

IV. Effects of Human Exposure

Formaldehyde primarily affects the mucous membranes of the upper airways and eyes. Exposed populations that have been studied include embalmers, residents in houses insulated with urea-formaldehyde foam, anatomy class students, histology technicians, wood and pulpmill workers, and asthmatics. The voluminous body of data describing these effects has been briefly summarized below. For the sake of brevity, only the studies that best represent the given effects are presented.

Kerfoot and Mooney (1975) reported that estimated formaldehyde exposures of 0.25-1.39 ppm evoked numerous complaints of upper respiratory tract and eye irritation among 7 embalmers at 6 different funeral homes. Three of the 7 embalmers in this study reportedly had asthma. Levine *et al.* (1984) examined the death certificates of 1477 Ontario undertakers. Exposure measurements taken from a group of West Virginia embalmers were used as exposure estimates for the embalming process, ranging from 0.3-0.9 ppm (average 1-hour exposure) and 0.4-2.1 ppm (peak 30-minute exposure). Mortality due to non-malignant diseases was significantly elevated due to a two-fold excess of deaths related to the digestive system. The authors suggest increased alcoholism could have contributed to this increase.

Ritchie and Lehnen (1987) reported a dose-dependent increase in health complaints (eye and throat irritation, and headaches) in 2000 residents living in 397 mobile and 494 conventional homes, that was demonstrated by logistic regression. Complaints of symptoms of irritation were noted at concentrations of 0.1 ppm formaldehyde or above. Similarly, Liu *et al.* (1991) found that exposure to 0.09 ppm (0.135 mg/m³) formaldehyde exacerbated chronic respiratory and allergy problems in residents living in mobile homes.

Employees of mobile day-care centers (66 subjects) reported increased incidence of eye, nose and throat irritation, unnatural thirst, headaches, abnormal tiredness, menstrual disorders, and increased use of analgesics as compared to control workers (Olsen and Dossing, 1982). The mean formaldehyde concentration in these mobile units was 0.29 ppm (0.43 mg/m³) (range = 0.24 - 0.55 mg/m³). The exposed workers were exposed in these units a minimum of 3 months. A control group of 26 subjects in different institutions was exposed to a mean concentration of 0.05 ppm (0.08 mg/m³) formaldehyde.

Occupants of houses insulated with urea-formaldehyde foam insulation (UFFI) (1726 subjects) were compared with control subjects (720 subjects) for subjective measures of irritation, pulmonary function (FVC, FEV₁, FEF₂₅₋₇₅, FEF₅₀), nasal airway resistance, odor threshold for pyridine, nasal cytology, and hypersensitivity skin-patch testing (Broder *et al.*, 1988). The mean length of time of exposure to UFFI was 4.6 years. The mean concentration of formaldehyde in the UFFI-exposed group was 0.043 ppm, compared with 0.035 ppm for the controls. A significant increase in symptoms of eye, nose and throat irritation was observed in subjects from UFFI homes, compared with controls. No other differences from control measurements were observed.

An increase in severity of nasal epithelial histological lesions, including squamous epithelium, keratosis, and metaplasia of the nasal epithelium was observed in 75 wood products workers

VI. Derivation of Chronic Reference Exposure Level (REL)

<i>Studies</i>	Wilhelmsson and Holmstrom, 1992; Holmstrom and Wilhelmsson, 1988
<i>Study population</i>	Human chemical plant workers (66 subjects)
<i>Exposure method</i>	Discontinuous occupational exposure
<i>Critical effects</i>	Nasal and eye irritation, nasal obstruction, and lower airway discomfort
<i>LOAEL</i>	Mean of 0.26 mg/m ³ (range = 0.05 to 0.6 mg/m ³) (described as exposed group)
<i>NOAEL</i>	Mean of 0.09 mg/m ³ (described as control group)
<i>Exposure continuity</i>	8 hours/day, 5 days/week (assumed)
<i>Exposure duration</i>	10 years (average); range = 1-36 years
<i>Average occupational concentration</i>	0.032 mg/m ³ for NOAEL group (0.09 x 10/20 x 5/7)
<i>Human equivalent concentration</i>	0.032 mg/m ³
<i>LOAEL uncertainty factor</i>	1
<i>Subchronic uncertainty factor</i>	1
<i>Interspecies uncertainty factor</i>	1
<i>Intraspecies uncertainty factor</i>	10
<i>Cumulative uncertainty factor</i>	10
<i>Inhalation reference level</i>	0.003 mg/m ³ (3 µg/m ³ ; 0.002 ppm; 2 ppb)

Sixty-six workers in a formaldehyde-producing plant were surveyed for symptoms of upper respiratory and eye irritation, in addition to examination of serum IgE antibodies to formaldehyde. Sixty six percent of the non-atopic workers experienced general nasal discomfort compared with only 17% discomfort in the 36 controls ($p < 0.001$). In addition, the workers exposed to formaldehyde experienced significantly increased incidence of lower airway discomfort as measured by cough, wheezing, and symptoms of bronchitis ($p < 0.01$). Formaldehyde-exposed workers also had significantly increased incidence of annoying dermatitis (39%) compared with controls (8%, $p < 0.01$). Twenty four percent of the formaldehyde-exposed workers also complained of eye irritation, compared with 6% in the control group ($p < 0.05$).

The study by Horvath *et al* (1988) supports the results of the study by Wilhelmsson and Holmstrom (1992) and can be used to further support the selection of the 0.09 mg/m³ value as a NOAEL for irritation. The 254 control subjects in the Horvath *et al.* (1988) study were exposed to a mean concentration of 0.05 ppm (0.08 mg/m³). The ambient outdoor concentration of formaldehyde in this study was 0.04 ppm (0.06 mg/m³). The prevalence of symptoms in the 2 control groups appears to be similar (e.g., 17% general nasal discomfort vs. 14.2% stuffy nose, 2% burning of the nose, and 8% itching of the nose). In the Horvath *et al.* study, concentrations ranging from ambient to 0.05 ppm (0.08 mg/m³) were considered the baseline for exposure comparisons.

The strengths of the inhalation REL include the use of human exposure data from workers exposed over a period of years and the observation of a NOAEL. The major areas of uncertainty are the uncertainty in estimating exposure and the potential variability in exposure concentration.

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CHRONIC TOXICITY SUMMARY

N-HEXANE

(normal hexane)

CAS Registry Number: 110-54-3

I. Chronic Toxicity Summary

<i>Inhalation reference exposure level</i>	200 g/m³ (U.S. EPA RfC) This document summarizes the evaluation of non-cancer health effects by U.S. EPA for the RfC.
<i>Critical effect(s)</i>	Neurotoxicity; electrophysiological alterations in humans
<i>Hazard index target(s)</i>	Nervous system

II. Physical and Chemical Properties (HSDB, 1995)

<i>Description</i>	Colorless liquid, gas
<i>Molecular formula</i>	C ₆ H ₁₄
<i>Molecular weight</i>	86.10
<i>Density</i>	0.660 g/cm ³ @ 20° C
<i>Boiling point</i>	68.95°C
<i>Vapor pressure</i>	150 mm Hg @ 25° C
<i>Solubility</i>	Insoluble in water; soluble in most organic solvents; very soluble in alcohol
<i>Conversion factor</i>	1 ppm = 3.52 mg/m ³ @ 25° C

III. Major Uses or Sources

n-Hexane is used in the extraction of vegetable oil from seeds such as safflower, soybean, cotton, and flax (HSDB, 1995). It is also used as a alcohol denaturant and as a paint diluent. The textile, furniture and leather industries use n-hexane as a cleaning agent. Many petroleum and gasoline products contain n-hexane.

IV. Effects of Human Exposure

An epidemiologic study was performed on workers employed in a factory producing tungsten carbide alloys 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).

V. Effects of Animal Exposure

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 were 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.

Dose-related signs of neurotoxicity, as measured by electromyography, were observed in male mice continuously exposed to 250, 500, 1000, or 2000 ppm commercial grade hexane (65-70% n-hexane) 6 days per week for 1 year (Miyagaki, 1967). Abnormal posture and muscle atrophy were also observed in a dose-related manner in mice exposed to 250 ppm n-hexane or higher. No adverse effects were detected in the 100 ppm exposure group.

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).

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.

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). 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 U.S. EPA RfC

<i>Study</i>	Sanagi <i>et al.</i> , 1980; U.S. EPA, 1995
<i>Study population</i>	Tungsten carbide alloy production workers
<i>Exposure method</i>	Discontinuous inhalation (occupational)
<i>Critical effects</i>	Decreased motor nerve conduction velocity; increased residual latency
<i>LOAEL</i>	58 ppm (204 mg/m ³)
<i>NOAEL</i>	Not observed
<i>Exposure continuity</i>	8 hours/day (10 m ³ /day occupational inhalation rate), 5 days/week
<i>Exposure duration</i>	6.2 years
<i>Average occupational exposure</i>	20.7 ppm (73 mg/m ³) for LOAEL group
<i>Human equivalent concentration</i>	20.7 ppm (73 mg/m ³)
<i>Subchronic uncertainty factor</i>	1
<i>Interspecies uncertainty factor</i>	1
<i>Intraspecies uncertainty factor</i>	10
<i>LOAEL uncertainty factor</i>	10
<i>Modifying factor</i>	3 (database deficiencies for reproductive effects)
<i>Cumulative uncertainty factor</i>	300
<i>Inhalation reference exposure level</i>	0.2 mg/m ³ (200 µg/m ³ ; 60 ppb; 0.06 ppm)

A statistically significant decrease in mean motor nerve conduction velocity (MMCV) and a statistically significant increase in residual latency (RL) was observed in exposed workers compared to unexposed workers (Sanagi *et al.*, 1980). The LOAEL for electrophysiological alterations in exposed workers was 58 ppm n-hexane. No NOAEL was apparent from this study.

The major strength of the RfC is the use of human health effects data. The major limitations are the lack of dose-response information or the observation of a NOAEL, the uncertainties associated with the pattern and magnitude of exposures, and the limited range of possible health effects that were addressed.

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CHRONIC TOXICITY SUMMARY

HYDROGEN CHLORIDE

(Hydrochloric acid; anhydrous hydrogen chloride; muriatic acid)

CAS Registry Number: 7647-01-0

I. Chronic Reference Exposure Level

<i>Inhalation reference exposure level</i>	7 $\mu\text{g}/\text{m}^3$ (U.S. EPA RfC) This document summarizes the evaluation of non-cancer health effects by U.S. EPA for the RfC.
<i>Critical effect(s)</i>	Hyperplasia of nasal mucosa, larynx, and trachea in rats
<i>Hazard index target(s)</i>	Respiratory system

II. Physical and Chemical Properties (HSDB, 1994)

<i>Description</i>	Colorless gas
<i>Molecular formula</i>	HCl
<i>Molecular weight</i>	36.46
<i>Density</i>	1.49 g/L @ 25° C
<i>Boiling point</i>	-84.9° C (HCl gas)
<i>Melting point</i>	-114.8° C (HCl gas)
<i>Vapor pressure</i>	760 mm Hg @ -84.3° C
<i>Solubility</i>	Soluble in water, alcohol, benzene, ether; insoluble in hydrocarbons
<i>Conversion factor</i>	1 ppm = 1.49 mg/m^3 at 25°C

III. Major Uses or Sources

Hydrogen chloride (HCl) is used in the manufacture of vinyl chloride, fertilizers, dyes, artificial silk, and pigments for paints. It is also used in electroplating, soap refining, and leather tanning. Other consumers of HCl include the photographic, textile and rubber industries (HSDB, 1994).

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).

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).

V. Effects of Animal Exposure

Male rats were exposed to 10 ppm HCl 6 hours per day, 5 days per week for their lifetime (Sellakumar *et al.*, 1985). No differences in body weights or survival were observed between exposed and control animals. Increased incidences of hyperplasia of the nasal mucosa, larynx, and trachea were observed in exposed rats compared to controls.

A 90-day inhalation study in mice and rats exposed the animals to 10, 20, or 50 ppm HCl 6 hours per day, 5 days per week for 90 days (Toxigenics, 1984). 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 identified a LOAEL in 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 (Sellakumar *et al.*, 1985).

Female rats 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.

