Health

There are no reliable studies indicating that sulfuric acid is a developmental or reproductive toxicant. Children are likely to be at greater risk from long-term exposures because their bodies are growing, and their developmental processes, especially in the lung, may well be impacted by air pollution exposures. Elevated sulfate levels (1.6 ppb or 6.6 μg/m$^3$) have been associated with statistically significant decrements in FVC and FEV1 in a cohort of Canadian children (Stern et al., 1989). The chronic REL for sulfuric acid of 1 μg/m$^3$ is below the level associated with those decrements in pulmonary function. However, in a study of moderately to severe asthmatic children (ages 7-13) (Thurston et al., 1997), a sensitive subpopulation for sulfate effects, approximately 1 μg/m3 was the lowest level of ambient sulfate measured. The mean daily morning to afternoon peak airflow change, the use of beta-agonist medication, and the number of chest symptoms versus sulfate concentration in these children extrapolated linearly down to 1 μg/m3. Thurston et al. (1997) also examined earlier data from Ontario (Burnett et al., 1994) on respiratory admissions to hospitals, and concluded that the sulfate threshold of effects, if it exists, lies below 5 μg/m$^3$, perhaps at about 2 μg/m$^3$. It should be noted that the sulfate and hydrogen ion effects are difficult to disentangle from each other and from the effcts of other PM constituents. The chronic REL of 1 μg/m$^3$ appears to have a relatively low margin of safety with respect to the epidemiological studies, but these observations are consistent with the proposed REL of 1 μg/m$^3$ since asthmatic children appear to be the critically sensitive human population for exposure to sulfuric acid (or sulfate).

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*CHRONIC TOXICITY SUMMARY*

# TOLUENE

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

**CAS Registry Number: 108-88-3**

I. Chronic Toxicity Summary

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

## II. Physical and Chemical Properties (HSDB (1999) except as noted)

| | |
|---|---|
| *Description* | Colorless liquid |
| *Molecular formula* | $C_7H_8$ |
| *Molecular weight* | 92.13 g/mol |
| *Density* | 0.8661 g/cm$^3$ @ 20°C |
| *Boiling point* | 110.6 ° C (CRC, 1994) |
| *Melting point* | –94.9° C (CRC, 1994) |
| *Vapor pressure* | 28.1 torr @ 25°C (U.S. EPA, 1984) |
| *Solubility* | miscible in most organic solvents |
| *Conversion factor* | 1 ppm = 3.76 mg/m$^3$ @ 25°C |

## III. Major Uses or Sources

Toluene occurs naturally as a component of crude oil and is produced in petroleum refining and coke oven operations; toluene is a major aromatic constituent of gasoline (HSDB, 1999). It is used in household aerosols, nail polish, paints and paint thinners, lacquers, rust inhibitor, adhesives and solvent based cleaning agents. Toluene is also utilized in printing operations, leather tanning and chemical processes. Benzene and other polycyclic aromatic hydrocarbons are common contaminants of toluene. Toluene is considered a sentinel chemical for benzene in the context of air and water sample monitoring. In 1996, the latest year tabulated, the statewide mean outdoor monitored concentration of toluene was approximately 2.2 ppb. For 1998, annual statewide industrial emissions of toluene from facilities reporting under the requirements of the Air Toxics Hot Spots Act in California were estimated to be 5,176,626 pounds (CARB, 1999). Note that this estimate is for stationary sources, and does not include emissions from mobile sources.

## IV. Effects of Human Exposures

*Neurological Effects*
Most studies reporting adverse effects due to chronic toluene exposures involve either toluene-containing solvent abuse or occupational exposure to toluene. Solvent abusers are generally exposed to higher levels of toluene than are workers. A continuum of neurotoxic effects ranging from frank brain damage to degraded performance on psychometric tests which roughly track exposure levels has been observed.

Solvent abusers
Chronic toluene abuse has been shown to cause permanent changes in brain structure (loss of grey and white matter differentiation; cerebral, cerebellar and brainstem atrophy) which correlated with brain dysfunction as measured by magnetic resonance imaging (MRI) and brainstem auditory evoked response (BAER) evaluations (Caldemeyer *et al.*, 1996; Filley *et al.*, 1990; Ikeda and Tsukagoshi, 1990; Rosenberg *et al.*, 1988a; Rosenberg *et al.*, 1988b; Yamanouchi *et al.*, 1995; reviewed by Agency for Toxic Substances and Disease Registry (ATSDR), 1999).

Eleven chronic solvent (spray lacquer; ≈ 60% toluene, 10% dichloromethane) abusers were examined using MRI and BAER tests (Rosenberg *et al.*, 1988b). Neurological abnormalities were seen in four of 11 subjects and included brainstem, cerebellar, cognitive and pyramidal findings. Brain MRIs were abnormal in three of 11 subjects and indicated the occurrence of diffuse cerebral, cerebellar, and brainstem atrophy and loss of differentiation between the gray and white matter throughout the CNS. BAERs were abnormal in five of 11 individuals. All three individuals with abnormal MRI scans also had abnormal neurological examinations and BAERs. However, two of five individuals with abnormal BAERs had normal neurological examinations and MRI scans. The authors suggested that BAERs may detect early CNS injury from toluene inhalation, even at a time when neurological examination and MRI scans are normal.

Two subjects of a group of 22 hospitalized solvent abusers (primarily abusing toluene-based solvents) demonstrated decreases in intelligence quotient (IQ) as measured by the comparison of tests administered before the commencement of solvent abuse with tests administered during hospitalization for long-term solvent abuse (Byrne *et al.*, 1991).

Filley *et al.* (1990) studied 14 chronic toluene abusers using MRI and neuropsychological evaluations. The neuropsychological testing indicated that three patients functioned normally, three were in a borderline range, and eight were impaired. Independent analyses of white matter changes on MRI demonstrated that the degree of white matter abnormality was strongly correlated ($p < 0.01$) with neuropsychological impairment. The authors concluded that dementia in toluene abuse appears to be related to the severity of cerebral white matter involvement.

Six chronic toluene abusers were examined using MRI by Caldemeyer *et al.* (1996). All patients examined demonstrated white matter atrophy and T2 hyperintensity (T2: “Spin-spin” relaxation time; a time constant that reflects the rate at which protons stop rotating in phase with each other because of the local magnetic fields of adjacent nuclei; OTA, 1984), and five of six demonstrated

T2 hypointensity of the basal ganglia and thalami. The authors noted a correlation between the severity of white matter degeneration and degree of neurological dysfunction. However, there was no correlation between the severity of imaged white matter changes and the presence of T2 hypointensity or duration of toluene abuse. Additionally, no definite clinical evidence of damage to the basal ganglia and thalami was found despite the MR imaging finding of T2 hypointensity..

Ungar *et al*. (1994) developed a physical bilayered model of dipalmitoylphosphatidylcholine (DPPC) and toluene, and subjected DPPC control and toluene-mixed bilayers to MRI. T1 (T1: "Spin-lattice" relaxation time; a time constant that reflects the rate at which excited protons exchange energy with the surrounding environment; OTA, 1984) and T2 were measured as a function of toluene and lipid concentrations. Measurements of the DPPC-toluene model indicated that toluene-containing lipid bilayers substantially shortened T2 and had little effect on T1. By comparison, DPPC alone had little effect on either T1 or T2. The authors believe that these results suggest that partitioning of toluene into the lipid membranes of cells in cerebral tissue may be responsible for the hypointensity of basal ganglia noted on T2-weighted MR images of brains of toluene abusers.

Occupational exposure

Solvent workers exposed to 42.8 ppm toluene (estimated as a time-weighted average) for an average duration of 6.8 years reported a significantly greater incidence of sore throat, dizziness and headache than controls; the sore throat and headache incidence demonstrated a rough dose-response (Yin *et al.*, 1987).

Orbaek and Nise (1989) examined the neurological effects of toluene on 30 rotogravure printers, 33-61 years of age (mean 50), employed at two Swedish printing shops for 4-43 years (median 29) in 1985. Mean exposure levels at the two printing shops were 43 and 157 mg/m$^3$ of toluene, respectively; however, before 1980 the exposure levels had exceeded 300 mg/m$^3$ in both shops. The authors noted that rotogravure printing provides an occupational setting with practically pure toluene exposure. Comparisons were made to a reference group of 72 men aged 27-69 (mean 47). The alcohol consumption of both the workers and referents was also determined (< 200 g/week or > 200 g/week). Neurological function in the workers and referents was evaluated using interviews and psychometric testing; the results from each of the two printing shops were pooled. The printers reported statistically significantly higher occurrences of fatigue (60%), recent short-term memory problems (60%), concentration difficulties (40%), mood lability (27%), and other neurasthenic symptoms. The printers also scored significantly worse than referents in a number of psychometric tests, including synonym, Benton revised visual retention and digit symbol tests, even after adjustment for age. For all comparisons, tests of interaction between the effects of toluene exposure and alcohol consumption were not statistically significant.

A battery of neurobehavioral tests was performed in 30 female workers exposed to toluene vapors in an electronic assembly plant (Foo *et al.*, 1990). The average number of years worked was 5.7 ± 3.2 for the exposed group and 2.5 ± 2.7 years for the controls. Study subjects did not smoke tobacco or drink alcohol, were not taking any medications, and had no prior history of central or peripheral nervous system illness or psychiatric disorders. The exposed group of workers inhaled a time-weighted average (TWA) of 88 ppm (330 mg/m$^3$) toluene while the

control workers inhaled 13 ppm (49 mg/m$^3$). A significant decrease in neurobehavioral performance was observed in the exposed workers in 6 out of 8 tests. Irritant effects were not examined, and concurrent exposures to other chemicals were not addressed. In this study, 88 ppm was considered a LOAEL for central nervous system effects. However, the workers designated by the authors to be controls did not comprise a true control group, since they were exposed to 13 ppm toluene. This may have resulted in an underestimation of the effects of exposure to 88 ppm toluene. Similar effects were noted in a follow-up study by Boey *et al.* (1997).

Abbate *et al.* (1993) evaluated alterations induced in the auditory nervous system by exposure to toluene in a group of rotogravure workers. A sample of 40 workers of normal hearing ability was selected from a group of 300 workers who were apparently in good health but were professionally exposed to toluene (12 – 14 years exposure, 97 ppm average exposure, exposure assessment not described). They were subjected to an adaptation test utilizing a BAER technique with 11 and 90 stimulus repetitions a second. The results were compared with an age and sex-matched control group not professionally exposed to solvents. A statistically significant alteration in the BAER results was noted in the toluene-exposed workers with both 11 and 90 stimuli repetitions. The authors suggested that these results can be explained as a toluene-induced effect on physiologic stimulus conduction mechanisms, even in the absence of any clinical sign of neuropathy. Furthermore, this effect could be observed in the responses of the entire auditory system, from peripheral receptors to brainstem nuclei.

A group of 49 printing-press workers occupationally exposed to toluene for approximately 21.6 years was studied by Vrca *et al.* (1997). Toluene exposure levels were determined from blood toluene and urinary hippuric acid levels, and were estimated to range from 40-60 ppm. No control group was used. Brain evoked auditory potential (BEAP; similar to BAER) and visual evoked potential (VEP) measurements were performed on a Monday morning after a nonworking weekend. There was a significant increase in the latencies of all the BEAP waves examined, except for P2 waves, as well as in the interpeak latency (IPL) P3-P4, while IPL P4-P5 decreased significantly with the length of exposure. No correlation was noted between the amplitude of BEAP waves and the length of exposure. The amplitude but not the latency of all the VEPs examined decreased significantly with the length of exposure.

The effects of acute and chronic toluene exposure on color vision were studied in a group of eight rotogravure printing workers (Muttray *et al.*, 1999). The workers had been employed as printers for an average of 9.8 years. The color vision acuity of the workers before and after an acute toluene exposure (28 – 41 minutes in duration, concentration 1115 – 1358 mg/m$^3$) was evaluated using the Farnsworth panel D-15 test, the Lanthony desaturated panel D-15 test, and the Standard Pseudoisochromatic Plates part 2. A control group of 8 unexposed workers was also tested. Acute toluene exposure had no effect on color vision. Print worker performance prior to acute toluene exposure (chronic effects) was similar to controls on the Farnsworth panel D-15 and Standard Pseudoisochromatic Plates part 2 tests. Print worker performance on the Lanthony desaturated panel D-15 test was worse than that of controls (median scores of 1.18 and 1.05 for exposed and controls (higher number indicates degraded performance), respectively, but not significantly ($p = 0.06$). The authors noted that the small number of subjects limited the statistical power of the study.

Zavalic *et al.* (1998) examined the effects of chronic occupational toluene exposure on color vision using a group of 45 exposed workers (mean toluene exposure concentration = 120 ppm) and 53 controls. Color vision was evaluated using the Lanthony desaturated panel D-15 test; test scores were age and alcohol consumption-adjusted. Color vision was significantly impaired in toluene-exposed workers ($p < 0.0001$) compared to controls. It was also observed that there was no significant difference between test scores on Monday morning (prework) and Wednesday morning. The authors stated that the effect of toluene on color vision can be chronic and that the possible recovery period is longer than 64 hours.

*Hepatic Effects*

Greenburg *et al.* (1942) reported liver enlargement in 32 of 106 (30.2%) painters employed in an aircraft factory compared to 7% in a control group. However, there was some exposure to other solvents (ethanol, ethyl acetate, butyl acetate) and paint ingredients such as zinc chromate.

Liver toxicity has been reported in toluene solvent abusers (Fornazzari *et al.*, 1983). Eight of 24 solvent abusers demonstrated abnormal results in three liver function tests; however, the tests used were not specified. The test parameters returned to normal after two weeks of toluene abstinence, suggesting that any liver damage caused by toluene abuse in those patients was not long lasting.

A cross-sectional study by Boewer *et al.* (1988) showed no significant correlation between toluene exposure and the levels of serum enzymes (serum aspartate aminotransferase (ASAT), alanine aminotransferase (ALAT), γ-glutamyltransferase (GGT)) considered to be indicators of hepatic damage. In another cross-sectional study of 289 printing workers exposed to less than 200 ppm for 8 hours/day, 8 workers had significantly elevated serum enzymes (ALT/AST ratio, mean = 1.61) potentially indicative of liver damage. In each case, liver biopsy indicated a mild pericentral fatty change (Guzelian *et al.*, 1988). However, the mean toluene exposure concentration was not reported (only an upper bound), and no control group was included in the study.

