CHRONIC TOXICITY SUMMARY

AMMONIA

(Anhydrous ammonia; aqueous ammonia)

CAS Registry Number: 7664-41-7

I. Chronic Toxicity Summary

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

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

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

III. Major Uses or Sources

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

IV. Effects of Human Exposures

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

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

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

Group	Exposure	Week 1	Week 2	Week 3	Week 4	Week 5	Week 6
A	ppm NH_3 hours	25 2	50 4	100 6	25 2	50 4	100 6
B	ppm NH_3 hours	50 6	50 6	50 6	50 6	50 6	50 6
C	ppm NH_3 hours	100 6	50 4	25 2	25 6	50 4	100 2

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

V. Effects of Animal Exposures

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

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

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

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

VI. Derivation of Chronic Reference Exposure Level

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

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

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

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

VII. Data Strengths and Limitations for Development of the REL

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

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

VIII. References

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CHRONIC TOXICITY SUMMARY

BENZENE

(Benzol; Benzole; Cyclohexatriene)

CAS Registry Number: 71-43-2

I. Chronic Toxicity Summary

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

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

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

III. Major Uses or Sources

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

IV. Effects of Human Exposure

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

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

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

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

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

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

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

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

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

V. Effects of Animal Exposure

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

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

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

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

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

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

Ward *et al*. (1985) exposed 50 Sprague-Dawley rats and 150 CD-1 mice of both sexes to 0, 1, 10, 30, or 300 ppm benzene, 6 hours/day, 5 days/week for 13 weeks. Serial sacrifices were conducted at 7, 14, 28, 56, and 91 days. No hematological changes were found for mice and rats at 1, 10, or 30 ppm in this study. Significant increases in mean cell volume and mean cell hemoglobin values and decreases in hematocrit, hemoglobin, lymphocyte percentages, and decreases in red cell, leukocyte and platelet counts were observed in male and female mice at 300 ppm. The changes were first observed after 14 days of exposure. Histological changes in mice included myeloid hypoplasia of the bone marrow, lymphoid depletion in the mesenteric lymph node, increased extramedullary hematopoiesis in the spleen, and periarteriolar lymphoid sheath depletion. Effects were less severe in the rats.

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

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

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

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

gestation resulted in neonatal suppression of erythropoietic precursor cells and persistent, enhanced granulopoiesis. This effect was considered significant bone-marrow toxicity by the authors. No hematotoxicity was seen in this study at 10 ppm (32 mg/m^3).

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

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

VI. Derivation of Chronic Reference Exposure Level (REL)

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

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

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

VII. Data Strengths and Limitations for Development of the REL

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

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

Ward *et al*. (1996) determined a relationship between occupational exposures to benzene and decreased red and white cell counts. A modeled dose-response relationship indicated a possibility for hematologic effects at concentrations below 5 ppm. However, no specific

measures of the actual effects at concentrations below 2 ppm were taken, and the Tsai *et al*. (1983) data were not considered in their analysis. The purpose of this study was to characterize the trend for effects at low concentrations of benzene. A NOAEL or LOAEL was not identified in the study. The selection of a NOAEL of 0.53 ppm is therefore not inconsistent with the results of the Ward *et al*. (1996) study.

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

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

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CHRONIC TOXICITY SUMMARY

CHLORINATED DIBENZO-P-DIOXINS AND CHLORINATED DIBENZOFURANS

(INCLUDING 2,3,7,8-TETRACHLORODIBENZO-P-DIOXIN)

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

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

I. Chronic Toxicity Summary

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

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

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

III. Major Uses and Sources

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

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

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

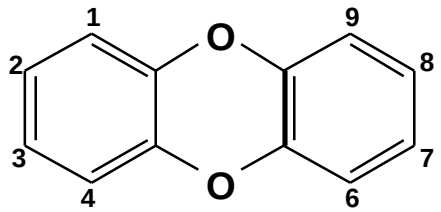
Ah receptor as well as on relative toxicity of different congeners. Values for the international system of toxic equivalents are provided in Table 1 (U.S. EPA, 1989).

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

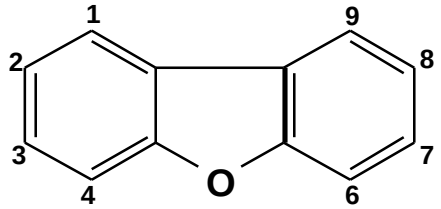
Compound[1,2]	**I-TEF**
Mono-, Di-, and Tri-CDDs and CDFs	0
TetraCDD	
2,3,7,8-substituted	1.0
Others	0
PentaCDD	
2,3,7,8-substituted	0.5
Others	0
HexaCDD	
2,3,7,8-substituted	0.1
Others	0
HeptaCDD	
2,3,7,8-substituted	0.01
Others	0
OctaCDD	0.001
TetraCDF	
2,3,7,8	0.1
Others	0
PentaCDF	
1,2,3,7,8-PentaCDF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

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

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



Dibenzo-p-dioxin



Dibenzofuran

IV. Effects of Human Exposure

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

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

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

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

V. Effects of Animal Exposure

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

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

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

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

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

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

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

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

VI. Derivation of Chronic Reference Exposure Level (REL)

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

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

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

VII. Data Strengths and Limitations for Development of the REL

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

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

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CHRONIC TOXICITY SUMMARY

CHLORINE

CAS Registry Number: 7782-50-5

I. Chronic Toxicity Summary

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

II. Physical and Chemical Properties (HSDB, 1995; 1999; CRC, 1994)

Description	Yellow/green gas
Molecular formula	Cl_2
Molecular weight	70.905
Density	2.9 g/L @ 25°C and 1 ATM
Boiling point	-34.6°C
Melting point	-100.98°C
Vapor pressure	5 atm @ 10.3°C; 5830 torr @ 25°C
Solubility	Slightly soluble in water (310 mL per 100 mL water at 10° C; 1.46 g per 100 mL water at 0° C)
Conversion factor	1 ppm = 2.9 mg/m^3 @ 25° C

III. Major Uses and Sources

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

IV. Effects of Human Exposure

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

workers who were employed/ exposed for 10-25 years. Group B consisted of 133 workers employed for less than 10 years. Both groups of workers were exposed to a range of 2.60-11.0 mg/m^3 (0.37-1.75 ppm) chlorine. The control group's average age was 39.7 years (ranging from 26-55 years), and it consisted of 192 workers not exposed to chlorine, but working within the same plant. For all the groups, respiratory symptoms and smoking habits were evaluated as well as clinical examinations, ENT examinations, chest x-rays and pulmonary function tests. Groups A and B showed 3-8 times higher incidence of upper airway complaints than the control workers. Current smokers in groups A and B experienced the highest incidence of pulmonary symptoms and group A workers had a higher prevalence of rhino-pharyngeal signs than the control workers. Abnormalities in chest x-rays were seen in 8.6% of group A workers and in 2.8% of group B workers, compared to 2.3% of the control workers. Groups A and B showed significantly impaired pulmonary function in tests of V50/H and FEF_{25-75} (forced expiratory flow between 25 and 75% of forced vital capacity (FVC), the total amount of air the subject can expel during a forced expiration) - compared with the control group, and group A showed reduced FEV_1 (forced expiratory volume in 1 second) results compared to the control group.

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

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

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

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

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

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

V. Effects of Exposure to Animals

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

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

VI. Derivation of Chronic Reference Exposure Level (REL)

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

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

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

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

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

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

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

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

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

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

VII. Data Strengths and Limitations for Development of the REL

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

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CHRONIC TOXICITY SUMMARY

ETHYLBENZENE

(Phenylethane; NCI-C56393)

CAS Registry Number: 100-41-4

I. Chronic Toxicity Summary

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

II. Physical and Chemical Properties (HSDB, 1994)

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

III. Major Uses or Sources

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

IV. Effects of Human Exposure

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

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

V. Effects of Animal Exposure

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

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

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

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

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

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

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

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

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

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

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

VI. Derivation of the Chronic Reference Exposure Level

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

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

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

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

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

VII. Data Strengths and Limitations for Development of the REL

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

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CHRONIC TOXICITY SUMMARY

ETHYLENE GLYCOL MONOETHYL ETHER

(2-ethoxyethanol; EGEE)

CAS Registry Number: 110-80-5

I. Chronic Toxicity Summary

Inhalation reference exposure level	**70 µg/m^3 (20 ppb)**
Critical effect(s)	Testicular degeneration and decreased hemoglobin in rabbits
Hazard index target(s)	Reproductive system; hematopoietic system

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

Description	Colorless liquid; sweet, pleasant, ether-like odor
Molecular formula	$C_4H_{10}O_2$
Molecular weight	90.12
Boiling point	135°C
Vapor pressure	3.8 torr @ 20°C; 5.31 torr at 25°C
Solubility	Miscible with water and organic solvents
Conversion factor	3.69 µg/m^3 per ppb at 25ºC

III. Major Uses and Sources

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

IV. Effects of Human Exposure

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

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

Welch and Cullen (1988) evaluated shipyard painters exposed to ethylene glycol ethers (EGEE and EGME). Air concentrations at the workplace were estimated based on 102 samples over six shifts in Sparer *et al*. (1988). Time –weighted average (TWA) exposures to EGEE ranged from 0 to 80.5 mg/m^3 with a mean of 9.9 mg/m^3. TWA exposures to EGME ranged from 0 to 17.7 mg/m^3 (mean = 2.6 mg/m^3). The authors note that during the time period of measurement, painting activities were unusually low and previous NIOSH analyses indicated considerably higher exposures. Ninety-four painters and 55 controls answered a medical and environmental exposure questionnaire including work history and provided blood, urine, and in some cases semen samples. Mean hemoglobin levels, total cell counts and differential counts did not differ between exposed and control. However, the authors found that the lowest quartile of hemoglobin was mostly painters and the lowest polymorphonuclear leukocyte counts were in painters. Nine painters were considered anemic and five were considered granulocytopenic. The authors note that the absence of a significant difference in the group as a whole and the inability to detect a dose-response pattern in the exposed group make a strong conclusion unwarranted.

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

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

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

V. Effects of Animal Exposure

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

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

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

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

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

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

VI. Derivation of Reference Exposure Level

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

The reproductive effects observed in the subchronic inhalation study of Barbee *et al.* (1984) were determined by the US EPA (U.S. EPA, 1990) to be the most sensitive endpoints due to EGEE exposure and resulted in a reference concentration (RfC) of 0.2 mg/m^3 (0.06 ppm). OEHHA staff concurred regarding the basis of the U.S. EPA RfC but differed in the application of the interspecies uncertainty factor. Reduced testes weight and testicular degeneration were found in rabbits exposed to EGEE at 403 ppm for 13 weeks. Changes in hematological parameters including decreased hemoglobin, hematocrit, and erythrocyte count were also observed at this dose. A gas:extrarespiratory effect ratio of 1.0 was used to calculate a human equivalency concentration (HEC) in the absence of information relating the effect in rabbits relative to humans.

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

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

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

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

VII. Data Strengths and Limitations for Development of the REL

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

VIII. References

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CHRONIC TOXICITY SUMMARY

ETHYLENE GLYCOL MONOETHYL ETHER ACETATE

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

CAS Registry Number: 111-15-9

I. Chronic Toxicity Summary

Inhalation reference exposure level	**300 µg/m^3 (60 ppb)**
Critical effect(s)	Teratogenicity and fetotoxicity in rabbits
Hazard index target(s)	Development

II. Chemical Property Summary (HSDB, 1996)

Description	Colorless liquid
Molecular formula	$C_6H_{12}O_3$
Molecular weight	132.16 g/mol
Boiling point	156°C
Vapor pressure	2 torr @ 20°C
Solubility	Soluble in water (229 g/L at 20°C); soluble in alcohol, ether, acetone; miscible with olive oil, aromatic hydrocarbons
Conversion factor	5.41 µg/m^3 per ppb at 25ºC

III. Major Uses and Sources

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

IV. Effects of Human Exposure

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

Ten male volunteers were exposed to EGEEA by inhalation. Five were exposed to 14, 28, and 50 mg EGEEA/m^3 and five to 28 mg/m^3 for 4 hours (Groeseneken *et al.*, 1987a). Twenty-two percent of the absorbed dose was eliminated in the urine as ethoxyacetic acid within 42 hours. In another study, male volunteers exposed to EGEEA by inhalation under various conditions were found to eliminate some in the form of ethylene glycol monoethyl ether (EGEE) (Groeseneken *et al.*, 1987b).

V. Effects of Animal Exposure

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

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

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

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

weight gain, decreased gravid uterine weight, increased number of dams with non-viable implants, and increased number of non-viable implants per litter (200 and 300 ppm); increased occult blood, increased mean corpuscular volume, decreased corpora lutea/litter and increased early resorptions/litter (300 ppm). Visceral and skeletal malformations were observed in the 100, 200, and 300 ppm EGEEA dose groups.

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

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

VI. Derivation of Chronic Reference Exposure Level (REL)

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

A review of the literature on the toxicity of EGEEA indicates that the most sensitive endpoint of toxicity is that seen in experimental animals showing developmental effects from inhalation exposure during pregnancy. There are no adequate data associating exposures in humans with toxic effects for the development of a chronic reference exposure level. Separate studies in animals have demonstrated developmental toxicity. Reduced fetal weights were observed in rats exposed to 130 ppm EGEEA on gestational days 7-15 (Nelson *et al.*, 1984). Skeletal and cardiovascular defects were observed at the next higher dose of 390 ppm EGEEA, and all litters were resorbed in the high-dose group. Visceral and skeletal defects were observed in all but the low-dose group (50 ppm EGEEA) in the litters of rabbit dams exposed to EGEEA on gestational

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

VII. Data Strengths and Limitations for Development of the REL

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

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CHRONIC TOXICITY SUMMARY

ETHYLENE GLYCOL MONOMETHYL ETHER

(EGME; 2-methoxyethanol; 1-hydroxy-2-methoxyethane; methyl cellosolve)

CAS Registry Number: 109-86-4

I. Chronic Toxicity Summary

Inhalation reference exposure level	**60 µg/m^3 (20 ppb)**
Critical effect(s)	Testicular toxicity in rabbits
Hazard index target(s)	Reproductive system

II. Physical and Chemical Properties (HSDB, 1995)

Description	Colorless liquid
Molecular formula	$C_3H_8O_2$
Molecular weight	76.09
Density	0.965 g/cm^3 @ 20° C
Boiling point	125°C
Melting point	-85.1°C
Vapor pressure	6.2 torr @ 20°C
Solubility	Miscible with water, alcohol, benzene, ether, acetone
Conversion factor	1 ppm = 3.1 mg/m^3 @ 25°C

III. Major Uses and Sources

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

IV. Effects of Human Exposure

Human exposures to ethylene glycol monomethyl ether have been associated with hematological and neurological abnormalities. To determine whether employees potentially exposed to ethylene glycol monomethyl ether during manufacturing and packaging had a higher prevalence

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

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

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

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

The painters moved frequently from one exposure area to the next, making exposure assessment particularly difficult in this cohort.

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

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

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

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

V. Effects of Animal Exposure

A concentration dependent decrease in testes weight was observed in male rabbits exposed to 30, 100, or 300 ppm EGME 6 hours per day, 5 days per week for 13 weeks (Miller *et al.*, 1983). Degenerative changes in the germinal epithelium were observed in male rabbits of <u>all</u> exposed groups, but were not statistically significant at 30 ppm. Two of five male rabbits exposed to 300 ppm EGME died during the course of the study. Female rabbits were also exposed; two of five female rabbits exposed to 100 or 300 ppm EGME died during the course of the study. The animals died at different times of different causes and thus the authors were uncertain if the

deaths were treatment related. Reduced body weight gain, pancytopenia (abnormal depression of all the cellular elements of the blood), and thymic atrophy were observed in rabbits of both sexes exposed to 300 ppm EGME. No effects on the reproductive organs of the female rabbits were observed.

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

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

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

Pregnant mice were exposed to 100, 150, or 200 mg/kg/day EGME on days 10-17 of gestation (Holladay *et al.*, 1994). Thymic atrophy and inhibition of fetal thymocyte maturation were observed in EGME-treated offspring examined on day 18 of gestation. Also, the ability of the EGME-treated fetal mouse liver cells to repopulate the spleen of irradiated mice was significantly impaired as compared to that of control fetal mouse liver cells.

VI. Derivation of Reference Exposure Level

Study	Miller *et al.*, 1983; U.S. EPA, 1995
Study population	Rats and rabbits
Exposure method	Inhalation (0, 30, 100, or 300 ppm)
Critical effects	Decreased testes weight and degenerative hanges in the testicular germinal pithelium.
LOAEL	100 ppm
NOAEL	30 ppm
Exposure continuity	6 hours/day, 5 days/week
Average experimental exposure	5.4 ppm for NOAEL group
Human equivalent concentration	5.4 ppm for NOAEL group (gas with stemic effects, based on RGDR = 1.0 using fault assumption that lambda (a) = lambda)
Exposure duration	13 weeks
LOAEL uncertainty factor	1
Subchronic uncertainty factor	10
Interspecies uncertainty factor	3
Intraspecies uncertainty factor	10
Cumulative uncertainty factors	300
Inhalation reference exposure level	0.02 ppm (20 ppb; 0.06 mg/m^3; 60µg/m^3)

The REL is based on the same study on which U.S. EPA based its RfC. However, OEHHA declined to use a modifying factor because the criteria for use of such factors are not well described by U.S. EPA. However, since rabbits were the more sensitive species and live 6 years (312 weeks), a 13 week study in rabbits merits a subchronic UF of 10.

A comparison with the proposed REL for EGME of 20 ppb (60 µg/m^3) can be made using the occupational study of Welch *et al*. (1988) of the semen of shipyard painters exposed to both EGEE and EGME. The men supplied demographic characteristics, medical conditions, personal habits, and reproductive history; underwent a physical examination; and provided a semen sample. The painters were exposed to EGEE at a TWA concentration of 0 to 80.5 mg/m^3 (mean = 9.9 mg/m^3, and to EGME at a TWA concentration of 0 to 17.7 mg/m^3 (mean = 2.6 mg/m^3). The painters had an increased prevalence of oligospermia and azoospermia and an increased odds ratio for a lower sperm count per ejaculate compared to shipyard employees who were not painters. (The results were controlled for smoking.) Adding the mean exposure levels together results in a total glycol ether concentration (EGME + EGEE) of 12.5 mg/m^3, equivalent to a continuous exposure of 4.5 mg/m^3. Division by a UF of 10 for a LOAEL and by another of 10 for human intraspecies variability results in a REL of 40 µg/m^3 (10 ppb), similar to the REL based on rabbits. Since exposure was primarily to EGEE with co-exposure to EGME, and exposure assessment ws difficult to quantify, this study was not deemed suitable for developing a REL. Nonetheless, the REL developed using this study is close in value to the proposed REL of 20 ppb.

VII. Data Strengths and Limitations for Development of the REL

The strengths of the inhalation REL for EGME include the availability of subchronic inhalation exposure data from a well-conducted study with histopathological analysis and the observation of a NOAEL. In addition, there are a number of human studies showing similar toxicological endpoints to those demonstrated in animal studies. Major areas of uncertainty are the lack of adequate human exposure data, and the lack of chronic inhalation exposure studies.

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CHRONIC TOXICITY SUMMARY

ETHYLENE GLYCOL MONOMETHYL ETHER ACETATE

(EGMEA; 2-methoxyethanol acetate; 2-methoxyethylester acetic acid; methyl glycol acetate; methyl Cellosolve® acetate)

CAS Registry Number: 110-49-6

I. Chronic Toxicity Summary

Inhalation reference exposure level	**90 mg/m^3 (20 ppb)**
Critical effect(s)	Reproductive (testicular) toxicity in rabbits (EGME)
Hazard index target(s)	Reproductive system

II. Chemical Property Summary (HSDB, 1995)

Description	Colorless liquid
Molecular formula	$C_5H_{10}O_3$
Molecular weight	118.3 g/mol
Boiling point	144-145°C
Vapor pressure	2 torr @ 20°C
Solubility	Miscible with water, organic solvents, oils
Conversion factor	4.83 µg/m^3 per ppb at 25ºC

III. Major Uses and Sources

Ethylene glycol monomethyl ether acetate (EGMEA) is used as a solvent for nitrocellulose, cellulose acetate, and various other gums, resins, waxes, and oils (HSDB, 1995). It is also used in the semiconductor industry and in textile printing, photographic films, lacquers, and silk-screening inks. The annual specific statewide industrial emissions of EGMEA from facilities reporting under the Air Toxics Hot Spots Act in California based on the most recent inventory were estimated to be 3,060 pounds (CARB, 1999).