Derivation of U.S. EPA Reference Concentration

<i>Study</i>	Sellakumar <i>et al.</i> , 1985
<i>Study population</i>	Rats (100 males)
<i>Exposure method</i>	Discontinuous whole-body inhalation (0 or 10 ppm)
<i>Critical effects</i>	Hyperplasia of the nasal mucosa, larynx and trachea
<i>LOAEL</i>	10 ppm
<i>NOAEL</i>	Not identified
<i>Exposure continuity</i>	6 hours per day, 5 days per week
<i>Average experimental exposure</i>	1.8 ppm for LOAEL group
<i>Human equivalent concentration</i>	4.6 ppm (gas with extrathoracic and tracheobronchial respiratory effects, RGDR = 2.6, based on MV = 0.5 m ³ /day, SA(ET+TB) = 49.2 cm ²) (U.S. EPA, 1990)
<i>Exposure duration</i>	Lifetime
<i>LOAEL uncertainty factor</i>	10
<i>Subchronic uncertainty factor</i>	1
<i>Interspecies uncertainty factor</i>	10
<i>Intraspecies uncertainty factor</i>	10
<i>Cumulative uncertainty factor</i>	1,000
<i>Reference Concentration (RfC)</i>	0.005 ppm (5 ppb; 0.007 mg/m ³ ; 7 µg/m ³)

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).

VII. References

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CHRONIC TOXICITY SUMMARY

HYDROGEN CYANIDE

(Formonitrile; hydrocyanic acid; prussic acid)

CAS Registry Number: 74-90-8

I. Chronic Toxicity Summary

<i>Inhalation reference exposure level</i>	3 µg/m³ (U.S. EPA RfC) This document summarizes the evaluation of non-cancer health effects by U.S. EPA for the RfC.
<i>Critical effect(s)</i>	CNS effects, thyroid enlargement, and hematological disorders in workers
<i>Hazard index target(s)</i>	Nervous system; endocrine system; cardiovascular system

II. Physical and Chemical Properties (HSDB, 1994)

<i>Description</i>	Colorless liquid/gas
<i>Molecular formula</i>	HCN
<i>Molecular weight</i>	27.03
<i>Boiling point</i>	25.6 °C
<i>Melting point</i>	-13.4 °C
<i>Vapor pressure</i>	630 mm Hg @ 20°C
<i>Solubility</i>	Miscible in water, alcohol; slightly soluble in ether
<i>Conversion factor</i>	1 ppm = 1.10 mg/m ³ @ 25 °C

III. Major Uses or Sources

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

Another common source of hydrogen cyanide is cigarette smoke. Levels in inhaled mainstream cigarette smoke range from 10 to 400 µg per cigarette (U.S. brands); 0.6% to 27% (w/w) of these mainstream levels are found in secondary or sidestream smoke (Fiskel *et al.*, 1981).

IV. Effects of Human Exposure

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

El Ghawabi *et al.* (1975) studied 36 male electroplating workers in three Egyptian factories exposed to plating bath containing 3% copper cyanide, 3% sodium cyanide, and 1% sodium carbonate. Breathing zone cyanide concentrations ranged from 4.2 to 12.4 ppm (4.6 to 13.7 mg/m³), with means from 6.4 to 10.4 ppm (7.1 to 11.5 mg/m³), in the three factories at the time of this cross-sectional study. The men were exposed for a duration of 5 to 10 years, except for one man with 15 years exposure. Twenty non-exposed male volunteers were used as controls. None of the subjects, controls or workers, currently smoked cigarettes. Complete medical histories were taken, and medical exams were performed. Urinary levels of thiocyanate (a metabolite of cyanide) were utilized as a biological index of exposure. Thyroid function was measured as the uptake of radiolabeled iodine, since thiocyanate may block the uptake of iodine by the thyroid leading to iodine-deficiency goiters. Frequently reported symptoms in the exposed workers included headache, weakness, and altered sense of taste or smell. Lacrimation, abdominal colic, and lower stomach pain, salivation, and nervous instability occurred less frequently. Twenty of the thirty six exposed workers had thyroid enlargements, and the 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. This study reported a LOAEL of 6.4 ppm (7.1 mg/m³) for the CNS symptoms and thyroid effects.

Dyspnea was observed in workers chronically exposed (5 to 15 years) to 6.4 to 10.4 ppm (7.0 to 11.4 mg/m³) of an unspecified cyanide form produced from sodium cyanide and copper cyanide during electroplating (El Ghawabi *et al.*, 1975). Symptoms persisted in 50% of the dyspneic workers in a 10-month nonexposure follow up period. These cyanide levels were associated with headache, weakness, giddiness, altered taste and smell, throat irritation, vomiting, lacrimation, thyroid enlargement and hematological disorders. Thyroid enlargement to a mild or moderate degree was found in 20 workers, although there was no correlation between the duration of exposure with either the incidence or the degree of enlargement. 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 (El Ghawabi *et al.*, 1975).

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.

V. Effects of Animal Exposures

There is little animal data for chronic inhalation exposure to hydrogen cyanide; only two subchronic studies were noted by U.S. EPA, one in rabbits (Hugod, 1979, 1981) and the other in dogs (Valade, 1952). Continuous exposure of rabbits to 0.5 ppm HCN (0.55 mg/m³) for either 1 or 4 weeks produced no microscopically detectable morphological changes of the lungs, pulmonary arteries, coronary arteries or aorta. This study observed a subacute inhalation NOAEL for HCN in rabbits of 0.5 ppm (Hugod, 1979, 1981). Four dogs exposed to 50 mg/m³ (45 ppm) hydrogen cyanide in a series of 30-minute inhalation periods conducted at 2-day intervals demonstrated extensive CNS toxicity, including dyspnea and vomiting, with vascular and cellular CNS lesions identified post-mortem (Valade, 1952).

No information was found regarding developmental and reproductive effects in humans for any route of hydrogen cyanide exposure. No animal studies utilizing inhalation or dermal exposure have been reported for either hydrogen cyanide or cyanide salts. Naturally occurring plant cyanogenic glycosides produce hydrogen cyanide when hydrolyzed. 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 U.S. EPA RfC

<i>Study</i>	El Ghawabi <i>et al.</i> (1975); U.S. EPA (1994)
<i>Study population</i>	36 male electroplating workers
<i>Exposure method</i>	Discontinuous occupational inhalation exposures
<i>Critical effects</i>	CNS effects, thyroid enlargement, and hematological disorders
<i>LOAEL</i>	7.1 mg/m ³
<i>NOAEL</i>	Not observed
<i>Exposure continuity</i>	8 hr/day (10/20 m ³ /day), 5 days/week
<i>Average occupational exposure</i>	2.5 mg/m ³ for LOAEL group
<i>Human equivalent concentration</i>	2.5 mg/m ³ for LOAEL group
<i>Exposure duration</i>	5 to 15 years
<i>LOAEL uncertainty factor</i>	10
<i>Subchronic uncertainty factor</i>	3
<i>Interspecies uncertainty factor</i>	1
<i>Intraspecies uncertainty factor</i>	10
<i>Modifying factor</i>	3 (lack of chronic and multi-generational reproduction studies)
<i>Cumulative uncertainty factor</i>	1,000
<i>Inhalation reference exposure level</i>	0.003 ppm (3 ppb, 0.003 mg/m ³ , 3 µg/m ³)

U.S. EPA used a 3-fold subchronic uncertainty factor because the exposures continued over a significant fraction of an average human lifetime (>20% in some subjects).

The major strength of the RfC 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

(sulfur hydride; sulfuretted hydrogen; H₂ S)

CAS registry number: 7783-06-4

I. Chronic Toxicity Summary

<i>Inhalation reference exposure level</i>	9 g/m³
<i>Critical effect(s)</i>	Nasal histological changes in B6C3F1 mice
<i>Hazard index target(s)</i>	Respiratory system

II. Physical and Chemical Properties (AIHA, 1991)

<i>Description</i>	Colorless gas
<i>Molecular formula</i>	H ₂ S
<i>Molecular weight</i>	34.08
<i>Density</i>	1.4 g/L @ 25° C (air = 1)
<i>Boiling point</i>	-60.7° C
<i>Vapor pressure</i>	1 atmosphere @ -60.4° C
<i>Solubility</i>	Soluble in water, hydrocarbon solvents, ether, and ethanol
<i>Odor threshold</i>	8.1 ppb (11 µg/m ³) (Amoore and Hautala, 1985)
<i>Odor description</i>	Resembles rotten eggs
<i>Conversion factor</i>	1 ppm = 1.4 mg/m ³ @ 25° C

III. Major Uses or Sources

Hydrogen sulfide (H₂S) is used as a reagent and an intermediate in the preparation of other reduced sulfur compounds. 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).

IV. Effects of Human Exposure

Although numerous case studies of acutely toxic effects of H₂S exist, there is inadequate occupational or epidemiological information for specific chronic effects in humans exposed to H₂S.

Bhambhani and Singh (1991) showed that 16 healthy subjects exposed for short durations to 5 ppm (7 mg/m³) H₂S under conditions of moderate exercise exhibited impaired lactate and oxygen uptake in the blood. Bhambhani and Singh (1985) reported that exposure of 42 individuals to 2.5 to 5 ppm (3.5 to 7 mg/m³) H₂S caused coughing and throat irritation after 15 minutes.

In another study, ten asthmatic volunteer subjects were exposed to 2 ppm H₂S for 30 minutes and pulmonary function was tested (Jappinen *et al.*, 1990). All subjects reported detecting “very unpleasant” odor but “rapidly became accustomed to it.” Three subjects reported headache following exposure. No significant changes in mean FVC or FEV₁ were reported. Although individual values for specific airway resistance (SR_{aw}) were not reported, the difference following exposure ranged from -5.95% to +137.78%. The decrease in specific airway conductance, SG_{aw}, ranged from -57.7% to +28.9%. The increase in mean SR_{aw} and decrease in mean SG_{aw} were not statistically significant.

Kilburn and Warshaw (1995) investigated whether people exposed to sulfide gases, including H₂S, as a result of working at or living downwind from the processing of "sour" crude oil demonstrated persistent neurobehavioral dysfunction. They studied thirteen former workers and 22 neighbors (of a California coastal oil refinery) who complained of headaches, nausea, vomiting, depression, personality changes, nosebleeds, and breathing difficulties. Their neurobehavioral functions and a profile of mood states were compared to 32 controls (matched for age and educational level). The exposed subjects' mean values were statistically significantly different (abnormal) compared to controls for several tests (two-choice reaction time; balance (as speed of sway); color discrimination; digit symbol; trail-making A and B; immediate recall of a story). Their profile of mood states scores were much higher than those of controls. Visual recall was significantly impaired in neighbors, but not in the former workers. The authors concluded that neurophysiological abnormalities were associated with exposure to reduced sulfur gases, including H₂S from crude oil desulfurization.

Xu *et al.* (1998) conducted a retrospective epidemiological study in a large petrochemical complex in Beijing, China in order to assess the possible association between petrochemical exposure and spontaneous abortion. The facility consisted of 17 major production plants divided into separate workshops, which allow for the assessment of exposure to specific chemicals. Married women (n = 2853), who were 20-44 years of age, had never smoked, and who reported at least one pregnancy during employment at the plant, participated in the study. According to their employment record, about 57% of these workers reported occupational exposure to petrochemicals during the first trimester of their pregnancy. There was a significantly increased risk of spontaneous abortion for women working in all of the production plants with frequent exposure to petrochemicals compared with those working in nonchemical plants. Also, when a comparison was made between exposed and non-exposed groups within each plant, exposure to petrochemicals was consistently associated with an increased risk of spontaneous abortion (overall odds ratio (OR) = 2.7 (95% confidence interval (CI) = 1.8 to 3.9) after adjusting for potential confounders). When the analysis was performed with the exposure information obtained from interview responses for (self reported) exposures, the estimated OR for spontaneous abortions was 2.9 (95% CI = 2.0 to 4.0). When the analysis was repeated by excluding those 452 women who provided inconsistent reports between recalled exposure and

work history, a comparable risk of spontaneous abortion (OR 2.9; 95% CI = 2.0 to 4.4) was found. In analyses for exposure to specific chemicals, an increased risk of spontaneous abortion was found with exposure to most chemicals. There were 106 women (3.7% of the study population) exposed only to hydrogen sulfide, and the results for hydrogen sulphide (OR 2.3; 95% CI = 1.2 to 4.4) were significant. No hydrogen sulfide exposure concentration was reported.

V. Effects of Animal Exposure

Rats (Fischer and Sprague-Dawley, 15 per group) were exposed to 0, 10.1, 30.5, or 80 ppm (0, 14.1, 42.7, or 112 mg/m³, respectively) H₂S for 6 hours/day, 5 days/week for 90 days (CIIT, 1983a,b). Measurements of neurological and hematological function revealed no abnormalities due to H₂S exposure. A histological examination of the nasal turbinates also revealed no significant exposure-related changes. A significant decrease in body weight was observed in both strains of rats exposed to 80 ppm (112 mg/m³).