## V. Effects of Animal Exposures

*Neurotoxic Effects*

Sprague-Dawley rats (15/sex/group) were exposed to 0, 100, or 1481 ppm toluene for 6 hours/day, 5 days/week for 26 weeks (API, 1981). Neurohistopathological examinations were conducted in 3-5 rats/sex/group at weeks 9, 18, and 27. No significant treatment-related effects were reported. The study usefulness was limited because there were no other neurohistopathological examinations or organ weight measurements conducted on the animals.

Forkman *et al.* (1991) studied the potential neurotoxicity of toluene inhalation exposure (3700 $mg/m^3$ (1000 ppm), 21 hours/day, 5 days/week for 4 weeks) in male Sprague-Dawley rats. The rats were either trained in behavior meant to be performance tested and then exposed to toluene, or exposed and then trained. The rats were then subjected to several behavioral tests, including an operant test with baseline performance and extinction, motor coordination, and exploratory

activity. All tests were performed from 11 to 35 days after the end of the exposure. Exposure of trained rats to toluene resulted in a significantly different overall test performance when compared to controls. Rats trained after toluene exposure also had test performances different from controls, but the difference was not statistically significant.

Rats exposed to toluene concentrations of 1000 ppm or 100 ppm, 6 h/day, 5 days/week, for 3 or 6 months, respectively, demonstrated statistically significant decreased motor function as measured by degraded performance (approximately 60% and 65% of control at 1000 and 100 ppm toluene, respectively) on a rotarod performance test and decreases in spontaneous motor activity (approximately 62% of control at 100 ppm toluene) (Korsak *et al*., 1992).

von Euler *et al.* (1993) studied the effects of subchronic toluene inhalation exposure (80 ppm, 4 weeks, 5 days/week, 6 hours/day) on spatial learning and memory, dopamine-mediated locomotor activity and dopamine $D_2$ agonist binding in rats. Spatial learning (postexposure days 3-6) and memory (postexposure day 14) were tested using a water maze. Spontaneous and apomorphine-induced locomotor activity was evaluated on postexposure day 17. Effects on binding parameters of the dopamine D2 agonist S(-)[N-propyl- $^3$H(N)]-propylnorapomorphine ([H]NPA) were determined using membrane preparations of the neostriatum of the rat brain. Toluene exposure caused a statistically significant impairment in spatial learning and memory. Toluene also significantly increased apomorphine-induced locomotion and motility but not rearing. Spontaneous locomotion, motility and rearing were not affected by toluene. Toluene exposure significantly increased the $B_{max}$ and $K_D$ values for [$^3$H]NPA binding. These results indicate that subchronic toluene exposure of rats to toluene causes persistent deficits in spatial learning and memory, a persistent increase in dopamine-mediated locomotor activity and an increase in the number of dopamine $D_2$ receptors in the neostriatum.

Male rat exposure to toluene (0, 40, 80, 160 or 320 ppm, 4 weeks, 6 hours/day, 5 days/week) , followed by a postexposure period of 29-40 days, resulted in decreased brain wet weights of the caudate-putamen (trend test for dose-response significant at $p < 0.05$) and subcortical limbic areas (trend test for dose-response significant at $p < 0.01$; significantly less than controls ($p < 0.001$) at concentrations of 80 ppm and higher) (Hillefors-Berglund *et al.*, 1995). Toluene exposure also significantly altered dopamine receptor activity (trend test for dose-response) as indicated by decreased $IC_{50}$ (inhibition constant) (significantly less than controls ($p < 0.05$) at 80 ppm), $K_H$ (inhibition constant for high-affinity receptor sites), $K_L$ (inhibition constant for low-affinity receptor sites), and $R_H$% (high-affinity receptor site specific binding) values for dopamine competitive inhibition of [$^3$H]raclopride-binding in the caudate-putamen. Toluene exposure did not significantly affect the wet weights of the whole brain, serum prolactin levels, the $K_D$ (disassociation constant) or the $B_{max}$ (maximal specific binding) values of [$^3$H]raclopride-binding in the caudate-putamen and the subcortical limbic area, or the effect of dopamine on $IC_{50}$ values at [$^3$H]raclopride-binding sites in the subcortical limbic area. Exposure to xylene or styrene (80 and 40 ppm, respectively; 4 weeks, 6 h/day, 5 days/week) followed by a postexposure period of 26-32 days had no effect on the parameters described above. The authors concluded that long-term exposure to low concentrations of toluene (≥ 80 ppm), but not xylene (80 ppm) or styrene (40 ppm), leads to persistent increases in the affinity of dopamine $D_2$ agonist binding in the rat caudate-putamen. The authors also suggested that the enhancement of

apomorphine-induced locomotor activity seen after toluene exposure by von Euler *et al.* (1993) may be related to the increased $D_2$ agonist activity described above ($IC_{50}$, $K_H$, $K_L$ values).

*Respiratory Effects*
A study of the chronic effects of toluene in rats (5-20 animals per group) exposed for 106 weeks to 0, 30, 100, or 300 ppm (0, 113, 375, or 1125 mg/m$^3$) toluene showed no treatment-related effects on histopathology of major organs, including the nasal turbinates (CIIT, 1980). In this study, the nasal histopathology examination sampling may have been inadequate to demonstrate the nasal lesions reported by the NTP (1990).

Rats (20 per group) exposed for 2 years to 0, 600, or 1200 ppm (0, 2261, or 4523 mg/m$^3$) toluene 6.5 hours/day, 5 days/week for 103 weeks were examined for hematological and histopathological effects in addition to gross observations of toxicity (NTP, 1990). Significant erosion of the olfactory epithelium was observed in male rats while degeneration of the respiratory and nasal epithelium was observed in both sexes at 600 ppm.

Mice were exposed chronically to 0, 120, 600, or 1200 ppm (0, 452, 2261, or 4523 mg/m$^3$) toluene 6.5 hours/day, 5 days/week, for 2 years (NTP, 1990). The only treatment-related effect was a significant increase in the number of animals with hyperplasia of the bronchial epithelium in the 1200 ppm exposure group.

*Reproductive and Developmental Toxicity*
Reproductive toxicity to maternal rats was observed during exposure to 1500 ppm toluene, 24 hours/day on days 9 to 14 of gestation (Hudak and Ungvary, 1978). Two dams out of 19 died during exposure. Fetuses from the 1500 ppm group showed increased incidence of sternebral alterations, extra ribs and missing tails. The same exposure on days 1 through 8 of gestation resulted in 5 deaths out of 14 dams. Fetuses in this regimen showed increased incidence of hydrocephaly and growth retardation compared to controls. A third regimen that exposed maternal rats to 1000 ppm on days 1 through 21 of gestation resulted in no maternal deaths or toxicity, and an increase in the incidence of skeletal variations in the fetuses. When exposed to 1500 ppm continuously, maternal mice died within 24 hours of exposure whereas exposure to 500 ppm had no apparent effect. Examination of the fetal mice showed significant growth retardation in the 500 ppm group.

A 2-generation study of the effects of 0, 100, 500, or 2000 ppm (0, 377, 1885, or 7538 mg/m$^3$) toluene in rats (males, 10-40 per group; females, 20-80 per group) was done by the American Petroleum Institute (API)(1985). Rats were exposed for 6 hours/day, 7 days/week for 80 days and a 15 day mating period. The mated females were then exposed to the same concentrations during days 1-20 of gestation and days 5-20 of lactation. After weaning, the $F_1$ pups were exposed 80 times to the appropriate exposure level and then randomly mated to members of the same exposure group. The $F_1$ generation showed significantly decreased body weight which persisted throughout lactation. No effects were observed on histopathology. No data were presented for the $F_2$ generation.

Da Silva *et al.* (1990) exposed rats and hamsters to 0 or 800 mg/m$^3$ toluene for 6 hours/day on gestation days 14-20 (rats), or days 6-11 (hamsters). Exposed rats demonstrated a significant

exposure-related decrease in birth weight compared with controls. In addition to low birth weight, the number of live pups was significantly lower in the 800 ppm group. No deficits in any parameter were noted in the hamsters. In this study, no neurobehavioral effects were noted in the offspring.

Hass *et al*. (1999) exposed rats to 0 or 1200 ppm toluene for 6 h per day from day 7 of pregnancy until day 18 postnatally. Developmental and neurobehavioral effects in the offspring were investigated using a test battery including assessment of functions similar to those in the proposed Organization for Economic Cooperation and Development (OECD) Testing Guidelines for Developmental Neurotoxicity Study (physical development, reflex development, motor function, motor activity, sensory function, and learning and memory). The exposure did not cause maternal toxicity or decreased offspring viability. However, lower birth weight, delayed development of reflexes, and increased motor activity in the open field was noted in the exposed offspring. The exposed female offspring had poorer scores on a Morris water maze test (they took longer to locate a hidden platform after platform relocation) at the age of 3.5 months indicating impaired cognitive function. The difference was not related to impaired swimming capabilities since swim speeds were similar to control values. The authors stated that exposure to 1200 ppm toluene during brain development caused long-lasting developmental neurotoxicity in rats.

Toluene has been listed under Proposition 65 as being known to the State of California to cause reproductive toxicity (OEHHA, 1999). Its NSRL is 7,000 micrograms per day.

## VI. Derivation of Chronic Reference Exposure Level (REL)

| | |
|---|---|
| **Study** | Hillefors-Berglund *et al*. (1995); supported by Orbaek and Nise (1989), Foo *et al.* (1990) |
| *Study population* | Male Sprague-Dawley rats |
| *Exposure method* | Inhalation |
| *Critical effects* | Decreased brain (subcortical limbic area) weight, altered dopamine receptor (caudate-putamen) binding |
| *LOAEL* | 80 ppm |
| *NOAEL* | 40 ppm |
| *Exposure continuity* | 6 hours/day, 5 days/week |
| *Exposure duration* | 4 weeks, followed by 29-40 days recovery |
| *Average experimental exposure* | 7 ppm (40 × 6/24 hours × 5/7 days) |
| *Human equivalent concentration* | 7 ppm (gas with systemic effects, based on RGDR = 1.0 using default assumption that $\lambda_a = \lambda_h$ |
| *Subchronic uncertainty factor* | 10 |
| *Interspecies uncertainty factor* | 1 (see below) |
| *Intraspecies uncertainty factor* | 10 |
| *Cumulative uncertainty factor* | 100 |
| *Inhalation reference exposure level* | 0.07 ppm (70 ppb; 0.3 mg/m$^3$; 300 µg/m$^3$) |

| | |
|---|---|
| *Supportive human study* | Foo *et al.*, 1990 |
| *Study population* | 30 female workers in an electronic assembly plant |
| *Exposure method* | Occupational inhalation |
| *Critical effects* | Neurobehavioral deficits in 6 out of 8 tests |
| *LOAEL* | 88 ppm |
| *NOAEL* | Not observed |
| *Exposure continuity* | 10 m$^3$/day occupational inhalation rate, 5 days/week |
| *Average occupational exposure* | 31.4 ppm (88 ppm x 10/20 x 5/7) |
| *Exposure duration* | 5.7 $\pm$ 3.2 years (exposed group); 2.5 $\pm$ 2.7 years (controls) |
| *LOAEL uncertainty factor* | 10 |
| *Subchronic uncertainty factor* | 3 |
| *Interspecies uncertainty factor* | 1 |
| *Intraspecies uncertainty factor* | 10 |
| *Cumulative uncertainty factor* | 300 |
| *Inhalation reference exposure level* | 0.1 ppm (100 ppb; 0.4 mg/m$^3$; 400 µg/m$^3$) |

The critical animal study (Hillefors-Berglund *et al*., 1995) used to derive an REL for toluene describes adverse neurological effects in rats after a well characterized inhalation exposure to toluene. The study results contain both a LOAEL and a NOAEL. Decreased brain (subcortical limbic area) weight and altered dopamine receptor binding compared to controls were noted at the NOAEL, but the changes were not statistically significant; this suggests that if a threshold for

adverse neurological effects exists in this study, it would be at or below the observed NOAEL. The study LOAEL for altered dopamine receptor binding agrees qualitatively with results from similar studies (von Euler *et al.*, 1994). Additionally, toluene-induced neurotoxicity has been described in many studies by a variety of endpoints in both animals and humans (ATSDR, 1999). The adverse neurotoxic effects associated with toluene exposure in the rat study by Hillefors-Berglund *et al*. (1995), decreased brain (subcortical limbic area) weight and altered dopamine receptor binding, occur in areas of the rat brain that are structurally and functionally similar to brain areas (basal ganglia, thalami) of some human toluene abusers that demonstrate MRI alterations (T2 hypointensity). The altered MRI parameters may be the result of the partitioning of toluene into the lipid membranes of brain cells (Ungar *et al*., 1994). Table 1 lists several Reference Exposure Levels (RELs) calculated from the most sensitive animal and human neurotoxicity studies available. These RELs are also protective for other adverse endpoints, such as respiratory tract damage and teratogenicity.

Table 1: Reference Exposure Levels (RELs) from Selected Neurotoxicity Studies

| Study | Duration | Effect | LOAEL (ppm) | LOAEL (ppm) (TWA) | NOAEL (ppm) | NOAEL (ppm) (TWA) | total UF[a] | REL (ppb) | REL (µg/m$^3$) |
|---|---|---|---|---|---|---|---|---|---|
| VonEuler *et al*. (1988) | 4 weeks | rat: altered brain dopamine receptor binding | 80 | 14.3 | | | 1000 | 14 | 54 |
| Orbaek and Nise[b] (1989) | 29 years | human: impairment on neuropsychometric tests | 11.2 - 41 | 4 - 14.6 | | | 100 | 40 - 146 | 150 - 551 |
| Foo (1990) | 5.7 years | human: neurobehavioral tests | 88 | 31.4 | | | 300 | 105 | 394 |
| Korsak (1992) | 6 months | rat: impaired motor function | 100 | 17.9 | | | 100 | 179 | 671 |
| Hillefors-Berglund (1995) | 4 weeks | rat: decreased brain (subcortical limbic area) weight; altered brain dopamine receptor binding | 80 | 14.3 | 40 | 7.1 | 100 | 71 | 271 |

LOAEL: Lowest Observable Effect Level; NOAEL: No Observable Effect Level
REL: Reference Exposure Levels; TWA: time-weighted average

a: Uncertainty Factors used to derive RELs

| | |
|---|---|
| VonEuler *et al*. (1988) | LOAEL to NOAEL UF = 10, subchronic to chronic UF = 10, animal to human UF = 1, intraspecies variability = 10; total UF = 1000. |
| Orbaek and Nise (1989) | LOAEL to NOAEL UF = 10, intraspecies variability = 10; total UF = 100 |
| Foo *et al*. (1990) | LOAEL to NOAEL UF = 10, subchronic to chronic UF = 3, intraspecies variability = 10; total UF = 300 |
| Korsak *et al*. (1992) | LOAEL to NOAEL UF = 10, animal to human UF = 1, intraspecies variability = 10; total UF = 100. |
| Hillefors-Berglund *et al*. (1995) | subchronic to chronic UF = 10, animal to human UF = 1, intraspecies variability = 10; total UF = 100. |

b: Pooled psychometric data from two printing plants with different toluene concentrations (11.2 and 41 ppm) were used to determine significant neurotoxic effects by Orbaek and Nise (1989). The range of RELs derived from that study lists the upper and lower bounds for risk associated with the pooled population exposures. ATSDR (1999) used the Orbaek and Nise (1989) study data, assuming an exposure concentration of 11.2 ppm, to derive a chronic inhalation minimal risk level (MRL).