IV. Effects of Human Exposure

Developmental defects have been described in the offspring of a mother who was occupationally exposed to EGMEA during pregnancy (Bolt and Golka, 1990). The mother was exposed during pregnancy by skin absorption and inhalation for approximately 1-4 hours/day to 1-2 liters of EGMEA. Her first child was born with congenital hypospadia, chordee, micropenis, and scrotum bifida and her second child (3 years later) was born with chordee, cryptorchidism, penile

hypospadia and scrotum bifida. Both children had normal karyotypes. No estimates of exposure were made.

A single case report described allergic dermatitis which may have developed from contact with EGMEA (Jordan and Dahl, 1971). A 58-year-old woman developed dermatitis on the nose possibly from contact with EGMEA on her eyeglasses. Ethylene glycol monoethyl ether acetate (EGEEA) was also present.

V. Effects of Animal Exposure

Cats, rabbits, guinea pigs, and mice were repeatedly exposed by inhalation for 8 hours daily to 500 and 1000 ppm EGMEA (Gross, 1943; as described by Gingell *et al.*, 1982). This exposure regimen was fatal to cats at 500 ppm EGMEA. Death occurred after the animals showed slight narcosis. Similarly, exposure to 1000 ppm EGMEA produced deaths among rabbits, guinea pigs, and mice within a few days. Kidney toxicity was observed in animals in both dose groups. Repeated 4- and 6-hour exposure of cats to 200 ppm EGMEA resulted in decreased "blood pigments" and red blood cell counts.

The toxic effects of EGMEA were examined in male mice treated by gastric intubation 5 days/week for 5 weeks with 0, 62.5, 125, 250, 500, 1000, or 2000 mg EGMEA/kg/day (Nagano *et al.*, 1984). Dose-related testicular atrophy was observed at doses above 250 mg EGMEA/kg/day. Decreased white blood cell counts were observed in all EGMEA-exposed groups.

EGMEA was readily converted in vitro to ethylene glycol monomethyl ether (EGME) by the nasal mucosal carboxylesterases of mice and rabbits (Stott and McKenna, 1985). The enzyme activity in the nasal mucosa was equal to that of the liver and greater than that of the kidney and lung.

A concentration dependent decrease in testes weight was observed in male rabbits exposed to 30, 100, or 300 ppm ethylene glycol monomethyl ether (EGME) 6 hours/day, 5 days/week for 13 weeks (Miller *et al.*, 1983). Degenerative changes in the germinal epithelium were observed in male rabbits of all exposed groups, but the changes were not statistically significant at 30 ppm. Two of five male rabbits exposed to 300 ppm EGME died during the course of the study. Female rabbits were also exposed; two of five female rabbits exposed to 100 or 300 ppm EGME died during the course of the study. Reduced body weight gain, pancytopenia (abnormal depression of all the cellular elements of the blood), and thymic atrophy were observed in rabbits of both sexes exposed to 300 ppm EGME. No effects on the reproductive organs of the female rabbits were observed.

In the same study male and female rats were exposed to 30, 100, or 300 ppm EGME 6 hrs/day, 5 days/week for 13 weeks. Moderate to severe degeneration of the germinal epithelium and seminiferous tubules was observed in male rats exposed to 300 ppm EGME. A significant decrease in body weight was observed in male rats exposed to 300 ppm and in female rats exposed to concentrations of EGME of 100 ppm or greater. Pancytopenia, lymphoid tissue

atrophy, and decreased liver weights were observed in animals of both sexes exposed to the highest concentration. Also in the highest exposure group, mean values for total serum protein, albumin and globulins were lower than control values.

VI. Derivation of Chronic Reference Exposure Level (REL)

Study	Miller *et al.*, 1983 (see below)
Study population	Rabbits
Exposure method	Discontinuous inhalation exposure (0, 30, 100, or 300 ppm EGME)
Critical effects	Testicular effects
LOAEL	100 ppm EGME
NOAEL	30 ppm EGME
Exposure continuity	6 hr/day, 5 days/week
Exposure duration	13 weeks
Average experimental exposure	5.4 ppm EGME for NOAEL group (30 x 6/24 x 5/7)
Human equivalent concentration	5.4 ppm EGME for NOAEL group (gas with systemic effects, based on RGDR = 1.0 using default assumption that lambda (a) = lambda (h))
LOAEL uncertainty factor	1
Subchronic uncertainty factor	10
Interspecies factor	3
Intraspecies factor	10
Cumulative uncertainty factor	300
Inhalation reference exposure level	0.02 ppm (20 ppb, 0.06 mg/m^3, 60 µg/m^3) EGME 90 µg/m^3 EGMEA (20 ppb) (60 x MW_{EGMEA} / MW_{EGME})

Data relating specific EGMEA exposure levels to toxicity in humans are not available for the development of a chronic REL. Data from experimental animals indicate that EGMEA is toxic to the hematopoietic and reproductive systems (Gross, 1943; Nagano *et al.*, 1984), however good, quantitative data relating chronic exposure to toxicity are lacking. Because of evidence that EGMEA is readily converted to EGME by several organ systems (Stott and McKenna, 1985) and since the scant data on EGMEA toxicity in animals indicate that the spectrum of toxicity of the two compounds is similar, the chronic REL was derived based upon the assumption of equimolar toxicity of EGMEA and EGME.

VII. Data Strengths and Limitations for Development of the REL

The strengths of the inhalation REL for EGMEA include the availability of subchronic inhalation exposure data from a well-conducted study of EGME as well as a number of supportive human studies on EGME showing the same toxicological endpoint, and the observation of a NOAEL. Major areas of uncertainty are the assumption that EGMEA toxicity is comparable to that of

EGME, the lack of adequate human exposure data, and the lack of chronic inhalation exposure studies.

VII. References

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CHRONIC TOXICITY SUMMARY

FORMALDEHYDE

(methanal; oxoymethane; oxomethylene; methylene oxide; formic aldehyde; methyl aldehyde)

CAS Registry Number: 50-00-0

I. Chronic Toxicity Summary

Inhalation reference exposure level	**3 mg/m^3 (2 ppb)**
Critical effect(s)	Upper and lower airway irritation and eye irritation in humans; degenerative, inflammatory and hyperplastic changes of the nasal mucosa in humans and animals
Hazard index target(s)	Respiratory system; eyes

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

Description	Colorless gas
Molecular formula	CH_2O
Molecular weight	30.03 g/mol
Density	0.815 g/L @ -20°C
Boiling point	-19.1°C
Melting point	-92°C
Vapor pressure	220 kPa @ 0°C
Solubility	Soluble in water, ethanol, ether, acetone
Conversion factor	1 ppm = 1.23-1.25 mg/m^3 @ 25° C

III. Major Uses or Sources (CARB, 1992; HSDB, 1995)

Formaldehyde is used in the manufacture of melamine, polyacetal, and phenolic resins. Phenol-formaldehyde resins are used in the production of plywood, particleboard, foam insulation, and a wide variety of molded or extruded plastic items. Formaldehyde is also used as a preservative, a hardening and reducing agent, a corrosion inhibitor, a sterilizing agent, and in embalming fluids. Indoor sources include upholstery, permanent press fabrics, carpets, pesticide formulations, and cardboard and paper products. Outdoor sources include emissions from fuel combustion (motor vehicles), industrial fuel combustion (power generators), oil refining processes, and other uses (copper plating, incinerators, etc.). In 1997, the population-weighted annual average exposure in the South Coast Air Basin was estimated (using a model calibrated against actual atmospheric measurements) to be 4.7 ppb formaldehye (CARB, 1999a). The annual statewide industrial

emissions from facilities reporting under the Air Toxics Hot Spots Act in California based on the most recent inventory were estimated to be 1,589,810 pounds of formaldehyde (CARB, 1999b).

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/m^3) 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/m^3) (range = 0.24 - 0.55 mg/m^3). 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/m^3) 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, FEV_1, FEF_{25-75}, FEF_{50}), 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 loss of cilia and goblet cell hyperplasia (11%), squamous metaplasia (78%), and mild dysplasia (8%), was observed in 75 wood products workers exposed to between 0.1 and 1.1 mg/m^3 formaldehyde for a mean duration of 10.5 years (range = 1 - 39 years), compared to an equal number of control subjects (Edling *et al.*, 1988). Only three exposed men had normal mucosa. A high frequency of symptoms relating to the eyes and upper airways was reported in exposed workers. Nasal symptoms included mostly a runny nose and crusting. The histological grading showed a significantly higher score for nasal lesions when compared with the referents (2.9 versus 1.8). Exposed smokers had a higher, but non-significant, score than ex-smokers and non-smokers. When relating the histological score to duration of exposure, the mean histological score was about the same regardless of years of employment. In addition, no difference in the histological scores was found between workers exposed only to formaldehyde and those exposed to formaldehyde and wood dust.

Alexandersson and Hedenstierna (1989) evaluated symptoms of irritation, spirometry, and immunoglobulin levels in 34 wood workers exposed to formaldehyde over a 4-year period. Exposure to 0.4 - 0.5 ppm formaldehyde resulted in significant decreases in FVC, FEV_1, and FEF_{25-75}. Removal from exposure for 4 weeks allowed for normalization of lung function in the non-smokers.

Kriebel *et al.* (1993) conducted a subchronic epidemiological study of 24 anatomy class students exposed to a range of formaldehyde of 0.49 to 0.93 ppm (geometric mean = 0.73 $\pm$ 1.22 ppm) for 3 hours per week for 10 weeks. One subject was a smoker, 2 reported current asthma, and 3 reported childhood asthma without current symptoms. Eye and throat irritation was significantly elevated in the students after classes compared with pre-laboratory session exposures. In addition, peak expiratory flow measurements declined by an average of 10 L/minute (2% of baseline), but returned to normal after 14 weeks of non-exposure.

Histology technicians (280 subjects) were shown to have reduced pulmonary function, as measured by FVC, FEV_1, FEF_{25-75}, and FEF_{75-85}, compared with 486 controls (Kilburn *et al.*, 1989). The range of formaldehyde concentrations was 0.2 - 1.9 ppm, volatilized from formalin preservative solution.

Malaka and Kodama (1990) investigated the effects of formaldehyde exposure in plywood workers (93 exposed, 93 controls) exposed for 26.6 years, on average, to 1.13 ppm (range = 0.28 - 3.48 ppm). Fifty-three smokers were present in both study groups. Exposure assessment was divided into 3 categories: high (> 5 ppm), low (< 5 ppm), and none (reference group). Subjective irritation and pulmonary function tests were performed on each subject, and chest x-rays were taken of 10 randomly selected volunteers from each group. Respiratory symptoms of irritation were found to be significantly increased in exposed individuals, compared with controls. In addition, exposed individuals exhibited significantly reduced FEV_1, FEV_1/FVC, and $FEF_{25-75,}$ compared with controls. Forced vital capacity was not significantly reduced. Pulmonary function was not found to be different after a work shift, compared to the same measurement taken before the shift. No differences in chest x-rays were observed between exposed and control workers.

Occupational exposure to formaldehyde concentrations estimated to be 0.025 ppm (0.038 mg/m^3) for greater than 6 years resulted in complaints by 22 exposed workers of respiratory, gastrointestinal, musculoskeletal, and cardiovascular problems, and in elevated formic acid excretion in the urine (Srivastava *et al.*, 1992). A control group of 27 workers unexposed to formaldehyde was used for comparison. A significantly higher incidence of abnormal chest x-rays was also observed in formaldehyde-exposed workers compared with controls.

Chemical plant workers (70 subjects) were exposed to a mean of 0.17 ppm (0.26 mg/m^3) formaldehyde for an unspecified duration (Holmstrom and Wilhelmsson, 1988). Compared with 36 control workers not exposed to formaldehyde, the exposed subjects exhibited a higher frequency of eye, nose, and deep airway discomfort. In addition, the exposed subjects had diminished olfactory ability, delayed mucociliary clearance, and decreased FVC.

Alexandersson *et al.* (1982) compared the irritant symptoms and pulmonary function of 47 carpentry workers exposed to a mean concentration of formaldehyde of 0.36 ppm (range = 0.04 - 1.25 ppm) with 20 unexposed controls. The average length of employment for the exposed workers was 5.9 years. Symptoms of eye and throat irritation as well as airway obstruction were more common in exposed workers. In addition, a significant reduction in FEV_1, FEV_1/FVC, and MMF was observed in exposed workers, as compared with controls.

Horvath *et al.* (1988) compared subjective irritation and pulmonary function in 109 workers exposed to formaldehyde with similar measures in a control group of 254 subjects. The formaldehyde concentrations for the exposed and control groups were 0.69 ppm (1.04 mg/m^3) and 0.05 ppm (0.08 mg/m^3), respectively. Mean formaldehyde concentration in the pre-shift testing facility and the state (Wisconsin) ambient outdoor - formaldehyde level were both 0.04 ppm (0.06 mg/m^3). Duration of formaldehyde exposure was not stated. Subjects were evaluated pre- and post work-shift and compared with control subjects. Significant differences in symptoms of irritation, FEV_1, FEV_1/FVC ratio, FEF_{50}, FEF_{25}, and FEF_{75} were found when comparing exposed subjects' pre- and post work-shift values. However, the pre-workshift values were not different from controls.

The binding of formaldehyde to endogenous proteins creates haptens that can elicit an immune response. Chronic exposure to formaldehyde has been associated with immunological hypersensitivity as measured by elevated circulating IgG and IgE autoantibodies to human serum albumin (Thrasher *et al.*, 1987). In addition, a decrease in the proportion of T-cells was observed, indicating altered immunity. Thrasher *et al.* (1990) later found that long-term exposure to formaldehyde was associated with autoantibodies, immune activation, and formaldehyde-albumin adducts in patients occupationally exposed, or residents of mobile homes or of homes containing particleboard sub-flooring. The authors suggest that the hypersensitivity induced by formaldehyde may account for a mechanism for asthma and other health complaints associated with formaldehyde exposure.

Symptoms of irritation were reported by 66 workers exposed for 1 - 36 years (mean = 10 years) to a mean concentration of 0.17 ppm (0.26 mg/m^3) formaldehyde (Wilhelmsson and Holmstrom,

1992). Controls (36 subjects) consisted of office workers in a government office and were exposed to a mean concentration of 0.06 ppm (0.09 mg/m^3) formaldehyde. The significant increase in symptoms of irritation in exposed workers did not correlate with total serum IgE antibody levels. However, 2 exposed workers, who complained of nasal discomfort, had elevated IgE levels. In another occupational health study, 37 workers, who were exposed for an unspecified duration to formaldehyde concentrations in the range of 0.003 to 0.073 ppm, reported ocular irritation; however, no significant serum levels of IgE or IgG antibodies to formaldehyde-human serum albumin were detected (Grammer *et al.*, 1990).

An epidemiological study of the effects of formaldehyde on 367 textile and shoe manufacturing workers employed for a mean duration of 12 years showed no significant association between formaldehyde exposure, pulmonary function (FVC, FEV_1, and PEF) in normal or asthmatic workers, and occurrence of specific IgE antibodies to formaldehyde (Gorski and Krakowiak, 1991). The concentrations of formaldehyde did not exceed 0.5 ppm (0.75 mg/m^3).

Workers (38 total) exposed for a mean duration of 7.8 years to 0.11 - 2.12 ppm (mean = 0.33 ppm) formaldehyde were studied for their symptomatology, lung function, and total IgG and IgE levels in the serum (Alexandersson and Hedenstierna, 1988). The control group consisted of 18 unexposed individuals. Significant decrements in pulmonary function (FVC and FEV_1) were observed, compared with the controls. Eye, nose, and throat irritation was also reported more frequently by the exposed group, compared with the control group. No correlation was found between duration of exposure, or formaldehyde concentration, and the presence of IgE and IgG antibodies.

The effects of formaldehyde on asthmatics appears to be dependent on previous, repeated exposure to formaldehyde. Burge *et al.* (1985) found that 3 out of 15 occupationally exposed workers challenged with formaldehyde vapors at concentrations from 1.5 ppm to 20.6 ppm for brief duration exhibited late asthmatic reactions. Six other subjects had immediate asthmatic reactions likely due to irritant effects. Asthmatic responses (decreased PEF, FVC, and FEV_1) were observed in 12 occupationally-exposed workers challenged with 1.67 ppm (2.5 mg/m^3) formaldehyde (Nordman *et al.*, 1985). Similarly, asthmatic responses were observed in 5 of 28 hemodialysis workers occupationally exposed to formalin and challenged with formaldehyde vapors (concentration not measured) (Hendrick and Lane, 1977). In asthmatics not occupationally exposed to formaldehyde, Sheppard *et al.* (1984) found that a 10-minute challenge with 3 ppm formaldehyde coupled with moderate exercise did not induce significant changes in airway resistance or thoracic gas volume.

V. Effects of Animal Exposure

Fischer-344 rats and B6C3F1 mice (120 animals/sex) were exposed to concentrations of 0, 2.0, 5.6, or 14.3 ppm formaldehyde vapor for 6 hours/day, 5 days/week for 24 months (Kerns *et al.*, 1983). The exposure period was followed by up to 6 months of non-exposure. Interim sacrifices were conducted at 6, 12, 18, 24, 27, and 30 months. Both male and female rats in the 5.6 and 14.3 ppm groups demonstrated decreased body weights over the 2-year period. At the 6 month sacrifice, the rats exposed to 14.3 ppm formaldehyde had non-neoplastic lesions of epithelial

dysplasia in the nasal septum and turbinates. As the study progressed, epithelial dysplasia, squamous dysplasia, and mucopurulent rhinitis increased in severity and distribution in all exposure groups. In mice, cumulative survival decreased in males from 6 months to the end of the study. Serous rhinitis was detected at 6 months in the 14.3 ppm group of mice. Metaplastic and dysplastic changes were noted at 18 months in most rats in the 14.3 ppm group and in a few mice in the 5.6 ppm exposure group. By 24-months, the majority of mice in the 14.3 ppm group had metaplastic and dysplastic changes associated with serous rhinitis, in contrast to a few mice in the 5.6 ppm group and a few in the 2 ppm group (exact number not given).

Woutersen *et al*. (1989) exposed male Wistar rats (60 animals/group) 6 hr/day for 5 days/week to 0, 0.1, 1.0 and 10 ppm formaldehyde vapor for 28 months. Compound-related nasal lesions of the respiratory and olfactory epithelium were observed only in the 10 ppm group. In the respiratory epiethlium, the lesions consisted of rhinitis, squamous metaplasia and basal cell/pseudoepithelial hyperplasia. In the olfactory region, the lesions included epithelial degeneration and rhinitis. No differences in behavior or mortality were noted among the various groups. However, growth retardation was observed in the 10 ppm group from day 14 onwards. In a parallel study, male Wistar rats were exposed to 0, 0.1, 1.0 and 10 ppm formaldehyde for 3 months followed by a 25-month observation period. Compound-related histopathological changes were found only in the noses of the 10 ppm group and comprised of increased incidences of squamous metaplasia of the respiratory epithelium and rhinitis.

In a chronic exposure study that primarily investigated aspects of nasal tumor development, Monticello *et al*. (1996) examined nasal cavities of male F-344 rats (0 - 10 ppm, 90 animals/group; 15 ppm, 147 animals) following exposure to 0, 0.7, 2, 6, 10, and 15 ppm formaldehyde for 6 hours/day, 5 days/week for 24 months. Treatment-related decreases in survival were apparent only in the 15 ppm group. Nasal lesions at the two highest doses included epithelial hypertrophy and hyperplasia, squamous metaplasia, and a mixed inflammatory cell infiltrate. Lesions in the 6 ppm group were minimal to absent and limited to focal squamous metaplasia in the anterior regions of the nasal cavity. No formaldehyde-induced lesions were observed in the 0.7 or 2 ppm groups.

Kamata *et al*. (1997) exposed 32 male F-344 rats/group to gaseous formaldehyde at 0, 0.3, 2, and 15 ppm 6 hr/day, 5 days/week for up to 28 weeks. A room control, non-exposed group was also included in the study. Five animals per group were randomly selected at the end of the 12th, 18th, and 24th months, and surviving animals at 28 months were sacrificed for full pathological evaulation. Behavioural effects related to sensory irritation were evident in the 15 ppm group. Significant decreases in food consumption, body weight and survival were also evident in this group. No exposure-related hematological findings were observed. Biochemical and organ weight examination revealed decreased triglyceride levels and absolute liver weights at the highest exposure, but was likely related to reduced food consumption. Abnormal histopathological findings were confined to the nasal cavity. Inflammatory cell infiltration, erosion or edema of the nasal cavity was evident in all groups, including controls. Significantly increased incidence of non-proliferative (squamous cell metaplasia without epithelial cell hyperplasia) and proliferative lesions (epithelial cell hyperplasia with squamous cell metaplasia) were observed in the nasal cavities beginning at 2 ppm. In the 0.3 ppm group, a non-significant

increase in proliferative nasal lesions (4/20 animals) were observed in rats that were either sacrificed or died following the 18th month of exposure.