In a companion study, the Chemical Industry Institute of Toxicology conducted a 90-day inhalation study in mice (10 or 12 mice per group) exposed to 0, 10.1, 30.5, or 80 ppm (0, 14.1, 42.7, or 112 mg/m³, respectively) H₂S for 6 hours/day, 5 days/week (CIIT, 1983c). Neurological function was measured by tests for posture, gait, facial muscle tone, and reflexes. Ophthalmological and hematological examinations were also performed, and a detailed necropsy was included at the end of the experiment. The only exposure-related histological lesion was inflammation of the nasal mucosa of the anterior segment of the noses of mice exposed to 80 ppm (112 mg/m³) H₂S. Weight loss was also observed in the mice exposed to 80 ppm. Neurological and hematological tests revealed no abnormalities. The 30.5 ppm (42.5 mg/m³) level was considered the NOAEL for histological changes in the nasal mucosa. (Adjustments were made by U. S. EPA to this value to calculate an RfC of 0.9 µg/m³.)

Male rats were exposed to 0, 10, 200, or 400 ppm H₂S for 4 hours (Lopez *et al.*, 1987). Samples of bronchoalveolar and nasal lavage fluid contained increased inflammatory cells, protein, and lactate dehydrogenase in rats treated with 400 ppm. Lopez and associates later showed that exposure to 83 ppm (116 mg/m³) for 4 hours resulted in mild perivascular edema (Lopez *et al.*, 1988).

A study by Saillenfait *et al.* (1989) investigated the developmental toxicity of H₂S in rats exposed 6 hours/day on days 6 through 20 of gestation to concentrations up to 150 ppm (210 mg/m³). No developmental defects were observed at any concentration of H₂S. However, maternal weight gain was depressed at 150 ppm (210 mg/m³). No maternal effects were noted at 100 ppm (140 mg/m³).

VI. Derivation of Chronic REL

<i>Study</i>	CIIT, 1983c
<i>Study population</i>	B6C3F1 mice (10-12 per group)
<i>Exposure method</i>	Discontinuous inhalation
<i>Critical effects</i>	Histopathological inflammatory changes in the nasal mucosa
<i>LOAEL</i>	80 ppm (112 mg/m ³)
<i>NOAEL</i>	30.5 ppm (42.5 mg/m ³)
<i>Exposure continuity</i>	6 hours/day, 5 days/week
<i>Exposure duration</i>	90 days
<i>Average experimental exposure</i>	5.4 ppm for NOAEL group
<i>Human equivalent concentration</i>	0.66 ppm (gas with extrathoracic respiratory effects, RGDR = 0.12, based on mouse MV = 0.04 m ³ , SA(ET) = 2.9 cm ²)
<i>LOAEL uncertainty factor</i>	1
<i>Subchronic uncertainty factor</i>	3
<i>Interspecies uncertainty factor</i>	3
<i>Intraspecies uncertainty factor</i>	10
<i>Cumulative uncertainty factor</i>	100
<i>Inhalation reference exposure level</i>	7 ppb (9 µg/m ³)

The adverse effects reported in chronic animal studies occur at higher concentrations than effects seen in acute human exposures. For example, human irritation was reported at concentrations of 2.5-5 ppm for 15 minutes (Bhambhani and Singh, 1985), yet no effects on laboratory animals were observed at concentrations up to 80 ppm for 90 days. This suggests either that humans are more sensitive to H₂S, or that the measurements in laboratory animals are too crude to detect subtle measures of irritation. However, the uncertainty factor and HEC attempt to account for these interspecies differences.

U.S.EPA used the CIIT (1983) study as a basis of an RfC. In the IRIS narrative for H₂S, USEPA justified use of a factor of 3 for subchronic to chronic exposure extrapolation. “It is concluded that progression of lesions on inhalation of hydrogen sulfide progresses minimally between acute and subchronic durations in rats. It is reasonable to extend this conclusion to mice and to the subchronic-to-chronic time frame because the effect is likely to be a nonspecific reactivity, rather than some other mechanism that might have some aspect of species specificity. On this basis, the standard uncertainty factor of 10 for subchronic-to-chronic extrapolation is reduced by half to a threefold factor.” OEHHA accepted this rationale and chose to use a UF of 3 for subchronic to chronic extrapolation in this instance. However, U.S. EPA actually used a subchronic UF of 10 in its final calculation of the RfC.

In addition USEPA used a modifying factor of 3 due to a “lack of reproductive and developmental toxicity data.” OEHHA has not generally used modifying factors in deriving RELs. Use of a modifying factor in this case is now unnecessary since there is not a lack of reproductive and developmental data. The Xu *et al.* (1998) study on spontaneous abortions in

humans was not available when USEPA published its RfC. The study in animals by Saillenfait *et al.* (1989) is summarized above. In addition, other studies on the reproductive and developmental toxicity of hydrogen sulfide in animals are summarized and referenced in the RfC documentation (Hannah and Roth, 1991; Hannah *et al.*, 1989; Hayden *et al.*, 1990).

The strengths of the inhalation REL include the availability of controlled exposure inhalation studies in multiple species at multiple exposure concentrations, adequate histopathological analysis, and the observation of a NOAEL. The REL is very close to the the level of 7 µg/m³ estimated by a Task Force of the International Programme on Chemical Safety (1981) to not produce odor nuisance in most situations (although the value was based on a 30 minute averaging time). A major area of uncertainty is the lack of adequate long-term human exposure data.

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CHRONIC TOXICITY SUMMARY

ISOPROPANOL

(2-propanol; dimethylcarbinol; isopropyl alcohol)

CAS Registry Number: 67-63-0

I. Chronic Toxicity Summary

<i>Inhalation reference exposure level</i>	7,000 g/m³
<i>Critical effect(s)</i>	Kidney lesions in mice and rats
<i>Hazard index target(s)</i>	Kidney

II. Chemical Property Summary (HSDB, 1995)

<i>Description</i>	Colorless liquid at room temperature (25°C) with a pleasant odor. Slightly bitter taste.
<i>Molecular formula</i>	C ₃ H ₈ O
<i>Molecular Weight</i>	60.09
<i>Boiling point</i>	82.5°C
<i>Vapor Pressure</i>	44.0 mm Hg at 25°C
<i>Solubility</i>	Miscible in water and most organic solvents; insoluble in salt solutions.
<i>Conversion factor</i>	1 ppb = 2.45 µg/m ³ at 25°C

III. Major Uses and Sources

Isopropyl alcohol is used as a solvent and in making many commercial products (HSDB, 1995). The annual production volume of isopropyl alcohol has been in excess of one billion pounds since 1956; it was ranked 50th among chemicals produced in the U.S. in 1994 (C&EN, 1995). Rubbing alcohol is a solution of 70% isopropyl alcohol 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. Isopropyl alcohol 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 isopropyl alcohol as a volatile solvent. An odor threshold has been estimated as 22 ppm (Amoore and Hautala, 1983), which is 3-fold higher than the chronic REL proposed.

IV. Effects of Human Exposures

Currently, there are no adequate chronic exposure data in humans. However, many other solvents and petroleum-based chemicals have been shown to cause brain or other nerve damage with prolonged exposure. While isopropyl alcohol 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 isopropyl alcohol (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 isopropyl alcohol was exhaled as acetone, CO₂ and the unmetabolized alcohol (Slauter *et al.*, 1994). Approximately 3-8% of the administered dose was excreted in urine as isopropyl alcohol, acetone, and a metabolite tentatively identified as isopropyl glucuronic acid. Isopropyl alcohol is readily absorbed from the GI tract and persists in the circulation longer than ethyl alcohol. Alcohol dehydrogenase oxidizes most isopropyl alcohol to acetone. Acetone may be further metabolized to acetate, formate, and finally CO₂. In another metabolism study, the amount of acetone in the blood stream was found to be directly related to the air concentration of isopropyl alcohol (Laham *et al.*, 1980). This finding indicated that the acetone metabolite could be used as a biochemical indicator of isopropyl alcohol 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 isopropyl alcohol (Burleigh-Flayer *et al.*, 1994). Three different routes of exposure have been used by researchers for isopropyl alcohol toxicity studies: inhalation, oral gavage and presence in drinking water. The following 5 subchronic studies exposed experimental animals to isopropyl alcohol by the inhalation route:

The most comprehensive recent report investigated subchronic and neurobehavioral endpoints in rats and mice following 13-week inhalation exposure (6 hr/day, 5 days/week) to 0, 100, 500, 1500 or 5000 ppm isopropyl alcohol (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 isopropyl alcohol 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 this microscopic change was most pronounced in the 5000 ppm group. The hyaline droplets found in kidneys of male rats have been shown to be a male rat-specific phenomenon and is not considered to be relevant to human risk assessment (Phillips and Cockrell, 1984; Beyer, 1992).

In a similar 13-week behavioral/neurotoxicity study by the same investigators, increased motor activity in female Fischer 344 rats was also seen during exposure to 5000 ppm (12,300 mg/m³) isopropyl alcohol (Union Carbide Corp., 1990). Increased motor activity was characterized as the summation of ambulation, rearing and fine movements. Complete recovery was apparent within 42 days post-exposure. Other effects included a significant increase in body weight and an increased incidence of swollen periocular tissue in isopropyl alcohol-exposed animals.

In a study conducted to investigate neurochemical and behavioural effects, 20 male Wistar rats/group were exposed to 0 or 300 ppm isopropyl alcohol 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). Isopropyl alcohol 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 isopropyl alcohol. 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 isopropyl alcohol 7 hr/day on gestation days 1-19 (Nelson *et al.*, 1988). At the highest exposure level, maternal body-weight gain and food consumption were reduced. Narcosis was also evident. At 7000 ppm isopropyl alcohol, only body-weight gain was slightly reduced. Increased fetal resorptions and reduced fetal weights (41%) occurred at the highest exposure level. Fetal weights were also significantly reduced at 7000 ppm (15%) and at 3500 ppm (4%), showing a dose-dependent relationship. Skeletal malformations 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.

In 1997 an inhalation study spanning the lifetime of rats and mice was published. 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, 2,500, or 5,000 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 5,000 ppm group. Increases in body weight and/or body weight gain were observed for both sexes of mice and rats from the 2,500 and 5,000 ppm groups throughout the study. Urinalysis and changes in urine chemistry, indicative of impaired kidney function, were noted for male rats in the 2,500 ppm group and for male and female rats in the 5,000 ppm group. At necropsy, an increased incidence of seminal vesicle enlargement was observed grossly for male mice in the 2,500 and 5,000 ppm groups. Some of the lesions included an increased incidence of ectasia of the seminal vesicles for male mice in the 2,500 and 5,000 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. In rats a number of lesions were observed in males and females in the 2500 and 5000 ppm groups. The most significant lesions were observed in the kidney, were associated with chronic renal disease, and included, in order of increased severity and/or frequency, 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 5,000 ppm and to account for much of the mortality observed for male rats exposed to 2,500 ppm. The no-observed-adverse-effect level (NOAEL) for toxic effects in the kidney for both rats and mice was 500 ppm. Unlike the subchronic study, anemia was not observed in the chronic study. (The study was designed mainly to investigate the neoplastic effects of isopropanol. However, the only tumor type increased in incidence was interstitial cell adenoma of the testes in male rats. The authors did not believe it to be exposure-related since there was an unusually low incidence of the adenomas observed in the control group.)

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 isopropyl alcohol 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 isopropyl alcohol 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 were 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

isopropyl alcohol 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 isopropyl alcohol.

In a multi-generation study carried out to investigate potential reproductive and developmental effects of isopropyl alcohol, Sprague-Dawley rats were administered 0, 100, 500 or 1000 mg/kg body wt-day of isopropyl alcohol by oral gavage (Beyer, 1992). P1 and P2 rats were dosed daily for 10 weeks prior to mating and throughout the mating, 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 isopropyl alcohol were not seen at any dose level. Statistically significantly reduced 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 isopropyl alcohol to experimental animals in drinking water:

In a study designed to investigate neurotoxicological effects, 22 male SPF rats/group were administered isopropyl alcohol in drinking water at concentrations of 0, 1, 2, 3, 4 or 5% (w/v) for 12 weeks (Pilegaard and Ladefoged, 1993). Average daily intake 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% isopropyl alcohol in water for up to 18 weeks (USEPA/OTS, 1986). The doses are equivalent to 0, 325, 711, 1002, and 1176 mg/kg body wt-day, respectively, for males; to 0, 517, 1131, 1330, and 1335 mg/kg body wt-day, respectively, for females during the pre-mating phase; and to 0, 1167, 2561, 2825, and 2722 mg/kg body wt-day, respectively, in females during the post-partum phase. Exposure periods were: 70 days pre-mating, plus 15 days during mating, plus 42 days for males; 21 days pre-mating plus 15 days during mating, plus 21 days gestation, plus 21 days rearing in females; and 21 days for the F₁ generation. At the highest two levels, body weights of males during the first two weeks were reduced and the body weights of females during the post-partum period were reduced. Water consumption and food ingestion were generally lower at the top three dose levels. The authors concluded that these effects were related to the unpalatability of drinking water containing isopropyl alcohol 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% isopropyl alcohol 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% isopropyl alcohol 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 isopropyl alcohol, 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 1.25 and 2.5% 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 isopropyl alcohol 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% isopropyl alcohol 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 isopropyl alcohol imbibed by the dogs could not be determined from data provided in the report.