If both human and animal adverse effect data on a chemical are available, OEHHA prefers to use the human data to develop a REL when possible. However, the study by Hillefors-Berglund *et al*. (1995) provides data (decreased brain [subcortical limbic area] weight and altered brain dopamine receptor binding) which are specific and sensitive measures of neurotoxicity that would not be obtainable in human studies. In contrast, the psychometric tests used to generate the neurotoxicity data in the human occupational exposure studies described above tend to be less sensitive and suffer from greater measurement uncertainty. Additionally, the Hillefors-Berglund *et al*. (1995) study has better exposure characterization than the human occupational exposure studies. Nonetheless, the human studies are useful in supporting the derivation of the REL for toluene. Ordinarily, an interspecies uncertainty factor of 3 would be applied, in addition to the human equivalent concentration calculation, to reflect the uncertainty associated with extrapolating from animals to humans. However, in this case the uncertainty in the interspecies extrapolation is reduced by the availability of human epidemiological data with generally consistent effect levels, after appropriate duration corrections. Based on comparison of the data in both animals and humans, it appears that a REL of 271 $\mu g/m^3$ (rounded to 300 $\mu g/m^3$ in the final derivation) would protect exposed humans from experiencing chronic neurotoxic effects.

## VII. Data Strengths and Limitations for Development of the REL

The major strength of the REL for toluene is the use of an animal study with accurate exposure characterization and both LOAEL and NOAEL observations for an effect (neurotoxicity), supported by observations from other animal and human studies. A weakness is the uncertainty in predicting human health risk from animal adverse effect data. However, this is mitigated by the availability of human data showing effect levels that are, after appropriate corrections, broadly consistent with the animal data.

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*CHRONIC TOXICITY SUMMARY*

# 2,4- and 2,6-TOLUENE DIISOCYANATE

*(2,4- and 2,6-TDI; 2,4- and 2,6-diisocyanato-1-methylbenzene; 2,4- and 2,6-diisocyanatoluene)*

**CAS Registry Number: 584-84-9 or 26471-62-5 (mixture)**

## I. Chronic Toxicity Summary

| | |
|---|---|
| *Inhalation reference exposure level* | **0.07 µg/m$^3$** (0.01 ppb) |
| *Critical effect(s)* | Decreased lung function in occupationally exposed workers |
| *Hazard index target(s)* | Respiratory system |

## II. Chemical Property Summary (HSDB, 1995; CRC, 1994)

| | |
|---|---|
| *Description* | Colorless to pale yellow liquid |
| *Molecular formula* | $C_9H_6N_2O_2$ |
| *Molecular weight* | 174.15 g/mol |
| *Boiling point* | 2,4-TDI: 251°C |
| *Melting point* | 2,4-TDI: 20.5°C<br>2,6-TDI: 18.3°C |
| *Vapor pressure* | 2,4-TDI: 0.008 torr @ 20°C |
| *Solubility* | Miscible with ether, acetone, benzene, carbon tetrachloride, chlorobenzene, diglycol monomethyl ether, kerosene, olive oil, alcohol; soluble in ethyl acetate |
| *Conversion factor* | 7.1 µg/m$^3$ per ppb at 25ºC |

## III. Major Uses and Sources

Commercial toluene diisocyanate is comprised of approximately 80% 2,4-TDI and 20% 2,6-TDI. TDI is used in the manufacture of polyurethane foams, elastomers, and coatings (HSDB, 1995; Howard, 1989). It is also used in the manufacture of floor and wood finishes, lacquers, foam plastics, polyurethane foam coated fabrics, and insulation materials (HSDB, 1995; Howard, 1989; Duncan *et al.*, 1962). Emissions of TDI to the atmosphere can occur during production, handling, and processing of polyurethane foam (Howard, 1989) and coatings. The annual statewide industrial emissions from facilities reporting under the Air Toxics Hot Spots Act in California based on the most recent inventory were estimated to be 13,223 pounds of toluene diisocyanates, 35,663 pounds of toluene-2,4-diisocyanate, and 754 pounds of toluene-2,6-diisocyanate (CARB, 1999).

## IV. Effects of Human Exposures

Diem *et al.* (1982) conducted a prospective study beginning in 1973 of 277 male workers involved in the production of TDI. The study examined pulmonary function, with nine examinations conducted over a five year period. A large group of workers (168) with no previously reported TDI exposure was examined 6 months prior to TDI production in the plant to provide baseline pulmonary function measurements. Personal sampling by continuous tape monitors provided exposure levels, but was not used until 2 years after the study was initiated. Sampling information resulted in a division of the workers into two groups: those exposed to levels below 68.2 ppb-months (which reflects the level of exposure of a worker for the entire 5 year duration in the low-exposure area (geometric mean = 1.1 ppb)) and those above this level. The arithmetic mean exposure level for the non-smokers was 1.9 ppb TDI in the high-exposure group and 0.9 ppb TDI in the low-exposure group (calculated by Hughes, 1993). The higher exposure group was further limited to those individuals who showed a normal $FEV_1$ to height ratio. Data were analyzed by the maximum likelihood weighted regression approach (Diem and Liukkonen, 1988). Both $FEV_1$ and forced expiratory flow (25-75%) [FEF (25-75%)] among workers who never smoked were found to be significantly reduced in the high-exposure group (n = 21) compared to the low-exposure group (n = 35). Categorizing workers based on time spent at exposure levels above 20 ppb demonstrated a significant difference in $FEV_1$ and FEF(25-75%) and this effect was also observed among current smokers. Among low-exposure workers, a smoking effect was observed, with smokers showing a significant decline in $FEV_1$.

A similar longitudinal study of lung function was conducted among workers exposed to TDI during the course of polyurethane foam production (Jones *et al.*, 1992). Participants (181 males and 46 females) were required to have 3 or more spirometric examinations over the 5 year study period. Exposure of males was evaluated by personal monitors and resulted in arithmetic mean low exposure levels of 0.3, 0.4, and 0.4 ppb TDI for never-smokers, ex-smokers, and current smokers, respectively. Among workers with high-level exposure, mean TDI levels were reported to be 1.3, 1.2, and 1.2 ppb for never-, ex-, and current smokers, respectively. Stepwise multiple linear regression methods (excluding asthmatics) were used in evaluating the data (Diem and Liukkonen, 1988). No relationship between TDI exposure and change in lung function was observed, although the prevalence of chronic bronchitis was significantly associated with exposure.

A longitudinal study of 780 workers exposed to TDI in the production of polyurethane foam was also conducted (Bugler *et al.*, 1991; unpublished). Exposure levels were established using continuous-tape personal monitoring devices. The mean exposure level was 1.2 ± 1.1 (SD) ppb TDI among 521 workers and 0.3 ± 0.18 ppb TDI in the control group. Another control group who handled cold urethane products had an 8 hour time-weighted average exposure of 0.6 ppb TDI. No significant longitudinal changes in $FEV_1$ were found after regression analysis, although $FEV_1$ decline was high among the control group. Exposure levels among the different groups were close, limiting the power of the study to detect changes. Approximately 3% of the 780 workers showed signs of TDI sensitization and, of these, over 80% were in the group exposed to 1.2 ppb.

Meta-analysis of the three data sets (Jones *et al.*, 1992; Bugler *et al.*, 1991; Diem *et al.*, 1982) showed that the difference in significance among the findings of each of the studies could have been due to chance. The change in the probability density for the decline in $FEV_1$ shifted in the same direction for all data sets and the smoker/non-smoker slope difference became less meaningful with the data set combination (Hasselblad, 1993).

Another toxicological area of concern with exposure to TDI is the development of sensitization, resulting in a well-documented condition known as "isocyanate asthma" of either immediate or delayed-type onset (Moscato *et al.*, 1991). The level of exposure required to either develop or trigger a sensitization reaction is not well documented, however. Weaknesses of studies showing pulmonary effects of TDI exposure include use of area sampling vs. breathing-zone measurement of exposure, poor statement of criteria for evaluating hypersensitivity, and the presence of other compounds in the environment which may influence lung function.

## V. Effects of Animal Exposures

Mice were exposed to TDI concentrations ranging from 0.007 to 1.18 ppm for 3 hours/day for 5 days consecutively (Sangha and Alarie, 1979); decreased respiratory rate was observed in groups exposed to levels higher than 0.023 ppm TDI. Groups of four mice were also exposed to 0.031 and 0.250 ppm TDI for 3 hours/day for 3 days. Lesions of the external nares and respiratory epithelium were observed in the high dose group.

Female guinea pigs were exposed to 0.12, 0.36, 0.61, 0.96, and 10.00 ppm TDI (head-only) for 3 hours/day for 5 consecutive days (short protocol) or to 0.02 ppm TDI (whole body) plus controls for 6 hours/day, 5 days/week for 70 days (long protocol). The animals showed decreased respiration rate two hours into exposure at levels above 0.12 ppm TDI and had a cytophilic antibody response at 0.96 ppm and above (Karol, 1983). All animals exposed to 10 ppm died. Dermal sensitivity was evident among animals in the short protocol down to 0.12 ppm TDI. No antibody response or dermal sensitivity developed in the animals exposed to 0.02 ppm TDI in the long protocol.

Similarly, guinea pigs (8 females) were exposed head only to 1.40 ppm TDI for 3 hours/day for 4 days (no control group). In a second exposure regimen, animals (n = 24) were exposed to 0.02 ppm TDI for 6 hours/day, 4 days/week for 70 days (whole body) including a control group (n = 8) exposed to room air in a similar manner (Wong *et al.*, 1985). Half the animals (4/8) exposed to 1.40 ppm TDI showed pulmonary hypersensitivity (measured on days 37 and 38) and all developed TDI-specific IgE antibodies, whereas none of the animals in the 0.02 ppm TDI group showed either of these effects. Histopathological effects in the 1.40 ppm TDI group included interstitial inflammation, pleural thickening, and peripheral lymphoid hyperplasia. Interstitial inflammation was noted in 2/24 animals exposed to 0.02 ppm TDI.

SD rats and CD-1 mice were exposed to 0.05 or 0.15 ppm TDI for 6 hours/day, 5 days/week for 2 years (Loeser, 1983; nasal histopathology reported by Owen, 1984). Among female rats at both dose levels and male rats at the high dose level, histopathological effects observed included necrotic rhinitis, metaplasia, and inflammation of the respiratory epithelium. Female animals

showed dose-dependent increases in incidence and severity of this effect. Similar lesions were reported in mice, although they were not well characterized.

Reproductive toxicity of TDI was evaluated in a two-generation study conducted in rats (Tyl and Neeper-Bradley, 1989). Weanling rats (28/sex/dose) were exposed to 0, 0.020, 0.079, and 0.290 ppm TDI for 6 hours/day, 5 days/week, for 10 weeks, at which time the animals were randomly mated. Exposure of the females continued through gestation (excepting gestational day 20 through the fourth day postpartum), and exposure of the males continued only until the delivery of the $F_1$ generation. Weanlings in the $F_1$ generation were exposed in a manner similar to the parental ($P_0$) generation and bred after weaning to produce the $F_2$ generation. Body weights were significantly reduced among animals of both sexes in the highest dose group and weight gain was reduced among males in the highest dose group. Effects on the respiratory system in the $P_0$ generation animals included rhinitis of the epithelium in the two highest dose groups of both male and female animals. Hyperplasia of the respiratory epithelium was also increased in the high dose groups of both sexes among $P_0$ animals. Among males in the $F_1$ generation, the incidence of rhinitis was significantly increased at all exposure levels and the incidence of submucosal lymphoid infiltrates of the larynx and trachea was increased in the highest dose group. $F_2$ generation animals showed reduced pup weight and weight gain during the lactation period in the two highest dose groups.

Developmental toxicity of TDI was evaluated by exposing pregnant Sprague-Dawley rats (25/group) for 6 hours/day on gestational days 6-15 to 0, 0.021, 0.120, or 0.48 ppm TDI (Tyl, 1988). Reduced maternal body weight, decreased food consumption, and rales occurred among the dams in the 0.48 ppm TDI dose group. A significant fetal effect, a statistically significant increase in a specific skeletal malformation, was reported in the highest dose group.

## VI. Derivation of the Chronic Reference Exposure Level

| | |
|---|---|
| *Study* | Diem *et al.*, 1982 |
| *Study population* | Human TDI production workers (n = 168) |
| *Exposure method* | Occupational inhalation exposure |
| *Critical effects* | Decreased lung function |
| *LOAEL* | 0.014 mg/m$^3$ (1.9 ppb) |
| *NOAEL* | 0.006 mg/m$^3$ (0.9 ppb) (non-smokers) |
| *Exposure continuity* | 8 h/day (10 m$^3$/day occupational exposure), 5 d/wk |
| *Exposure duration* | 5 years |
| *Average occupational exposure* | 0.002 mg/m$^3$ for NOAEL group (0.006 x 10/20 x 5/7) |
| *Human equivalent concentration* | 0.002 mg/m$^3$ for NOAEL group |
| *LOAEL uncertainty factor* | 1 |
| *Subchronic uncertainty factor* | 3 |
| *Interspecies uncertainty factor* | 1 |
| *Intraspecies uncertainty factor* | 10 |
| *Cumulative uncertainty factor* | 30 |
| *Inhalation reference exposure level* | 0.00007 mg/m$^3$ (0.07 µg/m$^3$; 0.01 ppb) |

The chronic REL is equivalent to the U.S. EPA RfC. OEHHA agreed with the U.S. EPA analysis and the selection of Diem *et al*. (1982) as the most appropriate study to use for the REL. The rationale for selection of this study is as follows. This study presented evidence of a decline in lung function, as indicated by decrements in $FEV_1$, among workers involved in TDI production. Other factors supporting its quality include:

(1) the absence of other confounding compounds in the work environment,
(2) the establishment of baseline lung function prior to exposure to TDI,
(3) a "parallel internal comparison" of study groups for lung function,
(4) an appropriate statistical analysis which took into account interindividual variability,
(5) breathing zone measurement of TDI (although commenced 2 years into the study), and
(6) a smoking effect on lung function.