Rusch *et al.* (1983) exposed groups of 6 male cynomolgus monkeys, 20 male or female rats, and 10 male or female hamsters to 0, 0.2, 1.0, or 3.0 ppm (0, 0.24, 1.2, or 3.7 mg/m^3) formaldehyde vapor for 22 hours/day, 7 days/week for 26 weeks. There was no treatment-related mortality during the study. In monkeys, the most significant findings were hoarseness, congestion and squamous metaplasia of the nasal turbinates in 6/6 monkeys exposed to 2.95 ppm. There were no signs of toxicity in the lower exposure groups. In the rat, squamous metaplasia and basal cell hyperplasia of the nasal epithelia were significantly increased in rats exposed to 2.95 ppm. The same group exhibited decreased body weights and decreased liver weights. In contrast to monkeys and rats, hamsters did not show any signs of response to exposure, even at 2.95 ppm.

Kimbell et al. (1997) exposed male F-344 rats ($\leq$ 6/group) to 0, 0.7, 2, 6, 10, and 15 ppm 6 hr/day, 5 days/week for 6 months. Squamous metaplasia was not observed in any regions of the nasal cavity in any of the control, 0.7, or 2 ppm groups. However, the extent and incidence of squamous metaplasia in the nasal cavity increased with increasing dose beginning at 6 ppm.

In subchronic studies, Wilmer *et al.* (1989) found that intermittent (8 hours/day, 5 days/week) exposures of rats to 4 ppm formaldehyde for 13 weeks resulted in significant histological changes in the nasal septum and turbinates. In contrast, continuous exposure of rats for 13 weeks to 2 ppm formaldehyde did not produce significant lesions. This study revealed the concentration dependent nature of the nasal lesions caused by formaldehyde exposure. Zwart *et al.*, (1988) exposed male and female Wistar rats (50 animals/group/sex) to 0, 0.3, 1, and 3 ppm formaldehyde vapor for 6 hr/day, 5 days/week for 13 weeks. Compound related histopathological nasal changes varying from epithelial disarrangement to epithelial hyperplasia and squamous metaplasia were found in the 3 ppm group, and were restricted to a small area of the anterior respiratory epithelium. These changes were confirmed by electron microscopy and were not observed in other groups. Wouterson *et al.* (1987) exposed rats (20 per group) to 0, 1, 10, or 20 ppm formaldehyde 6 hours/day, 5 days/week for 13 weeks. Rats exposed to 20 ppm displayed retarded growth, yellowing of the fur, and significant histological lesions in the respiratory epithelium. Exposure to 10 ppm did not affect growth, but resulted in significant histological lesions in the respiratory tract. No effects on specific organ weights, blood chemistries, liver glutathione levels, or urinalysis were detected at any level. No significant adverse effects were seen at the 1.0 ppm exposure level.

Appelman *et al.* (1988) found significant nasal lesions in rats (20 per group; 0, 0.1, 1.0, or 10.0 ppm) exposed to 10 ppm formaldehyde 6 hours/day, 5 days/week for 52 weeks, but exposure to 1.0 ppm or less for this period did not result in nasal histological lesions. However, the rats exposed to formaldehyde displayed decreased body weight in all groups compared with controls.

Apfelbach and Weiler (1991) determined that rats (5 exposed, 10 controls) exposed to 0.25 ppm (0.38 mg/m^3) formaldehyde for 130 days lost the olfactory ability to detect ethyl acetate odor.

Maronpot *et al.* (1986) exposed groups of 20 mice to 0, 2, 4, 10, 20, or 40 ppm formaldehyde 6 hours/day, 5 days/week, for 13 weeks. Histological lesions in the upper respiratory epithelium were seen in animals exposed to 10 ppm or greater. Exposure to 40 ppm was lethal to the mice.

A six-month exposure of rats to 0, 0.5, 3, and 15 ppm formaldehyde (3 rats per group) resulted in significantly elevated total lung cytochrome P450 in all formaldehyde-exposed groups (Dallas *et al.*, 1989). The degree of P450 induction was highest after 4 days exposure and decreased slightly over the course of the experiment.

A developmental toxicity study on formaldehyde was conducted by Martin (1990). Pregnant rats (25 per group) were exposed to 0, 2, 5, or 10 ppm formaldehyde for 6 hours/day, during days 6-15 of gestation. Although exposure to 10 ppm formaldehyde resulted in reduced food consumption and body weight gain in the maternal rats, no effects on the number, viability or normal development of the fetuses were seen. In addition, Saillenfait *et al.* (1989) exposed pregnant rats (25 per group) to 0, 5, 10, 20, or 40 ppm formaldehyde from days 6 - 20 of gestation. Maternal weight gain and fetal weight were significantly reduced in the 40 ppm exposure group. No significant fetotoxicity or teratogenic defects were observed.

VI. Derivation of Chronic Reference Exposure Level (REL)

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

The Wilhelmsson and Holmstrom (1992) study was selected because it was a human occupational study that contained a LOAEL and a NOAEL, was recent, and contained a

reasonable number of subjects. The supporting occupational study by Edling *et al*. (1988) noted similar sensory irritation results due to long-term formaldehyde exposure. In addition, nasal biopsies from exposed workers in the Edling *et al*. (1988) study exhibited nasal epithelial lesions similar to those found in subchronic and chronic animal studies.

For comparison with the proposed REL of 3 $\mu g/m^3$, we estimated a REL from Edling *et al*. (1988). A median concentration of 0.6 mg/m^3 was determined for the LOAEL from the TWA range of 0.1-1.1 mg/m^3. A NOAEL was not reported. The average continuous occupational concentration was 0.2 mg/m^3 (0.6 x 10/20 x 5/7) and the exposure duration was 10.5 years (range = 1 – 39 years). Application of a UF of 10 for intraspecies variability and a UF of 10 for estimation of a NOAEL from the LOAEL would result in a REL of 2 $\mu g/m^3$ (2 ppb).

Table 1 presents a summary of potential RELs based on chronic and subchronic animal studies. The toxicological endpoint was nasal lesions, consisting principally of rhinitis, squamous metaplasia, and dyplasia of the respiratory epithelium.

Table 1. Summary of Chronic and Subchronic Formaldehyde Studies in Experimental Animals

Study	Animal	Exposure Duration	LOAEL/ NOAEL (mg/m^3)	HEC adj. (mg/m^3)	Cumulative UF	REL ($\mu g/m^3$)
Woutersen *et al*., 1989	rat	28 mo	9.8 / 1.0	0.06	30	2
Kerns *et al*., 1983	rat	24 mo	2.0 / NA	0.1	300	0.3
Monticello *et al*., 1996	rat	24 mo	6.01 / 2.05	0.1	30	4
Kamata *et al*., 1997	rat	24-28 mo	0.30 / NA	0.02	100	0.2
Appelman *et al*., 1988	rat	52 wk	9.4 / 1.0	0.06	30	2
Rusch *et al*., 1983	rat	26 wk	2.95 / 0.98	0.2	30	7
Kimbell *et al*., 1997	rat	26 wk	6 / 2	0.1	30	3
Wilmer *et al*., 1989	rat	13 wk	4 / 2	0.2	300	0.7
Woutersen *et al*., 1987	rat	13 wk	9.7 / 1.0	0.03	100	0.3
Zwart *et al*., 1988	rat	13 wk	2.98 / 1.01	0.2	300	0.7
Kerns *et al*., 1983	mouse	24 mo	2.0 / NA	0.05	100	0.5
Maronpot *et al*., 1986	mouse	13 wk	10.1 / 4.08	0.09	100	0.9
Rusch *et al*., 1983	monkey	26 wk	2.95 / 0.98	none	300	4

The most striking observation is the similarity of potential RELs among the rat chronic studies (exposures $\geq$ 26 weeks) that contain a NOAEL. The range of RELs from these animal studies, 2 – 7 $\mu g/m^3$, is comparable to the proposed REL based on a human study. Another related observation is that the NOAEL and LOAEL are similar among all the studies, regardless of exposure duration. The NOAEL and LOAEL are generally in the range of 1-2 mg/m^3 and 2-10 mg/m^3, respectively, with the exception of the study by Kamata *et al*. (1997). These results indicate that the formation of formaldehyde-related nasal lesions are more concentration dependent than time, or dose, dependent.

A limitation of a majority of the occupational studies is their high reliance on surveys and other methods that focus on sensory irritation. Such sensory irritant results, as exhibited in the Wilhelmsson and Holmstrom (1992) study, may be more related to recurrent acute injury rather than a true chronic injury. The concentration dependent nature of the nasal lesions in the supporting animal studies, and suggested in the supporting human nasal biopsy study, would also imply that the nasal cavity endpoint may be a recurrent acute effect. However, Kerns *et al*. (1983) and Kamata *et al*. (1997) clearly demonstrated that near the LOAEL, increasing exposure durations would result in nasal lesions at lower formaldehyde concentrations. Also, the rat study by Woutersen *et al*. (1989) demonstrated that subchronic exposure to formaldehyde concentrations that produce nasal lesions could result in lifelong changes of the nasal epithelium. These findings substantiate the chronic nature of the nasal/upper airway injury that results from long-term formaldehyde exposure.

VII. Data Strengths and Limitations for Development of the REL

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. In addition, a number of well-conducted animal studies supported the derivation of the REL. The major areas of uncertainty are the uncertainty in estimating exposure in the occupational studies and the potential variability in exposure concentration.

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CHRONIC TOXICITY SUMMARY

HYDROGEN CHLORIDE

(Hydrochloric acid; anhydrous hydrogen chloride; muriatic acid)

CAS Registry Number: 7647-01-0

I. Chronic Reference Exposure Level

Inhalation reference exposure level	**9 µg/m^3 (6 ppb)**
Critical effect(s)	Hyperplasia of nasal mucosa, larynx, and trachea in rats
Hazard index target(s)	Respiratory system

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

Description	Colorless gas or colorless liquid
Molecular formula	HCl
Molecular weight	36.46
Density	1.49 g/L @ 25° C
Boiling point	-84.9° C (HCl gas)
Melting point	-114.8° C (HCl gas)
Solubility	Soluble in water, alcohol, benzene, ether; insoluble in hydrocarbons
Conversion factor	1 ppm = 1.49 mg/m^3 at 25°C

III. Major Uses or Sources

Hydrogen chloride (HCl) is used in the manufacture of vinyl chloride, fertilizers, dyes, artificial silk, and pigments for paints. It is also used in electroplating, soap refining, and leather tanning. Other consumers of HCl include the photographic, textile and rubber industries (HSDB, 1999).

Hydrogen chloride is produced in large quantities during combustion of most materials and especially materials with a high chlorine content. Thus, HCl is a major product formed during the thermal decomposition of polyvinyl chloride, a commonly used plastic polymer (Burleigh-Flayer *et al.*, 1985). It is also released in large quantities during the test firing of some rocket and missile engines (Wohlslagel *et al.*, 1976). Since HCl is extremely hygroscopic, it generally exists as an aerosol in the ambient atmosphere. The annual statewide industrial emissions from facilities reporting under the Air Toxics Hot Spots Act in California based on the most recent inventory were estimated to be 2,570,888 pounds of HCl (CARB, 1999b).

IV. Effects of Human Exposure

Few reports are available on the effects of chronic HCl exposure on humans. Bleeding of the nose and gums and ulceration of the mucous membranes was observed following repeated occupational exposure to HCl mist at high but unquantified concentrations (Stokinger, 1981).

In another report, workers exposed to various mineral acids, including HCl, exhibited etching and erosion of the front teeth (Ten Bruggen Cate, 1968). Dental erosion was noted in 176 of 555 (32%) workers examined between 1962 and 1964, and progressive erosion was reported in 66 of 324 (20%) workers examined repeatedly. Rates of active erosion were highest (50%) in the most highly-exposed category (battery formation workers), intermediate (23%) in an intermediate-exposure category (picklers), and low (7%) in a low-exposure category (other processes). Grade 1 erosion (enamel loss) was noted in workers exposed for greater than 3 months; grade 2 erosion (loss of enamel and dentine) was noted after 2.5 to 5 years exposure; and grade 3 (loss of enamel and dentine with exposure of secondary dentine) was noted after six or more years of exposure.

V. Effects of Animal Exposure

Male Sprague-Dawley rats were exposed to 10 ppm HCl for 6 hours per day, 5 days per week over their lifetime (Sellakumar *et al.*, 1985). No differences in body weights or survival were observed between 99 exposed and 99 control animals. Increased incidences of hyperplasia of the nasal mucosa (62/99 vs. 51/99), larynx (22/99 vs. 2/99), and trachea (26/99 vs. 2/99) were observed in exposed rats compared to air-exposed controls.

A 90-day inhalation study using B6C3F1 mice and Sprague-Dawley and Fisher 344 rats exposed the animals (groups of 31 males and 31 females for each species and strain) to 10, 20, or 50 ppm HCl for 6 hours per day, 5 days per week over 90 days (Toxigenics, 1984). Several animals died during the study, though the deaths were not considered to be exposure related. A slight but significant decrease in body weight gain was reported in male and female mice and in male Fischer 344 rats in the high-exposure groups. No effect were noted in hematology, clinical chemistry, or urinalysis. Minimal or mild rhinitis was observed in both strains of rats Concentration- and time-related lesions were noted in the anterior portion of the nasal cavity of exposed rats. Cheilitis, eosinophilic globules in the nasal epithelium and accumulation of macrophages in the peripheral tissues were observed in mice of all exposed groups. This study thus observed a LOAEL for both mice and rats of 10 ppm. The U.S. EPA considered this study supportive of the portal-of-entry effects observed at 10 ppm in the lifetime rat study (USEPA, 1999). Female rats (8-15/group) exposed to 302 ppm HCl for 1 hour either 12 days prior to mating or on day 9 of gestation exhibited severe dyspnea and cyanosis; the exposure was lethal to one-third of the exposed animals (Pavlova, 1976). Fetal mortality was significantly higher in rats exposed during pregnancy. Organ functional abnormalities observed in offspring exposed at 2-3 months of age were reported to be similar to those observed in the exposed dams.

Female rats were exposed to 302 ppm HCl for 1 hour prior to mating (GEOMET Technologies, 1981). Exposure killed 20 to 30% of the rats. In rats surviving 6 days after exposure, a decrease in blood oxygen saturation was reported, as were kidney, liver, and spleen effects. Estrus cycles

were also altered. In rats mated 12-16 days postexposure and killed on day 21 of pregnancy, a decrease in fetal weight, an increase in relative fetal lung weights, and reduced numbers of live fetuses were observed.

VI. Derivation of Chronic Reference Exposure Level

Study	Sellakumar *et al.*, 1985
Study population	Sprague-Dawley rats (100 males)
Exposure method	Discontinuous whole-body inhalation (0 or 10 ppm)
Critical effects	Hyperplasia of the nasal mucosa, larynx and trachea
LOAEL	10 ppm
NOAEL	Not identified
Exposure continuity	6 hours per day, 5 days per week
Average experimental exposure	1.8 ppm for LOAEL group
Human equivalent concentration	0.57 ppm (gas with extrathoracic respiratory effects, RGDR =0.32, based on rat MV_a = 0.33 L/min, MV_h = 13.8 L/min, $SA_a(ET)$ = 15 cm^2); Sa_h = 200 cm^3) (U.S. EPA, 1994)
Exposure duration	Lifetime
LOAEL uncertainty factor	3 (<30% incidence; mild effect)
Subchronic uncertainty factor	1
Interspecies uncertainty factor	3
Intraspecies uncertainty factor	10
Cumulative uncertainty factor	100
Reference Concentration (RfC)	0.006 ppm (6 ppb; 0.009 mg/m^3; 9 $\mu g/m^3$)

Both extrathoracic and tracheobronchial effects have been associated with exposures to hydrogen chloride. The REL was based on extrathoracic effects as humans are predicted to be relatively more susceptible to the effects of hydrogen chloride in that region. An intermediate LOAEL factor was used as the effects were both mild and occurring at a low incidence at the dose tested.

VII. Data Strengths and Limitations for Development of the REL

The USEPA based its RfC of 7 $\mu g/m^3$ on the same study. U.S. EPA evaluated this RfC as a having a low level of confidence because of (1) the use of only one dose; (2) limited toxicity evaluation; (3) the lack of reproductive toxicity data; and (4) the lack of chronic exposure studies (U.S. EPA, 1994). OEHHA agrees with this assessment. The database for chronic exposure to this common chemical is limited.

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CHRONIC TOXICITY SUMMARY

ISOPROPANOL

(2-propanol; dimethylcarbinol; isopropyl alcohol)

CAS Registry Number: 67-63-0

I. Chronic Toxicity Summary

Inhalation reference exposure level	**7,000 mg/m^3 (3000 ppb)**
Critical effect(s)	Kidney lesions in mice and rats; fetal growth retardation and developmental anomalies in rats
Hazard index target(s)	Kidney; development

II. Chemical Property Summary (HSDB, 1995)

Description	Colorless liquid at room temperature (25°C) with a pleasant odor. Slightly bitter taste.
Molecular formula	C_3H_8O
Molecular Weight	60.09
Boiling point	82.5°C
Vapor Pressure	44.0 torr at 25°C
Solubility	Miscible in water and most organic solvents; insoluble in salt solutions.
Conversion factor	1 ppb = 2.45 μg/m^3 at 25°C

III. Major Uses and Sources

Isopropanol is used as a solvent and in making many commercial products (HSDB, 1995). The annual production volume of isopropanol has been in excess of one billion pounds since 1956; it was ranked 50th among chemicals produced in the U.S. in 1994 (C&EN, 1995). Rubbing alcohol is a solution of 70% isopropanol in water. Specific uses and sources include: a component of antifreeze; a solvent for gums, shellac, essential oils, creosote and resins; extraction of alkaloids; component of quick drying oils and inks; component of denaturing alcohol; antiseptic for hand lotions; rubefacient; component of household products (after-shave lotions, cosmetics, etc.); the manufacture of acetone; deicing agent for liquid fuels; dehydrating agent and synthetic flavoring adjuvant. Isopropanol can enter the environment as emissions from its manufacture and use as a solvent. It naturally occurs as a plant volatile and is released during the microbial degradation of animal wastes. Human exposure will be both in occupational atmospheres and from use of consumer products containing isopropanol as a volatile solvent. An odor threshold has been estimated as 22 ppm (Amoore and Hautala, 1983), which is 7-fold higher than the chronic REL.

The annual statewide industrial emissions from facilities reporting under the Air Toxics Hot Spots Act in California based on the most recent inventory were estimated to be 525,826 pounds of isopropanol (CARB, 1999b).

IV. Effects of Human Exposures

Currently, there are no adequate chronic exposure data for isopropanol in humans. While isopropanol is not considered a dermal irritant, it is a defatting agent and can cause dermatitis with prolonged exposure to skin (IARC, 1977). A subacute study of daily oral intake of isopropanol (2.6 or 6.4 mg/kg body weight) by groups of 8 men for 6 weeks had no effect on blood cells, serum or urine and produced no subjective symptoms (Wills *et al.*, 1969). A pharmacokinetic study of men occupationally exposed to isopropyl alcohol revealed that uptake occurs readily via the inhalation route; acetone is the major metabolite (Brugnone *et al.*, 1983). Acetone was eliminated mainly by the lung but was also eliminated in the urine.

V. Effects of Animal Exposures

In metabolism studies with rats and mice, up to 92% of the administered dose (via i.v. or inhalation) of isopropanol was exhaled as acetone, CO_2 and the unmetabolized alcohol (Slauter *et al.*, 1994). Approximately 3-8% of the administered dose was excreted in urine as isopropanol, acetone, and a metabolite tentatively identified as isopropyl glucuronic acid. Isopropanol is readily absorbed from the GI tract and persists in the circulation longer than ethyl alcohol. Alcohol dehydrogenase oxidizes most isopropanol to acetone. Acetone may be further metabolized to acetate, formate, and finally CO_2. In another metabolism study, the amount of acetone in the blood stream was found to be directly related to the air concentration of isopropanol (Laham *et al.*, 1980). This finding indicated that the acetone metabolite could be used as a biochemical indicator of isopropanol exposure.