Many studies of isopropanol noted increased liver and kidney weights in exposed animals but no observable relevant pathology. This weight change may be considered to be more of a metabolic response rather than a toxic effect of the alcohol. Several studies noted hyaline droplets and other lesions in kidneys characteristic of a male rat-specific phenomenon, which is not relevant to human toxicity. 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 isopropyl alcohol. 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.

Sensitive indicators of chronic adverse effects from isopropyl alcohol exposure include development of tolerance to narcosis, blood chemistry changes and reduced fetal body weights. Burleigh-Flayer *et al.* (1994) noted a development of tolerance in rats to the narcotic effects of isopropyl alcohol at 5000 ppm. Burleigh-Flayer *et al.* (1994) and USEPA/OTS (1986) observed

blood changes characteristic of anemia in rats at 5000 ppm and 2561 mg/kg body wt-day (equivalent to 3660 ppm), respectively. The most sensitive indicator of chronic oral isopropyl alcohol exposure appears to be the reduction of fetal body weights in developmental studies. This effect occurred in studies using exposure routes via inhalation, drinking water and oral gavage. In addition, reduced fetal body weights occurred at doses lower than the observed reduction of maternal body weights. In studies by Nelson *et al.* (1988), Tyl *et al.* (1994), USEPA/OTS (1986), USEPA/OTS (1992a,b) and Beyer (1992), LOAELs for significantly reduced fetal body weights occurred at 3500 ppm, 400 mg/kg body wt-day (equivalent to 570 ppm), 2560 mg/kg body wt-day (equivalent to 3660 ppm), 1240 mg/kg body-wt day (equivalent to 1770 ppm) and 1000 mg/kg body wt-day (equivalent to 1430 ppm), respectively. The study by Tyl *et al.* (1994) resulted in the smallest LOAEL (570 ppm) for chronic adverse effects to isopropyl alcohol, while the Burleigh-Flayer *et al.* (1997) chronic inhalation study yielded the highest NOAEL of 500 ppm. (Note that skeletal malformations, probably related to reduced fetal weight, were observed in 2 studies (Nelson *et al.*, 1988; USEPA/OTS, 1992a,b)).

VI. Derivation of Chronic Reference Exposure Level (REL)

<i>Study</i>	Burleigh-Flayer <i>et al.</i> (1997)
<i>Study population</i>	Rats and mice
<i>Exposure method</i>	Discontinuous whole-body inhalation (0, 500, 2,500 or 5,000 ppm)
<i>Critical effects</i>	Kidney lesions including mineralization, tubular dilation, glomerulosclerosis, interstitial nephritis, interstitial fibrosis, hydronephrosis, and transitional cell hyperplasia
<i>LOAEL</i>	2,500 ppm
<i>NOAEL</i>	500 ppm
<i>Exposure continuity</i>	6 hours/day, 5 days/week
<i>Exposure duration</i>	78 weeks in mice; 104 weeks in rats
<i>Average experimental exposure</i>	89 ppm for NOAEL group (500 x 6/24 x 5/7)
<i>Human equivalent concentration</i>	89 ppm for NOAEL group (gas with systemic effects, based on RGDR = 1.0 using default assumption that lambda (a) = lambda (h))
<i>LOAEL uncertainty factor</i>	1
<i>Subchronic uncertainty factor</i>	1
<i>Interspecies uncertainty factor</i>	3
<i>Intraspecies factor</i>	30
<i>Cumulative uncertainty factor</i>	30
<i>Inhalation reference exposure level</i>	2.97 ppm (7.0 mg/m ³ , 7,000 µg/m ³)

Strengths of the database for isopropyl alcohol, besides similar toxicological endpoints among different studies, include pharmacokinetic similarities between humans and experimental animals. These studies show that isopropyl alcohol is metabolized through a similar pathway to acetone and CO₂.

Weaknesses of the database for isopropyl alcohol 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 isopropyl alcohol. Another weakness is that, while most developmental studies observed maternal and fetal effects, one recent study found no such effects at equivalent doses (Bates *et al.*, 1994).

VII. References

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CHRONIC TOXICITY SUMMARY

MANGANESE AND COMPOUNDS

<i>Molecular Formula</i>	<i>Synonyms</i>	<i>Molecular Weight</i>	<i>CAS Reg. No.</i>
Mn	elemental manganese; colloidal manganese; cutaval	54.94 g/mol	7439-96-5
MnO	manganese oxide; manganese monoxide; manganosite	70.94 g/mol	1344-43-0
MnO ₂	manganese dioxide; black manganese oxide	86.94 g/mol	1313-13-9
Mn ₃ O ₄	manganese tetroxide; trimanganese tetraoxide; manganomanganic oxide	228.82 g/mol	1317-35-7
MnCl ₂	manganese chloride; manganese dichloride; manganous chloride	125.84 g/mol	7773-01-5

I. Chronic Toxicity Summary

<i>Inhalation reference exposure level</i>	0.05 µg/m³ (U.S. EPA RfC) This document summarizes the evaluation of non-cancer health effects by U.S. EPA for the RfC.
<i>Critical effect(s)</i>	Impairment of neurobehavioral function in humans
<i>Hazard index target(s)</i>	Nervous system

II. Physical and Chemical Properties (HSDB, 1995)

<i>Description</i>	Lustrous, gray-pink metal (Mn); green (MnO), black (MnO ₂) or pink (MnCl ₂) crystals; brownish-black powder (Mn ₃ O ₄)
<i>Molecular formula</i>	See above
<i>Molecular weight</i>	See above
<i>Density (in g/cm³)</i>	7.21-7.4 (Mn - depending on allotropic form); 5.43-5.46 (MnO); 4.88 (Mn ₃ O ₄); 2.977 @ 25°C (MnCl ₂)
<i>Boiling point</i>	1962°C (Mn); not available (MnO); unknown (Mn ₃ O ₄); 1190°C (MnCl ₂)
<i>Melting point</i>	1244 ± 3°C (Mn); 1650°C (MnO); 2847°C (Mn ₃ O ₄ - NIOSH Pocket Guide™, 1995); 650°C (MnCl ₂)
<i>Vapor pressure</i>	1 mm Hg @ 1292°C (Mn); 0 mm Hg (Mn ₃ O ₄);

<i>Solubility</i>	not available (MnO; MnCl ₂) Sol. in dil. acids and aq. solns. of Na- or K- bicarbonate (Mn); sol. in NH ₄ Cl, insol. in H ₂ O (MnO); insol. in H ₂ O, HNO ₃ , or cold H ₂ SO ₄ (MnO ₂ - Reprotext®, 1995); insol. in H ₂ O, sol. in HCl (Mn ₃ O ₄); 72.3 g/100 ml H ₂ O @ 25°C (MnCl ₂)
<i>Conversion factor</i>	Not applicable (dusts or powders)

III. Major Uses or Sources

Metallic manganese is used in the manufacturing of steel, carbon steel, stainless steel, cast iron, and superalloys to increase hardness, stiffness, and strength (HSDB, 1995). Manganese chloride is used in dyeing, disinfecting, batteries, and as a paint drier and dietary supplement. Manganese oxide (MnO) is used in textile printing, ceramics, paints, colored glass, fertilizers, and as food additives. Manganese dioxide is used in batteries and may also be generated from the welding of manganese alloys. Manganese tetroxide may be generated in situations where other oxides of manganese are heated in air (NIOSH Pocket Guide, 1995).

IV. Effects of Human Exposure

Male workers (n=92, plus 101 matched controls) in an alkaline battery plant in Belgium exposed to manganese dioxide were the subject of a cross-sectional epidemiological investigation (Roels *et al.*, 1992). Evaluation of the subjects included tests for neurobehavioral function, lung function, hematological parameters, and urinalysis. Exposed workers showed significant differences in performance on tests of visual reaction time, eye-hand coordination, and hand tremor. Occupational-lifetime integrated respiratory dust (IRD) levels ranged from 0.04-4.43 mg Mn/m³-yr with a geometric mean of 0.793 mg Mn/m³-yr. Average exposure time was 5.3 years, with a range of 0.2-17.7 years. The authors grouped the workers into three exposure groups based on the IRD levels: <0.6, 0.6-1.2, and >1.2 mg Mn/m³-yrs. Although there was an indication of a linear dose-related trend for visual reaction time and hand steadiness, the authors concluded that “analysis of the data on a group basis...does not permit us to identify a threshold effect level for airborne Mn.” A daily average exposure level of 0.15 mg Mn/m³ was derived by dividing the geometric mean of the IRD (0.793 mg Mn/m³-yr) by the average exposure time (5.3 yr).

In an earlier study, 141 male workers plus 104 matched control workers were examined for effects of exposure to MnO₂, manganese tetroxide (Mn₃O₄), and other manganese salts (Roels *et al.*, 1987). Tests measuring visual reaction time, eye-hand coordination, hand tremor, and short-term memory were found to be significantly different in the manganese-exposed group. Statistically significant clinical symptoms (as evaluated in a questionnaire) included fatigue, tinnitus, finger trembling and irritability. Self-reported prevalence of coughs, colds and acute bronchitis were increased in the manganese exposed group relative to controls. Mean time of

employment was 7.1 years, with a range of 1-19 years. Total airborne manganese dust levels had an arithmetic mean of 1.33 mg/m³ and a geometric mean of 0.94 mg/m³.

Several other studies have identified neurobehavioral endpoints of manganese toxicity in human populations. A matched-pair cross-sectional study investigated 74 pairs of manganese alloy workers (Mergler *et al.*, 1994). Matched pairs were found to be discordant in reporting a number of adverse clinical symptoms including the following areas: fatigue, emotional state, memory, attention, concentration difficulty, nightmares, unusual sweating, sexual dysfunction, lower back pain, joint pain, and tinnitus. Motor function tests also revealed deficits in the manganese exposed group. Olfactory perception was enhanced in the manganese exposed group. Exposure levels were estimated at a geometric mean of 0.035 mg Mn/m³ for respirable dust and 0.225 mg Mn/m³ for total dust. Mean duration of exposure was 16.7 years.

Workers in two Swedish foundries were evaluated for potential neurobehavioral effects from exposure to manganese (Iregren, 1990). Exposure levels ranged from 0.02-1.4 mg Mn/m³ with a mean of 0.25 mg Mn/m³. Simple reaction time, standard deviation of reaction time, finger-tapping speed, digit-span short term memory, speed of mental addition, and verbal understanding were significantly different from controls among manganese exposed workers.

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

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

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

Junior high school students exposed to manganese were examined for potential effects on the respiratory system (Nogawa *et al.*, 1973). Measurement of atmospheric manganese levels showed a 5-day average level of 0.0067 mg Mn/m³ 300 m from the school.

V. Effects of Animal Exposure

Toxic effects have been described in animals exposed to manganese compounds by inhalation (Shiotsuka, 1984; Suzuki *et al.*, 1978; Moore *et al.*, 1975). Shiotsuka *et al.* (1984) demonstrated increased incidence of pneumonia among rats exposed for 2 weeks to manganese dioxide concentrations ranging from 68-219 mg/m³. Monkeys exposed to manganese dioxide concentrations ranging from 0.7-3.0 mg/m³ for 10 months showed increased incidence of

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pulmonary emphysema (Suzuki *et al.*, 1978). Hamsters and rats exposed for 56 days to 0.117 mg Mn₃O₄/m³ showed bronchial lesions (Moore *et al.*, 1975).

High concentrations of manganese (>10 mg/m³) have decreased host resistance in exposed animals (Adkins *et al.*, 1980; Bergstrom, 1977; Maigetter *et al.*, 1976).