## VII. Data Strengths and Limitations for Development of the REL

The major strengths of the chronic REL for TDI are the use of human exposure data from workers exposed over a period of years and the observation of a NOAEL. The major weaknesses are the uncertainty in estimating exposure, the potential variability in exposure concentration, and the limited nature of the study that focused on lung effects.

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*CHRONIC TOXICITY SUMMARY*

# TRICHLOROETHYLENE

*(trichloroethylene; 1,1-2-trichloroethylene, 1,1-dichloro-2-chloroethylene, acetylene trichloride, and ethylene trichloride*) )

**CAS Registry Number: 79-01-6**

## I. Chronic Toxicity Summary

| | |
|---|---|
| *Inhalation reference exposure level* | **600 µg/m$^3$ (100 ppb)** |
| *Critical effect(s)* | Neurotoxicological effects (drowsiness, fatigue, headache) and eye irritation in workers. |
| *Hazard index target(s)* | Nervous system; eyes |

## II. Physical and Chemical Properties (Fan, 1988; CRC, 1994)

| | |
|---|---|
| *Description* | Colorless liquid/vapor; sweetish, chloroform-like odor |
| *Molecular formula* | $C_2HCl_3$ |
| *Molecular weight* | 131.4 |
| *Density* | 1.47 g/cm$^3$ @ 20°C |
| *Boiling point* | 87.2 °C |
| *Melting point* | –84.7°C |
| *Vapor pressure* | 77 torr @ 25°C |
| *Vapor density* | 4.5 (air = 1) |
| *Solubility* | Soluble in alcohol, ethers, petroleum distillates and other halogenated solvents |
| *Conversion factor* | 1 ppm = 5.37 mg/m$^3$ @ 25° C |

## III. Major Uses or Sources

Trichloroethylene was once used as an extractant in food processing and has been used as an anesthetic and analgesic for medical purposes (Waters *et al.* 1977). Currently, it is widely used as a solvent in the industrial degreasing of metals, with secondary solvent uses in adhesive paint and polyvinyl chloride production (U.S. EPA, 1985). Trichloroethylene is used as a solvent in the textile industry, as a solvent for adhesives and lubricants, and as a low-temperature heat transfer fluid (IARC, 1979). Trichloroethylene is also implemented in the manufacturing of pesticides and other chemicals (Feldman, 1979). In 1996, the latest year tabulated, the statewide mean outdoor monitored concentration of trichloroethylene was approximately 0.035 ppb (CARB, 1999a). The annual statewide emissions from facilities reporting under the Air Toxics Hot Spots Act in California based on the most recent inventory were estimated to be 176,908 pounds (CARB, 1999b)

## IV. Effects of Human Exposure

An occupational study of trichloroethylene (TCE) vapor emissions in a pump room was conducted by Vandervort and Polnkoff (1973). Workers were an average age of 40 and had been employed for an average of 8 years. For 11-day shift workers, individual 8 hour time weighted average (TWA) TCE exposure concentrations were extrapolated from two area samples; these averages ranged from 170-420 mg/m$^3$ (32-78 ppm). Nineteen workers (including the 11 workers whose work areas were sampled) completed a questionnaire and reported the following symptoms: 73% eye irritation, 70% drowsiness, 58% heart palpitations, 58% cough, 53% weakness and 52% dizziness. About half of the 19 exposed workers reported that consumption of small amounts of alcohol outside of work resulted in changes of skin color and severe intoxication. One worker of the 19 reported no adverse effects from the occupational exposure. Nine control workers experienced none of the above symptoms. Urine samples from the 19 exposed and 9 unexposed workers were collected before and after the work shift and examined for the TCE metabolites trichloroacetic acid (TCA) and trichloroethanol (TRI). TRI levels ranged from 4-260 mg/l and TCA levels ranged from 4-197 mg/l. Results of the urine assays showed a range of TCE metabolite concentrations and, therefore, confirmed that the workers were exposed to a variety of concentrations in their environments.

Nomiyama *et al.* (1977) examined 36 trichloroethylene workers, of which 9 males and 12 females were occupationally exposed to a constant concentration of trichloroethylene (TCE) and 18 males were exposed to variable concentrations (duration of exposure unspecified). The control group consisted of 6 males and 10 females who were of similar educational, sociologic and economic status to the trichloroethylene workers. Researchers used urinary excretion of TCE metabolites as an indicator of the level of TCE exposure in the working environment; total excreted trichloro-compounds of 100 mg in 4 hours corresponded to 100 ppm TCE present in the working environment (Bardodej, 1958; Medek, 1958). Of the 36 exposed workers, 5 were exposed to 0-25 ppm; 14 were exposed to 25-50 ppm; 6 were exposed to 50-100 ppm; 8 were exposed to 100-150 ppm; and 3 were exposed to 150-200 ppm TCE. In the low exposure group, workers experienced mucous membrane irritation in the eyes, nose and throat, in addition to drowsiness, fatigue and headache. These symptoms were persistent through the higher concentration exposures with an increase in eye irritation, headache, fatigue, and nasal obstruction above 100 ppm TCE. Increases in rhinorrhea and drowsiness were seen above 50 ppm TCE exposure.

Kimmerle and Eben (1973) exposed 4 human subjects (3 males and 1 female) to a subacute regimen of 48 $\pm$ 3 ppm trichloroethylene (TCE) for 4 hours a day over a period of 5 days. Levels of TCE and the metabolites trichlororethanol (TRI) and trichloroacetic acid (TCA) were determined. Trichloroethanol-blood levels were elevated immediately after exposure, and detection of trichloroethanol occurred up to 7 days after the last exposure. TCE-blood concentration increased slightly over the 5 days. Levels of urinary excreted trichloroethanol, as well as the TCA-concentration, increased throughout the study, with the female showing a significantly higher excretion of TCA. Levels of TCA were detected up to 12 days after the final exposure.

Okawa and Bodner (1973) studied the occupational exposure of 24 electrical plant workers to trichloroethylene (TCE). The plant worker group consisted of 22 males and 2 females ranging in age from 21-52 years old. Environmental samples of TCE were collected over three days and yielded varying concentrations of TCE related to the task performed in certain areas (duration of exposure unspecified). Spray booth operators were exposed to an average of 25.3 ppm TCE (13-40 ppm range) in addition to averages of 15.2 ppm n-propyl acetate (NPA) and 6 ppm toluene (TOL). Workers involved in washing board units were exposed to an average value of 39 ppm (6-82 ppm range) TCE. Although the workers wore respiratory protection during the washing procedure, the overall average of airborne TCE in this area was 48.3 ppm. In the testing area of the plant, researchers report that the amounts of toluene and n-propyl acetate were insignificant. Here, TCE levels were an average of 24.4 ppm (range = 8-44 ppm). The solder machine operators were exposed to an average of 44.0 ppm TCE (range = 23-87 ppm) with no NPA or TOL present. During the cleaning of the soldering machines, TCE levels rose to an average of 70.5 ppm (range = 30-106 ppm). Concentrations were only at these elevated levels for 20-30 minutes a day. Researchers note that although other agents were used in the work area, TCE was the only chemical found in significant amounts throughout the work area and that the levels of NPA and TOL were insignificant. An analysis of urinary TCE metabolites indicated that the workers were exposed to a time weighted average concentration of <50 ppm TCE. Three of the 24 workers reported that they were unaffected by their working conditions, but the most prominent complaints consisted of 70.8% workers experiencing nausea, 54.2% headache, 33.3% dizziness, 25.0% fatigue, 25% nose and throat irritation, and 20.8% eye irritation. Workers reported that these symptoms were alleviated hours after leaving the work environment. Researchers collected 8 hour urine samples from 20 of the workers and from 9 controls and analyzed them for TCE metabolites. Results of urinary analysis showed that the controls had exposure to an unspecified amount of TCE. TCA levels in exposed workers were elevated from that of the controls and correlated to the different exposures in specific work areas.

Phoon *et al.* (1984) reported on 5 cases of Stevens-Johnson syndrome (erythema multiforme major) with liver involvement which followed exposure to TCE. In two cases, reactions to the exposure began with a fever followed by an itchy rash on the face spreading over the body. Lesions were observed on the face, arms and in the mouth. Liver function tests were abnormal. One of the two developed jaundice with hepatomegaly. Case #3 developed a similar reaction after 5 weeks of exposure to 216-912 $mg/m^3$ TCE (40-170 ppm) as did case #5 after two weeks of exposure to 370 $mg/m^3$ TCE (69 ppm). Case #4 involved a 39 year old man exposed to <50 $mg/m^3$ TCE (< 9.3 ppm) for three weeks who developed the characteristic rash, lesions and jaundice with slight hepatomegaly. Upon returning to work over the next three weeks, he developed generalized erythrodermia and facial oedema, hepatosplenomegaly and liver failure with septicemia from which he died 14 days later.

Stewart *et al.* (1974) studied the effects of subacute trichloroethylene (TCE) exposure in combination with alcohol consumption. Seven men exposed to 200 ppm TCE ingested 1 quart of beer or 90 ml of 100-proof vodka and developed red blotches on their faces 30-40 minutes later. These lesions enlarged with time until they reached a peak intensity, whereupon they faded. One subject experienced facial flush with the consumption of alcohol for three weeks after the last TCE exposure, while another showed flushing six weeks after the last exposure.

## V. Effects of Animal Exposure

Kjellstrand *et al.* (1983) studied the effects of both intermittent and continuous exposures of various concentrations of trichloroethylene on male and female mice over a period of 30 days. The concentrations used range from 37-3600 ppm, and 7 of the 14 groups were continuously or intermittently exposed to lower concentrations of 37, 75, 150, 225 and 300 ppm TCE. Continuous exposure studies were conducted over a period of 30 days for exposure groups of 37, 75, 150 and 300 ppm TCE. All groups consisted of 10 males and 10 females (except the 37 ppm group, consisting of 20 males and 20 females) and were compared to identical groups of air-exposed controls. Liver weights increased in a non-linear fashion as the concentration level of TCE increased. All groups exhibited statistically significant increases in liver weights as compared to the controls. In both the 37 and the 75 ppm groups, the increase in females was less than in males. No increase in spleen weight was detected at either the 37 or 75 ppm exposure level. At the 37 ppm level, a slight increase in plasma butyrylcholinesterase (BuChE) activity (not statistically significant) was also detected. A significant increase in kidney weight was seen in the male 75 ppm group and was more pronounced with increasing concentration. Male mice in the 75 ppm group also showed statistically significant increases in BuChE activity. In the 150 ppm group, male and female liver weight increases were statistically significant and of equal magnitude. A statistically significant increase was seen in the BuChE activity of the 150 ppm male mice. It was not until female mice were exposed to 300 ppm, that they showed slight increase in BuChE activity, while the males increased 3.5 times the controls. Liver weight increases for the 300 ppm group were close to the maximum with females showing greater increase than the males. Ten male and 10 female mice were continuously exposed to 150 ppm TCE for 30 days, but then allowed a 120 day rehabilitation period. Following rehabilitation, liver weights returned to levels comparable to the controls. The elevated BuChE activity returned to a normal level. No significant effects were seen after the period of rehabilitation. A continuous study was performed on 10 male and 10 female mice for 120 days at an exposure level of 150 ppm TCE. No further increase in liver weight occurred beyond the level reached in the 30 day study. Body weight gain was slightly decreased, and the same level of BuChE activity was seen as in the 30 day exposure. The intermittent study consisted of 30 days exposure to 225 ppm TCE for 16 hours a day, 7 days a week. A significant increase was seen in the BuChE activity of male mice, while females did not exhibit an increase in BuChE activity. Both males and females showed statistically significant increases in liver weight. Kidney weight increased in the same manner as in the continuous exposures. The authors noted that "extrapolation of the concentration-effect curve suggests that both liver weight and BuChE activities are influenced at still lower concentration."

Briving *et al.* (1986) examined neurotoxicity as a result of chronic trichloroethylene (TCE) inhalation exposure. Two groups of gerbils (6 in each group) were exposed to 50 or 150 ppm TCE for a period of 12 months. Two equivalent groups were used as controls. Two areas of the brain were specifically observed, the hippocampus and the posterior part of the cerebellar vermis. These discrete brain areas were previously shown to be sensitive towards chlorinated aliphatic solvents (Haglid *et al.*, 1981). Following exposure, gerbils were decapitated and measurements were made of total free tissue amino acids as well as high-affinity uptake and release of $^{3}$H-aminobutyric acid (GABA) and $^{14}$C-glutamate. A significant increase in

glutathione was seen in the hippocampus of the 150 ppm gerbils, but amino acid levels were not significantly affected. In the posterior part of the cerebellar vermis, glutamate and GABA accumulation levels increased in a dose-dependent manner, with significant increases seen at both 50 and 150 ppm TCE. Evaluation of the hippocampus revealed no significant changes. The authors suggest that the stimulation of transport functions for GABA and glutamate may be triggered by the presence of the TCE metabolite, trichloroethanol. Therefore, the levels of GABA and glutamate are indicative of the amount of trichloroethanol from TCE in the brain.

Kligerman *et al.* (1994) exposed 20 male CD rats to 0, 5, 50, or 500 ppm trichloroethylene (TCE) for 6 hours a day, over a period of 4 days. Groups at each concentration consisted of 5 rats. One of the cytogenetic effects measured was peripheral blood lymphocytes (PBLs), abnormal with regard to sister chromatid exchanges. Also analyzed, were the cell cycle, bone marrow micronuclei in polychromatic erythrocytes (MN-PCEs/1000) and micronuclei in cytochalasin B-blocked binucleated cells (MN-BN/1000). The 5 ppm and 500 ppm exposure groups showed a decrease (not statistically significant) in cell cycle. In addition, the 50 ppm group exhibited a statistically significant decrease in cell cycle. For all concentrations, there was an overall increase in the PCE percentage. The number of PCEs with micronuclei also rose with the increasing concentrations of 50 ppm and 500 ppm TCE (not statistically significant due to high control values). The researchers conclude that the resulting increase of MN in exposed rats is indicative of aneuploidy induction as opposed to chromosomal breakage, and that the lack of chromosome aberrations corresponds to spindle effects such as aneuploid induction. Concurrent results of increased levels of leukocyte aneuploidy were also found by Konietzko *et al.* (1978) in degreasing workers occupationally exposed to TCE.