Subchronic studies by Guseinov and Abasov (1982) and Baikov *et al.* (1974) reported changes in certain hematologic and clinical chemistry parameters, as well as increases in some organ weights. But the Environmental Protection Agency deemed these studies insufficient to reasonably predict subchronic toxicity of isopropanol (Burleigh-Flayer *et al.*, 1994). Three different routes of exposure have been used by researchers for isopropanol toxicity studies: inhalation, oral gavage and presence in drinking water. The following subchronic and chronic studies exposed experimental animals to isopropanol by the inhalation route:

Toxicological and neurobehavioral endpoints were investigated in rats and mice following 13-week inhalation exposure (6 hr/day, 5 days/week) to 0, 100, 500, 1500 or 5000 ppm isopropanol (Burleigh-Flayer *et al.*, 1994). In rats, clinical signs observed following exposures included swollen periocular tissue (females) at the highest dose and perinasal encrustation (males) at 500 ppm and above. Narcosis was observed in a few animals of both species during exposure to 5000 ppm and possibly 1500 ppm as well. However, the animals became tolerant to the narcotic effects of isopropanol after week 2. No neurobehavioral changes were observed in any parameters of the functional observational battery. However, increased motor activity was noted

at week 9 of exposure in female rats of the 5000 ppm group. After an initial drop in body weight gain in the first week of exposure at the high dose (5000 ppm), rats in the 1500 and 5000 ppm groups had significant increases in body weight gain and/or body weight throughout most of the exposure period. But only the 5000 ppm group had greater than 10% body weight gain compared to controls. Increases in body weight and body weight gain greater than 10% were also noted in female mice in the 5000 ppm group. Consistent clinical pathology changes included an increase in mean corpuscular volume (rats; female mice) and mean corpuscular hemoglobin (male rats; female mice) at the 5000 ppm exposure level. Other changes noted include a slight anemia in rats at week 6 only and a slight dehydration in female mice at the end of the study. Relative liver weight in rats was elevated no more than 8% in the 5000 ppm groups. However, a 10 and 21% increase in relative liver weight was observed in female mice at 1500 and 5000 ppm, respectively. No gross lesions were observed in any organs. The only microscopic change observed was hyaline droplets within kidneys of all male rats. This change was not clearly concentration related, although it was most pronounced in the 5000 ppm group.

In a follow-up inhalation study spanning the lifetime of rats and mice, Burleigh-Flayer *et al*. (1997) exposed four groups of animals, each consisting of 75 CD-1 mice/sex and 75 Fischer 344 rats/sex, to 0, 500, 2500, or 5000 ppm isopropanol vapor. Of these, 55 mice/sex/group and 65 rats/sex/group were exposed 6 hr/day, 5 days/week for at least 78 weeks (mice) or 104 weeks (rats). Transient signs of narcosis were observed at the higher doses. Increased mortality and a decreased mean survival time (577 days versus 631 days for controls) were noted for male rats in the 5000 ppm group. Increases in body weight and/or body weight gain were observed for both sexes of mice and rats from the 2500 and 5000 ppm groups throughout the study. Concentration-related increases in absolute and relative liver weight were observed for male and female mice. In addition, increased absolute and/or relative liver and kidney weight were observed for male and/or female rats from the 2500- and 5000-ppm groups. Urinalysis and changes in urine chemistry, indicative of impaired kidney function (i.e. decreased osmolality and increased total protein, volume, and glucose), were noted for male rats in the 2500 ppm group and for male and female rats in the 5000 ppm group. At necropsy, the most significant noncancer lesions in rats were observed in the kidney, and were associated with an exacerbation of spontaneous chronic renal disease. The kidney lesions noted with increased severity and/or frequency included mineralization, tubular dilation, glomerulosclerosis, interstitial nephritis, interstitial fibrosis, hydronephrosis, and transitional cell hyperplasia. The authors considered chronic renal disease to be the main cause of death for male and female rats exposed to 5000 ppm and to account for much of the mortality observed for male rats exposed to 2500 ppm. Unlike the subchronic study, anemia was not observed in rats in the chronic study. In mice, an increased incidence of seminal vesicle enlargement was observed grossly in males in the 2500 and 5000 ppm groups. Microscopically, the lesions in mice included an increased incidence of ectasia (dilation) of the seminal vesicles for male mice in the 2500 and 5000 ppm groups, minimal renal tubular proteinosis for male and female mice from all isopropanol groups, and renal tubular dilation for female mice in the 5000-ppm group. The seminal vesicle effects did not have any associated inflammatory or degenerative changes. The enlargement may have been the result of either increased secretion or decreased evacuation of the secretory product by these glands. Microscopic evaluation of the livers of rats and mice revealed no exposure-related lesions.
In a 13-week behavioral/neurotoxicity study by the same investigators, the reproducibility and reversibility of increased motor activity in isopropanol-exposed female Fischer 344 rats was

investigated (Burleigh-Flayer *et al.* 1998). Rats were exposed to 0 or 5000 ppm isopropanol for 6 hr/day, 5 days/week. Increased motor activity was characterized as the summation of ambulation, rearing and fine movements and was first observed 4 weeks following exposure to 5000 ppm isopropanol. Reversibility of this effect was observed 2 days following cessation of exposure in a subgroup of rats exposed to isopropanol for only 9 weeks. In the subgroup exposed for 13 weeks, reversal of the increased motor activity did not occur until 2 weeks following cessation of exposure. However, complete reversibility of the time versus activity profile, or habituation curve, was not noted until 42 days following exposure to isopropanol for 13 weeks.. Other effects included a significant increase in body weight and an increased incidence of swollen periocular tissue in isopropanol-exposed animals.

In a study conducted to investigate neurochemical and behavioural effects, 20 male Wistar rats/group were exposed to 0 or 300 ppm isopropanol 6 hr/day, 5 days/week for up to 21 weeks (Savolainen *et al.*, 1979). Enzyme activity of superoxide dismutase and azoreductase in cerebellar homogenate was decreased at week 20-21. Acid protease activity in glial cells was increased up to week 10. Open-field tests indicated sporadic changes in urination (10th week) and defecation (15th week). Isopropanol also appeared to depress caffeine stimulation activity at 15 weeks.

In a subchronic neurotoxicity study by Teramoto *et al.* (1993), motor and sensory nerve conduction velocity increased significantly following a 20-week exposure (8 hr/day, 5 days/week) of Jcl-Wistar rats to 8000 ppm isopropanol. Low dose (1000 ppm) exposure had no effect on conduction velocity. Conduction velocities returned to normal following the end of exposure. The sex of the rats in this study was not specified.

A developmental study in rats exposed pregnant dams (15/group) to 0, 3500, 7000 or 10,000 ppm isopropanol 7 hr/day on gestation days 1-19 (Nelson *et al.*, 1988). At the two highest exposure levels, feed intake (weeks 1 and 2 of exposure) and maternal body-weight gain were reduced. Narcosis was evident only at the 10,000 ppm level.Increased fetal resorptions and reduced fetal weights (59% of controls) occurred at the highest exposure level. Fetal weights were also significantly reduced (85% of controls) at 7000 ppm. A slight reduction in fetal weight (96% of controls) occurred at 3500 ppm but was significant in the sense that a dose-dependent relationship in fetal weight reduction was present across all exposed groups. Skeletal malformations (primarily rudimentary cervical ribs) were seen only in the presence of maternal toxicity at the two highest exposure levels. No detectable teratogenic effects were observed in the 3500 ppm group. The authors noted that the developmental effects at 3500 ppm were considered very slight, indicating that this exposure level is close to the LOAEL for isopropanol.

The following studies administered isopropyl alcohol to experimental animals by oral gavage:

In a developmental study, pregnant (VAF)CD(SD) rats (25/group) were gavaged with either 0, 400, 800 or 1200 mg/kg body wt-day of isopropanol daily on gestational days 6 through 15 (Tyl *et al.*, 1994). In the same study, pregnant New Zealand white rabbits (15/group) were dosed orally with either 0, 120, 240 or 480 mg/kg body wt-day of isopropanol daily during gestational days 6 through 18. In rats, fetal body weight exhibited a linear downward trend with increasing dose and was significantly lower at the highest dose compared to controls. However, the fetal

body weight differences at each dose level was less than 10% of controls. Maternal weight gain during gestation was significantly reduced at the highest dose level. In rabbits, maternal weight gain and food consumption was reduced during gestation at 480 mg/kg body wt-day. Four rabbits died after dosing at this level. No differences were observed in reproduction indices or in fetal development. No teratogenic effects were seen in either species.

In another developmental study performed to investigate neurotoxicity in rat pups, 64 time-mated Sprague-Dawley rats/group were administered 0, 200, 700 or 1200 mg/kg body wt-day isopropanol by oral gavage from gestational day 6 through postnatal day 21 (Bates *et al.*, 1994). One high-dose dam died on postnatal day 15, but there were no other clinical observations of effects on maternal weight, food consumption or gestation length. All fetal developmental indices were unaffected at the dose levels used. Developmental neurotoxicity, in the form of motor activity, auditory startle and active avoidance tests, was not found at any dose of isopropanol.

In a multi-generation study carried out to investigate potential reproductive and developmental effects of isopropanol, Sprague-Dawley rats were administered 0, 100, 500 or 1000 mg/kg body wt-day of isopropanol by oral gavage (Bevan *et al*., 1995). P1 and P2 rats were dosed daily for 10 weeks prior to mating, throughout the mating, and during the gestation and lactation period for the F1 and F2 litters, respectively. In adult rats, centrilobular hepatocyte hypertrophy and increased relative liver weight (>10%) was observed in P2 males at 1000 mg/kg. A general increase in absolute and relative liver and kidney weights was observed (less than 10% in most cases) in treated animals in both P1 and P2 generations. However, with the exception of hepatocellular hypertrophy in P2 males, no histopathological effects relevant to human risk were present. Reproductive effects due to isopropanol were not seen at any dose level. Statistically significant reduction of body weights (5-12%) in F1 and F2 offspring and increased mortality (14%) in F1 offspring were observed at the 1000 mg/kg dose level.

The following toxicology studies administered isopropanol to experimental animals in drinking water:

In a study designed to investigate neurotoxicological effects, 22 male SPF rats/group were administered isopropanol in drinking water at concentrations of 0, 1, 2, 3, or 5% (*w*/*v*) for 12 weeks (Pilegaard and Ladefoged, 1993). Average daily intake of isopropanol was 0, 870, 1280, 1680 and 2520 mg/kg body wt, respectively. Water intake and body weights were consistently lower at the two highest doses. Relative weights of liver, kidney and adrenals were increased in a dose-dependent manner. However, histopathology revealed no treatment-related changes in organs other than the male rat-specific kidney lesions. Evidence of astrogliosis, in the form of increased glial fibrillary acidic protein in dorsal hippocampus, was not found in exposed rats.

In a 1-generation study, 10 Wistar-derived rats/sex/group were exposed to 0, 0.5, 1.25, 2.0, and 2.5% isopropanol in water for up to 18 weeks (USEPA/OTS, 1986). The doses are equivalent to 0, 325, 711, 1002, and 1176 mg/kg body wt-day, respectively, for males; to 0, 517, 1131, 1330, and 1335 mg/kg body wt-day, respectively, for females during the pre-mating phase; and to 0, 1167, 2561, 2825, and 2722 mg/kg body wt-day, respectively, in females during the post-partum phase. Exposure periods were: 70 days pre-mating, plus 15 days during mating, plus 42 days for

males; 21 days pre-mating plus 15 days during mating, plus 21 days gestation, plus 21 days rearing in females; and 21 days for the F_1 generation. At the highest two levels, body weights of males during the first two weeks were reduced and the body weights of females during the post-partum period were reduced. Water consumption and food ingestion were generally lower at the top three dose levels. The authors concluded that these effects were related to the unpalatability of drinking water containing isopropanol and not due to a toxic effect of the alcohol itself. Anemia was present in post-partum females. Red cell numbers were reduced in a dose-related manner at doses of 1.25% isopropanol or higher. Hematocrit was lower at the two highest doses while hemoglobin was lower at the highest dose. In males, mean cell volume was reduced at 1.25% isopropanol or higher. Absolute and relative liver and kidney weights were higher in most exposure groups at 2.0% or higher in both sexes, but no relevant pathology was seen. Absolute liver weight of females was also higher in the 1.25% group. Fetal weight gain was depressed in a dose-related fashion in the 1.25% and higher groups. Mean pup weights and pup survival were lower than controls at the two highest doses. Fewer pups were born per animal in the 2.5% exposure group. A teratogenic examination was not performed on the pups.

In a similar exposure study investigating the potential teratogenic effects of isopropanol, 20 pregnant Wistar-derived rats/group were exposed to 0, 0.5, 1.25 or 2.5% of the alcohol in drinking water (equivalent to 0, 596, 1242 and 1605 mg/kg body wt-day) during gestational days 6 to 16 (USEPA/OTS, 1992a,b). Water and feed consumption were reduced at the two highest doses while maternal body weight was significantly reduced at the highest dose. Fetal body weights were decreased in the two highest dose groups. Minor abnormalities and variants (reduced ossification of the skeleton) were present in fetuses of exposed groups in a dose-related manner. However, the authors concluded that the reduced fetal weights are probably a consequence of maternal growth retardation during the critical period of organogenesis. Similarly, the fetal abnormalities are probably due to small fetal size, related to slightly retarded development. Therefore, the study found no indication of teratogenesis.

A multi-generation study performed in ‘white’ rats also observed reduced body weights in F1 offspring (Lehman *et al.*, 1945). Body weights of F2 offspring were the same as controls. The adult rats had imbibed an average of 1.9 ml/kg (1470 mg/kg body wt) of isopropanol per day in drinking water 80 days prior to mating. No other developmental or reproductive effects were seen. In the same study, several dogs were given 4% isopropanol in drinking water for approximately 7 months. Histopathology at the end of exposure revealed a decrease in the number of nephrons with hydropic changes and necrosis of some of the tubular epithelium. Some capillary hemorrhages were also noted in the brains of two of the dogs. Average daily dose of isopropanol imbibed by the dogs could not be determined from data provided in the report.

VI. Derivation of Chronic Reference Exposure Level (REL)

Study	Burleigh-Flayer *et al.* (1997)
Study population	Rats and mice
Exposure method	Discontinuous whole-body inhalation (0, 504, 2,509 or 5,037 ppm)
Critical effects	Kidney lesions in mice and rats
LOAEL	2,509 ppm
NOAEL	504 ppm
Exposure continuity	6 hours/day, 5 days/week
Exposure duration	78 weeks in mice; 104 weeks in rats
Average experimental exposure	90 ppm for NOAEL group (500 x 6/24 x 5/7)
Human equivalent concentration	90 ppm for NOAEL group (gas with systemic effects, based on RGDR = 1.0 using default assumption that lambda (a) = lambda (h))
LOAEL uncertainty factor	1
Subchronic uncertainty factor	1
Interspecies uncertainty factor	3
Intraspecies factor	10
Cumulative uncertainty factor	30
Inhalation reference exposure level	3 ppm (3000 ppb, 7 mg/m^3, 7000 μg/m^3)

The Burleigh-Flayer et al. (1997) study was selected because it was a chronic study, was recent, and was published in a respected, peer-reviewed journal. While numerous subchronic studies have been performed, this was the only study that conducted lifetime animal exposures. In addition, the chronic kidney effects observed in rats and mice were not seen in the subchronic studies, indicating that chronic exposure is necessary for development of these lesions.

The lesions observed in the kidneys of male rats in some of the studies described above is typical of a male rat-specific chronic renal disease and is not considered to be relevant to human risk assessment (Phillips and Cockrell, 1984; Beyer, 1992). However, the exacerbation of chronic renal disease in male and female rats, and the slight kidney damage observed in mice of both sexes following chronic isopropanol exposure indicates that the kidney is a sensitive indicator for nonneoplastic effects (Burleigh-Flayer *et al*., 1997). Suggestive evidence also exists for kidney damage in dogs following subchronic exposure to isopropanol in drinking water (Lehman *et al*., 1945).

Some isopropanol exposure studies noted increased liver and kidney weights in exposed animals but no observable relevant pathology. With particular relevance to the liver, this weight change may be considered to be more of a metabolic response rather than a toxic effect of the alcohol. The changes noted in the neurochemical and behavioural study by Savolainen *et al.* (1979) may have also been more of a metabolic response to the increased load of isopropanol. It is also possible that these changes reflected the development of tolerance. The changes in behavior were small and unconvincing. This study would have benefited from additional dose levels to analyze for dose-response trends.

Other possible sensitive indicators of isopropanol toxicity include blood chemistry changes and reduced fetal body weights. However, the blood chemistry findings were conflicting among the various studies that investigated this endpoint. Reduced fetal weights at doses below maternal body weight reductions were minor (<10% compared to controls), but consistent, suggesting that reduced fetal weights are a manifestation of isopropanol developmental toxicity.

A comparative REL was calculated from the only reproduction/developmental study that utilized inhalation as the route of exposure (Nelson *et al*., 1988). Exposure of pregnant rats to isopropanol during gestation caused dose-dependent reduction in fetal body weights across all treatment groups, resulting in a LOAEL of 3500 ppm (average measured concentration = 3510 ppm). A NOAEL was not observed for this effect. Skeletal malformations probably related to reduced fetal weight was observed at 7000 ppm and 10,000 ppm. The average exposure duration at the LOAEL for this study is 1024 ppm (7hr/24hr x 3510 ppm). Use of an RGDR of 1 and a cumulative uncertainty factor of 100 (3 for LOAEL to NOAEL, 3 for interspecies, and 10 for intraspecies) resulted in a REL of 10 ppm (25 mg/m^3). Since the endpoint is a function of exposure only during gestation, no subchronic to chronic UF was used. This developmental REL is within an order of magnitude of the chronic REL for kidney lesions, and therefore, is also considered to be a critical effect.

The oral dose developmental studies by Tyl *et al*. (1994), Bevan *et al*. (1995), USEPA/OTS (1986), and USEPA/OTS (1992 a, b) provide supportive evidence that reduced fetal weights is a sensitive developmental endpoint. The USEPA/OTS (1992a,b) study provides supportive evidence for skeletal malformations in exposed rat fetuses.

VII. Data Strengths and Limitations for Development of the REL

Strengths of the database for isopropanol include availability of a well-conducted chronic study in two species, similar toxicological endpoints among different studies, and pharmacokinetic similarities between humans and experimental animals. Isopropanol is metabolized through a similar pathway to acetone and CO_2.

Weaknesses of the database for isopropanol include a lack of literature regarding chronic toxicity endpoints in humans. The deficiency of chronic toxicity cases in humans may be related to the relatively low chronic toxicity of isopropanol. Another weakness is that, while most developmental studies observed maternal and fetal effects, only one study was performed via the inhalation route.

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CHRONIC TOXICITY SUMMARY

MERCURY, INORGANIC

(liquid silver; hyfarargyrum; colloidal mercury)

CAS Registry Number: 7439-97-6

I. Chronic Toxicity Summary

Inhalation reference exposure level	**0.09 mg/m^3 - This REL is intended for use in assessing mercury salts as well as elemental mercury.**
Critical effects	Hand tremor, memory disturbances, neurobehavioral and autonomic dysfunction in humans
Hazard index target(s)	Nervous system

II. Chemical Property Summary (HSDB, 1995)

Description	Silvery, odorless, heavy liquid
Molecular formula	Hg
Molecular weight	200.59 g/mol
Boiling point	Hg°: 356.7 °C; $HgCl_2$: 302°C
Vapor pressure	0.002 torr @ 25°C
Solubility	Soluble in concentrated nitric and hot sulfuric acids; dissolves to some extent in lipids
Conversion factor	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). The annual

statewide industrial emissions in California based on the most recent inventory were estimated to be 9714 pounds of mercury (CARB, 1999).

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 $\mu g/m^3$ 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 glomular changes, albuminuria, glomerulosclerosis, and increased urinary N-acetyl-β-glucosaminidase have been seen in workers exposed to approximately 25-60 $\mu g/m^3$ 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. Fawer *et al*. (1983) measured intention tremor in 26 male workers (mean age of 4 years) exposed to low concentrations of mercury vapor. The men worked either in a chloralkali plant (n = 12), a fluroescent tube manufacturing plant (n = 7), or in acetaldehyde production (n = 7) Twenty-five control subjects came from different parts of the same plants and were not occupationally exposed to mercury. The average exposure as measured by personal air sampling was 0.026 mg/m^3 and the average duration of exposure was 15 years. The measurements of intention tremor were significantly higher in exposed workers than in controls. Using the average exposure as a LOAEL and adjusting for occupational ventilation rates and workweek, the resultant LOAEL is 0.009 mg/m^3.

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 μg/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. The authors used a conversion factor calculated by Roels *et al*. (1987) to extrapolate from blood mercury levels of 12 μg/L to an air concentration of 0.025 mg/m^3.