Nine month inhalation toxicity studies in rats and monkeys exposed to levels as high as 1.15 mg Mn₃O₄/m³ produced no significant pulmonary effects (Ulrich *et al.*, 1979a; Ulrich *et al.*, 1979b; Ulrich *et al.*, 1979c)

VI. Derivation of U.S. EPA Reference Concentration

<i>Study</i>	Roels <i>et al.</i> , 1992 (evaluated by U.S. EPA, 1993)
<i>Study population</i>	Occupationally-exposed humans
<i>Exposure method</i>	Discontinuous occupational inhalation exposure to manganese dioxide (0.2, 1.0, and 6.0 mg/m ³)
<i>Critical effects</i>	Impairment of neurobehavioral function
<i>LOAEL</i>	0.15 mg respirable manganese dust/m ³ (geometric mean from exposures of 0.040 to 4.4 mg Mn/m ³ -years)
<i>NOAEL</i>	Not observed
<i>Study continuity</i>	8 hours per day, 5 days per week
<i>Average occupational exposure</i>	0.05 mg/m ³ for LOAEL group (based on an 8-hour TWA occupational exposure to 10 m ³ manganese contaminated air per day out of 20 m ³ total air inhaled per day over 5 days per week)
<i>Human equivalent concentration</i>	0.05 mg/m ³ for LOAEL group
<i>Study duration</i>	5.3 years (average)
<i>LOAEL uncertainty factor</i>	10
<i>Subchronic uncertainty factor</i>	3
<i>Interspecies uncertainty factor</i>	1
<i>Intraspecies uncertainty factor</i>	10
<i>Modifying factor</i>	3 (lack of developmental data and potential differences in toxicity for different forms of manganese)
<i>Cumulative uncertainty factor</i>	1,000
<i>Inhalation reference exposure level</i>	0.05 µg/m ³

In the derivation of its reference concentration for manganese and compounds, the U.S. EPA selected the Roels *et al.* (1992) study for establishing the exposure level associated with adverse health effects. Although this study did not establish a no-observed-adverse-effect-level (NOAEL), clear evidence of toxicity was established at the level of exposure which was found in the facility studied, and was therefore taken to be a LOAEL. This study offers several advantages over the other available studies of manganese toxicity. (1) The study population was

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human. (2) The workers were only exposed to a single manganese compound. (3) The study population was well controlled for with matching for age, height, weight, work schedule, coffee and alcohol consumption, and smoking. (4) The exposure duration was relatively long and work practice continuity suggests exposure levels changed little over time. (5) The effects observed were consistent with those observed among other workers occupationally exposed to manganese.

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

Derivation of U.S. EPA Reference Dose

<i>Study</i>	Freeland-Graves <i>et al.</i> , 1987; NRC, 1989; WHO, 1973
<i>Study population</i>	Various human populations
<i>Exposure method</i>	Chronic ingestion of foodstuffs
<i>Critical effects</i>	CNS effects
<i>LOAEL</i>	Not determined
<i>NOAEL</i>	0.14 mg/kg-day
<i>Exposure continuity</i>	
<i>Exposure duration</i>	Chronic
<i>Average experimental exposure</i>	Up to lifetime
<i>LOAEL uncertainty factor</i>	1
<i>Subchronic uncertainty factor</i>	1
<i>Interspecies uncertainty factor</i>	1
<i>Intraspecies factor</i>	1
<i>Cumulative uncertainty factor</i>	1
<i>Oral reference exposure level</i>	0.14 mg/kg-day (U.S. EPA RfD)

*Conversion Factors and Assumptions -- The NOAEL of 10 mg/day (0.14 mg/kg-day for 70 kg adult) for chronic human consumption of manganese in the diet is based on a composite of data from several studies.

The oral Reference Exposure Level (REL) for manganese is the U.S. EPA's Oral RfD (IRIS, 1996). The principal studies used were Freeland-Graves *et al.*, 1987; NRC, 1989; and WHO 1973.

Manganese is a ubiquitous element that is essential for normal physiologic functioning in all animal species. Several disease states in humans have been associated with both deficiencies and excess intakes of manganese. Thus any quantitative risk assessment for manganese must take into account aspects of both the essentiality and the toxicity of manganese. In humans, data are available providing information about the range of essentiality for manganese. In addition, there are many reports of toxicity to humans exposed to manganese by inhalation; much less is

known, however, about oral intakes resulting in toxicity. Rodents do not provide a good experimental model for manganese toxicity, and only one limited study in primates by the oral route of exposure is available. The following assessment, therefore, focuses more on what is known to be a safe oral intake of manganese for the general human population. Finally, it is important to emphasize that individual requirements for, as well as adverse reactions to, manganese may be highly variable. The reference dose is estimated to be an intake for the general population that is not associated with adverse health effects; this is not meant to imply that intakes above the reference dose are necessarily associated with toxicity. Some individuals may, in fact, consume a diet that contributes more than 10 mg Mn/day without any cause for concern.

The Food and Nutrition Board of the National Research Council (NRC, 1989) determined an “estimated safe and adequate daily dietary intake” (ESADDI) of manganese to be 2-5 mg/day for adults. The lower end of this range was based on a study by McLeod and Robinson (1972), who reported equilibrium or positive balances at intakes of 2.5 mg Mn/day or higher. The range of the ESADDI also includes an “extra margin of safety” from the level of 10 mg/day, which the NRC considered to be safe for an occasional intake. While the NRC determined an ESADDI for manganese of 2-5 mg/day, some nutritionists feel that this level may be too low. Freeland-Graves *et al.* (1987) have suggested a range of 3.5-7 mg/day for adults based on a review of human studies. It is noted that dietary habits have evolved in recent years to include a larger proportion of meats and refined foods in conjunction with a lower intake of whole grains. The net result of such dietary changes includes a lower intake of manganese such that many individuals may have suboptimal manganese status.

The World Health Organization (WHO, 1973) reviewed several investigations of adult diets and reported the average daily consumption of manganese to range from 2.0-8.8 mg Mn/day. Higher manganese intakes are associated with diets high in whole-grain cereals, nuts, green leafy vegetables, and tea. From manganese balance studies, the WHO concluded that 2-3 mg/day is adequate for adults and 8-9 mg/day is “perfectly safe.” Evaluations of standard diets from the United States, England, and Holland reveal average daily intakes of 2.3-8.8 mg Mn/day. Depending on individual diets, however, a normal intake may be well over 10 mg Mn/day, especially from a vegetarian diet. While the actual intake is higher, the bioavailability of manganese from a vegetarian diet is lower, thereby decreasing the actual absorbed dose.

From this information taken together, EPA concludes that an appropriate reference dose for manganese is 10 mg/day (0.14 mg/kg-day). In applying the reference dose for manganese to a risk assessment, it is important that the assessor consider the ubiquitous nature of manganese, specifically that most individuals will be consuming about 2-5 mg Mn/day in their diet. This is particularly important when one is using the reference dose to determine acceptable concentrations of manganese in water and soils. There is one epidemiologic study of manganese in drinking water, performed by Kondakis *et al.* (1989). Three areas in northwest Greece were chosen for this study, with manganese concentrations in natural well water of 3.6-14.6 µg/L in area A, 81.6-252.6 µg/L in area B, and 1600-2300 µg/L in area C. The total population of the three areas studied ranged from 3200 to 4350 people. The study included only individuals over the age of 50 drawn from a random sample of 10% of all households (n=62, 49 and 77 for areas A, B and C, respectively). The authors reported that “all areas were similar with respect to social

and dietary characteristics,” but few details were reported. The three areas are located within a 200-square km region. Although the amount of manganese in the diet was not reported, the authors indicated that most of the food was purchased from markets and is expected to be comparable for all three areas. Chemicals other than manganese in the well water were reported to be within Economic Community (EC) standards, except for hardness (120-130 mg calcium carbonate per liter). The individuals chosen were submitted to a neurologic examination; the score represents a composite of the presence and severity of 33 symptoms (e.g., weakness/fatigue, gait disturbances, tremors, dystonia). Whole blood and hair manganese concentrations also were determined. The mean concentration of manganese in hair was 3.51, 4.49 and 10.99 µg/g dry weight for areas A, B and C, respectively ($p < 0.0001$ for area C versus A). The concentration of manganese in whole blood did not differ between the three areas, but this is not considered to be a reliable indicator of manganese exposure. The mean (\bar{x}) and range (r) of neurologic scores were as follows: Area A (males: $\bar{x} = 2.4$, $r = 0-21$; females: $\bar{x} = 3.0$, $r = 0-18$; both $\bar{x} = 2.7$, $r = 0-21$); Area B (males $\bar{x} = 1.6$, $r = 0-6$; females: $\bar{x} = 5.7$, $r = 0-43$; both: $\bar{x} = 3.9$, $r = 0-43$); and Area C (males: $\bar{x} = 4.9$, $r = 0-29$; females: $\bar{x} = 5.5$, $r = 0-21$; both $\bar{x} = 5.2$, $r = 0-29$). The authors indicate that the difference in mean scores for area C versus A was significantly increased (Mann-Whitney $z = 3.16$, $p = 0.002$ for both sexes combined). In a subsequent analysis, logistic regression indicated that there is a significant difference between areas A and C even when both age and sex are taken into account (Kondakis, 1990).

The individuals examined in the Kondakis study also had exposure to manganese in their diet. This was originally estimated to be 10-15 mg/day because of the high intake of vegetables (Kondakis, 1990). This estimate was subsequently lowered to 5-6 mg/day (Kondakis, 1993). Because of the uncertainty in the amount of manganese in the diet and the amount of water consumed, it is impossible to estimate the total oral intake of manganese in this study. These limitations preclude the use of this study to determine a quantitative dose-response relationship for the toxicity of manganese in humans.

This study, nevertheless, raises significant concerns about possible adverse neurological effects at doses not far from the range of essentially. Because of this concern, it is recommended that a modifying factor of 3 be applied when assessing risk from manganese in drinking water or soil.

The information used to determine the RfD for manganese was taken from many large populations consuming normal diets over an extended period of time with no adverse health effects. As long as physiologic systems are not overwhelmed, humans exert an efficient homeostatic control over manganese such that body burdens are kept constant with variation in the manganese content of the diet. The information providing a chronic NOAEL in many cross-sections of human populations, taken in conjunction with the essentiality of manganese, warrants an uncertainty factor of 1.

When assessing exposure to manganese from food, the modifying factor is 1; however, when assessing exposure to manganese from drinking water or soil, a modifying factor of 3 is recommended. There are four reasons for this recommendation. First, while the data suggest that there is no significant difference between absorption of manganese as a function of the form in which it is ingested (i.e., food versus water), there is some degree of increased uptake of manganese from water in fasted individuals. Second, the study by Kondakis *et al.* (1989) raises

some concern for possible adverse health effects associated with a lifetime consumption of drinking water containing about 2 mg/L of manganese. Third, although toxicity has not been demonstrated, there is concern for infants fed formula that typically has a much higher concentration of manganese than does human milk. If powdered formula is made with drinking water, the manganese in the water would represent an additional source of intake. Finally, there is some evidence that neonates absorb more manganese from the gastrointestinal tract, that neonates are less able to excrete absorbed manganese, and that in the neonate the absorbed manganese more easily passes the blood-brain barrier. These findings may be related to the fact that manganese in formula is in a different ionic form and a different physical state from that in human milk. These considerations concerning increased exposure in an important population group, in addition to the likelihood that any adverse neurological effects of manganese are likely to be irreversible and not manifested for many years after exposure, warrant caution until more definitive data are available.

U.S. EPA stated its confidence in the RfC as: Study - Medium; Data Base - Medium; and RfD - Medium. Many studies have reported similar findings with regard to the normal dietary intake of manganese by humans. These data are considered to be superior to any data obtained from animal toxicity studies, especially as the physiologic requirements for manganese vary quite a bit among different species, with man requiring less than rodents. There is no single study used to derive the dietary RfD for manganese. While several studies have determined average levels of manganese in various diets, no quantitative information is available to indicate toxic levels of manganese in the diet of humans. Because of the homeostatic control humans maintain over manganese, it is generally not considered to be very toxic when ingested with the diet. It is important to recognize that while the RfD process involves the determination of a point estimate of an oral intake, it is also stated that this estimate is associated “with uncertainty spanning perhaps an order of magnitude.” Numerous factors, both environmental factors (e.g., the presence or absence of many dietary constituents) and biological or host factors (e.g., age, alcohol consumption, anemia, liver function, general nutritional status) can significantly influence an individual’s manganese status. As discussed in the Additional Studies / Comments Section, there is significant variability in the absorption and elimination of manganese by humans. Confidence in the data base is medium and confidence in the dietary RfD for manganese is also medium.