Haglid *et al.* (1981) continuously exposed gerbils to 60 ppm or 320 ppm trichloroethylene (TCE) for 3 months. Following the exposure period, gerbils were maintained for 4 months in TCE-free conditions in order to observe any restoration of neuronal function. Both of the exposed groups as well as the control group consisted of six pairs of males and females. Brain samples were collected from the gerbils after the 4 month non-exposure period and used for determination of DNA and proteins. In order to determine areas of the brain that were sensitive to TCE, researchers examined biochemical and morphological changes in the hippocampus, the posterior part of the cerebellar vermis, and the brain stem. In addition to the biochemical tests, the cerebellum, brain stem, and cerebrum of two gerbils from each group, including the control, were used for neuropathological examination. Brain tissue from 2 gerbils in the control group and the 320 ppm group were examined under the electron microscope. No difference was seen in the body and brain weights of the exposed gerbils compared with controls. A slight but significant increase in soluble proteins was detected in the frontal cerebral cortex of the 60 ppm group, and a more significant elevation was seen in the visual cerebral cortex of both the 60 ppm and 320 ppm groups. In the 60 ppm group, a slight but significant decrease was seen in the soluble proteins of the sensory-motor cortex. Both groups exhibited significant decreases in levels of soluble proteins in the hippocampus, the brain stem, and in the posterior part of the cerebellar vermis. Soluble protein levels in the cerebellar hemisphere and anterior part of the vermis of gerbils in both exposed groups did not differ from the controls. The 320 ppm group showed significantly increased DNA levels in the posterior part of the sensory motor cortex and cerebellar vermis. The glial cytoplasmic protein (S 100 fraction) level of the 60 ppm group was decreased in the frontal and visual cerebral cortex, but increased in the posterior part of the

cerebellar hemisphere and the sensory-motor cortex. However, only a slight decrease of S 100 protein was observed in the visual cerebral cortex of 320 ppm exposed gerbils. The most notable S 100 increase occurred in the hippocampus, brain stem and the posterior part of the cerebellar vermis, indicating that either the glial cells were directly affected or that damage to surrounding neuronal cells caused an indirect response. There was an increase in DNA in the posterior part of the cerebellar vermis in the exposed gerbils, suggesting that TCE induced astroglial cell mitosis. Light microscopy revealed shrinkage of cell bodies and axon swelling occurred in various parts of the brain. The electron microscopy performed on control and 320 ppm brain tissues revealed increased levels of filament bundles in the cytoplasm of some Purkinje and Golgi cell perikarya, lysosomes, myelin bodies and lipid containing lysosomal structures in the exposed gerbils. Unique arrangements of filament bundles were seen in Purkinje and Golgi cell dendrites of the exposed group. A significant decrease in the number of microtubules was observed as well as a decrease in the number of synaptic vesicles in the granular layer. Also, the granular layer had decreased maximal nerve cell surface area. Nerve cells were affected by the exposure as several types were reduced in size with fewer organelles and more lysosomes and myelin bodies. Many axons and dendrites had reduced numbers of microtubles, and there were filament bundles observed that were not present in the controls. Lysosomal structures were increased in the synaptic terminals.

Kimmerle and Eben (1973) performed a subchronic study on 20 male rats for a period of 14 weeks. Rats were exposed to a mean concentration of 55.0 $\pm$4 ppm trichloroethylene (TCE) for 8 hours a day, 5 days a week. The control group consisted of 20 rats who in similar inhalation chambers under similar conditions to that of the exposed rats. Ten exposed rats were analyzed for TCE metabolite excretion on a daily basis. Blood levels of trichloroacetic acid (TCA), trichloroethanol (TRI) and chloral hydrate (CH) were measured during the 2nd, 3rd, 4th, 6th, 9th and 14th weeks. Weekly measurements of body weights were recorded. Macroscopic examinations were performed on the thyroid gland, heart, lungs, liver, kidneys, testes and adrenal glands. Hematological evaluations, liver function tests, and renal function tests were also conducted following exposure. Urinary levels of TRI varied individually among the rats, but a continuous increase in TRI was observed through the 10th week. TCA levels remained fairly constant throughout the duration of the experiment. TCE was not detectable in the blood or the tissues of exposed rats. Although liver and renal function tests did not reveal abnormalities, there was an increase in the liver weights of the exposed rats. The weights of the other organs examined were similar to the controls.

Norpoth *et al.* (1974) observed an increase in liver cytochrome $P_{450}$ activity in 9 rats exposed to 50 ppm trichloroethylene for 28 days, compared with 9 control rats.

## VI. Derivation of Chronic Reference Exposure Level

| | |
|---|---|
| *Study* | Vandervort and Polnkoff (1973) |
| *Study population* | 19 workersand 9 controls |
| *Exposure method* | Discontinuous occupational inhalation exposure |
| *Critical effects* | Drowsiness, fatigue, headache, and eye irritation |
| *LOAEL* | 32 ppm (170 mg/m$^3$) in the heavy assembly area |
| *NOAEL* | Not observed |
| *Exposure continuity* | 8 hours a day (10 m$^3$/day occupational inhalaton rate), 5 days a week |
| *Exposure duration* | 8 years |
| *Average occupational exposure* | 11.4 ppm for LOAEL group (32 x 10/20 x 5/7) |
| *Human equivalent concentration* | 11.4 ppm for LOAEL group |
| *LOAEL uncertainty factor* | 10 |
| *Subchronic uncertainty factor* | 1 |
| *Interspecies uncertainty factor* | 1 |
| *Intraspecies uncertainty factor* | 10 |
| *Cumulative uncertainty factor* | 100 |
| *Inhalation reference exposure level* | 0.1 ppm (100 ppb; 0.6 mg/m$^3$; 600 µg/m$^3$) |

The Vandervort and Polnkoff (1973) study accounted for 8 years of human occupational exposure to TCE vapors. Sensitive, non-specific neurotoxicological endpoints were exhibited by a majority of those workers exposed. Although the time-weighted averages (TWAs) included a wide range of concentrations, the TWA of 32 ppm (170 mg/m$^3$) was shown to contribute to the high incidence (52 - 73%) of adverse effects experienced by the workers. Many of the symptoms reported by the workers may have been due to short-term fluctuations in the concentrations in the workplace. The symptoms were not reported separately for the various TWAs, therefore, the lowest TWA (32 ppm) was chosen as a LOAEL. Uncertainty includes the small number of workers studied, the limited extent of the effects mentioned, and the lack of a NOAEL. Strengths include the use of human data, the demonstration of a dose-response relationship, and exposure estimates correlated with urinary excretion measurements.

This study was the best chronic account of the non-carcinogenic effects of TCE on humans, but several other studies show similar results. Nomiyama *et al.* (1977) found similar endpoints of drowsiness, fatigue and eye irritation in 36 workers occupationally exposed to trichloroethylene. Okawa *et al.* (1973) also saw non-specific neurological endpoints in 24 electrical plant workers who were similarly exposed to TCE.

For comparison with the proposed REL of 100 ppb based on human studies, the LOAEL of 50 ppm trichloroethylene obtained by Briving *et al*. (1986) in gerbils exposed continuously for 12 months was used to estimate a REL based on animal data. Use of a LOAEL UF of 3, a subchronic UF of 1, an interspecies UF of 10, and an intraspecies UF of 10 resulted in an estimated REL of 200 ppb for trichloroethylene.

## VII. Data Strengths and Limitations for Development of the REL

The strengths of the inhalation REL for trichloroethylene include the use of human exposure data from workers exposed over a period of years. Major areas of uncertainty are the lack of reproductive and developmental toxicity studies and the lack of observation of a NOAEL.

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*CHRONIC TOXICITY SUMMARY*

# TRIETHYLAMINE

*(diethylaminoethane; ethanamine; N,N-diethylethanamine)*

**CAS Registry Number: 121-44-8**

## I. Chronic Toxicity Summary

| | |
|---|---|
| *Inhalation reference exposure level* | **200 μg/m$^3$** (40 ppb) |
| *Critical effect(s)* | Eye effects in rats and humans |
| *Hazard index target(s)* | Eyes |

## II. Physical and Chemical Properties (Nelson and Bull, 1990, except as noted)

| | |
|---|---|
| *Description* | Colorless, volatile liquid |
| *Molecular formula* | $C_6H_{15}N$ |
| *Molecular weight* | 101.9 g/mol |
| *Density* | 0.726 g/cm$^3$ @ 25°C |
| *Boiling point* | 89.3°C |
| *Melting point* | –114.7°C (CRC, 1994) |
| *Vapor pressure* | 400 torr @ 31.5°C |
| *Odor threshold* | 480 ppb (Amoore and Hautala, 1983) |
| *Solubility* | soluble in acetone, benzene and chloroform |
| *Conversion factor* | 1 ppm = 4.14 mg/m$^3$ 25°C |

## III. Major Uses or Sources

Triethylamine (TEA) is primarily used as a cross-linking catalyst in the production of polyurethane foam used in the manufacture of cores for metal castings (Albrecht and Stephenson, 1988). Triethylamine is also used as a catalyst for epoxy resins, and as a corrosion inhibitor for polymers (Nelson and Bull, 1990). TEA is one of the amines emitted from cattle feedlots (Mosier *et al*., 1973). In the gas phase TEA can react with nitric acid to form amine nitrates that become part of atmospheric particulates. The annual statewide industrial emissions from facilities reporting under the Air Toxics Hot Spots Act in California based on the most recent inventory were estimated to be 4152 pounds of triethylamine (CARB, 2000).

## IV. Effects of Human Exposures

Acute, high level triethylamine exposures (20 mg/m$^3$ (4.8 ppm) for 8 hours) resulted in reversible ocular effects that included corneal swelling and halo vision in 4 out of 5 volunteer subjects (Akesson *et al.*, 1988). Jarvinen *et al*. (1999) reported exposure chamber studies of ocular

responses to TEA in volunteer subjects, who were industrial workers exposed to TEA during the course of their normal jobs (but had good general and ocular health). Four people were exposed for 4 hours to 3.0, 6.5, or 40.6 mg/m$^3$ triethylamine. Corneal thickness was measured by ultrasonography, clinical observations were recorded using ocular microscopy, and statistical analysis of the size, density and distribution of corneal endothelial cells was performed by automated analysis of photographs. Visual acuity and contrast sensitivity were evaluated using test charts. After exposure to 40.6 mg/m$^3$ there was a marked edema in the corneal epithelium, and subepithelial microcysts. However, corneal thickness increased only minimally. Vision was blurred in all subjects and visual acuity and contrast sensitivity decreased in three of the four. After exposure to 6.5 mg/m$^3$ two subjects experienced symptoms (e.g., blurred vision), and contrast sensitivity decreased in three of the four. There were no symptoms or decreases in contrast sensitivity after exposure to 3.0 mg/m$^3$ triethylamine for 4 hours.

A medical examination of 19 workers exposed at a polyurethane foam production plant to a time-weighted average concentration of 13 mg/m$^3$ (3.1 ppm) TEA showed reversible corneal edema in 5 workers (Akesson *et al.*, 1986). Peak concentrations were up to twice the time-weighted average level. A questionnaire on self-reported symptoms of visual disturbances revealed repeated occurrences of temporary eye irritation and "foggy vision." Small quantities of dimethylethanolamine, toluene diisocyanate, and methylene diphenyl isocyanate were also present in the workplace atmosphere.

Jarvinen and Hyvärinen (1997) reported loss of visual acuity and contrast sensitivity in 41 foundry workers (core makers) exposed to TEA. Concentrations of TEA were reported to have a mean of 46 mg/m$^3$ and a maximum of 486 mg/m$^3$, but were highly variable with numerous large excursions above a background of about 20 mg/m$^3$ during a two-hour period of continuous monitoring. It is therefore difficult to determine an effect level for the observed symptoms. Jarvinen (1998) also reported that cold box core makers exposed to TEA had a somewhat increased incidence of mild headaches.

## V. Effects of Animal Exposures

Lynch *et al.* (1990) exposed male and female Fischer 344 rats to triethylamine at concentrations of 0, 25, or 247 ppm (0, 103.4, or 1022.2 mg/m$^3$) for 6 hours/day, 5 days/week. Groups of rats were necropsied at approximately 30, 60, and 120 days of exposure. The last corresponds to an elapsed time of 28 weeks. Endpoints examined included gross and histopathological examination of all major organs, including the lungs, nasal passages, and eyes. Clinical enzyme and nitrogen levels (BUN, ALT, AST, CPK, and creatinine), and hematological values (hemoglobin, RBC count) were also measured. No gross or histological effects in any organ were observed in any group. Clinical and hematological parameters were unchanged with exposure. However, all rats exposed to 247 ppm TEA manifested irritation. "At 247 ppm TEA the rats kept their eyes closed and noses buried in their fur during the entire exposure period." Thus 247 ppm is a LOAEL and 25 ppm is a NOAEL for eye and nose irritation in the rat.

In a short-term study by the same authors for Virginia Chemicals (1987), necrotizing inflammation of the nasal cavity, metaplasia of the trachea, and thymic atrophy were observed

after exposure to 1000 ppm (4140 mg/m$^3$) triethylamine 6 hours per day for 10 days. Two of five males and one of five females died from pulmonary edema after the seventh day. Thymic atrophy was noted in 7 out of 10 animals, and all animals exhibited necrotizing inflammation in the nasal epithelium.

Rabbits (6-12 per group), exposed to 48 or 100 ppm (199 or 414 mg/m$^3$) triethylamine for 7 hours/day, 5 days/week, for 6 weeks, showed concentration-dependent pathology in the eyes, lungs, liver, kidney, and heart (Brieger and Hodes, 1951). The eyes showed multiple punctate erosions of the corneal epithelium, and corneal edema at 48 ppm. Lung lesions included thickening of vascular walls; liver lesions included parenchymal degeneration. Overall the lesions in the 48 ppm group were less severe than those seen in the 100 ppm group. No control animals were included in this study, nor were the incidences of histologic effects among the exposed animals reported. All animals did survive the exposures. The lesser effects at 48 ppm in the rabbit (compared to those at 100 ppm) are consistent with the findings of Lynch *et al*. (1990) where 25 ppm was a NOAEL in the rat for eye and nose irritation.

A chronic 3-generation reproductive study in rats (10/sex/group) was inconclusive due to excessive mortality in controls (Davison *et al.*, 1965). In this study, rats were exposed to 0, 2, or 200 ppm triethylamine. The third generation of the 200 ppm group was changed to 500 ppm since no effects were noted in the 200 ppm group. Exposure of this group to 500 ppm resulted in decreased body weight and decreased water consumption.