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. The exposed workers had mean blood levels of 11.6 μg/L corresponding to a TWA exposure of 25 μg Hg/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 μg Hg/m^3 (extrapolated from blood level based on methods of Roels *et al.* (1987), which is indicative of autonomic reflex dysfunction.

Piikivi and Hanninen (1989) studied subjective symptoms and psychological performance on a computer-administered test battery in 60 chloralkali workers. The workers were exposed to approximately 0.025 mg/m^3 mercury vapor for a mean of 13.7 years. The vapor concentration was extrapolated from blood mercury levels based on the conversion factor in Roels *et al*. (1987). A statistically significant increase in subjective symptoms of sleep disturbance, and memory disturbance was noted in the exposed workers, although there was no difference in objective measures of motor, memory, or learning abilities.

A more recent study by Ngim *et al.* (1992) assessed neurobehavorial performance in a cross-sectional study of 98 dentists exposed to a TWA concentration of 14 μg Hg/m^3 (range 0.7 to 42 μg/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/m^3 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 neurobehavorial tests measuring motor speed (finger tapping), visual scanning, visuomotor coordination and concentration, visual memory, and visuomotor coordination speed. These neurobehavorial 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/m^3 across worksites. The average level of exposure was estimated at 33 μg Hg/m^3 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/m^3 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/m^3 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/m^3 mercury vapor for 6 weeks, 100 hours/week (Gage, 1961). Rats exposed intermittently to 3 mg/m^3 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/m^3 mercury vapor for 21 days demonstrated prolongation of the estrous cycle and a decrease in the number of living fetuses (Baranski and Szymezyk, 1973), however, no differences in developmental abnormalities were observed.

VI. Derivation of Reference Exposure Levels

Derivation of Chronic Reference Exposure Level

Study	Piikivi and Hanninen (1989); Fawer *et al*. (1983); Piikivi and Tolonen (1989); Piikivi (1989); Ngim *et al*. (1992); supported by Liang *et al*. (1993)
Study population	Humans
Exposure method	Inhalation of workplace air
Critical Effects	Neurotoxicity as measured by :intention tremor; memory and sleep disturbances; decreased performance on neurobehavioral tests (finger tapping, visual scan, visuomotor coordination, visual memory); decreased EEG activity
LOAEL	25 μg/m^3 (3 ppb)
NOAEL	Not observed
Exposure continuity	8 hours per day (10 m^3/workday), 5 days/week
Average experimental exposure	8.9 μg/m^3 for LOAEL group
Human equivalent concentration	8.9 μg/m^3
Exposure duration	13.7 to 15.6 year average
Subchronic uncertainty factor	1
LOAEL uncertainty factor	10
Interspecies uncertainty factor	1
Intraspecies uncertainty factor	10
Cumulative uncertainty factor	100
Inhalation reference exposure level	0.09 μg/m^3 (0.0 1 ppb)

The USEPA (1995) based its RfC of 0.3 $\mu g/m^3$ on the same study but used an intraspecies uncertainty factor of 3, a LOAEL uncertainty factor of 3 and included a Modifying Factor (MF) of 3 for database deficiencies (lack of developmental and reproductive toxicity data). The criteria for use of modifying factors are not well specified by U.S. EPA. Such modifying factors were not used by OEHHA

The studies chosen to calculate the chronic REL all point to a LOAEL of approximately 0.025 mg/m3. When adjusted for worker ventilation and workweek exposure, the LOAEL becomes 0.009 mg/m3.

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 slightly above the other studies, but for a shorter duration. It is possible that mercury could cause neurotoxic effects after a shorter exposure period than that used in derivation of the chronic REL.

VII. Data Strengths and Limitations for Development of the REL

The strengths of the inhalation REL include the use of human exposure data from workers exposed over a period of years in a number of studies and occupations. The studies were consistent in terms of finding a LOAEL at about 0.025 mg/m^3. Major areas of uncertainty are the lack of observation of a NOAEL, the uncertainty in estimating exposure and the potential variability in exposure concentration, and the lack of reproductive and developmental toxicity studies.

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

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

Study	U.S. EPA, 1987
Study population	Brown Norway rats
Exposure method	feeding and subcutaneous application
Critical effects	autoimmune effects in kidney
LOAEL	0.226 mg/kg-day (feeding); 0.317 mg/kg-day (subcutaneous)
NOAEL	none
Exposure continuity	
Exposure duration	up to 60 days
Average experimental exposure	
LOAEL uncertainty factor	10

Subchronic uncertainty factor	10
Interspecies uncertainty factor	10
Intraspecies factor	(The intraspecies and interspecies factors were combined into one factor of 10 to avoid an exceedingly large uncertainty factor.)
Cumulative uncertainty factor	1000
Oral reference exposure level	0.0003 mg/kg-day

Factors and Assumptions -- Dose conversions in the three studies employed a 0.739 factor for $HgCl_2$ to Hg^{2+}, 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 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 Hg^{2+}-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) Hg^{2+} 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 ($HgCl_2$) 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 $HgCl_2$ ingestion portion of the study involved the forcible feeding of either 0 or 3000 µg/kg-week of $HgCl_2$ 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 $HgCl_2$ 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 $HgCl_2$ 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 $HgCl_2$ (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 $HgCl_2$-treated rats started to lose weight and hair. Two of the $HgCl_2$-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. OEHHA believes that the RfD is an acceptable oral REL until better data become available.

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CHRONIC TOXICITY SUMMARY

METHYL BROMIDE

(bromomethane; monobromomethane)

CAS Registry Number: 74-83-9

I. Chronic Toxicity Summary

Inhalation reference exposure level	**5 mg/m^3 (1 ppb)**
Critical effect(s)	Histological lesions of the olfactory epithelium of the nasal cavity in rats
Hazard index target(s)	Respiratory system; nervous system; development

II. Physical and Chemical Properties (HSDB, 1994)

Description	Colorless gas
Molecular formula	CH_3Br
Molecular weight	94.95 g/mol
Density	3.89 g/L @ 25°C
Boiling point	3.6°C
Vapor pressure	1420 torr @ 20°C
Solubility	Soluble in ethanol, benzene, carbon disulfide, and 1.75% (w/w) in water
Odor threshold	20.6 ppm
Odor description	Sweetish odor
Metabolites	Methanol, bromide, 5-methylcysteine
Conversion factor	1 ppm = 3.89 mg/m^3 @ 25° C

III. Major Uses and Sources

Methyl bromide (MeBr) was used historically as an industrial fire extinguishing agent and was introduced in the U.S. from Europe in the 1920s. Current uses of MeBr include the fumigation of homes and other structures for termites and other pests. Methyl bromide is also used to fumigate soil before planting and fruits and vegetables after harvest. In 1981, 6.3 million pounds of MeBr were reportedly used in California (Alexeeff and Kilgore, 1983). By 1991, its use had grown to 18.7 million pounds in the state (Cal/EPA, 1993). The annual statewide industrial emissions from facilities reporting under the Air Toxics Hot Spots Act in California, based on the most recent inventory, were estimated to be 75,575 pounds of methyl bromide (CARB, 1999). This does not include emissions of methyl bromide during its use as a pesticide.

IV. Effects of Human Exposure

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

V. Effects of Animal Exposure

The first experimental animal study on repeated MeBr exposures was carried out and reported by Irish and associates (1940). In this study, rats (135 per group), rabbits (104 per group), or female rhesus monkeys (13 per group) were exposed to 0, 17, 33, 66, 100, or 220 ppm (0, 66, 128, 256, 388, or 853 mg/m^3) 7-8 hours/day, 5 days/week for 6 months or until the majority of the animals exhibited severe signs of toxicity. Mortality was seen in rats, guinea pigs, and monkeys at 100 ppm. Rabbits began to die at 33 ppm. Severe effects, including paralysis, were seen after exposure to 66 ppm in rabbits and monkeys. None of the species exhibited adverse effects after exposure to 17 ppm.

Kato and associates (1986) observed focal lesions in the brain and heart in rats (10-12 per group) after inhalation of 150 ppm (585 mg/m^3) MeBr 4 hours/day, 5 days/week for 11 weeks. In another experiment, rats were exposed to 0, 200, 300, or 400 ppm (0, 777, 1160, or 1550 mg/m^3) MeBr 4 hours/day, 5 days/week for 6 weeks. In this experiment, rats exposed to any concentration of MeBr exhibited coronary lesions, and exposures of 300 ppm or greater resulted in neurological dysfunction, including ataxia and paralysis. Testicular atrophy was noted in 6 of the 8 animals exposed to 400 ppm.

Anger *et al.* (1981) determined that rabbits are more sensitive than rats to neurotoxicity of MeBr. In this study, rats or rabbits were exposed to 0 or 65 ppm (0 or 254 mg/m^3) MeBr for 7.5 hours/day, 4 days/week, for 4 weeks. Nerve conduction velocity and eyeblink reflex were impaired in the rabbits but not rats exposed to 65 ppm MeBr. Similarly, rats did not exhibit neurological signs after exposure to 55 ppm (215 mg/m^3) MeBr for 36 weeks. Rabbits exposed to 26.6 ppm (104 mg/m^3) did not display any neurological effects after 8 months exposure (Russo *et al.*, 1984).

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

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

The National Toxicology Program (NTP) conducted a 13-week and a chronic study on the toxicology and carcinogenesis of methyl bromide in rats and mice (NTP, 1990). In the 13-week study, 18 rats/sex/group were exposed to 0, 30, 60, or 120 ppm (0, 117, 233, or 466 mg/m^3) MeBr 6 hours/day, 5 days/week. The mice were exposed to 0, 10, 20, 40, 80, or 120 ppm (0, 39, 78, 155, 311, or 466 mg/m^3) 6 hours/day, 5 days/week. Hematological parameters and selected organ weights were measured in both species, in addition to histopathological changes. Pseudocholinesterase activity and neurobehavioral tests were conducted in the mice. Serious effects, including 58% body weight loss, 17% mortality and severe curling and crossing of the hindlimbs were observed in mice exposed to 120 ppm MeBr. Exposure of males to 40 ppm or higher resulted in significant effects on several hematological parameters, including decreased mean cell hemoglobin and increased red blood cell count. The only exposure-related histological effect was olfactory epithelial dysplasia and cysts in the rats of both sexes exposed to 120 ppm.

A 6-week study in rats and mice (5 animals/sex/group) exposed to 0 or 160 ppm (0 or 624 mg/m^3) showed high mortality rates, loss in body weight and histological changes in multiple organ systems including brain, kidney, nasal cavity, heart, adrenal gland, liver, and testes (NTP, 1990).

An exposure of mice (86 animals/group) to 0, 10, 33, or 100 ppm (0, 38.8, 128, or 388 mg/m^3) MeBr for 6 hours/day, 5 days/week, for 103 weeks was also conducted by NTP (1990). In this study, high mortality rates in both males and females in the 100 ppm group resulted in a discontinuation of exposure after 20 weeks. A low incidence of sternal dysplasia and a significant decrease in locomotor activity were noted in the 10 ppm group.

A 5-day exposure of rats (10 animals/group) to 0, 90, 175, 250, or 325 ppm (0, 350, 680, 971, or 1260 mg/m^3) resulted in lesions in the nasal olfactory sensory cells, the cerebellum and adrenal gland beginning at 175 ppm (Hurtt *et al.*, 1987). Hurtt and Working (1988) later observed severe histological damage to the nasal epithelium following a single exposure to 90 or 200 ppm (351 or 780 mg/m^3) MeBr. Olfactory function, measured by the ability to locate buried food, was impaired at the 200 ppm exposure. In this study, reduced testosterone and testicular glutathione levels were observed in the male rats exposed to 200 ppm, but no effects on spermatogenesis, sperm quality, or testes histopathology were noted.

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

Another developmental toxicity study was conducted in rabbits by Breslin *et al.* (1990). In this study, rabbits were exposed to 0, 20, 40, or 80 ppm (0, 78, 156, or 312 mg/m^3) MeBr for 6 hours/day on gestation days 6-19. Maternal toxicity was observed at 80 ppm and included reduced body weight gain and signs of neurotoxicity. In addition to the maternal effects observed, a significant increase in incidence of gall bladder agenesis and fused sternebrae were observed in the offspring exposed to 80 ppm. No adverse effects were observed at 40 ppm or lower concentrations.

A 2-generation reproduction and developmental toxicity study on MeBr in rats was conducted by American Biogenics Corporation (1986). Groups of rats (25/sex/concentration) were exposed to 0, 3, 30, or 90 ppm (0, 12, 117, or 350 mg/m^3) MeBr 6 hours/day, 5 days/week during premating, gestation, and lactation through 2 generations. Significant decreases in body weight during the pre-mating period and at the end of the study were observed in the males exposed to 90 ppm. Although some adult organ weights were affected in the 90-ppm group, there was no evidence of histopathology in these organs. Neonatal body weights were decreased by exposure to 30 ppm. There was a decreased cerebral cortex width in the 90 ppm F_1 group, reduced brain weight in 30 ppm F_1 females, and reduced fertility in the 30 and 90 ppm F_{2b} groups.

VI. Derivation of Chronic Reference Exposure Level

Study	Reuzel *et al.*, 1987; 1991
Study population	Male and female Wistar rats (50 and 60 per group, respectively)
Exposure method	Discontinuous inhalation exposures (0, 3, 30, or 90 ppm) over 29 months
Critical effects	Basal cell hyperplasia of the olfactory epithelium of the nasal cavity
LOAEL	3 ppm
NOAEL	Not observed
Exposure continuity	6 hr/day, 5 days/week
Exposure duration	29 months
Average experimental exposure	0.54 ppm for the LOAEL group
Human equivalent concentration	0.12 ppm for the LOAEL group (gas with extrathoracic respiratory effects, RGDR = 0.23, based on MV = 0.03 m^3/min, SA = 11.6 cm^2)
LOAEL uncertainty factor	3 (20% extra risk of a mild effect)
Subchronic uncertainty factor	1
Interspecies uncertainty factor	3
Intraspecies uncertainty factor	10
Cumulative uncertainty factor	100
Inhalation reference exposure level	0.001 ppm (1 ppb, 0.005 mg/m^3, 5 μg/m^3)

The chronic REL for methyl bromide is also the U.S EPA RfC.

VII. Data Strengths and Limitations for Development of the REL

The major strengths of the REL for methyl bromide are the use of a comprehensive, long-term, multiple dose study with large sample sizes, and the availability of supporting data including long-term studies in other species and reproductive and developmental studies. The major uncertainties are the lack of human data and the lack of a NOAEL observation for the critical effect.

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

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CHRONIC TOXICITY SUMMARY

METHYL CHLOROFORM

(1,1,1-trichloroethane, methyltrichloromethane)

CAS Registry Number: 71-55-6

I. Chronic Toxicity Summary

Inhalation reference exposure level	**1,000 µg/m^3 (200 ppb)**
Critical effect(s)	Astrogliosis in the sensorimotor cortex (brain) of gerbils
Hazard index target(s)	Nervous system

II. Chemical Property Summary (HSDB, 1999)

Description	Colorless liquid
Molecular formula	$C_2H_3Cl_3$
Molecular weight	133.42 g/mol
Density	1.3376 g/cm^3 @ 20° C
Boiling point	74.1° C
Melting point	-30.4° C
Vapor pressure	127 torr @ 25° C
Solubility	Soluble in acetone, benzene, methanol, carbon tetrachloride
Conversion factor	5.47 µg/m^3 per ppb at 25ºC

III. Major Uses and Sources

Methyl chloroform is used as a solvent for adhesives and for metal degreasing (ACGIH, 1992). It is also used in the manufacture of vinylidene chloride and in textile processing and dry cleaning. The annual statewide industrial emissions from facilities reporting under the Air Toxics Hot Spots Act in California, based on the most recent inventory, were estimated to be 25,316,458 pounds of methyl chloroform (CARB, 1999a). Statewide monitored median and mean concentrations of methyl chloroform have been generally declining; decreasing from 0.8 or 1.71 ppb in 1990 to 0.12 or 0.30 ppb in 1996 (CARB, 1999b).

IV. Effects of Human Exposure

A 44-year old woman was diagnosed with peripheral neuropathy following 18 months of occupational exposure to methyl chloroform in a solvent bath (House *et al.*, 1994). There was no

identified exposure to agents known to cause peripheral neuropathy, such as n-hexane or trichloroethylene. The worker reported that she wore protective gloves and a respirator, both of which frequently leaked. Seven months following removal from exposure, the worker showed improved nerve conduction.

Other case reports have identified the nervous system as a target of methyl chloroform toxicity in similar exposure scenarios. Three workers developed distal sensory neuropathy after working with methyl chloroform in a degreasing operation with repeated dermal exposure (Liss, 1988; Howse *et al.*, 1989). Changes were observed in nerve conduction in the upper extremities accompanied by both axonopathy and myelonopathy.

Twenty-eight workers with chronic exposure to high (but unquantified) concentrations of 1,1,1-trichloroethane had significant deficits in memory, intermediate memory, rhythm, and speed based on the Luria-Nebraska Neuropsychological Battery (Kelafant *et al.*, 1994). Deficits in vestibular, somatosensory, and ocular components of balance were noted.

A 13-year-old male died after intentional inhalation of 1,1,1-trichloroethane (Winek *et al.*, 1997). Autopsy findings included tissue congestion of lung, liver and kidney.

Cardiac arrhythmia resulting from heightened cardiac sensitivity to epinephrine has been reported in several case reports of high acute inhalation exposures to methyl chloroform (ATSDR, 1990). There are case reports of arrhythmias persisting for two weeks or more after cessation of exposure to methyl chloroform (McLeod *et al.*, 1987).

An epidemiological study of workers chronically exposed to low levels of methyl chloroform (<250 ppm) found no changes in blood pressure, heart rate, or electrocardiogram (Kramer *et al.*, 1978). This study consisted of 151 workers who had been exposed for more than one year. No neurophysiological testing was done.

Another study of 22 female workers exposed to methyl chloroform (plus 7 unexposed control workers) at concentrations ranging from 110-345 ppm in air for a mean of 6.7 years failed to identify neurotoxicity resulting from methyl chloroform exposure (Maroni *et al.*, 1977). The examination included evaluation for neurologic symptoms, changes in nerve conduction, and psychomotor tests.

Liver disease was observed in a worker exposed to methyl chloroform in a clothing factory screen printing room (Cohen and Frank, 1994). The worker was exposed for a total of 4 years before occupational exposure was identified as the cause of the liver disease. The worker sprayed an adhesive (containing 65% methyl chloroform, 25% propane and dimethyl ether, and 10% inert ingredients) during which the worker reported often feeling dizzy or intoxicated. Three months following removal of the worker from exposure, liver function tests, although still abnormal, were significantly improved. Other case reports support these findings (Hodgson *et al.*, 1989; Halevy *et al.*, 1980).

Six male volunteers were exposed to 35 and 350 ppm methyl chloroform for 6-hours on two separate occasions (Nolan *et al.*, 1984). Absorption was determined to be 25% of the inhaled

dose. Of the absorbed dose, 91% was excreted unchanged in the expired air. Although the odor was perceptible for the duration of the exposure, no subjective symptoms were reported by the volunteers.

V. Effects of Animal Exposure

Gerbils (4/sex/dose plus 24 sex-matched control animals) were continuously exposed to 70, 210, or 1000 ppm methyl chloroform for 3 months (Rosengren *et al.*, 1985). A 4-month (solvent-free) recovery period following exposure was included to evaluate "lasting or permanent changes." Body weights were not changed significantly as a result of exposure. Brain weights in the animals in the 1000 ppm dose group were significantly decreased. Fibrillary astrocytes are formed in the brain in response to injury. Brain injury in methyl chloroform exposed gerbils was evaluated by detection of glial fibrillary acidic (GFA) protein, the main protein subunit of astroglial filaments. Increased levels of GFA protein were detected in the sensorimotor cerebral cortex of animals exposed to 210 or 1000 ppm methyl chloroform.

A later study in gerbils examined the effects of a 3-month continuous exposure to 70 ppm methyl chloroform followed by a 4-month recovery period (Karlsson *et al.*, 1987). DNA content was significantly decreased in three areas of the brain: posterior cerebellar hemisphere, anterior cerebellar vermis, and hippocampus. The authors contended that depressions in DNA content reflect decreased cell density.

No evidence of peripheral neuropathy or other neurotoxicity was detected in rats exposed to 200, 620, or 2000 ppm methyl chloroform 6 hours per day, 5 days per week for 13 weeks (Mattson *et al.*, 1993). The study included a functional observational test battery and measured visual, somatosensory, auditory and caudal nerve-evoked potentials. Histopathology of the brain, spinal cord, peripheral nerves and limb muscles was also examined at the end of the 13-week exposure.