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CHRONIC TOXICITY SUMMARY

INORGANIC MERCURY

(liquid silver; hyfarargyrum; colloidal mercury)

CAS Registry Number: 7439-97-6

I. Chronic Toxicity Summary

<i>Inhalation reference exposure level</i>	0.3 g/m³ (U.S. EPA RfC) This document summarizes the evaluation of non-cancer health effects by U.S. EPA for the RfC.
<i>Critical effects</i>	Hand tremor, memory disturbances, and autonomic dysfunction in humans
<i>Hazard index target(s)</i>	Nervous system

II. Chemical Property Summary (HSDB, 1995)

<i>Description</i>	Silvery, odorless, heavy liquid
<i>Molecular formula</i>	Hg
<i>Molecular weight</i>	200.59 g/mol
<i>Boiling point</i>	Hg ⁰ : 356.7 °C; HgCl ₂ : 302°C
<i>Vapor pressure</i>	0.002 mm Hg @ 25°C
<i>Solubility</i>	Soluble in concentrated nitric and hot sulfuric acids; dissolves to some extent in lipids
<i>Conversion factor</i>	1 ppm = 8.2 mg/m ³

III. Major Uses or Sources

The uses of mercury and mercury containing compounds are considerable. Because it has uniform volume expansion with increasing temperature over the entire temperature range of its liquid state, it is widely used in barometers, thermometers, hydrometers, and pyrometers. It is used in mercury arc lamps producing ultraviolet rays, in fluorescent lamps, as a catalyst in oxidation of organic compounds, extracting gold and silver from ores, electric rectifiers, the making of mercury fulminate, for Millon's Reagent, and as a cathode in electrolysis. It is also used in pulp and paper manufacturing, as a component of batteries, in amalgams (dental preparations), and in the manufacture of switching devices such as oscillators, the manufacture of chlorine and caustic soda, as a lubricant, and a laboratory reagent.

To lesser extent is has been used to fumigate and protect grain from insect infestation, in pharmaceuticals, agricultural chemicals, and antifouling paints (ACGIH, 1991).

IV. Effect of Exposure to Humans

The primary effects of chronic exposure to mercury vapor are on the central nervous system. Chronic duration exposures to elemental mercury have resulted in tremors (mild or severe), unsteady walking, irritability, poor concentration, short-term memory deficits, tremulous speech, blurred vision, performance decrements, paresthesia, and decreased nerve conduction (Albers *et al.*, 1988; Chaffin *et al.*, 1973; Fawer *et al.*, 1983; Kishi *et al.*, 1993; Langolf *et al.*, 1978; Piikivi *et al.*, 1984; Smith *et al.*, 1970). Motor system disturbance can be reversible upon cessation of exposure, however, memory deficits may be permanent (Chaffin *et al.*, 1973). Studies have shown effects such as tremor and decreased cognitive skills in workers exposed to approximately 25 $\mu\text{g}/\text{m}^3$ mercury vapor (Piikivi *et al.*, 1984; Piikivi and Hanninen, 1989; Piikivi and Toulonen, 1989) (see discussion below).

The kidney is also a sensitive target organ of mercury toxicity. Effects such as proteinuria, proximal tubular and glomerular changes, albuminuria, glomerulosclerosis, and increased urinary N-acetyl- β -glucosaminidase have been seen in workers exposed to approximately 25-60 $\mu\text{g}/\text{m}^3$ mercury vapor (Barregard *et al.*, 1988; Bernard *et al.*, 1987; Roels *et al.*, 1982; Piikivi and Ruokonen, 1989).

Chronic exposure to mercury vapors has also resulted in cardiovascular effects such as increased heart and blood pressure (Fagala and Wigg, 1992; Taueg *et al.*, 1992; Piikivi, 1989) and in leukocytosis and neutrophilia (Fagala and Wigg, 1992).

A number of other studies with similar exposure levels also found adverse psychological and neurological effects in exposed versus unexposed individuals. Piikivi and Tolonen (1989) used EEGs to study the effects of long-term exposure to mercury vapor in 41 chloralkali workers exposed for a mean of 15.6 years as compared to matched controls. They found that exposed workers who had blood mercury levels of 12 $\mu\text{g}/\text{L}$ tended to have an increased number of EEG abnormalities when analyzed by visual inspection. When analyzed by computer, brain activity was found to be significantly lower than matched controls. The changes were most prominent in the parietal cortex, but absent in the frontal cortex.

Another study by Piikivi (1989) examined subjective and objective symptoms of autonomic dysfunction in 41 chloralkali workers exposed to mercury vapor for an average of 15.6 years as compared with matched controls. Similar to the above studies, the exposed workers had mean blood levels of 11.6 $\mu\text{g}/\text{L}$ corresponding to a TWA exposure of 25 $\mu\text{g Hg}/\text{m}^3$ in air (Roels *et al.*, 1987). The workers were tested for pulse rate variation in normal and deep breathing, the Valsalva maneuver, vertical tilt, and blood pressure responses during standing and isometric work. The only significant difference in subjective symptoms was an increased reporting of palpitations in exposed workers. The objective tests demonstrated an increase in pulse rate variations at 30 $\mu\text{g Hg}/\text{m}^3$ (extrapolated from blood level based on methods of Roel *et al.* (1987)), which is indicative of autonomic reflex dysfunction.

A more recent study by Ngim *et al.* (1992) assessed neurobehavioral performance in a cross-sectional study of 98 dentists exposed to a TWA concentration of 14 $\mu\text{g Hg}/\text{m}^3$ (range 0.7 to 42 $\mu\text{g}/\text{m}^3$) compared to 54 controls with no history of occupational exposure to mercury. Exposed

dentists were adequately matched to the control group for age, amount of fish consumption, and number of amalgam fillings. Air concentrations were measured with personal sampling badges over typical working hours (8-10 hours/day) and converted to a TWA. Blood samples were also taken (average 9.8 µg/L). The average concentration in air was estimated at 23 µg Hg/m³ when the methods of Roels *et al.* (1987) were used. The average duration in this study of dentists was only 5.5 years, shorter than the above studies. The performance of the dentists was significantly worse than controls on a number of neurobehavioral tests measuring motor speed (finger tapping), visual scanning, visuomotor coordination and concentration, visual memory, and visuomotor coordination speed. These neurobehavioral changes are consistent with central and peripheral neurotoxicity commonly observed in cases of chronic mercury toxicity.

Liang *et al.* (1993) investigated workers in a fluorescent lamp factory with a computer-administered neurobehavioral evaluation system and a mood-inventory profile. The cohort consisted of 88 individuals (19 females and 69 males) exposed for at least 2 years prior to the study. Exposure was monitored with area samplers and ranged from 8 to 85 µg Hg/m³ across worksites. The average level of exposure was estimated at 33 µg Hg/m³ and the average duration of exposure was estimated at 15.8 years. The exposed cohort performed significantly worse than the controls on tests of finger tapping, mental arithmetic, two digit searches, switching attention, and visual reaction time. The effect of performance persisted after controlling for chronological age as a confounding factor.

V. Effects of Exposure to Animals

In laboratory animals mercury exposure resulted in adverse neurological and behavioral changes. Rabbits exposed to 28.8 mg/m³ mercury vapor for 1 to 13 weeks exhibited unspecified pathological changes, marked cellular degeneration, and necrosis in the brain (Ashe *et al.*, 1953). Rats exhibited a decline in conditioned avoidance response with exposure to 3 mg/m³ mercury vapor for 12 to 42 weeks. No histopathological changes were evident (Kishi *et al.*, 1978).

Congested lungs were observed in rats exposed to 1 mg/m³ mercury vapor for 6 weeks, 100 hours/week (Gage, 1961). Rats exposed intermittently to 3 mg/m³ mercury vapor for 12 to 42 weeks for 3 hours/day showed no changes in the respiratory system.

Rats exposed intermittently to 2.5 mg/m³ mercury vapor for 21 days demonstrated prolongation of the estrous cycle and a decrease in the number of living fetuses (Baranski and Szyrmezyk, 1973), however, no differences in developmental abnormalities were observed.

VI. Derivation of Reference Exposure Levels

Derivation of U.S. EPA Reference Concentration (RfC)

<i>Study</i>	Piikivi and Hanninen (1989)
<i>Study population</i>	Humans
<i>Exposure method</i>	Inhalation of workplace air
<i>Critical Effects</i>	Psychological disturbances
<i>LOAEL</i>	25 $\mu\text{g}/\text{m}^3$ (3 ppb)
<i>NOAEL</i>	Not observed
<i>Exposure continuity</i>	8 hours per day (10 $\text{m}^3/\text{workday}$), 5 days/week
<i>Average experimental exposure</i>	9 $\mu\text{g}/\text{m}^3$ for LOAEL group
<i>Human equivalent concentration</i>	9 $\mu\text{g}/\text{m}^3$
<i>Exposure duration</i>	14 year average
<i>Subchronic uncertainty factor</i>	1
<i>LOAEL uncertainty factor</i>	3
<i>Interspecies uncertainty factor</i>	1
<i>Intraspecies uncertainty factor</i>	3
<i>Modifying factor</i>	3 (lack of developmental and reproductive toxicity data)
<i>Cumulative uncertainty factor</i>	30
<i>Inhalation reference exposure level</i>	0.3 $\mu\text{g}/\text{m}^3$ (0.04 ppb)

This study was chosen to calculate the chronic REL because of the finding of a statistically significant increase in subjective psychological disturbances. Subjective symptoms and psychological performances were examined in 60 chloralkali workers using a computer administered test battery. Increases in memory disturbances and sleep disorders were found in exposed versus unexposed individuals, however, objective disturbance in perceptual motor activity, memory, or learning abilities were not found. One weakness of this study is that mercury concentrations in air were extrapolated from blood levels based on the conversion factor of Roel *et al.* (1987). A mean blood level of 10 $\mu\text{g}/\text{L}$ corresponded to an average air exposure level of 25 $\mu\text{g}/\text{m}^3$ for the group.

The human studies consistently demonstrate a LOAEL of approximately 25 $\mu\text{g}/\text{m}^3$ in air. It is noteworthy that none of the above studies discussed in sufficient detail a dose-response relationship between mercury vapor inhalation and the toxic effects measured. Because none of the studies mention a level below which toxic effects were not seen after evaluation (a NOAEL), the extrapolation from a LOAEL to a NOAEL should be regarded with caution. Secondly, one study (Ngim *et al.*, 1992) demonstrated neurotoxic effects from mercury inhalation at an exposure level similar to the above studies, but for a much shorter duration. No adjustment was made for lifetime average exposure since one study demonstrated effects after 5 years. It is possible, however, that mercury could cause neurotoxic effects after a shorter exposure period than that used in derivation of the chronic REL.

The strengths of the inhalation REL include the use of human exposure data from workers exposed over a period of years. Major areas of uncertainty are the lack of observation of a

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NOAEL, the limited nature of the study, the uncertainty in estimating exposure and the potential variability in exposure concentration, and the lack of reproductive and developmental toxicity studies.

Derivation of U.S. EPA Reference Dose (RfD)

<i>Study</i>	U.S. EPA, 1987
<i>Study population</i>	Brown Norway rats
<i>Exposure method</i>	feeding and subcutaneous application
<i>Critical effects</i>	autoimmune effects in kidney
<i>LOAEL</i>	0.226 mg/kg-day (feeding); 0.317 mg/kg-day (subcutaneous)
<i>NOAEL</i>	none
<i>Exposure continuity</i>	
<i>Exposure duration</i>	up to 60 days
<i>Average experimental exposure</i>	
<i>LOAEL uncertainty factor</i>	10
<i>Subchronic uncertainty factor</i>	10
<i>Interspecies uncertainty factor</i>	10
<i>Intraspecies factor</i>	(The intraspecies and interspecies factors were combined into one factor of 10 to avoid an exceedingly large uncertainty factor.)
<i>Cumulative uncertainty factor</i>	1000
<i>Oral reference exposure level</i>	0.0003 mg/kg-day

Factors and Assumptions -- Dose conversions in the three studies employed a 0.739 factor for HgCl₂ to Hg²⁺, a 100% factor for subcutaneous (s.c.) to oral route of exposure, and a time-weighted average for days/week of dosing. This RfD is based on the back calculations from a Drinking Water Equivalent Level (DWEL), recommended to and subsequently adopted by the U.S. EPA, of 0.010 mg/L: (RfD = 0.010 mg/L x 2 L/day/70 kg bw = 0.0003 mg/kg bw/day). The LOAEL exposure levels, used in the three studies selected as the basis of the recommended DWEL, are from Druet *et al.* (1978), Bernaudin *et al.* (1981) and Andres (1984), respectively.

The oral Reference Exposure Level for mercuric chloride is the U.S. EPA's RfD (IRIS, 1996). The principal study used was U.S. EPA (1987). A panel of mercury experts met at a Peer Review Workshop on Mercury Issues in Cincinnati, Ohio, on October 26-27, 1987, and reviewed outstanding issues concerning the health effects and risk assessment of inorganic mercury. The following five consensus conclusions and recommendations were agreed to as a result of this workshop: 1) The most sensitive adverse effect for mercury risk assessment is formation of mercuric-mercury-induced autoimmune glomerulonephritis. The production and deposition of IgG antibodies to the glomerular basement membrane can be considered the first step in the formation of this mercuric-mercury-induced autoimmune glomerulonephritis. 2) The Brown Norway rat should be used for mercury risk assessment. The Brown Norway rat is a good test species for the study of Hg²⁺-induced autoimmune glomerulonephritis. The Brown Norway rat is not unique in this regard (since this effect has also been observed in rabbits). 3) The Brown

Norway rat is a good surrogate for the study of mercury-induced kidney damage in sensitive humans. For this reason, the uncertainty factor used to calculate criteria and health advisories (based on risk assessments using the Brown Norway rat) should be reduced by 10-fold. 4) Hg²⁺ absorption values of 7% from the oral route and 100% from the s.c. route should be used to calculate criteria and health advisories. 5) A DWEL of 0.010 mg/L was recommended based on the weight of evidence from the studies using Brown Norway rats and limited human tissue data.