## VI. Derivation of Reference Exposure Level

| | |
|---|---|
| *Study* | Lynch *et al.*, 1990; Brieger and Hodes, 1951 |
| *Study population* | Rats; rabbits |
| *Exposure method* | Discontinuous whole-body inhalation |
| *Critical effects* | Eye irritation; lung and liver toxicity |
| *LOAEL* | 48 ppm (Brieger and Hodes, 1951) |
| *NOAEL* | 25 ppm (Lynch *et al.*, 1990) |
| *Exposure continuity* | 6 or 7 hours/day, 5 days/week |
| *Exposure duration* | 28 weeks; 6 weeks |
| *Average experimental exposure* | 4.46 ppm for NOAEL group (25 x 6/24 x 5/7) |
| *Human equivalent concentration* | 4.46 ppm (18.5 mg/m$^3$) for NOAEL group |
| *LOAEL uncertainty facto* | 1 |
| *Subchronic uncertainty factor* | 1 (NOAEL is based on a 28 wk study in rats) |
| *Interspecies uncertainty factor* | 10 |
| *Intraspecies uncertainty factor* | 10 |
| *Cumulative uncertainty factor* | 100 |
| *Reference exposure level* | 0.04 ppm (40 ppb; 200 μg/m$^3$) |

The U.S. EPA (1995) based its Reference Concentration (RfC) of 7 μg/m$^3$ (2 ppb) for triethylamine on Lynch *et al*. (1990) but included a Modifying Factor (MF) of 10 for "database deficiencies" - "lack of developmental and reproductive effects, and of appropriate data in a second species." The criteria for use of modifying factors are not well specified by U.S. EPA.

Such modifying factors were not used by OEHHA. In addition OEHHA applied a subchronic UF of 1 since 24 male and 24 female rats in the NOAEL group were exposed to 25 ppm TEA for 28 weeks, while USEPA used a subchronic UF of 10. U.S. EPA considered 247 ppm to be a NOAEL. However, the Lynch *et al*. (1990) study indicates that the animals closed their eyes and buried their noses in their fur, likely to prevent the irritant effects of TEA on their eyes and respiratory tract. Thus adverse effects occurred at 247 ppm, although they could be considered repeated acute effects. Brieger and Hodes (1951) observed adverse effects in the eyes, lungs, and livers of rabbits after six weeks of discontinuous exposure to TEA. Thus 48 ppm is a LOAEL in this study.

For comparison, the five affected workers studied by Akesson *et al*. (1986) showed symptoms at 12-13 mg/m$^3$ TEA, which is equivalent to 4.5 mg/m$^3$ continuous exposure. (Other tasks were at 4-5 mg/m$^3$ TEA or 1.6 mg/m$^3$ continuous exposure.) Selection of a LOAEL UF of 3 (26% incidence of a reversible effect), a subchronic UF of 1 since the workers had been employed for 9.7 years (range = 4-11), and an intraspecies UF of 10 results in an estimated REL of 200 μg/m$^3$ based on human data. These workers experienced some short-term peak exposures to TEA and were also exposed to dimethylethanolamine (<0.1 mg/m$^3$), toluene diisocyanate, and methylene diphenyl isocyanate.

## VII. Data Strengths and Limitations for Development of the REL

The major strengths of the triethylamine REL are the observation of a NOAEL in a controlled exposure experiment and finding of the same adverse effect, eye irritation, in humans and animals. The major weaknesses are the minimal amount of adequate human health effects information, the lack of dose-response data in a single experiment, and the lack of long-term exposure data.

## VIII. Potential for Differential Impacts on Children's Health

There is no direct evidence in the literature to quantify a differential effect of TEA in infants and children. However, it is a respiratory irritant and thus has the potential to exacerbate asthma. In addition, other alkylamines are known to be associated with occupational asthma (Bernstein *et al*., 1999). There is some concern that TEA could have a similar effect.

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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

| | |
|---|---|
| *Inhalation reference exposure level* | **200 µg/m$^3$** (50 ppb) |
| *Critical effect(s)* | Nasal epithelial lesions in rats and mice |
| *Hazard index target(s)* | Respiratory system |

## II. Physical and Chemical Properties (HSDB, 1994)

| | |
|---|---|
| *Description* | Colorless liquid |
| *Molecular formula* | $C_4H_6O_2$ |
| *Molecular weight* | 86.09 g/mol |
| *Density* | 0.932 g/cm$^3$ @ 20°C |
| *Boiling point* | 72.7° C |
| *Melting point* | –93.2°C |
| *Vapor pressure* | 115 torr @ 25°C |
| *Solubility* | Slightly soluble in water, soluble in ethane, acetone, chloroform; >10% soluble in ethanol and benzene |
| *Conversion factor* | 1 ppm = 3.52 mg/m$^3$ @ 25°C |

## III. Major Uses and Sources

The major use of vinyl acetate monomer is in the manufacture of polyvinyl and vinyl acetate copolymers, which are used in water-based paints, adhesives, paper coatings, and applications not requiring service at extreme temperatures (HSDB, 1994). It is also used in safety glass interlayers and in hair sprays (HSDB, 1994). In the atmosphere vinyl acetate breakdown can result in formation of acetaldehyde. The annual statewide industrial emissions from facilities reporting under the Air Toxics Hot Spots Act in California based on the most recent inventory were estimated to be 3855 pounds of vinyl acetate (CARB, 2000).

## IV. Effects of Human Exposures

Deese and Joyner (1969) conducted an occupational study of 21 chemical workers with a mean length of employment of 15.2 years and exposed to a time-weighted average of 8.6 ppm (30.3 mg/m$^3$) VA. No adverse effects were noted following chest x-ray, electrocardiogram, blood

chemistry, and urinalysis. The control group (sample size unspecified) consisted of workers in units not exposed to VA. Deese and Joyner (1969) also showed intolerable eye irritation in 3 out of 3 subjects exposed for an unspecified extended period of time to 21.6 ppm (76 mg/m$^3$) VA. Upper respiratory irritation was also experienced by a majority of 5 subjects. Odor was detected at 0.4 ppm (1.4 mg/m$^3$) in 3 out of 3 subjects.

## V. Effects of Animal Exposures

A 104-week inhalation study in rats and mice (90/sex/group) was conducted using concentrations of 0, 50, 200, or 600 ppm (0, 176, 704, or 2113 mg/m$^3$) vinyl acetate (VA) (Owen, 1988). The study was later published by Bogdanffy *et al.* (1994). Exposures were for 6 hours/day, 5 days/week. Histology was performed on all major organs. There was no mortality resulting from these exposures. A close examination of the effects of VA on the lung and nasal passages showed significant lesions in the nasal cavity, bronchi, and lungs of rats exposed to 600 ppm VA. Lesions included olfactory epithelial metaplasia/atrophy (see table below) and nest-like epithelial folds in the nasal cavity, exfoliation of bronchial epithelium, fibrous intraluminal projections in the bronchi, and pigmented histiocyte accumulation in the lungs. Body weight gain of rats was significantly decreased in the 600 ppm VA group. Rats treated with 200 ppm VA showed some evidence of epithelial atrophy and metaplasia in the nasal cavity. No effects were observed in the rats exposed to 50 ppm VA.

Number of male rats with olfactory epithelial atrophy (Bogdanffy *et al*. 1994)

| VA (ppm) | N in group | Very slight | Slight | Moderate | Severe |
|---|---|---|---|---|---|
| 0 | 58 | 0 | 0 | 0 | 0 |
| 50 | 59 | 1 | 2 | 0 | 0 |
| 200 | 60 | 4 | 47*** | 2 | 0 |
| 600 | 60 | 0 | 7* | 33*** | 10*** |

* $p<0.05$; ***$p<0.001$ by Fisher's pair-wise test compared to control group

Mice also exhibited significant histological lesions in the respiratory tract following exposure to 200 ppm VA or greater. The lesions included atrophy of the olfactory epithelium and submucosal gland. At 600 ppm, hyperplasia of the trachea was observed, in addition to exfoliation/flattening of the bronchial epithelium and decreased body weight gain. Relative brain and kidney weights were increased in the 600 ppm group at the end of the study, and absolute liver, heart and kidney weights were also significantly elevated. No adverse effects were observed in the 50 ppm group.

A 13-week study on the effects of VA in mice was conducted by Owen (1980a). Mice (10/sex/concentration) were exposed to 0, 50, 200, or 1000 ppm (0, 176, 704, or 3520 mg/m$^3$) VA for 6 hours/day, 5 days/week for 13 weeks. A concentration-dependent increase in the incidence of diffuse rhinitis, beginning at the 200 ppm concentration, was detected using histopathological examination. Focal pneumonitis was observed in the 1000 ppm treatment group. No adverse effects were seen in the 50 ppm treatment group. An identical study in rats was also conducted by Owen (1980b). In this study, body weight gain was significantly reduced

in male and female rats exposed to 1000 ppm VA. An increase in the incidence of mild histiocytic alveolitis was observed in the 1000 ppm group.

Irvine (1980) conducted a study on the developmental toxicity of VA in rats. Groups of 24 pregnant female rats were exposed to 0, 52, 198, or 1004 ppm (0, 182, 696, or 3533 mg/m$^3$) VA for 6 hours/day on days 6-15 of gestation. Significant maternal toxicity, as measured by reduced weight gain from day 10 through day 15, was observed in animals exposed to 1004 ppm. Fetotoxicity, as measured by reduced crown-rump length, reduced body weight, and increased incidence of ossification defects in the sternebrae and occipital regions, was observed in the 1004 ppm group. No maternal or fetal effects were seen at the lower two VA treatments.

In another developmental toxicity study, groups of 23-24 Crl:CD(SD)BR rats were given 0, 200, 1000, or 5000 ppm VA in drinking water or exposed 6 hr/day to 0, 50, 200, or 1000 ppm VA on gestation days 6-15 of gestation. The authors (Hurtt *et al*., 1995) estimated that the doses by both routes were approximately 0, 25, 100, or 500 mg/kg/day. VA in the drinking water produced no evidence of maternal or developmental toxicity at any dose. In the inhalation study, maternal toxicity was indicated by a reduction in weight gain of dams exposed to 1000 ppm. Fetal toxicity was evident by a significant decrease in mean fetal weight and mean crown-rump length in fetuses from the 1000-ppm group and by a significant increase in the incidence of minor skeletal alterations (especially delayed ossification) in fetuses from dams exposed to 1000 ppm VA. These results indicated to the authors that VA is not uniquely toxic to the conceptus. The NOAEL was greater than 5000 ppm via the drinking water and 200 ppm by the inhalation route.

## VI. Derivation of Chronic Reference Exposure Level

| | |
|---|---|
| *Study* | Bogdanffy *et al*., 1994 |
| *Study population* | Male and female Sprague-Dawley rats and CD-1 mice (90/sex/group) |
| *Exposure method* | Discontinuous inhalation exposures (0, 50, 200, or 600 ppm) over 104 weeks |
| *Critical effects* | Histological lesions of the nasal epithelium |
| *LOAEL* | 200 ppm |
| *NOAEL* | 50 ppm |
| *Exposure continuity* | 6 hours/day, 5 days/week |
| *Exposure duration* | 104 weeks |
| *Average experimental exposure* | 8.9 ppm for NOAEL group (50 x 6/24 x 5/7) |
| *Human Equivalent Concentration (HEC)* | 1.4 ppm for NOAEL group (RGDR = 0.15 based on a gas with respiratory effects in both rats and mice) |
| *LOAEL uncertainty factor* | 1 |
| *Subchronic uncertainty factor* | 1 |
| *Interspecies uncertainty factor* | 3 |
| *Intraspecies uncertainty factor* | 10 |
| *Cumulative uncertainty factor* | 30 |
| *Inhalation reference exposure level* | 0.05 ppm (50 ppb, 0.2 mg/m$^3$, 200 μg/m$^3$) |

The chronic REL is the U.S. EPA RfC (U.S. EPA, 1995) for vinyl acetate. Acetaldehyde, a hydrolysis product of vinyl acetate, was present in the Owen (1988) study at a concentration of 49 ppm (89 mg/m$^3$). The duration-adjusted concentration for acetaldehyde was 16 mg/m$^3$, whereas the NOAEL for histological lesions in rats by Appleman *et al.* (1982) was 48.75 mg/m$^3$ acetaldehyde. Therefore, the concentration of acetaldehyde was not considered to account for significant irritation in the Owen (1988) study. OEHHA accepted the U.S. EPA analysis.

For comparison, Irvine (1980) obtained a NOAEL of 198 ppm for fetotoxicity in rats exposed 6 hours/day on days 6-15 of gestation. This is equivalent to 50 ppm continuous exposure during development. Multiplying by an RGDR of 1 and dividing by a total UF of 30 (3 for interspecies and 10 for intraspecies) results in a REL estimate based on fetotoxicity of 1.7 ppm. The results of Hurtt *et al*. (1995) also yield an estimate of 1.7 ppm.

## VII. Data Strengths and Limitations for Development of the REL

The strengths of the inhalation REL for vinyl acetate include the availability of controlled exposure lifetime inhalation studies in multiple species at multiple exposure concentrations and with adequate histopathological analysis, and the observation of a NOAEL. The major area of uncertainty is the lack of adequate human exposure data.

## VIII. Potential for Differential Impacts on Children's Health

Since the chronic REL (0.05 ppm) is lower than the comparison estimate based on developmental effects (1.7 ppm), the REL is likely to be protective of children. However, there is no direct evidence in the literature to quantify a differential effect of vinyl acetate in infants and children relative to adults.