Forty percent of all mice continuously exposed to 1000 ppm methyl chloroform for 14 weeks exhibited evidence of hepatocellular necrosis (McNutt *et al.*, 1975). A statistically significant increase in liver weight per body mass was observed throughout the study. Electron microscopy revealed accumulation of triglyceride droplets in the centrilobular hepatocytes following one week of exposure to 1000 ppm methyl chloroform. After 4 weeks of exposure, cytoplasmic alterations in centrilobular hepatocytes included a loss of polyribosomes and increased smooth endoplasmic reticulum. Similar changes observed occasionally in hepatocytes from mice exposed to 250 ppm were not as dramatic.

Mild hepatocellular changes were observed in rats exposed to 1500 ppm methyl chloroform 6 hours per day, 5 days per week for 6, 12, and 18 months (Quast *et al.*, 1988). At 24 months, these slight effects were no longer discernible due to confounding geriatric changes. No hepatocellular changes or other adverse effects were observed in rats exposed to 150 or 500 ppm methyl chloroform for up to 24 months.

The developmental toxicity of inhaled methyl chloroform was studied in CD-1 mice. Mice were exposed on gestation days 12 through 17 to either 2000 ppm methyl chloroform for 17 hours per

day or 8000 ppm methyl chloroform for 1 hour three times per day (Jones *et al*., 1996). There were no effects on pregnancy outcome, but exposed pups has reduced weight gain, had poorer results on motor coordination tests and showed delays in negative geotaxis (orienting towards the top of a sloped screen).

VI. Derivation of Chronic Reference Exposure Level (REL)

Study	Rosengren *et al.* (1985)
Study population	Mongolian gerbils (4/sex/dose)
Exposure method	Whole-body inhalation exposure
Critical effects	Astrogliosis in the sensorimotor cerebral cortex
LOAEL	210 ppm
NOAEL	70 ppm
Exposure continuity	Continuous
Average experimental exposure	70 ppm for NOAEL group
Human equivalent concentration	Not derived (species-specific data for gerbils unavailable to validate assumption of RGDR=1)
Exposure duration	3 months
LOAEL uncertainty factor	1
Subchronic uncertainty factor	3
Interspecies uncertainty factor	10
Intraspecies uncertainty factor	10
Cumulative uncertainty factor	300
Inhalation reference exposure level	0.2 ppm (200 ppb; 1 mg/m^3; 1,000 µg/m^3)

VII. Data Strengths and Limitations for Development of the REL

Case reports indicate that the nervous system and the liver are targets of the toxicity of methyl chloroform (House *et al.*, 1994; Liss, 1988; Howse *et al.*, 1989; Cohen and Frank, 1994). The largest of the epidemiological studies (Kramer *et al.*, 1978; Maroni *et al.*, 1977), however, did not identify adverse effects as a result of chronic methyl chloroform exposure. The Kramer *et al.* (1978) study limited its evaluation to changes in blood pressure, heart rate, or electrocardiogram and exposure levels were only characterized as less than 250 ppm. Maroni *et al.* (1977) conducted their study among 22 women exposed occupationally to methyl chloroform levels as low as 110 ppm. Although the subjects were evaluated specifically for signs of neurotoxicity, the small sample size limits conclusions that can be drawn from their failure to identify adverse effects in this population. If no effects are associated with the exposures in the 2 studies (Kramer *et al.*, 1978; Maroni *et al.*, 1977), the REL predicted would be approximately 3 ppm.

Data from animal studies generally support the findings of the case reports from human exposures. Both neurotoxicity and hepatotoxicity have been identified among animals exposed by inhalation to methyl chloroform. The adverse effect observed at the lowest level in these studies was the development of astrogliosis in the brains of gerbils exposed for 3 months to 210 ppm methyl chloroform (Rosengren *et al.*, 1985). A no-observed-adverse-effect-level (NOAEL) in this study was 70 ppm methyl chloroform. A subsequent study identified a more subtle change in the brains of gerbils exposed similarly to 70 ppm methyl chloroform, with slightly

decreased DNA content found in several discrete brain regions of exposed animals. However, the relationship between tissue DNA content and cell density as an indication of adverse effect in the brain was considered too tenuous for the development of a guidance value for chronic exposure to methyl chloroform.

The major strengths of the REL for methyl chloroform are the observation of the NOAEL and the continuous subchronic exposure regimen. The major uncertainties are the lack of human exposure data, the lack of dose-response information, and the lack of comprehensive multi-organ effects data.

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CHRONIC TOXICITY SUMMARY

METHYL t-BUTYL ETHER

(MTBE; 2-methoxy-2-methylpropane; tert-butyl methyl ether; methyl 1,1dimethyl ether)

CAS Registry Number: 1634-04-4

I. Chronic Toxicity Summary

Inhalation reference exposure level	**8000 mg/m^3 (2000 ppb)**
Critical effect(s)	Nephrotoxicity, prostration, periocular swelling in Fischer 344 rats
Hazard index target(s)	Kidney; eyes; alimentary system

II. Physical and Chemical Properties (HSDB, 1994)

Description	Colorless liquid
Molecular formula	$C_5H_{l2}0$
Molecular weight	88.15 g/mol
Density	0.7405 g/cm^3 @ 20°C
Boiling point	55.2°C @ 760 mm Hg
Vapor pressure	245 torr @ 20°C
Solubility	Soluble in alcohol, ether, and 5% soluble in water
Conversion factor	1 ppm = 3.61 mg/m^3 @ 25° C; 3.67 mg/m^3 @ 20° C

III. Major Uses or Sources

Methyl t-butyl ether (MTBE) is used as a gasoline additive to improve octane ratings and reduce emissions of some pollutants, in industry to improve miscibility of solvents, and in clinical medicine to dissolve cholesterol gall stones (Yoshikawa *et al.*, 1994). The annual statewide industrial emissions from facilities reporting under the Air Toxics Hot Spots Act in California,based on the most recent inventory, were estimated to be 215,182 pounds of MTBE (CARB, 1999).

IV. Effects of Human Exposure

Gasoline (with 10% MTBE) tanker drivers reported significantly higher fatigue at the end of the work week than before the work week, and those with longer exposure to gasoline with MTBE during the work week reported significantly higher fatigue than drivers with shorter exposure

(Hakkola *et al*., 1997). 20% of drivers reported symptoms such as headache, dizziness, nausea, and dyspnoea at the end of work week. No human chronic toxicity or chronic epidemiology information for MTBE without coexposure to gasoline was found.

Ten healthy male volunteers undergoing light physical work were exposed to 5, 25, and 50 ppm MTBE vapor for 2 hours (Nihlen *et al*., 1998). While a solvent smell was noted at these concentrations, there were no consistent concentration-related effects on reported ocular or nasal irritation. The blockage index (a measure of nasal airway resistance) increased significantly after exposure but was not correlated with exposure concentration.

V. Effects of Animal Exposure

Male and female rats (50/sex/group) were exposed by inhalation for 6 hours/day, 5 days/week to mean concentrations of 0, 403, 3023, or 7977 ppm (0, 1453, 10,900, or 28,760 mg/m^3) MTBE for 24 months (Chun *et al.*, 1992). Clinical signs, hematology, body weights and food consumption were monitored. Necropsy included measurements of organ weights and histopathology. Corticosterone levels were measured on 10 animals prior to sacrifice. Serum enzymes were not monitored. The NOAEL for several endpoints, including non-alpha-2μ-globulin induced nephrotoxicity, increased relative liver and kidney weights and prostration in females, and periocular swelling in both sexes was 403 ppm (1453 mg/m^3).

Mice were exposed for 6 hours/day, 5 days/week for 18 months to MTBE concentrations of 0, 402, 3014, or 7973 ppm (0, 111, 835, or 2208 mg/m^3) (Burleigh-Flayer *et al.*, 1992). The mice exposed to the highest concentration (7973 ppm) all exhibited ataxia. Prostration was also noted in 8 of 50 animals in this group. Liver weights were elevated in a concentration-dependent manner in the female mice but this change was not significant at the lowest concentration (402 ppm). Kidney weights were elevated in the female mice exposed to 7973 ppm. At the highest concentration, a significant increase in hepatocellular hypertrophy and adrenal gland weight was detected in the male mice. Spleen weights were increased in the females exposed to the highest concentration.

Moser *et al*. (1998) exposed female B6C3F$_1$ mice to 7924 ppm (2195 mg/m^3) MTBE for 4 months, or 7919 ppm (2194 mg/m^3) MTBE for 8 months; controls received plain air. Body weight increases for control and MTBE-exposed mice, respectively, were 57% and 37% at 4 months and 79% and 45% at 8 months: the reduced weight gain in MTBE-exposed mice was significantly different from the controls at both time points. In MTBE-exposed mice, mean uterine weight was 83% reduced relative to controls at 4 and 8 months. Ovary weight was also reduced in exposed mice, the mean weight being 55% of control at 4 months and 51% of control at 8 months. Pituitary weights were decreased by 44% and 31% at 4 and 8 months, relative to controls. Disturbances of the estrus cycle and histological changes in the reproductive organs were also noted. Although the changes in organ weights and histology were suggestive of an anti-estrogenic effect of MTBE, serum estrogen levels were unaffected. No changes in estrogen receptor (ER) immunoreactivity in reproductive system tissues were observed. Experiments *in vitro* failed to demonstrate any inhibition of estradiol binding to ER by MTBE or its metabolites. No inhibition of ER by MTBE was detected, nor was there any inhibition of the induction of ER by estradiol. The authors concluded that the apparent anti-estrogenic effects of MTBE were not

mediated via the ER, and drew a parallel with the anti-estrogenic effects of dioxins and chlorinated biphenyls.

Tests for histopathology in the respiratory tract, plasma corticosterone levels, motor activity and neurobehavioral endpoints were performed in rats exposed to MTBE at concentrations of 0, 797, 3920, or 8043 ppm (0, 2877, 14151, or 29035 mg/m^3), 6 hours/day, 5 days/week for 13 weeks (Dodd and Kintigh, 1989). Of these endpoints, the most significant finding was an elevation in plasma corticosterone in the high dose group. This finding was consistent with the elevated adrenal weights reported by Burleigh-Flayer *et al.* (1992). A clear dose-response for neurotoxic effects in these rats was not established.Biles *et al.* (1987) reported a NOAEL of 300 ppm (1083 mg/m^3) MTBE for decreased pup viability in rats exposed for 6 hours/day, 5 days/week for a total of 16 weeks. Animals exposed to 1240 ppm (4470 mg/m^3) or 2860 ppm (10,311 mg/m^3) MTBE exhibited slightly decreased pup survival.

Neeper-Bradley (1991) exposed rats to 0, 402, 3019, or 8007 ppm (0, 111, 836, and 2218 mg/m^3) MTBE over 2 generations. Exposures were for 6 hours/day, 5 days/week during the prebreeding period, and for 7 hours/day, 5 days/week during gestation and lactation. Parental effects of MTBE exposure were observed, including ataxia, blepharospasm, lack of startle reflex, and increased relative liver weights (F1 generation only). There were no histological changes in the organs from either parental generation. Reduced body weights were observed in the F1 and F2 pups at the 3019 and 8007 ppm concentrations. Reduced survivability to postnatal day 4 was observed in the 8007 ppm group. No adverse effects were noted at the 403 ppm (111 mg/m^3) concentration.

In a developmental and reproductive toxicity study, Conaway and associates (1985) found no significant increases in maternal or fetal toxicity, nor in pregnancy rates or in any gross toxicologic parameter tested with pregnant rats or mice exposed during gestation to concentrations of MTBE up to 3300 ppm (11,897 mg/m^3).

Maternal toxicity, in the form of hypoactivity and ataxia, was observed in pregnant mice exposed during gestation to 4076 ppm (14,690 mg/m^3) MTBE (Bushy Run Research Center, 1989a). Significant reductions in food intake and body weight gain were observed in dams exposed to 8153 ppm (29,390 mg/m^3). Fetal body weight was significantly reduced in the 4076 ppm group, and there were significant increases in the incidences of skeletal variations and unossified phalanges in the 4076 and 8153 ppm groups. Pregnant rabbits exposed to similar concentrations during gestation showed no significant maternal or fetal toxicity or developmental toxicity up to a concentration of 8021 ppm (28,918 mg/m^3) (Bushy Run Research Center, 1989b).

VI. Derivation of Chronic Reference Exposure Level

Study	Chun *et al.*, 1992; Bird 1997
Study population	Male and female rats (50 per sex/group)
Exposure method	Discontinuous whole-body inhalation exposures (0, 403, 3023, or 7977 ppm)
Critical effects	Nephrotoxicity, increased liver and kidney

	weight, prostration and periocular swelling
LOAEL	3023 ppm
NOAEL	403 ppm
Exposure continuity	6 hours per day, 5 days per week
Exposure duration	24 months
Average experimental exposure	72 ppm for the NOAEL group
Human equivalent concentration	72 ppm for the NOAEL group (gas with systemic effects, based on RGDR = 1.0 using default assumption that lambda (a) = lambda (h))
LOAEL uncertainty factor	1
Subchronic uncertainty factor	1
Interspecies uncertainty factor	3
Intraspecies uncertainty factor	10
Cumulative uncertainty factor	30
Inhalation reference exposure level	2 ppm (2000 ppb, 8 mg/m^3, 8000 μg/m^3)

The USEPA (1995) based its RfC of 3000 μg/m^3 on the same study but included a Modifying Factor (MF) of 3 for database deficiencies. The criteria for use of modifying factors are not well specified by U.S. EPA. Such modifying factors were not used by OEHHA.

VII. Data Strengths and Limitations for Development of the REL

The major strengths of the REL for MTBE are the use of a comprehensive, long-term multiple dose study with large sample sizes and the observation of a NOAEL. The major uncertainty is the lack of human data.

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CHRONIC TOXICITY SUMMARY

METHYLENE CHLORIDE

(dichloromethane, methylene dichloride)

CAS Registry Number: 75-09-2

I. Chronic Toxicity Summary

Inhalation reference exposure level	**400 µg/m^3 (100 ppb)**
Critical effect(s)	Carboxyhemoglobin formation above 2% in human workers
Hazard index target(s)	Cardiovascular system; nervous system

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

Description	Colorless liquid
Molecular formula	CH_2Cl_2
Molecular weight	84.93
Density	1.32 g/cm^3 @ 20° C (ACGIH, 1991)
Boiling point	39.75° C
Vapor pressure	400 torr @ 24.1° C
Solubility	Miscible with most organic solvents, slightly soluble in water (ACGIH, 1991)
Conversion factor	1 ppm = 3.47 mg/m^3 @ 25° C

III. Major Uses and Sources

Methylene chloride (MC) is used in paint and varnish remover, in aerosols as a cosolvent or vapor pressure depressant, and in solvent degreasing and metal cleaning. It is also used in plastics processing and in extraction of fats and oils from food products (HSDB, 1999). The annual statewide industrial emissions from facilities reporting under the Air Toxics Hot Spots Act in California, based on the most recent inventory, were estimated to be 3,504,271 pounds of methylene chloride (CARB, 1999a). Both mean and maximum monitored ambient methylene chloride concentrations have decreased slightly between 1990 and 1996 (CARB, 1999b). Median and maximum concentrations were 1.09 and 11 ppb in 1990 and 0.66 and 5.6 ppb in 1996.

IV. Effects of Human Exposure

Effects of a controlled 2-hour inhalation exposure to MC included CNS depression at concentrations of 1000 ppm (3500 mg/m^3) or more and increased blood carboxyhemoglobin (COHb) content at lower concentrations (500 ppm) due to metabolism of MC to carbon monoxide (Stewart *et al.*, 1972). High levels of COHb can be found in the blood hours after exposure to methylene chloride, due to its partitioning into fat and its slow release into circulation with subsequent metabolism, leading to formation of carbon monoxide (Engstrom and Bjurstrom, 1977). In situations of chronic exposure, carbon monoxide toxicity is also of concern. Barrowcliff (1978) documented the case of an adult male who developed an unsteady gait, a peculiar dysarthria and a loss of memory. The man had worked with 15-50 liters of methylene chloride daily for 3 years in a poorly ventilated room while cleansing road materials. No natural disease could be found to explain his conditions and the effects were attributed to chronic carbon monoxide poisoning.

Twelve women volunteer subjects were exposed to 0, 300, or 800 ppm methylene chloride for 4 hours (Fodor and Winneke, 1971). Neurobehavioral vigilance was measured by auditory discrimination of intensity of certain sound pulses against a background of continuous white noise. A significant interactive effect between methylene chloride concentration and duration of exposure using 2-way ANOVA ($p < 0.01$) was found.

Human erythrocytes enzymatically convert methylene chloride to formaldehyde in cell-culture experiments (Hallier *et al.*, 1994).

A subacute controlled exposure of eleven resting non-smokers to methylene chloride was conducted by DiVincenzo and Kaplan (1981a). The eleven subjects were exposed to 50, 100, 150, or 200 ppm methylene chloride for 7.5 hours on 5 consecutive days. Exposure to all concentrations led to dose-dependent elevation in COHb concentrations in the blood and elevated exhaled CO. The peak blood COHb saturations were 1.9, 3.4, 5.3, and 6.8%, respectively, for the 50, 100, 150, and 200 ppm groups.

Divencenzo and Kaplan (1981a) also measured COHb percentage in the blood of workers occupationally exposed to methylene chloride and a group of workers not exposed to methylene chloride. The 19 workers exposed to methylene chloride had mean blood COHb concentrations of 2.3% in the morning and 3.9% at the end of the work-shift. Ambient concentrations in the workplace were estimated from 57 samples, which ranged from 0 to 250 ppm, with a mean concentration of 40 ppm. Three exposed workers also wore monitors to estimate personal exposures. The time-weighted average exposure for these workers was 33 ppm. Controls (8 subjects) had significantly lower mean blood COHb concentrations of 0.8% in the AM and 1.3% in the PM compared with the exposed workers. The length of employment of the exposed workers was not given.

A companion study by DiVincenzo and Kaplan (1981b) showed that smoking and methylene chloride exposure result in an additive effect on COHb levels compared with levels in non-smokers. Similarly, light, moderate or heavy exercise workloads resulted in higher COHb levels.

Soden *et al.* (1996) showed a dose-response increase in carboxyhemoglobin levels in non-smokers with increasing methylene chloride exposure in workers involved in triacetate fiber production. Carboxyhemoglobin levels ranged from 1.77% to 4% from exposures ranging from 6.5 to 89.7 ppm, respectively. The number of employees in the study was not reported.

Although animal studies have shown COHb-induced cardiovascular effects following MC exposure (Aviado *et al.*, 1977), no data exist on this outcome in humans. However, studies of men with coronary artery disease and exercise-induced angina report a decrease in time to onset of exercise-induced angina following exposure to carbon monoxide (CO) at concentrations sufficient to result in blood COHb levels of about 2% (Kleinman *et al.*, 1989; Allred *et al.*, 1989). A physiologically based pharmacokinetic model of MC and CO estimated that a 1-hour exposure to 340 ppm (1200 mg/m^3) MC at a ventilation rate of 9 liters/min would result in a peak blood COHb level of 2% (Andersen *et al.*, 1991; Reitz, 1994). The California Ambient Air Quality Standard for CO is based on a blood COHb level of 2% (CARB, 1982).

An epidemiological study of 751 male workers in the Eastman Kodak Company exposed to daily 8-hour time-weighted average concentrations of 30-125 ppm methylene chloride for up to 30 years was conducted by Friedlander and associates (1978). A control group of workers in production but not exposed to methylene chloride was used together with New York state cause and age-specific mortality rates. The follow-up period for these workers was 13 years, with 97% success. The studies did not indicate any increase in risk of death from circulatory disease, cancer, or other causes due to methylene chloride exposure.

A study of female pharmaceutical workers in eight different factories exposed to a variety of organic solvents indicated that solvent exposure, and particularly methylene chloride exposure, resulted in an increase in spontaneous abortions (Taskinen *et al.*, 1986). In all, 1795 pregnancies were followed, with 142 spontaneous abortions occurring. The odds ratio for methylene chloride exposure was 1.0 to 5.7 (average = 2.3; $p < 0.06$). There was a significant effect of exposure to 4 or more solvents, compared with age-matched controls ($p < 0.05$). The concentrations of MC were not reported in the study.

The U.S. Occupational Safety and Health Administration reduced its permissible exposure limits (PEL) for MC from 500 ppm to 25 ppm in 1997 (U.S. CFR, 1997).

V. Effects of Animal Exposure

Nitschke *et al.* (1988) found that a 2-year exposure to 0, 50, 200, or 500 ppm MC for 6 hours/day, 5 days/week resulted in significant histopathologic lesions in the livers of rats exposed to 500 ppm. No significant adverse effects were observed at 200 ppm or lower. The predominant hepatocellular lesion was fatty vacuolization of hepatocytes.