Three studies using the Brown Norway rat as the test strain were chosen from a larger selection of studies as the basis for the panel's recommendation of 0.010 mg/L as the DWEL for inorganic mercury. The three studies are presented below for the sake of completeness. It must be kept in mind, however, that the recommended DWEL of 0.010 mg/L and back calculated oral RfD of 0.0003 mg/kg-day were arrived at from an intensive review and workshop discussions of the entire inorganic mercury data base, not just from one study. In the Druet *et al.* (1978) study, the duration of exposure was 8-12 weeks; s.c. injection was used instead of oral exposure. In this study the development of kidney disease was evaluated. In the first phase the rats developed anti-GBM antibodies. During the second phase, which is observed after 2-3 months, the patterns of fixation of antisera changed from linear to granular as the disease progressed. The immune response was accompanied by proteinuria and in some cases by a nephrotic syndrome. Both male and female Brown Norway rats 7-9 weeks of age were divided into groups of 6-20 animals each. The numbers of each sex were not stated. The animals received s.c. injections of mercuric chloride (HgCl₂) 3 times weekly for 8 weeks, with doses of 0, 100, 250, 500, 1000 and 2000 µg/kg. An additional group was injected with a 50 µg/kg dose for 12 weeks. Antibody formation was measured by the use of kidney cryostat sections stained with a fluoresceinated sheep anti-rat IgG antiserum. Urinary protein was assessed by the biuret method (Druet *et al.*, 1978). Tubular lesions were observed at the higher dose levels. Proteinuria was reported at doses of 100 µg/kg and above, but not at 50 µg/kg. Proteinuria was considered a highly deleterious effect, given that affected animals developed hypoalbuminemia and many died. Fixation of IgG antiserum was detected in all groups except controls (Druet *et al.*, 1978). Bernaudin *et al.* (1981) reported that mercurials administered by inhalation or ingestion to Brown Norway rats developed a systemic autoimmune disease. The HgCl₂ ingestion portion of the study involved the forcible feeding of either 0 or 3000 µg/kg-week of HgCl₂ to male and female Brown Norway rats for up to 60 days. No abnormalities were reported using standard histological techniques in either experimental or control rats. Immunofluorescence histology revealed that 80% (4/5) of the mercuric-exposed rats were observed with a linear IgG deposition in the glomeruli after 15 days of exposure. After 60 days of HgCl₂ exposure, 100% (5/5) of the rats had a mixed linear and granular pattern of IgG deposition in the glomeruli and granular IgG deposition in the arteries. Weak proteinuria was observed in 60% (3/5) of the rats fed HgCl₂ for 60 days. The control rats were observed to have no deposition of IgG in the glomeruli or arteries as well as normal urine protein concentrations. Andres (1984) administered HgCl₂ (3 mg/kg in 1 mL of water) by gavage to five Brown Norway rats and two Lewis rats twice a week for 60 days. A sixth Brown Norway rat was given only 1 mL of water by gavage twice a week for 60 days. All rats had free access to tap water and pellet food. After 2-3 weeks of exposure, the Brown Norway HgCl₂-treated rats started to lose weight and hair. Two of the HgCl₂-treated Brown Norway rats died 30-40 days after beginning the study. No rats were observed to develop detectable proteinuria during the 60-day study. The kidneys appeared normal in all animals when evaluated using standard histological techniques, but examination by immunofluorescence

showed deposits of IgG present in the renal glomeruli of only the mercuric-treated Brown Norway rats. The Brown Norway treated rats were also observed with mercury-induced morphological lesions of the ileum and colon with abnormal deposits of IgA in the basement membranes of the intestinal glands and of IgG in the basement membranes of the lamina propria. All observations in the Lewis rats and the control Brown Norway rat appeared normal.

The U.S. EPA reported its confidence in the RfD as: Data Base - High and RfD - High. No one study was found adequate for deriving an oral RfD; however, based on the weight of evidence from the studies using Brown Norway rats and the entirety of the mercuric mercury data base, an oral RfD of high confidence was derived.

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CHRONIC TOXICITY SUMMARY

METHANOL

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

CAS Registry Number: 67-56-1

I. Chronic Toxicity Exposure Level

<i>Inhalation reference exposure level</i>	10,000 g/m³
<i>Critical effect(s)</i>	Increased incidence of abnormal cervical ribs, cleft palate, and exencephaly in mice
<i>Hazard index target(s)</i>	Teratogenicity

II. Chemical Property Summary (HSDB, 1995)

<i>Description</i>	Colorless liquid
<i>Molecular formula</i>	CH ₃ OH
<i>Molecular Weight</i>	32.04 g/mol
<i>Vapor Pressure</i>	92 torr at 20°C
<i>Solubility</i>	Methanol is miscible with water, ethanol, ether and many other organic solvents.
<i>Color</i>	Colorless
<i>Conversion Factor</i>	1 ppm = 1.31 mg/m ³

III. Major Uses and Sources

Originally distilled from wood, methanol is now manufactured synthetically from carbon oxides and hydrogen. Methanol is used primarily for the manufacture of other chemicals and as a solvent. It is also added to a variety of commercial and consumer products such as windshield washing fluid and de-icing solution, duplicating fluids, solid canned fuels, paint remover, model airplane fuels, embalming fluids, lacquers, and inks. Methanol is also used as an alternative motor fuel.

IV. Effects of Human Exposure

The majority of the available information on methanol toxicity in humans relates to acute rather than chronic exposure. The toxic effects after repeated or prolonged exposure to methanol are believed to be qualitatively similar but less severe than those induced by acute exposure (Kavet and Nauss, 1990). These effects include CNS and visual disturbances such as headaches, dizziness, nausea and blurred vision. The role of formate, a metabolite of methanol, in chronic

toxicity is unclear. In one study, symptoms of blurred vision, headaches, dizziness, nausea and skin problems were reported in teachers aides exposed to duplicating fluid containing 99% methanol (Frederick *et al.*, 1984). Individual aides worked as little as 1 hr/day for 1 day a week to 8 hrs/day for 5 days/wk. The workers' total exposure duration was not mentioned. A dose-response relationship was observed between the self-reported amount of time spent at the duplicator and the incidence of symptoms. The concentrations of methanol in the breathing zones near the machines in 12 schools ranged from 485 to 4096 mg/m³ (365 to 3080 ppm) for a 15 minute sample.

Forty-five percent of duplicating machine operators experienced blurred vision, headache, nausea, dizziness and eye irritation (NIOSH, 1981). Air concentrations of methanol for 25 minutes near the machines averaged 1330 mg/m³.

Employees working in the proximity of direct process duplicating machines complained of frequent headaches and dizziness (Kingsley and Hirsch, 1954). Air concentrations of methanol ranged from 15 ppm (20 mg/m³) to 375 ppm (490 mg/m³).

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

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

Ubaydullayev (1968) exposed 3 to 6 subjects to methanol vapor for short durations (40 minutes for some subjects and others for an unspecified amount of time). Electrical brain cortex reflex activity was significantly altered upon exposures to 1.17 mg/m³ (0.89 ppm) or 1.46 mg/m³ (1.11 ppm). No effect was observed at 1.01 mg/m³ (0.77 ppm).

V. Effects of Animal Exposure

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

Two chronic studies have been conducted with monkeys. In one study, ultrastructural abnormalities of hepatocytes indicating alteration of RNA metabolism were observed in rhesus monkeys given oral doses of 3 to 6 mg/kg methanol for 3 to 20 weeks (Garcia and VanZandt, 1969; as cited in Rowe and McCollister, 1978). In a study aimed at examining ocular effects, cynomolgous monkeys were exposed by inhalation to methanol concentrations ranging from 680 mg/m³ (520 ppm) to 6650 mg/m³ (5010 ppm) for 6 hours per day, 5 days per week for 4 weeks (Andrews *et al.*, 1987). No deaths occurred and no treatment-related effects were found upon histopathologic examination.

Exposure to a mixture of methanol and other solvents has been associated with central nervous system birth defects in humans (Holmberg, 1979). However, because of mixed or inadequate exposure data, methanol is not considered a known human teratogen.

In two separate studies in male rats, inhalation exposure to methanol ranging from 260 to 13,000 mg/m³ for 6 to 8 hours per day for either 1 day or 1, 2, 4 or 6 weeks resulted in a significant reduction in testosterone levels (Cameron *et al.*, 1984; Cameron *et al.*, 1985).

Ubaydullayev (1968) exposed rats (15 per group) to 0, 0.57, or 5.31 mg/m³ methanol continuously for 90 days. Chronaxy ratios of flexor and extensor muscles were measured in addition to hematologic parameters and acetyl cholinesterase activity. No changes were apparent in the 0.57 mg/m³ group. Effects observed in the 5.31 mg/m³ group included decreased blood albumin content beginning 7 weeks after exposure, slightly decreased acetylcholinesterase activity, decreased coproporphyrin levels in the urine after 7 weeks, and changes in muscle chronaxy. (Chronaxy is the minimum time an electric current must flow at a voltage twice the rheobase to cause a muscle to contract. The rheobase is the minimal electric current necessary to produce stimulation (Dorland, 1981).

Pregnant rats were exposed by inhalation to methanol at concentrations ranging from 5000 to 20,000 ppm for 7 hours per day on days 1-19 gestation, and days 7-15 for the highest dose group (Nelson *et al.*, 1985). A dose-related decrease in fetal weight, an increase in extra or rudimentary cervical ribs, and urinary or cardiovascular defects were observed. Exencephaly and 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)

<i>Study</i>	Rogers <i>et al.</i> (1993)
<i>Study population</i>	Pregnant mice
<i>Exposure method</i>	Discontinuous inhalation, 7 hours/day on days 6-15 of gestation
<i>Critical effects</i>	Abnormal cervical ribs, exencephaly, cleft palate
<i>LOAEL</i>	5000 ppm
<i>NOAEL</i>	1000 ppm
<i>Exposure continuity</i>	7 hr/day
<i>Exposure duration</i>	10 days
<i>Average experimental exposure</i>	292 ppm for NOAEL group
<i>Human equivalent concentration</i>	292 ppm for NOAEL group (gas with systemic effects, based on RGDR = 1.0 using default assumption that lambda (a) = lambda (h))
<i>Subchronic uncertainty factor</i>	1 (see below)
<i>LOAEL uncertainty factor</i>	1
<i>Interspecies uncertainty factor</i>	3
<i>Intraspecies uncertainty factor</i>	10
<i>Cumulative uncertainty factor</i>	30
<i>Inhalation reference exposure level</i>	10 ppm (10,000 ppb, 10 mg/m ³ , 10,000 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 for subchronic to chronic was not required. The investigators calculated maximum likelihood estimates (MLEs) using a log-logistic model for both 1% and 5% added risks above background. The most sensitive developmental toxicity endpoint was an increase in the incidence of cervical ribs. The MLE₀₅ and BMC₀₅ for cervical ribs were 824 ppm (1079 mg/m³) and 305 ppm (400 mg/m³), respectively.

Andrews *et al.* (1987) have investigated the effects of chronic exposure to methanol in primates. In this study aimed at examining ocular toxicity, no treatment-related effects were observed in cynomolgous monkeys exposed by inhalation to 6650 mg/m³ (5010 ppm) methanol for 6 hours/day, 5 days/week for 4 weeks. 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). Teachers aides exposed to duplicating fluid containing 99% methanol reported symptoms of blurred vision, headaches, dizziness, nausea and skin problems (Frederick *et al.*, 1984) The measured methanol concentrations ranged from 485 to 4096 mg/m³ for a 15-minute sampling period. Exposure assessment in this study was poorly characterized and exposure duration was not specified.

The major strengths of the REL are the observation of a NOAEL and the demonstration of a dose-response relationship. The major uncertainties are the lack of human data for chronic

inhalation exposure, the lack of comprehensive, long-term multiple dose studies, and the difficulty in addressing reproductive short-term effects within the chronic REL framework.