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*CHRONIC TOXICITY SUMMARY*

# XYLENES

*(Xylol or commercial xylenes (mixture of 60-70% m- and remaining percentage is mix of o- and p- xylenes), technical grade xylenes or mixed xylenes (20% o-xylene, 40% m-xylene, 20% p-xylene, 20% ethyl benzene, and traces of toluene and C9 aromatics), o-xylene (1,2-dimethylbenzene or 2-xylene), m-xylene (1,3-dimethylbenzene or 3-xylene), p-xylene (1,4-dimethylbenzene or 4-xylene), also noted as methyltoluene, benzene-dimethyl, dimethylbenzene)*

**CAS Registry Numbers.: 1330-20-7 (technical mixture of o-, p-, and m-xylene); 95-47-6 (o-xylene); 108-38-3 (m-xylene); 106-42-3 (p-xylene)**

## I. Chronic Toxicity Summary

| | |
|---|---|
| *Inhalation reference exposure level* | **700 µg/m$^3$ (200 ppb)** (for technical or mixed xylenes or sum of individual isomers of xylene) |
| *Critical effect(s)* | CNS effects in humans; irritation of the eyes, nose, and throat |
| *Hazard index target(s)* | Nervous system; respiratory system |

## II. Physical and Chemical Properties (ATSDR, 1995; HSDB, 1995; CRC, 1994)

| | |
|---|---|
| *Description* | Colorless liquid |
| *Molecular formula* | $C_8H_{10}$ |
| *Molecular weight* | 106.16 g/mol |
| *Density* | 0.864 g/cm$^3$ @ 20°C(technical mixture); 0.881 (o-); 0.860 (m-); 0.861 (p-) |
| *Boiling point* | 137-140°C @ 760 TorrHg (technical mixture); 144.5 °C (o-); 139.1°C (m-); 138.3 °C (p-) |
| *Melting point* | –25.2 °C (o-); –47.8°C (m-); +13.2 °C (p-) |
| *Vapor pressure* | 6.6 torr (o-); 8.39 torr (m-); 8.87 torr (p-) all @ 25°C. |
| *Solubility* | Practically insoluble in water; miscible with absolute alcohol, ether and many other organic solvents |
| *Conversion factor* | 1 ppb = 4.34 µg/m$^3$ |

## III. Major Uses or Sources

Mixtures of o-, p-, and m-xylenes are extensively used in the chemical industry as solvents for products including paints, inks, dyes, adhesives, pharmaceuticals, and detergents (HSDB, 1995). In the petroleum industry xylenes are used as antiknock agents in gasoline, and as an intermediate in synthetic reactions. Of the three isomers, p-xylene is produced in the highest

quantities in the U.S. for use in the synthesis of phthalic, isophthalic, and terephthalic acid used in manufacture of plastics and polymer fibers including mylar and dacron. In 1996, the latest year tabulated, the statewide mean outdoor monitored concentration of meta/para-xylene was approximately 1 ppb (CARB, 1999a). The annual statewide emissions from facilities reporting under the Air Toxics Hot Spots Act in California based on the most recent inventory were estimated to be 3,568,318 pounds of xylenes (CARB, 1999b). Also reported were speciated emissions of p-xylene - 51,203 pounds, of o-xylene - 34,573 pounds, and of m-xylene - 30,440 pounds. (Xylenes are also present in motor vehicle exhaust.)

## IV. Effects of Human Exposure

Information on the toxicity of xylenes to humans is almost exclusively limited to case reports of acute exposures and studies of occupational exposures in which persons often inhaled a mixture of hydrocarbon solvents 8 hours per day, 5-6 days per week. These studies often have incomplete information on the airborne concentrations of xylene and other hydrocarbons. One study examining chronic effects in humans from inhalation of predominantly mixed xylenes was identified (Uchida *et al.*, 1993) and one 4-week controlled exposure study examining the effects of p-xylene exclusively was identified (Hake *et al.*, 1981). No studies examining the chronic effects of oral or dermal xylene exposure in humans were identified.

Pharmacokinetic studies have documented the absorption of xylene in humans through inhalation, oral, and dermal routes of exposure. Approximately 60% of inspired xylene is retained systemically (Sedivec and Flek, 1979). The majority of ingested xylene (~90%) is absorbed into the systemic circulation (ATSDR, 1995). Xylene is also absorbed dermally; the rate of absorption of xylene vapor is estimated as 0.1-0.2% of that by inhalation (Riihimaki and Pfaffli, 1978). Loizou *et al*. (1999) exposed human volunteers to 50 ppm *m*-xylene for 4 hours and determined that the dermal route of exposure contributed 1.8% of the total body burden. Measurement of the rate of absorption through direct contact with the skin produced variable results ranging from 2 $\mu g/cm^2/min$ (Engstrom *et al.*, 1977) to 75-160 $\mu g/cm^2/min$ (Dutkiewicz and Tyras, 1968).

Xylene exposure has been associated with effects in a number of organ systems including the lungs, skin and eyes; neurological system; heart and gastrointestinal system; kidney; and possibly the reproductive system.

Pulmonary effects have been documented in occupational exposures to undetermined concentrations of mixed xylenes (and other solvents) and include labored breathing and impaired pulmonary function (Hipolito 1980; Roberts *et al.*, 1988). High levels of xylene exposure for short periods are associated with irritation of the skin, eyes, nose and throat (ATSDR, 1995). Chronic exposure to xylenes has been associated with eye and nasal irritation (Uchida *et al.*, 1993).

The central nervous system is affected by both short term and long term exposure to high concentrations of xylene with: 100-200 ppm associated with nausea and headache; 200-500 ppm with dizziness, irritability, weakness, vomiting, and slowed reaction time; 800-10,000 ppm with

lack of muscle coordination, giddiness, confusion, ringing in the ears, and changes in sense of balance; and >10,000 ppm with loss of consciousness (HESIS, 1986). Other documented, neurological effects include impaired short term memory, impaired reaction time, performance decrements in numerical ability, and impaired equilibrium (dizziness) and balance (Carpenter *et al.*, 1975; Dudek *et al.*, 1990; Gamberale *et al.*, 1978; Riihimaki and Savolainen, 1980; Savolainen and Linnavuo, 1979; Savolainen and Riihimaki 1981; Savolainen *et al.*, 1979; 1984; 1985).

Chronic exposure to xylenes (with other hydrocarbons) has been associated with cardiovascular and gastrointestinal effects. Heart palpitations, chest pain, and abnormal electrocardiogram were noted (Hipolito, 1980; Kilburn *et al.*, 1985) as were effects on the gastrointestinal system producing nausea, vomiting and gastric discomfort in exposed workers (Goldie, 1960; Hipolito, 1980; Uchida *et al.*, 1993; Klaucke *et al.*, 1982; Nersesian *et al.*, 1985).

Results of studies of renal effects of xylene are mixed and come from case reports and occupational studies where multiple chemical exposures are common. The effects from subchronic exposure documented by Hake *et al.* (1981) and from chronic exposure documented by Uchida *et al.* (1993) did not include renal effects. However, Morley *et al.* (1970) found increased BUN and decreased creatinine clearance; Martinez *et al.* (1989) found distal renal tubular acidemia; Franchini *et al.* (1983) found increased levels of urinary β-glucuronidase; and Askergren (1981, 1982) found increased urinary excretion of albumin, erythrocytes, and leukocytes.

Reproductive effects were documented by Taskinen *et al.* (1994) who found increased incidence of spontaneous abortions in 37 pathology and histology workers exposed to xylene and formaldehyde in the work place. The multiple chemical exposures and the small number of subjects in this study limit the conclusions that can be drawn as to reproductive effects of xylene in humans.

No hematological effects have been identified in studies where exposure was to xylene only. Previous studies identifying hematological effects included known or suspected exposure to benzene (ATSDR, 1995; ECETOC, 1986). One series of case reports identified lowered white cell counts in two women with chronic occupational exposure to xylene (Hipolito, 1980; Moszczynsky and Lisiewicz, 1983; 1984), although they may also have had multiple chemical exposures.

Groups of male volunteers (1 to 4 subjects/group) were exposed to p-xylene in a controlled-environment chamber for 7.5, 3, or 1 hr/day, 5 days/week for 4-weeks (Hake *et al*., 1981). The p-xylene concentration was changed on a weekly basis starting at 100 ppm the first week, followed by 20 ppm, 150 ppm, and 100 ppm (average, with a range of 50 to 150 ppm) over subsequent weeks. In addition, groups of female volunteers (2 or 3/group) were exposed to 100 ppm p-xylene for 7.5, 3, or 1 hr/day for 5 days. The volunteers acted as their own controls, with exposure to 0 ppm p-xylene occurring for two days (males) or one day (females) the week before and the week after the xylene exposures. No serious subjective or objective health responses, including neurological tests, cognitive tests and cardiopulmonary function tests were observed. Odor was noted, but the intensity decreased usually within the first hour of exposure. The

authors concluded that p-xylene may have a weak irritating effect on the soft tissues starting at 100 ppm, but overall, the small sample size and high variability among the volunteers made all results difficult to interpret.

The Uchida *et al.* (1993) study included a relatively large number of workers studied, exposure for an average of 7 years to xylenes predominately and a comprehensive set of medical examinations to document potential effects. A survey of 994 Chinese workers involved in the production of rubber boots, plastic coated wire and printing processes employing xylene solvents was carried out. The survey consisted of fitting individual workers with diffusive samplers for an 8 hour shift. At the end of the 8 hour shift the samplers were recovered for analysis of solvent exposure, and urine samples were collected for analysis of xylene metabolites. The following day workers answered a questionnaire concerning subjective symptoms, and blood and urine were collected for analysis. Out of this group of xylene-exposed workers, 175 individuals (107 men and 68 women) were selected for further study and analysis based on completion of their health examinations and on results from diffusive samplers showing that xylene constituted 70% or more of that individual's exposure to solvents in the workplace. The control population consisted of 241 (116 men and 125 women) unexposed workers from the same factories or other factories in the same region, of similar age distribution, of similar time in this occupation (average of 7 years), and having a similar distribution of alcohol consumption and cigarette usage. The xylene-exposed and unexposed groups were given health examinations which evaluated hematology (red, white, and platelet cell counts, and hemoglobin concentration), serum biochemistry (albumin concentration, total bilirubin concentration, aspartate aminotransferase, alanine aminotransferase, gamma-glutamyl transferase, alkaline phosphatase, leucine aminopeptidase, lactate dehydrogenase, amylase, blood urea nitrogen, creatinine), and subjective symptoms (survey of symptoms occurring during work and in the previous three months).

Results of analysis of the diffusive samplers showed that workers were exposed to a geometric mean of 14.2 ± 2.6 ppm xylene (arithmetic mean of 21.3 ± 21.6 ppm). This was broken down into geometric means of 1.2 ppm o-xylene, 7.3 ppm m-xylene, 3.8 ppm p-xylene, 3.4 ppm ethyl benzene, and 1.2 ppm toluene. N-Hexane was rarely present and no benzene was detected. Analysis of data from the health examinations found no statistically significant difference ($p<0.10$) between hematology and serum biochemistry values for xylene-exposed and unexposed populations. The frequency of an elevated ratio of aspartate aminotransferase to alanine transferase and of elevated ratio of alkaline phosphatase to leucine aminopeptidase was significantly ($p<0.01$) higher in exposed men than in the control population of men. Results of the survey of subjective symptoms found differences in symptoms occurring during work and during a similar analysis over the preceding three month period, apparently related to effects on the central nervous system and to local effects on the eyes, nose and throat. The frequency of five symptoms experienced during work was significantly ($p<0.01$) elevated in either xylene-exposed men or women including: dimmed vision, unusual taste, dizziness, heavy feeling in the head, and headache. The frequency of four symptoms experienced during work were significantly ($p<0.01$) elevated in both men and women including irritation in the eyes, nasal irritation, sore throat, and floating sensation. Ten subjective symptoms occurring in the previous three months were significantly ($p<0.01$) elevated in exposed men and women including nausea, nightmare, anxiety, forgetfulness, inability to concentrate, fainting after suddenly standing up,

poor appetite, reduced grasping power, reduced muscle power in the extremities, and rough skin. Dose dependency appeared to exist for 3 subjective symptoms noted during work: irritation in the eyes, sore throat, floating sensation, and for one symptom occurring in the last three months, poor appetite.

## V. Effects of Animal Exposure

A limited number of chronic toxicity studies are available for xylene including two inhalation studies with o-xylene (Tatrai *et al.*, 1981; Jenkins *et al.*, 1970) and one oral chronic study with mixed xylenes (NTP, 1986). No chronic dermal studies could be identified. A spectrum of adverse effects has been documented in shorter term studies which potentially could occur with chronic exposure. These studies are presented here along with a brief description of the three chronic studies identified. Xylene affects a number of organ systems including the pulmonary system, the cardiovascular system, the gastrointestinal system, the hepatic system, the renal system, the dermis, and the eye, and it has numerous neurological effects and developmental effects.

Animal data are consistent with human data in documenting respiratory effects from xylene exposure. Acute and subacute exposures in mice, rats, and guinea pigs have been associated with decreased metabolic capacity of the lungs; decreased respiratory rate; labored breathing; irritation of the respiratory tract; pulmonary edema; and pulmonary inflammation (Carpenter *et al.*, 1975; De Ceaurriz *et al.*, 1981; Elovaara *et al.*, 1987; 1989; Furnas and Hine, 1958; Korsak *et al.*, 1988; 1990; Patel *et al.*, 1978; Silverman and Schatz, 1991; Toftgard and Nilsen, 1982).

Limited evidence is available in animal studies for cardiovascular effects resulting from xylene exposure. Morvai *et al.* (1976; 1987) conducted two studies. The first study observed rats following acute and intermediate duration inhalation exposure to very high (unspecified) levels of xylene and recorded ventricular repolarization disturbances, atrial fibrillation, arrhythmias, occasional cardiac arrest and changes in electrocardiogram (Morvai *et al.*, 1976). In a subsequent study morphological changes in coronary microvessels were seen in rats exposed to 230 ppm xylene (isomer composition unspecified) (Morvai *et al.*, 1987). However the chronic toxicity studies conducted by the National Toxicology Program (NTP, 1986) and by Jenkins *et al.* (1970), as well as other shorter term studies (Carpenter *et al.*, 1975; Wolfe, 1988), have not identified histopathological lesions of the heart.

Studies identifying adverse gastrointestinal effects, hematological effects, or musculoskeletal effects in animals were not identified. Studies reporting no hematological effects include Carpenter *et al.* (1975) (rats exposed to 810 ppm of mixed xylenes for 10 weeks, 5 days/week, 6 hours/day and dogs exposed for 13 weeks to 810 ppm mixed xylenes, 5 days/week, 6 hours/day) and Jenkins *et al.* (1970) (rats, guinea pigs and dogs exposed for 6 weeks to 780 ppm o-xylene, 5 days/week, 8 hours per day). Carpenter *et al.* (1975) and the NTP (1986) reported no effects on the musculoskeletal system.