Female B6C3F1 mice inhaling 2000 ppm MC for 1 to 26 weeks had 40 to 60% lower cell turnover rates of bronchiolar cells compared with controls (Kanno *et al.*, 1993). At this concentration no observable pathological changes were found in the lungs of MC exposed animals.

A continuous exposure of mice (16 per group) to 100 ppm MC for 1, 2, 3, 4 or 10 weeks resulted in signifcant elevation in liver triglycerides beginning at 2 weeks and lasting throughout the 10-week period (Weinstein and Diamond, 1972). Liver/body weight ratios were unaffected at any time point. After 1 week, small fat droplets were apparent in centrilobular hepatocytes and a decrease in hepatic glycogen was also noted. Necrosis was not observed during the 10-week period, but fat droplet size increased and glycogen depletion persisted.

Male and female Sprague-Dawley rats and Golden Syrian hamsters inhaled methylene chloride (0, 500, 1500, or 3500 ppm) for 6 hr per day, 5 days a week over 2 years (Burek *et al.*, 1984). The groups consisted of 129 rats per sex per concentration, and 107 to 109 hamsters per sex per concentration. Females rats inhaling 3500 ppm had an increased mortality rate while female hamsters inhaling 1500 or 3500 ppm had decreased mortality rates. Slight histopathological findings were noted in livers of rats exposed to 500, 1500, or 3500 ppm MC. Decreased amyloidosis was also found in livers and other organs of hamsters at each of the three MC concentrations. Overall, effects were more potent in rats compared with hamsters, which had fewer spontaneous age-related changes, decreased mortality (at least for females), and evidence of specific target organ toxicity was weak. Carboxyhemoglobin values were elevated in both rats and hamsters exposed to 500 ppm or more of MC, with the percentage increase greater in hamsters than in rats.
Monkeys were observed to be more susceptible subjects for methylene chloride induced COHb than dogs upon 14-week subchronic continuous exposure to 25 or 100 ppm (Haun *et al.*, 1972). At 25 ppm, approximately 1.5% COHb was reached in the 4 monkeys, compared to approximately 0.5% in 16 dogs. Monkeys exposed to 100 ppm MC had COHb levels of approximately 4% compared with 2% in the dogs.

Oral ethanol pretreatment in rats has been shown to suppress the COHb formation characteristic of methylene chloride exposure through inhibition of biotransformation of methylene chloride (Glatzel *et al.*, 1987).

Gerbils (10/sex per group; 60 controls) exposed continuously to MC concentrations of 210, 350, or 700 ppm for a period of 3 months, with a 4-month follow-up period, showed irreversible cellular and biochemical changes in brain (Rosengren *et al.*, 1986). A high mortality rate (19/20) was observed in the 700 ppm group, and this exposure was terminated after 7 weeks. The gerbils exposed to 350 ppm also had a high mortality rate (9/20) and this exposure was terminated after 10 weeks. The gerbils exposed to 210 ppm had no premature mortality and the exposure continued for the full 3 months. Four months after termination of exposure, the animals in the 350 and 210 ppm groups had significantly decreased brain DNA content in the hippocampus. The 350 ppm group exhibited elevated astroglial proteins in the frontal and sensory motor cerebral cortex, consistent with astrogliosis in these regions. In addition, the gerbils exposed to 350 ppm MC had significantly decreased DNA in the cerebellar hemispheres. Complimentary studies by these investigators showed that the formation of carboxyhemoglobin did not increase in gerbils between the 210 and 350 ppm exposures, indicating that the metabolism of MC to CO is saturable at concentrations below those in the study. On the other hand, the neurotoxic brain biochemical alterations were significantly greater in gerbils exposed to 350 ppm as compared

with the 210 ppm group, implying that carboxyhemoglobin induced cerebral hypoxia is not the major cause of MC-induced neurotoxicity in the brain.

Rats (50 per sex per group) were exposed to 0, 1000, 2000, or 4000 ppm methylene chloride 6 hours/day, 5 days/week for 102 weeks (NTP, 1986). Both sexes exhibited hemosiderin pigmentation in the liver in a dose-dependent fashion, beginning with the 1000 ppm concentration. Squamous metaplasia of the nasal cavity was observed in female rats, and thyroid C-cell hyperplasia was observed in males exposed to 2000 ppm or greater. Kidney tubule degeneration (not otherwise specified) was increased at all exposure levels.

Mice (50 per sex per group) exposed to 0, 2000, or 4000 ppm methylene chloride 6 hours/day, 5 days/week for 102 weeks showed increased incidence of liver cytologic degeneration and splenic atrophy at 4000 ppm (males) (NTP, 1986). Male and female mice also had an increased incidence of kidney tubule casts (not otherwise specified) at 2000 ppm or greater, and significant testicular atrophy was observed in males at 4000 ppm. Female mice showed cytologic degeneration in the liver at 2000 ppm or greater, and ovarian atrophy at 2000 ppm or greater.

A six month exposure to 5000 ppm MC of 8 guinea pigs for 7 hours/day, 5 days/week resulted in 3 deaths; 2 showed moderate centrilobular fatty degeneration of the liver and extensive pneumonia at necropsy (Heppel *et al.*, 1944). None of the 14 control animals died. Food consumption and body weight were lower in the exposed guinea pigs, compared with control pigs. One out of 12 rats died at this concentration, and the liver histology in this animal revealed multiple thrombi in renal vessels, associated with marked cortical infarction. By comparison, dogs and rabbits showed no signs of illness, nor were blood pressure or hematological values altered at the 5000 ppm concentration. At 10,000 ppm, 2 of 4 dogs showed moderate centrilobular congestion, narrowing of liver cell cords, and slight to moderate fatty degeneration. One of 2 monkeys revealed disseminated tuberculosis lesions, but no other histological alterations. Four out of 6 guinea pigs had moderate fatty degeneration of the liver at this concentration.

The offspring of rats (10 dams per group) exposed during gestation to 0 or 4500 ppm methylene chloride exhibited altered rates of behavioral habituation to novel environments (Bornschein *et al.*, 1980). This effect was observed beginning at 10 days of age but was still demonstrable in rats 150 days old. The authors concluded that elevated maternal COHb could have been a contributing factor in the developmental impairment.

In a study of the effects of methylene chloride on estrous cycle and serum prolactin, groups of 15 female rats were exposed to 0 or 3500 ppm for 6 hours/day for 15 to 19 consecutive days (Breslin and Landry, 1986). Males (15 per group) were exposed for 5 hours/day for 5 consecutive days. Female rats exhibited decreased body weight and increases in the estrous cycle duration and in serum prolactin. Males did not show any significant effects on serum prolactin from methylene chloride exposure.

Pregnant mice and rats were exposed to 0 or 1250 ppm MC 7 hours/day, on days 6 through 15 of gestation (Schwetz *et al.*, 1975). Significantly elevated absolute liver weights were seen in

maternal animals from both species. In addition, significantly increased incidences of delayed ossification of the sternebrae were seen in both species, compared to controls.

Methylene chloride exposure of female rats before or during gestation to 4500 ppm resulted in elevated maternal liver weights and decreased birth weights of the offspring, but no terata or skeletal/soft tissue anomalies (Hardin and Manson, 1980).

A 2-generation reproduction test was conducted by Dow Chemical Company (Nitschke *et al.*, 1985) which showed no significant reproductive or developmental effects in rats exposed to 0, 100, 500, or 1500 ppm MC 6 hours/day, 5 days/week, for 14 weeks. The exposure conditions were identical for the F_0 and F_1 generations.

VI. Derivation of Chronic Reference Exposure Level (REL)

Study	DiVincenzo and Kaplan (1981a)
Study population	19 workers, 8 controls
Exposure method	Occupational inhalation exposure
Critical effects	Significantly elevated carboxyhemoglobin levels (> 2%)
LOAEL	40 ppm (ambient workplace exposures averaged 40 ppm with a range of 0 to 250 ppm); controls = 0 ppm
NOAEL	Not observed
Exposure continuity	8 hours/day, 5 days/week
Exposure duration	Length of employment unspecified
Average occcupational exposure	14 ppm for LOAEL group (40 x 10/20 x 5/7)
LOAEL uncertainty factor	10
Subchronic uncertainty factor	1 (see following text for explanation)
Interspecies uncertainty factor	1
Intraspecies uncertainty factor	10
Cumulative uncertainty factor	100
Inhalation reference exposure level	0.1 ppm (100 pbb; 0.4 mg/m^3; 400 µg/m^3)

Workers were exposed to average measured concentrations of 40 ppm during the workday, and the personal monitors on 3 of the subjects indicated a 8-hour time-weighted average of 33 ppm over a 2-week period. The average COHb levels were 3.9% at the end of the work-shift. Elevated carboxyhemoglobin concentrations of above 2% are considered to aggravate angina in some individuals (CARB, 1982). In effect, 2% COHb can be considered a NOAEL for aggravation of angina. Therefore, the 33 ppm concentration was considered a LOAEL for the formation of greater than 2% COHb. The duration of the employment period was not specified. However, in the DiVincenzo and Kaplan (1981a) study, the levels of COHb did not appear to increase over a period of 5 days in experimental exposures using volunteers, therefore an uncertainty factor for subchronic exposure was not necessary. A number of factors contribute to

the uncertainty in determining the degree of sensitivity to methylene chloride, including activity level, metabolic enzyme activity, age, and background COHb status (e.g., from smoking, etc.).

The subchronic study by Haun *et al.* (1972) with monkeys reported a NOAEL of 25 ppm and a LOAEL of 100 ppm for 2% COHb formation following a 14-week exposure. These results are consistent with the LOAEL reported in the DiVincenzo and Kaplan study. However, the human occupational study likely contains less uncertainty, since the toxicokinetics of the effect, including rate of formation of CO and thus COHb is metabolism-dependent, resulting in considerable potential interspecies differences.

The study in hamsters by Burek *et al.*(1984) showed a LOAEL for elevated carboxyhemoglobin of 500 ppm. A time-weight average exposure and HEC of 89 ppm was calculated. Using a 10-fold LOAEL uncertainty factor, a 3-fold interspecies uncertainty factor for residual uncertainty not accounted for in the HEC calculation, and a 10-fold intraspecies uncertainty factor, a REL of 300 ppb or 1000 $\mu g/m^3$ was derived. Thus, the REL derived from the best available animal study is comparable to the 400 $\mu g/m^3$ REL derived from the best-available human study.

VII. Data Strengths and Limitations for Development of the REL

The major strength of the key study (DiVincenzo and Kaplan, 1981a) used to derive the REL for methylene chloride is that human health effects were observed. The major uncertainties from the key study itself are the lack of a NOAEL observation, the difficulty in estimating exposures, and the discontinuous and variable nature of the exposures.

The health effects database for methylene chloride includes, in addition to an adequate study of human occupational exposures (DiVincenzo and Kaplan, 1981a), an adequate lifetime inhalation exposure study in 2 species of laboratory animals (Burek *et al*., 1984). The REL values derived from these studies (400 $\mu g/m^3$ vs. 1,000 $\mu g/m^3$) are comparable. That both the human and animal studies measured the same endpoint and arrived at similar conclusions is a circumstance that is rarely found but one that considerably increases the weight of evidence from which the REL was derived. The two studies complement each other, as the animal study involved controlled, measured exposures over a lifetime but introduces the uncertainty of predicting human health effects from animal observations, and the human study involved poorly characterized human exposures but lacks the uncertainty inherent in interspecies extrapolation.

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CHRONIC TOXICITY SUMMARY

NICKEL AND NICKEL COMPOUNDS
NICKEL OXIDE

Molecular Formula	*Molecular Weight*	*Synonyms*	*CAS Registry Number*
Ni	59	elemental nickel	7440-02-0
NiO	74.69	nickel oxide	1313-99-1
$NiCl_2$	129.6	nickel chloride nickel dichloride	7718-54-9
$NiSO_4$	154.75	nickel sulfate nickelous sulfate	7786-81-4
$NiCO_3$	118.7	nickel carbonate carbonic acid nickel salt	3333-67-3
Ni_3S_2	240.19	nickel subsulfide trinickel disulfide heazlewoodite	12035-72-2

I. Chronic Toxicity Summary

A. Nickel and Nickel Compounds (except nickel oxide)

Inhalation reference exposure level	**0.05 mg Ni/m^3**
Critical effect(s)	Lung, nasal epithelial and lymphatic pathology in male and female rats
Hazard index target(s)	Respiratory system; hematopoietic system

B. Nickel Oxide

Inhalation reference exposure level	**0.10 mg Ni/m^3**
Critical effect(s)	Lung and lymphatic pathology in male and female rats
Hazard index target(s)	Respiratory system; hematopoietic system

II. Physical and Chemical Properties (from HSDB, 1995)

Description	Ni metal: Silvery metal; $NiCl_2$: deliquescent crystals (U.S.EPA, 1985)
Molecular formula	See above
Molecular weight	See above
Density	8.9 g/cm^3 @ 20°C (Ni)
Boiling point	2730°C (Ni)
Vapor pressure	Not applicable
Solubility	Elemental nickel, nickel subsulfide, and nickel oxide are insoluble in water, but are soluble in dilute nitric, hydrochloric, and sulfuric acids. The chloride and sulfate forms of nickel are water soluble.
Conversion factor	Not applicable for fumes and dusts

III. Major Uses and Sources

The most common airborne exposures to nickel compounds are to insoluble nickel compounds such as elemental nickel, nickel sulfide, and the nickel oxides from dusts and fumes. Contributions to nickel in the ambient air are made by combustion of fossil fuels, nickel plating, and other metallurgical processes. The most common oxidation state of nickel is the divalent (Ni^{2+}) form (U.S.EPA, 1985). Elemental nickel is a malleable, silvery-white metal that is highly resistant to strong alkali. Because of its corrosion resistance, nickel is used in the production of stainless steel, permanent magnets, and other alloys that require resistance to extremes of temperature or stress (U.S.EPA, 1985). Nickel is also used in electroplating baths, batteries, textile dyes, and catalysts (U.S.EPA, 1985). Nickel dust or powder is flammable (CDTSC, 1985). Due to its unique toxicological and physico-chemical properties, nickel carbonyl is not included in this summary. The annual statewide industrial emissions from facilities reporting under the Air Toxics Hot Spots Act in California, based on the most recent inventory, were estimated to be 110,334 pounds of nickel (CARB, 1999).

IV. Effects of Human Exposure

Several studies have indicated that occupational inhalation exposure to nickel aerosols can result in development of asthma specific to nickel. Davies (1986) found 3 cases of asthma among 53 nickel-plating workers without a history of asthma prior to employment. Novey *et al.* (1983) described biphasic metal-specific bronchial responses in an individual metal-plating worker exposed to nickel and chromium salts. In another case, immunological studies conducted in a 24-year old man showed nickel-specific antibodies in the serum after several weeks of working in a nickel-plating shop using nickel sulfate (McConnell *et al.*, 1973). Dermatitis was observed on exposed areas of his skin, and pulmonary function, measured by FEV_1 with and without isoproterenol challenge, was significantly impaired compared with a control subject and normal

values. Dyspnea, non-productive cough, chest-tightness, and wheezing were reported as symptoms by this worker during the work period.

A group of 7 metal plating workers with occupational asthma were evaluated for atopy and pulmonary function challenge in response to inhalational challenge with nickel and other metals (Cirla *et al.*, 1985). Three of the asthmatics tested positive for the presence of nickel-specific IgE antibodies. Positive reactions to skin testing with nickel were found in 3 of the asthmatic workers who also had dermatitis. Six out of the 7 asthmatics exhibited significantly decreased FEV_1 (> 15%) when exposed to 0.3 mg/m^3 nickel sulfate for 30 minutes. Control challenges with other metal salts did not reveal similar deficits in FEV_1.

Although asthma has been described in the above studies, occupational inhalation of nickel dusts has not been found to be associated with pulmonary fibrosis (Muir *et al.*, 1993). An occupational epidemiology report by Broder *et al.* (1989) found no significant effects on pulmonary function in relation to nickel exposure in a nickel smelter, however a healthy worker effect was observed in this study.

V. Effects of Animal Exposure

Early studies on the chronic non-cancer effects of metallic nickel dust were complicated by early mortality and cancer in guinea pigs and rats (Hueper, 1958).

A 2-year inhalation study of nickel oxide in rats and mice (65 per sex, per group) was conducted by the National Toxicology Program (NTP, 1994a). In the first study, rats were exposed to 0, 0.62, 1.25, or 2.5 mg nickel oxide/m^3 (0, 0.5, 1.0, or 2.0 mg Ni/m^3) 6 hours/day, 5 days/week for 104 weeks. In addition to the carcinogenic effects of nickel oxide, a number of non-cancerous lesions were observed, particularly in the lungs. The incidence of inflammatory pigmentation in the alveoli was significantly greater in all exposed groups, compared to controls. The severity of the lesions reportedly increased with increasing exposure. Atypical alveolar hyperplasia was also seen in all exposed groups. Lymphoid hyperplasia in the bronchial lymph nodes was observed in males and females exposed to 1 mg Ni/m^3 or greater at 7 and 15 months and the incidence generally increased with increasing concentration at the end of the 2-year study. Females had an increased incidence of adrenal medullary hyperplasia at all exposures of nickel oxide. Body weights were significantly lower in the groups exposed to 2.0 mg Ni/m^3 for both sexes, and in males exposed to 1.0 mg Ni/m^3.

A companion study on nickel oxide in mice conducted by NTP showed similar lung inflammatory changes as seen in the rats, in addition to pigmentation of the alveolar region at all exposure concentrations, compared with controls (NTP, 1994a). The mice were exposed to 0, 1.0, 2.0, or 3.9 mg Ni/m^3. Bronchial lymph-node hyperplasia was also evident in all nickel-exposed animals. Body weights were slightly but significantly lower in the 3.9 mg Ni/m^3 group, compared with controls.

A continuous exposure of rats (20 - 40 per group) to 0, 60, or 200 μg Ni/m^3 as nickel oxide for 2 years resulted in severe pulmonary damage and premature mortality so that carcinogenesis could not be evaluated (Glaser *et al.*, 1986). Pulmonary alveolar proteinosis and septal fibrosis were

observed in the animals exposed to nickel. Only 1 rat per group survived the nickel exposures to the end of the experiment.

A 2-year study on the effects of nickel subsulfide in rats and mice was conducted by NTP (1994b). Rats (52-53 per sex per group) were exposed to 0, 0.15, or 1 mg Ni_3S_2/m^3 (0, 0.11, or 0.73 mg Ni/m^3) for 6 hours/day, 5 days/week for 104 weeks. Body weights were lowered in rats exposed to 0.73 mg Ni/m^3 compared with controls. Lung inflammation, alveolar hyperplasia, macrophage hyperplasia, and pulmonary fibrosis were observed with a significantly increased incidence at both nickel concentrations. Female rats exposed to nickel had significantly increased adrenal medullary hyperplasia. In addition to the pulmonary lesions, nasal inflammation and olfactory epithelial atrophy was observed in both sexes exposed to 0.73 mg Ni/m^3.

In the second phase of the NTP study (NTP, 1994b), mice were exposed to 0, 0.6, or 1.2 mg Ni_3S_2/m^3 (0, 0.44, or 0.88 mg Ni/m^3) for 6 hours/day, 5 days/week for 104 weeks. The same pathological lesions were observed in the lung and nasal passages as in the rats in the above study. These lesions were evident at both the 0.44 mg Ni/m^3 and the 0.88 mg Ni/m^3 concentrations. The adrenal medullary hyperplasia seen in female rats was not observed in the mice.

An exposure of rats to either 0 or 0.97 mg Ni_3S_2/m^3 (0 or 0.71 mg Ni/m^3) for 6 hours/day, 5 days/week for 78-80 weeks resulted in decreased body weight, hyperplasia, metaplasia, and neoplasia in the lungs due to Ni (Ottolenghi *et al.*, 1974).

The NTP (1994c) studied the chronic non-cancer and carcinogenic effects of nickel sulfate hexahydrate on rats and mice. Rats were exposed to 0, 0.12, 0.25, or 0.5 mg $NiSO_4/m^3$ (0, 0.03, 0.06, or 0.11 mg Ni/m^3) for 6 hours/day, 5 days/week for 104 weeks. Chronic effects of nickel exposure in rats included inflammatory lesions in the lung, lung macrophage hyperplasia, alveolar proteinosis, and fibrosis, in addition to bronchial lymph node hyperplasia and nasal epithelial atrophy. The above effects were seen at exposures of 0.06 mg Ni/m^3 or greater.