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CHRONIC TOXICITY SUMMARY

METHYL BROMIDE

(bromomethane; monobromomethane)

CAS Registry Number: 74-83-9

I. Chronic Toxicity Summary

<i>Inhalation reference exposure level</i>	5 g/m³ (U.S. EPA RfC) This document summarizes the evaluation of non-cancer health effects by U.S. EPA for the RfC.
<i>Critical effect(s)</i>	Histological lesions of the olfactory epithelium of the nasal cavity in rats
<i>Hazard index target(s)</i>	Respiratory system; nervous system; teratogenicity

II. Physical and Chemical Properties (HSDB, 1994)

<i>Description</i>	Colorless gas
<i>Molecular formula</i>	CH ₃ Br
<i>Molecular weight</i>	94.95 g/mol
<i>Density</i>	3.89 g/L @ 25°C
<i>Boiling point</i>	3.6°C
<i>Vapor pressure</i>	1420 mm Hg @ 20°C
<i>Solubility</i>	Soluble in ethanol, benzene, carbon disulfide, and 1.75% (w/w) in water
<i>Odor threshold</i>	20.6 ppm
<i>Odor description</i>	Sweetish odor
<i>Metabolites</i>	Methanol, bromide, 5-methylcysteine
<i>Conversion factor</i>	1 ppm = 3.89 mg/m ³ @ 25° C

III. Major Uses and Sources

Methyl bromide (MeBr) was used historically as an industrial fire extinguishing agent and was introduced in the U.S. from Europe in the 1920s. Current uses of MeBr include the fumigation of homes and other structures for termites and other pests. Methyl bromide is also used to fumigate soil before planting and fruits and vegetables after harvest. In 1981, 6.3 million pounds of MeBr were reportedly used in California (Alexeeff and Kilgore, 1983). By 1991, its use had grown to 18.7 million pounds in the state (Cal/EPA, 1993).

IV. Effects of Human Exposure

Workers (n = 32) exposed to MeBr during fumigation of soil or structures were compared to a referent group of 29 workers not exposed to MeBr, but exposed to other fumigants (Anger *et al.*, 1986). Exposures to MeBr were not quantified. It was found that workers exposed to MeBr had a higher rate of neurological symptoms and performed less well on several behavioral tests. Several confounding factors were present in this study, including lack of adjustments for age, alcohol consumption, prescription medication, illegal drugs, education, or ethnic group between the exposed and the referent groups.

V. Effects of Animal Exposure

The first experimental animal study on repeated MeBr exposures was carried out and reported by Irish and associates (1940). In this study, rats (135 per group), rabbits (104 per group), or female rhesus monkeys (13 per group) were exposed to 0, 17, 33, 66, 100, or 220 ppm (0, 66, 128, 256, 388, or 853 mg/m³) 7-8 hours/day, 5 days/week for 6 months or until the majority of the animals exhibited severe signs of toxicity. Mortality was seen in rats, guinea pigs, and monkeys at 100 ppm. Rabbits began to die at 33 ppm. Severe effects, including paralysis, were seen after exposure to 66 ppm in rabbits and monkeys. None of the species exhibited adverse effects after exposure to 17 ppm.

Kato and associates (1986) observed focal lesions in the brain and heart in rats (10-12 per group) after inhalation of 150 ppm (585 mg/m³) MeBr 4 hours/day, 5 days/week for 11 weeks. In another experiment, rats were exposed to 0, 200, 300, or 400 ppm (0, 777, 1160, or 1550 mg/m³) MeBr 4 hours/day, 5 days/week for 6 weeks. In this experiment, rats exposed to any concentration of MeBr exhibited coronary lesions, and exposures of 300 ppm or greater resulted in neurological dysfunction, including ataxia and paralysis. Testicular atrophy was noted in 6 of the 8 animals exposed to 400 ppm.

Anger *et al.* (1981) determined that rabbits are more sensitive than rats to neurotoxicity of MeBr. In this study, rats or rabbits were exposed to 0 or 65 ppm (0 or 254 mg/m³) MeBr for 7.5 hours/day, 4 days/week, for 4 weeks. Nerve conduction velocity and eyeblink reflex were impaired in the rabbits but not rats exposed to 65 ppm MeBr. Similarly, rats did not exhibit neurological signs after exposure to 55 ppm (215 mg/m³) MeBr for 36 weeks. Rabbits exposed to 26.6 ppm (104 mg/m³) did not display any neurological effects after 8 months exposure (Russo *et al.*, 1984).

In the studies of Reuzel and associates (1987, 1991), groups of 50 male and 60 female Wistar rats were exposed to 0, 3, 30, or 90 ppm methyl bromide (98.8%) for 6 hours per day, 5 days per week. Three groups of animals (10/sex/exposure level) were killed for observations at 14, 53, and 105 weeks of exposure. Body weight, hematology, clinical chemistry, and urinalyses were examined throughout the experiment in addition to histopathology and organ weights at time of necropsy. Exposures of males and females to 90 ppm resulted in reduced body weight. Exposure to 90 ppm also resulted in significant lesions in the heart in the form of cartilaginous metaplasia and thrombus in the males, and myocardial degeneration and thrombus in the

females. Exposure of males to 30 or 90 ppm resulted in a decrease in relative kidney weight. Histological changes in the nose, heart, esophagus, and forestomach were the principal effects of methyl bromide toxicity. At the lowest concentration (3 ppm), very slight degenerative changes in the nasal epithelium, and olfactory basal cell hyperplasia were noted in both sexes at 29 months. Based on this study, a LOAEL of 3 ppm (11.7 mg/m³) was determined.

The National Toxicology Program (NTP) conducted a 13-week and a chronic study on the toxicology and carcinogenesis of methyl bromide in rats and mice (NTP, 1990). In the 13-week study, 18 rats/sex/group were exposed to 0, 30, 60, or 120 ppm (0, 117, 233, or 466 mg/m³) MeBr 6 hours/day, 5 days/week. The mice were exposed to 0, 10, 20, 40, 80, or 120 ppm (0, 39, 78, 155, 311, or 466 mg/m³) 6 hours/day, 5 days/week. Hematological parameters and selected organ weights were measured in both species, in addition to histopathological changes. Pseudocholinesterase activity and neurobehavioral tests were conducted in the mice. Serious effects, including 58% body weight loss, 17% mortality and severe curling and crossing of the hindlimbs were observed in mice exposed to 120 ppm MeBr. Exposure of males to 40 ppm or higher resulted in significant effects on several hematological parameters, including decreased mean cell hemoglobin and increased red blood cell count. The only exposure-related histological effect was olfactory epithelial dysplasia and cysts in the rats of both sexes exposed to 120 ppm.

A 6-week study in rats and mice (5 animals/sex/group) exposed to 0 or 160 ppm (0 or 624 mg/m³) showed high mortality rates, loss in body weight and histological changes in multiple organ systems including brain, kidney, nasal cavity, heart, adrenal gland, liver, and testes (NTP, 1990).

An exposure of mice (86 animals/group) to 0, 10, 33, or 100 ppm (0, 38.8, 128, or 388 mg/m³) MeBr for 6 hours/day, 5 days/week, for 103 weeks was also conducted by NTP (1990). In this study, high mortality rates in both males and females in the 100 ppm group resulted in a discontinuation of exposure after 20 weeks. A low incidence of sternal dysplasia and a significant decrease in locomotor activity were noted in the 10 ppm group.

A 5-day exposure of rats (10 animals/group) to 0, 90, 175, 250, or 325 ppm (0, 350, 680, 971, or 1260 mg/m³) resulted in lesions in the nasal olfactory sensory cells, the cerebellum and adrenal gland beginning at 175 ppm (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/m³) MeBr. Olfactory function, measured by the ability to locate buried food, was impaired at the 200 ppm exposure. In this study, reduced testosterone and testicular glutathione levels were observed in the male rats exposed to 200 ppm, but no effects on spermatogenesis, sperm quality, or testes histopathology were noted.

Sikov *et al.* (1981) examined the teratogenic potential of MeBr in rats and rabbits exposed to 0, 20, or 70 ppm (0, 78, or 272 mg/m³) 7 hours/day, 5 days/week for 3 weeks during days 1-19 (rats) or 1-24 (rabbits) of gestation. No maternal or fetal effects were observed in the rats, however, severe maternal neurotoxic effects were observed in the rabbits that resulted in 24/25 deaths. In this study, no significant maternal or fetal effects were observed at a concentration of 20 ppm.

Another developmental toxicity study was conducted in rabbits by Breslin *et al.* (1990). In this study, rabbits were exposed to 0, 20, 40, or 80 ppm (0, 78, 156, or 312 mg/m³) MeBr for 6 hours/day on gestation days 6-19. Maternal toxicity was observed at 80 ppm and included reduced body weight gain and signs of neurotoxicity. In addition to the maternal effects observed, a significant increase in incidence of gall bladder agenesis and fused sternbrae were observed in the offspring exposed to 80 ppm. No adverse effects were observed at 40 ppm or lower concentrations.

A 2-generation reproduction and developmental toxicity study on MeBr in rats was conducted by American Biogenics Corporation (1986). Groups of rats (25/sex/concentration) were exposed to 0, 3, 30, or 90 ppm (0, 12, 117, or 350 mg/m³) MeBr 6 hours/day, 5 days/week during pre-mating, gestation, and lactation through 2 generations. Significant decreases in body weight during the pre-mating period and at the end of the study were observed in the males exposed to 90 ppm. Although some adult organ weights were affected in the 90-ppm group, there was no evidence of histopathology in these organs. Neonatal body weights were decreased by exposure to 30 ppm. There was a decreased cerebral cortex width in the 90 ppm F₁ group, reduced brain weight in 30 ppm F₁ females, and reduced fertility in the 30 and 90 ppm F_{2b} groups.

VI. Derivation of U.S. EPA RfC

<i>Study</i>	Reuzel <i>et al.</i> , 1987; 1991; U.S. EPA, 1995
<i>Study population</i>	Male and female Wistar rats (50 and 60 per group, respectively)
<i>Exposure method</i>	Discontinuous inhalation exposures (0, 3, 30, or 90 ppm) over 29 months
<i>Critical effects</i>	Histological lesions of the olfactory epithelium of the nasal cavity
<i>LOAEL</i>	3 ppm
<i>NOAEL</i>	Not observed
<i>Exposure continuity</i>	6 hr/day, 5 days/week
<i>Exposure duration</i>	29 months
<i>Average experimental exposure</i>	0.54 ppm for the LOAEL group
<i>Human equivalent concentration</i>	0.12 ppm for the LOAEL group (gas with extrathoracic respiratory effects, RGDR = 0.23, based on MV = 0.03 m ³ /min, SA = 11.6 cm ²)
<i>LOAEL uncertainty factor</i>	3
<i>Subchronic uncertainty factor</i>	1
<i>Interspecies uncertainty factor</i>	3
<i>Intraspecies uncertainty factor</i>	10
<i>Cumulative uncertainty factor</i>	100
<i>Inhalation reference exposure level</i>	0.001 ppm (1 ppb, 0.005 mg/m ³ , 5 µg/m ³)

The major strengths of the REL are the use of a comprehensive, long-term multiple dose study with large sample sizes, and the availability of supporting data including long-term studies in

other species and reproductive and developmental studies. The major uncertainties are the lack of human data and the lack of a NOAEL observation for the critical effect.

The California Department of Pesticide Regulation used a different approach that adjusts for respiration rate differences between humans and animals and which uses 10-fold uncertainty factors for interspecies differences, for intraspecies variability, and for a LOAEL to NOAEL extrapolation. Applying these factors to the same 3 ppm LOAEL results in a level for children and adults of 1 and 2 ppb (4 and 8 $\mu\text{g}/\text{m}^3$), respectively.

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Determination of Noncancer Chronic Reference Exposure Levels

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CHRONIC TOXICITY SUMMARY

METHYL CHLOROFORM

(1,1,1-trichloroethane, methyltrichloromethane)

CAS Registry Number: 71-55-6

I. Chronic Toxicity Summary

<i>Inhalation reference exposure level</i>	1,000 µg/m³
<i>Critical effect(s)</i>	Astrogliosis in the sensorimotor cortex (brain) of gerbils
<i>Hazard index target(s)</i>	Nervous system

II. Chemical Property Summary (HSDB, 1995)

<i>Description</i>	Colorless liquid
<i>Molecular formula</i>	C ₂ H ₃ Cl ₃
<i>Molecular weight</i>	133.42 g/mol
<i>Density</i>	1.3376 g/cm ³ @ 20° C
<i>Boiling point</i>	74.1° C
<i>Melting point</i>	-30.4° C
<i>Vapor pressure</i>	127 torr @ 25° C
<i>Solubility</i>	Soluble in acetone, benzene, methanol, carbon tetrachloride
<i>Conversion factor</i>	5.47 µg/m ³ per ppb at 25°C

III. Major Uses and Sources

Methyl chloroform is used as a solvent for adhesives and for metal degreasing (ACGIH, 1992). It is also used in the manufacture of vinylidene chloride and in textile processing and dry cleaning.

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.