Hepatic effects have been documented after acute exposure to high concentrations of xylene (2,000 ppm) or subacute exposure to lower concentrations (345-800 ppm) of mixed xylene or individual isomers. These effects include increased cytochrome P-450 and b5 content, increased hepatic weight, increased liver to body weight ratios, decreased hepatic glycogen, proliferation

of endoplasmic reticulum, changes in distribution of hepatocellular nuclei, and liver degeneration (Bowers *et al.*, 1982; Condie *et al.*, 1988; Elovaara, 1982; Elovaara *et al.*, 1980; Muralidhara and Krishnakumari 1980; Patel *et al.*, 1979; Pyykko 1980; Tatrai and Ungvary, 1980; Tatrai *et al.*, 1981; Toftgard and Nilsen, 1981; 1982; Toftgard *et al.*, 1981; Ungvary *et al.*, 1980).

Renal effects have been identified in studies with rats, guinea pigs, dogs, and monkeys exposed to 50-2,000 ppm of xylenes. These effects include increased cytochrome P-450 content and increased kidney to body weight ratios (Condie *et al.*, 1988; Elovaara 1982; Toftgard and Nilsen, 1982). Condie *et al.* (1988) also noted tubular dilation, atrophy, and increased hyaline droplets in the kidney of Sprague-Dawley rats administered 150 mg/kg/day orally of mixed xylenes. This response is consistent with early nephropathy.

Xylene has been found to affect the dermis and eyes of animals. Hine and Zuidema (1970) found skin erythema and edema, epidermal thickening, and eschar formation in response to xylene exposure. Direct instillation of xylenes into the eyes of rabbits produces eye irritation (Hine and Zuidema, 1970; Smyth *et al.*, 1962)

Numerous neurological effects have been documented in response to acute and subchronic xylene exposures ranging from 100 to 2,000 ppm. This is consistent with effects on neurofunction documented in humans. These effects include narcosis, prostration, incoordination, tremors, muscular spasms, labored respiration, behavioral changes, hyperactivity, elevated auditory thresholds, hearing loss, and changes in brain biochemistry (Andersson *et al.*, 1981; Carpenter *et al.*, 1975; De Ceaurriz *et al.*, 1983; Furnas and Hine, 1958; Ghosh *et al.*, 1987; Gralewicz *et al*., 1995; Kyrklund *et al.*, 1987; Molnar *et al.*, 1986; NTP, 1986; Pryor *et al.*, 1987; Rank 1985; Rosengren *et al.*, 1986; Savolainen and Seppalainen, 1979; Savolainen *et al.*, 1978; 1979a; Wimolwattanapun *et al.*, 1987).

Developmental effects have been documented in pregnant animals exposed to xylenes. ATSDR (1995) concluded that the body of information available for developmental effects is consistent with the hypothesis that xylene is fetotoxic and many of the fetotoxic responses are secondary to maternal toxicity. However, the ATSDR also observed that there was a large variation in the concentrations of xylene producing developmental effects and of those producing no developmental effects. The ATSDR thought that these differences were influenced by a number of factors (strain and species of animal, purity of xylene, method of exposure, exposure pattern and duration, etc.). The two most common test species have been the rat and the mouse.

With respect to rats, Mirkova *et al*. (1983) exposed groups of pregnant rats (unspecified strain of white rats) to clean air or 2.3, 12, or 120 ppm of xylene (unspecified composition) for 6 h/day on days 1-21 of gestation. They reported increased postimplantation losses and fetotoxicity (reduced fetal weights) as well as a statistically increased incidence of visceral abnormalities (including ossification defects in bones of the skull) at xylene air concentrations of 12 ppm and above. The ATSDR has suggested that the Mirkova *et al*. (1983) study results may have been influenced by poor animal husbandry as indicated by the low conception rates and the high incidence of fetal hemorrhages seen in the controls. Hass and Jakobsen (1993) attempted to replicate the findings of Mirkova *et al*. (1983). Hass and Jakobsen (1993) exposed groups of 36 pregnant Wistar rats to clean air or 200 ppm of xylene for 6 h/day on days 4-20 of gestation.

Unlike Mirakova *et al*. (1983), there was no sign of maternal toxicity and no decrease in fetal weights and no increase in soft-tissue or skeletal malformations. A large increase in the incidence of delayed ossification of the *os maxillare* of the skull, however, was observed (53% of experimental fetuses as opposed to 2% of the controls). Potential neurological/muscular changes measured as performance on a rotorod were also noted upon testing of 2-day-old rat pups.

Ungvary *et al*. (1985) exposed CFY rats by inhalation to air concentrations of xylene (60 ppm, 440 ppm, 800 ppm) for 24 h/day on days 7-15 of gestation. Maternal toxicity was described as moderate and dose-dependent. They observed weight retarded fetuses at all air concentrations. However, there was no increase in malformations, and an increase in minor anomalies and resorbed fetuses occurred only at the highest concentration. In a separate study investigating the interactions between solvents and other agents, Ungvary (1985) exposed CFY rats to either 140 ppm or 440 ppm of xylene on days 10-13 of gestation and also reported increases for either condition in weight retarded and skeletal retarded fetuses without any increase in malformations. Hudak and Ungvary (1978) had earlier examined the effect of 230 ppm xylene (24 h/day, days 9-14 of pregnancy) in the CFY rat and reported effects on skeletal development (e.g., fused sternebrae). In contrast to the other Ungvary findings, no effect on fetal weight was observed. Bio/dynamics (1983) conducted an inhalation exposure study in the rat (CrL-CD (SD) BR strain). Rats were exposed 6 h/day during premating, mating, gestation and lactation. Exposure concentrations were 0, 60, 250, and 500 ppm. Most measures for adverse effects on fetal development were not significantly increased. Mean fetal weights at the highest exposure level were lower than controls, but this difference was significant only for the female fetuses. These depressed weights were, however, still significant on day 21 of lactation. Other adverse effects (such as increased soft tissue and skeletal abnormalities, increased fetal resorptions) were not increased significantly at any of the test concentrations.

Ungvary *et al*. (1980a) tested by inhalation the individual ortho, meta, and para isomers of xylene in the CFY rat. Pregnant rats were exposed 24 h/day on days 7 –14 of pregnancy to 35, 350, or 700 ppm of each isomer. An increased incidence of weight retarded fetuses was observed for each isomer at the 700 ppm level, and for the ortho isomer at the 350 ppm level. Post implantation losses were increased only at the 700 ppm level in the para-xylene exposed group. Skeletal anomalies were increased only at the 700 ppm level for the meta and para isomers of xylene. Rosen *et al*. (1986) evaluated the effects of prenatal exposure to para-xylene in the rat. They exposed pregnant Sprague-Dawley rats by inhalation to either 800 ppm or 1600 ppm of p-xylene from days 7-16 of gestation. Despite the high concentrations, no effects were seen on litter size or weight at birth or on the subsequent growth rates of the pups.

Hass *et al.* (1995) examined postnatal development and neurobehavioral effects in rats following prenatal exposure to 0 or 500 ppm technical xylene 6 hr/day on gestation days 7-20 of pregnancy. Xylene exposure caused no signs of maternal toxicity and no difference in the number of live or dead fetuses. The mean birth weight in exposed litters was about 5% lower compared to control litters but the difference was not statistically significant. Body weights were similar between groups during the preweaning and postweaning period but lower absolute brain weights were observed in exposed animals. Exposed offspring showed a delay in the ontogeny of the air righting reflex and exhibited impaired performance in behavioral tests for neuromotor

abilities (Rotorod) and for learning and memory (Morris water maze). In a follow-up study under the same exposure conditions, exposed offspring exhibited impaired performances in the Morris water maze at 16, 28, and 55 weeks of age, although the difference was not statistically significant at 55 weeks (Hass *et al*., 1997). These data indicate that xylene exposure during development may cause long-lasting deficits on learning and memory in offspring.

With respect to mice, Ungvary *et al*. (1985) exposed CFLP mice by inhalation to air concentrations of xylene (120 ppm, 230 ppm) for 24 h/day on days 7-15 of gestation. In the mouse, they observed increased incidences of weight-retarded fetuses and increased skeletal retarded fetuses at 230 ppm. Shigeta *et al*. (1983) exposed pregnant ICR mice to approximately 0, 120, 230, 460, and 920 ppm of xylene in an exposure chamber for 6 h/day on days 6-12 of gestation. Shigeta *et al.* (1983) reported significant decreases in fetal weight in the 460 ppm and 920 ppm dose groups only. There was no difference in the number of live or dead fetuses. Decreased weight gains and delayed development of body hair and teeth were observed at the 920 ppm exposure level. Dose-response relations were reported for delayed ossification of the sternebrae. Marks *et al*. (1982) noted that 2060 mg/kg/day of mixed xylene administered orally is associated with cleft palate and decreased fetal weight in the mouse.

Ungvary *et al*. (1985) also tested the individual ortho, meta, and para isomers of xylene at 120 ppm in the CFLP mouse. Each isomer of xylene also increased the incidence of weight-retarded fetuses and skeletal retarded fetuses at 120 ppm. There was no increase in malformations.

Of the three chronic studies available (Tatrai *et al.*, 1981; Jenkins *et al.*, 1970; NTP 1986) none comprehensively examined systemic effects. The study by Tatrai *et al.* (1981) exposed rats for one year, 7 days/week, 8 hours per day to 1096 ppm o-xylene. This exposure was a LOAEL for body weight gain in males and a NOAEL for hepatic effects in male rats. Jenkins *et al.* (1970) exposed rats, guinea pigs, squirrel monkeys, and beagle dogs for 90-127 days continuously to 78 ppm of o-xylene. The study examined body weight gain; hematological parameters including white cell counts, red blood cell counts, and hematocrit; serum biochemistry including bromosulfophthalein retention, blood urea nitrogen, alanine aminotransferase, aspartate aminotransferase, alkaline phosphatase, and creatinine and liver function including alkaline phosphatase, tyrosine aminotransferase, and total lipids. No effects were observed in any of the parameters examined in this study. This study found a NOAEL for all effects examined of 78 ppm o-xylene. The NTP (1986) study administered 0, 250, or 500 mg/kg/day doses of mixed xylene in corn oil by gavage 5 days/week for 103 weeks to groups of F344/N rats of both sexes, 50 animals per group. B6C3F1 mice were treated in a similar manner but given 0, 500 or 1000 mg/kg/day of mixed xylenes in corn oil by gavage. A complete histopathological examination of all tissues was made as well as determination of body weight gain. Based on histopathology of all organ systems, a NOAEL of 500 mg/kg/day was observed for rats and a NOAEL of 1000 mg/kg/day was observed for mice.

## VI. Derivation of Chronic Reference Exposure Level (REL)

| | |
|---|---|
| *Study* | Uchida *et al.* (1993) |
| *Study population* | 175 xylene-exposed factory workers and control population of 241 factory workers |
| *Exposure method* | Inhalation |
| *Critical Effects* | Dose related increase in the prevalence of eye irritation, sore throat, floating sensation, and poor appetite. |
| *LOAEL* | 14.2 ppm (geometric mean of exposure concentrations) |
| *NOAEL* | Not applicable |
| *Exposure continuity* | 8 hr/d (10 $m^3$/day occupational inhalation rate), 5 d/wk |
| *Exposure duration* | Occupational exposure for an average of 7 years |
| *Average occupational exposure* | 5.1 ppm for LOAEL group (14.2 x 10/20 x 5/7) |
| *Human equivalent concentration* | 5.1 ppm for LOAEL group |
| *LOAEL uncertainty factor* | 3 |
| *Subchronic uncertainty factor* | 1 |
| *Interspecies uncertainty factor* | 1 |
| *Intraspecies uncertainty factor* | 10 |
| *Cumulative uncertainty factor* | 30 |
| *Inhalation reference exposure level* | 0.2 ppm (200 ppb; 0.7 mg/$m^3$; 700 µg/$m^3$) for mixed xylenes or for total of individual isomers |

A number of issues are important in considering the uncertainty associated with this REL. For ATSDR (1995) the animal and human toxicity data suggest that mixed xylenes and the different xylene isomers produce similar effects, although different isomers are not equal in potency for producing a given effect. Therefore exposure of workers to a mix of xylenes in the Uchida *et al.* (1993) study would be expected to generate a similar spectrum of responses as exposure to single isomers, however the intensity of particular effects could be different. The use of a neurological endpoint for derivation of a REL is supported by the large number of inhalation and oral studies associating neurological effects with xylene exposure. ATSDR (1995) indicates that neurological effects are a sensitive endpoint. The observation that floating sensation is apparently related to dose further supports the concept that this subjective symptom related to neurological effects was due to xylene exposure.

A UF of 3, rather than 10, was applied for the LOAEL to NOAEL extrapolation due to the generally mild adverse effects observed and the principally low incidence (<50%) of the effects. A factor of 1 was used for subchronic uncertainty. Although the average occupational exposure was only 7 years, there were 176 xylene-exposed workers of average age 29.7±9.0 years (arithmetic mean ±SD) for whom, according to the report, there had been essentially no change in workplace in their working life. Thus, many workers would likely have been exposed for more than 8.4 years, the cut-off point for chronic human exposure. Another issue is the use of diffusive samplers in the Uchida *et al.* (1993) study. These samplers provide a time weighted

average concentration of hydrocarbon and cannot indicate the maximum concentrations a worker is exposed to. It is unknown whether peak concentrations alter the response to xylenes in humans.

For comparison with the proposed REL of 200 ppb based on human studies, (1) the free-standing NOAEL of 78 ppm o-xylene obtained by Jenkins *et al*. (1970) in rats and guinea pigs continuously exposed for 90 days was used to estimate a REL based on animal data. Use of an RGDR of 1, a subchronic UF of 3, an interspecies UF of 3, and an intraspecies UF of 10 result in a REL of 800 ppb for o-xylene for systemic effects. (2) Tatrai *et al*. (1981) found a free standing LOAEL of 1096 ppm o-xylene for body weight gain in male rats exposed every day for 8 hours. Time adjustment to continuous exposure and use of an RGDR of 1, a LOAEL UF of 3 for a mild effect, an interspecies UF of 3, and an intraspecies UF of 10 result in a REL of 4000 ppb. (3) Ungvary *et al*. (1985) exposed mice by inhalation continuously to 120 ppm or 230 ppm xylene for 24 h/day on days 7-15 of gestation. The LOAEL was 230 ppm and the NOAEL was 120 ppm. No time adjustment is needed. Use of an RGDR of 1, a subchronic UF of 1, an interspecies UF of 3, and an intraspecies UF of 10 results in a REL of 4000 ppb for xylene for developmental effects.

## VII. Data Strengths and Limitations for Development of the REL

The strengths of the inhalation REL for xylene include the use of human exposure data from 175 workers exposed over a period of years. Major areas of uncertainty are the uncertainty in estimating exposure, the potential variability in exposure concentration, and the lack of observation of a NOAEL in the key study.

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