Mice were exposed to a similar regimen that included 0, 0.06, 0.11, and 0.22 mg Ni/m^3 as nickel sulfate hexahydrate (NTP, 1994c). Similar pulmonary, lymphatic and nasal changes were observed in the mice as with the rats. Fibrosis was not reported, but an increased incidence of interstitial infiltration and alveolar proteinosis were observed at exposures of 0.11 mg Ni/m^3 or greater. No clinical findings or hematological effects were observed, but body weights were significantly depressed in all groups of nickel-exposed female mice. The body weights of males were reduced only in the group exposed to 0.22 mg Ni/m^3.

Rats and mice (10 per group) were exposed to nickel sulfate, nickel subsulfide, or nickel oxide 6 hours/day, 5 days/week, for 13 weeks (Dunnick *et al.*, 1989). Exposure-related increases in lung weight and histological lesions were observed in both species for all nickel exposures. Histological lesions included inflammatory changes, fibrosis, and alveolar macrophage hyperplasia. Nasal lesions were also observed in animals treated with nickel sulfate or nickel subsulfide. Lung weight changes were observed at exposures of 0.05 mg Ni/m^3 or greater in female rats. Macrophage hyperplasia in the alveolar region was observed at concentrations as

low as 0.02 mg Ni/m^3. Additional inflammatory lesions in the lungs were observed at 0.1 mg Ni/m^3.

A similar study by Haley *et al.* (1990) found that exposure of mice to nickel sulfate, nickel subsulfide, or nickel oxide resulted in various immunological effects. Mice were exposed to 0, 0.11, 0.45, or 1.8 mg Ni/m^3 as Ni_3S_2; 0.47, 2.0, or 7.9 mg Ni/m^3 as NiO; and 0.027, 0.11, and 0.45 mg Ni/m^3 as $NiSO_4$ for 6 hours/day, 5 days/week for 13 weeks. Nickel exposures consistently decreased splenic antibody-forming cell (AFC) responses, with significant decreases occurring at 1.8 mg Ni/m^3 as nickel subsulfide. In contrast, AFC responses in the lung-associated lymph nodes were consistently increased, indicating a possible indirect influence of inflammatory mediators released in the lung on local lymph nodes.

Rabbits (8 nickel exposed and 8 controls) exposed to 0.24 mg Ni/m^3 as nickel chloride 6 hours/day, 5 days/week for 4 weeks exhibited significantly decreased macrophage lysozyme activity in pulmonary lavage fluid and in macrophage cultures, compared with control animals (Lundborg and Camner, 1984). Similar exposures of rabbits to chlorides of cadmium, cobalt, or copper did not reduce lysozyme activity.

VI. Derivation of Chronic Reference Exposure Level (REL)

A. Nickel and Nickel Compounds (except nickel oxide)

Study	National Toxicology Program, 1994c
Study population	Male and female F344/N rats (52-53 per group)
Exposure method	Discontinuous inhalation
Critical effects	Pathological changes in lung, lymph nodes, and nasal epithelium: (1) active pulmonary inflammation, (2) macrophage hyperplasia, (3) alveolar proteinosis, (4) fibrosis, (5) lymph node hyperplasia, (6) olfactory epithelial atrophy
LOAEL	60 µg Ni/m^3 (as nickel sulfate hexahydrate)
NOAEL	30 µg Ni/m^3
Exposure continuity	6 hours/day, 5 days/week
Exposure duration	104 weeks
Average experimental exposure	5.4 µg Ni/m^3 for NOAEL group (30 x 6/24 x 5/7)
Human equivalent concentration	1.6 µg Ni/m^3 for NOAEL group males (particulate with respiratory effects, RDDR = 0.29 based on MMAD = 2.5, sigma g = 1.26, male rat body weight = 380 g, SA(PU) = 0.34 m^2, DEP(PU) = 0.024)
LOAEL uncertainty factor	1
Subchronic uncertainty factor	1
Interspecies uncertainty factor	3
Intraspecies uncertainty factor	10
Cumulative uncertainty factor	30
Inhalation reference exposure level	0.05 µg Ni/m^3

B. Nickel Oxide

Study	National Toxicology Program, 1994c
Study population	Male and female F344/N rats (52-53 per group)
Exposure method	Discontinuous inhalation
Critical effects	Pathological changes in lung and lymph nodes: (1) active pulmonary inflammation, (2) lymph node hyperplasia Adrenal medullary hyperplasia (females)
LOAEL	500 μg Ni/m^3
NOAEL	Not observed
Exposure continuity	6 hours/day, 5 days/week
Exposure duration	104 weeks
Average experimental exposure	89.5 μg Ni/m^3 for LOAEL group (500 x 6/24 x 5/7)
Human equivalent concentration	30 μg Ni/m^3 for LOAEL group males (particulate with respiratory effects, RDDR = 0.29 based on MMAD = 2.5, sigma g = 1.26, male rat body weight = 380 g, SA(PU) = 0.34 m^2, DEP(PU) = 0.024)
LOAEL uncertainty factor	10
Subchronic uncertainty factor	1
Interspecies uncertainty factor	3
Intraspecies uncertainty factor	10
Cumulative uncertainty factor	300
Inhalation reference exposure level	0.10 μg Ni/m^3

The studies conducted by NTP (1994 a,b, & c) all showed similar non-carcinogenic effects in rats and mice, regardless of the form of nickel administered. It therefore appears that soluble and insoluble forms of nickel cause similar effects in rodents. The human epidemiological literature predominantly describes cancer mortality rates from occupational exposures to nickel compounds, but does not specifically examine non-cancer effects. However, it is clear from many case reports that allergies and dermatitis can occur in exposed workers. Hypersensitive reactions to nickel have not been quantitatively studied in humans or in animals, therefore it is not possible to develop an REL based on immunological hypersensitivity at the present time. A host of subacute and subchronic animal studies have shown nickel to affect certain immunological responses unrelated to hypersensitivity, but the applicability of these results to chronic human exposures and responses involves considerable uncertainty. Furthermore, data show that nickel may precipitate onset of asthma in occupational settings.

The results of the NTP studies and these dose response analyses support the speciation of nickel oxide for noncancer effects. The health effects data for nickel oxide indicate that its adverse pulmonary effects were less severe (absence of fibrosis, lower chronic lung inflammation severity scores) at higher doses than the pulmonary effects observed for nickel sulfate and nickel subsulfide. The higher chronic REL value for nickel oxide of 0.1 μg/m^3 reflects these dose

response differences. Furthermore, while it is based upon a LOAEL, the lower severity of the adverse health effects at the LOAEL mitigates some of the uncertainty associated with use of a LOAEL rather than a NOAEL. OEHHA therefore concludes that 0.1 $\mu g/m^3$ is an appropriate REL for nickel oxide. However, in setting inhalation exposure RELs for groups of compounds, OEHHA uses the most sensitive strain, species, sex, chronic endpoint, and agent for each group of substances. Therefore, as the pulmonary toxicity of the relatively insoluble nickel subsulfide is greater than that of nickel oxide and closer to that of nickel sulfate, OEHHA proposes to use the chronic REL derived from nickel sulfate for all other nickel compounds.

VII. Data Strengths and Limitations for Development of the REL

The strengths of the inhalation REL include the availability of controlled lifetime exposure inhalation studies in multiple species at multiple exposure concentrations and with adequate histopathogical analysis and the observation of a NOAEL. The major areas of uncertainty are the lack of adequate human exposure data and the lack of lifetime toxicity studies in any non-rodent species.

In addition to being inhaled, airborne nickel can settle onto crops and soil and enter the body by ingestion. Thus an oral chronic refererence exposure level for nickel is also required.

Derivation of Oral Chronic Reference Exposure Level

Study	Ambrose *et al*., 1976
Study population	Rats
Exposure method	Diet
Critical effects	Decreased body and organ weights
LOAEL	1000 ppm (50 mg/kg-day)
NOAEL	100 ppm (5 mg/kg-day)
Exposure continuity	Continuous
Exposure duration	Lifetime
Average exposure	5 mg/kg-day
Human equivalent concentration	5 mg/kg-day
LOAEL uncertainty factor	1
Subchronic uncertainty factor	1
Interspecies uncertainty factor	10
Intraspecies uncertainty factor	10
Cumulative uncertainty factor	300
Oral reference exposure level	0.05 mg/kg-day

The oral REL for nickel used the same study used for the U.S. EPA's oral Reference Dose (RfD). U.S.EPA assumed that rat consumption of 1 ppm Ni in the feed resulted in a dose of 0.05 mg/kg/day. An uncertainty factor of 10 was used for interspecies extrapolation and another of 10 to protect sensitive human populations. An additional uncertainty factor of 3 was used by U.S. EPA to account for inadequacies in reproductive studies of nickel. OEHHA has not used such special uncertainty or modifying factor because the criteria for their use are not well presented.

In addition there is an extensive toxicologic database on nickel in general which includes studies on reproductive effects.

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CHRONIC TOXICITY SUMMARY

PHOSPHORIC ACID

(Orthophosphoric acid)

CAS Registry Number: 7664-38-2

I. Chronic Toxicity Summary

Inhalation reference exposure level	**7 µg/m^3**
Critical effect(s)	Bronchiolar fibrosis of the respiratory tract in rats
Hazard index target(s)	Respiratory system

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

Description	Clear syrupy liquid or unstable crystals; odorless
Molecular formula	H_3PO_4
Molecular weight	98
Boiling point	213°C
Melting point	42.35°C
Vapor pressure	0.03 torr @ 20°C
Solubility	Very soluble in hot water; 548 g/100 ml cold water; soluble in alcohol
Conversion factor	4.0 µg/m^3 per ppb at 25ºC

III. Major Uses and Sources

Phosphoric acid has varied uses (HSDB, 1995). In manufacturing, it is a chemical intermediate or reagent in the production of numerous phosphate fertilizers, agricultural feeds, waxes, polishes, soaps, and detergents. It is added to foods as a preservative, acidifying agent, flavor enhancer, and clarifying agent. Phosphoric acid is also used in processes such as the coagulation of rubber latex, electropolishing, soil stabilization, and as a catalyst in the production of propylene and butene polymers, ethylbenzene, and cumene. By far, largest use of phosphoric acid comes in the production of fertilizers (>75%). The annual statewide industrial emissions from facilities reporting under the Air Toxics Hot Spots Act in California, based on the most recent inventory, were estimated to be 81,103 pounds of phosphoric acid (CARB, 1999).

Airborne phosphoric acid can be produced by the hydrolysis of phosphorus oxides generated from either the spontaneous ignition of white phosphorus in air or the combustion of red phosphorus (Burton *et al.*, 1982; US Department of Defense (US DOD), 1981).

IV. Effects of Human Exposures

The toxic effects to 48 workers exposed (28 unexposed control workers) to oxidation products of phosphorus during the course of phosphorus production were reported (Hughes *et al.*, 1962). Exposure duration ranged from 1 to 17 years. No differences were observed between exposed and control workers with respect to leukocyte count, an effect observed in acute intoxications, or hand bone density, an effect observed in experimentally exposed animals (Inuzuka, 1956).

A prospective study of 131 workers exposed to several compounds including phosphoric acid, phosphorus pentoxide, fluorides and coal tar pitch in the air was conducted at an industrial refinery (Dutton *et al.*, 1993). Mean duration of exposure (employment) was 11.4 years and the maximum exposure level measured was 2.23 mg/m^3 (phosphorus pentoxide). Pulmonary function tests were performed annually over a 3 to 7 year period. No significant residual effect was found after adjusting for age and smoking status.

V. Effects of Animal Exposures

Two 13-week inhalation studies of the effects of exposure to the combustion products of 95% red phosphorus and 5% butyl rubber were conducted in male Sprague-Dawley rats, with the first group exposed to 0, 300, 750, or 1200 mg/m^3 combustion products, and the second exposed to 0, 50, 180, or 300 mg/m^3 combustion products (Aranyi *et al.*, 1988a; Aranyi *et al.*, 1988b). Group numbers in the first study were 176, 84, 176, and 176, respectively. The second study used 40 animals/group. Animals were exposed for 2¼ hours/day on 4 consecutive days/week. Control animals were exposed to filtered air only. Daily particle measurements showed MMADs of 0.49-0.65 µm and σ_gs of 1.56-1.83. Fractional content of phosphoric acid in the aerosol was 71-79%. Nineteen of the 176 animals in the 1200 mg/m^3 dose group died of treatment related effects. Post-mortem examination of animals that died during the course of the study showed damage to the laryngeal mucosa, which was probably contributory to mortality. The two highest dose groups in the first study also showed decreased weight gain. Twelve animals from each dose group in the first study were examined histologically and neurobehavioral studies were conducted on other animals. Half the animals in the second study were examined strictly for toxic effects on the respiratory tract, with examination of the trachea, 2 sections of the nasal turbinates, and 5 lobes of the lung. Surviving animals in the high-dose study were observed to have moderate to severe fibrosis of the terminal bronchioles, with minimal severity of this effect in the animals in the low-dose study. The reported incidence of this lesion was 9/20 at 300 mg/m^3, 4/20 at 180 mg/m^3, and 0/20 at 50 mg/m^3. Little to no involvement of pulmonary tissue was observed.

The effects of acid aerosols (particularly sulfuric and phosphoric acid) were studied by U.S. EPA (1989). The respiratory tract was the primary target of toxicity resulting from the irritational effect of the acid on the tissues of the larynx and trachea. The nature of the effect was dependent upon the aerosol particle size, duration of exposure, and the hygroscopic character of the acid.

Sprague-Dawley rats were exposed to the smoke and combustion products of white phosphorus in felt pellets at 192.5 (18 animals/sex), 589 (24 animals/sex), or 1161 mg/m^3 (34 males, 43 females) phosphoric acid equivalents for 15 minutes/day, 5 days/week, for 13 weeks (US Department of Defense (US DOD), 1981). Control animals numbering half the size of the treated groups were exposed to air only. Groups of animals were sacrificed at 6 and 13 weeks, and 4 weeks post-exposure. Endpoints examined included: hematology, clinical chemistry, gross- and histo-pathology, ECG, pulmonary function, and behavior. Of the animals in the highest dose group, 56% died as a result of exposure, with the only other death occurring in the control group. Findings were restricted to effects on the respiratory system, with tracheitis and laryngitis incidences of 2/35, 32/47, and 28/31among surviving animals in the three dose groups. In the post-exposure examination, bronchiolitis occurred with a frequency of 0/12, 5/24, and 6/16in the three dose groups.

The toxicity of the combustion products of 95% amorphous red phosphorus and 5% polyvinyl butyral BL18 to female Wistar rats, Porton-strain mice, and guinea pigs was reported (Marrs *et al.*, 1989). Rats (50/group), mice (100/group), and guinea pigs (42-48/group) were exposed to concentrations of 0, 16, or 128 mg/m^3 for 1 hour/day, 5 days/week for 36 weeks (mice) or 40 weeks (rats and guinea pigs), with an examination conducted at 19 months or when animals appeared unhealthy. All groups, including controls, showed high mortality. Mice showed accumulation of alveolar macrophages with incidences of 2/41, 9/37, and 9/22 in the control, low-, and high-dose groups, respectively. Guinea pigs appeared to be particularly intolerant to the effects of the smoke.

Female rabbits and rats (10/group) were examined for acute toxic effects of smoke generated by the combustion of either 95% red phosphorus / 5% butyl rubber (Smoke I) or 97% red phosphorus / 3% butadiene styrene (Smoke II) (Marrs, 1984). Animals were exposed for 30 minutes and examined one and 14 days later. Smoke I produced inflammation of the larynx and trachea in rats at 1 day with some inflammation still observed at 14 days. Tracheal inflammation was also reported in rabbits exposed to Smoke I. Four of the rats exposed to Smoke II died within the first day, with severe pulmonary congestion observed in the animals.

One hour exposure to the combustion products of 95% red phosphorus / 5% butyl rubber (plus 1% mineral oil) produced epiglottal deformation, laryngeal edema, and laryngeal and tracheal lesions in rats (Burton *et al.*, 1982). A four-hour exposure produced more severe effects of a similar nature plus some hemorrhaging.

Rats (number unspecified) exposed to 150-160 mg/m^3 elemental phosphorus for 30 minutes/day for 60 days were examined for toxic effects (Inuzuka, 1956). Limb bone abnormalities were noted and effects included delayed ossification, widening of the epiphysis, and abnormal axial development.

Two studies have addressed the reproductive and developmental toxicity from exposure to the combustion products of white phosphorus and felt for 15 minutes/day during gestational days 6-15 in rats (24/group) (US Department of Defense (US DOD), 1981; US Department of Defense (US DOD), 1982). Fetal effects included increased incidence of some visceral variations and hypoplasia of the xiphoid process although data were incompletely reported. Another study,

which exposed dams 3 weeks prior to mating, throughout gestation, and through lactation and males for 10 weeks prior to and during mating, showed decreased pup body weight, 24-hour and 21-day survival, and lactation. An oral study in which elemental phosphorus was administered to male and female rats by gavage in corn oil showed no statistically significant effects (Condray, 1985).

VI. Derivation of the Chronic Reference Exposure Level

Study	Aranyi *et al.*, 1988a
Study population	Male Sprague-Dawley rats (40-176/group)
Exposure method	Discontinuous whole body inhalation
Critical effects	Bronchiolar fibrosis of the respiratory tract
LOAEL	180 mg/m^3
NOAEL	50 mg/m^3
BMC_{05}	64 mg/m^3
Exposure continuity	2¼ hours/day, 4 days/week
Exposure duration	13 weeks
Average experimental exposure	2.7 mg/m^3 for NOAEL group (estimated as 3.5 mg/m^3 at BMC_{05})
Human equivalent concentration	2.2 mg/m^3 at BMC_{05} (particle with respiratory effects, RDDR = 0.63) (3.5 x 0.63)
LOAEL uncertainty factor	1 (BMC_{05} assumed to be similar to NOAEL)
Subchronic uncertainty factor	10
Interspecies uncertainty factor	3
Intraspecies uncertainty factor	10
Cumulative uncertainty factor	300
Reference exposure level	0.007 mg/m^3 (7 µg/m^3)

OEHHA has used the same study, which U.S. EPA used in the development of its Reference Concentration (RfC) of 10 µg/m^3. The U.S. EPA has used a benchmark dose methodology for the derivation of the RfC for phosphoric acid from the toxicity data in the Aranyi *et al.* (1988) study (U.S. EPA, 1995). The RfC is restricted to "aerosols of phosphoric acid and phosphorus oxidation products and does not apply to elemental phosphorus or other forms of phosphorus, such as phosphorus salts".

The U.S. EPA, using the Weibull model, estimated the lower 95% confidence level bound on the maximum likelihood estimate (MLE = 150 mg/m^3) resulting in 10% incidence of lesions in the tracheo-bronchiolar region to be 100 mg/m^3 (the BMC_{10}). The U.S. EPA considered 10% incidence level to be a correlate to the NOAEL, based on a precedent in the analysis of data with developmental toxicity endpoints (Allen *et al.*, 1994; Faustman *et al.*, 1994). After correction for exposure continuity, a regional deposited dose ratio (RDDR) for the tracheobronchial region of 0.64 was applied due to the availability of data concerning the growth and deposition of phosphoric acid aerosol particles in humans and the similarities in the effects of phosphoric and better-characterized sulfuric acid aerosols. Key assumptions in the generation of this factor include: (1) the lowest σ_g of 1.56 µm cited in the study was used in the calculation; (2) geometric

rather than aerodynamic diameter approximations were used; (3) particles of this size reach the deposition / lesion site (bronchioles); 4) these hygroscopic particles become more uniform with growth; and (5) particle growth is similar in humans and rodents. An uncertainty factor of 10 was applied because of the subchronic duration of the study. A factor of 3 was applied for interspecies extrapolation in light of the fact that some correction for human equivalency was made with the RDDR. Finally, a factor of 10 was applied for protection of potentially sensitive human subpopulations. The resulting RfC for phosphoric acid is 0.01 mg/m^3.

OEHHA uses a BMC_{05} for development of acute Reference Exposure Levels (OEHHA, 1999; Fowles *et al*., 1999). OEHHA staff believe that the BMC_{05} is more likely to approximate a NOAEL than a BMC_{10} since 5% is closer than 10% to the lower end of average risk levels associated with a NOAEL (Leisenring and Ryan, 1992). A BMC_{05} is more likely to represent a value close to the limit of most studies to detect an effect, and is therefore more like a NOAEL. In contrast, a BMC_{10} is more likely to represent a LOAEL since it is usually in the detectable range of responses. In the specific case of phosphoric acid the BMC_{10} of 100 mg/m^3 was twice the NOAEL of 50 mg/m^3. The BMC_{05} was calculated to be 64 mg/m^3, much closer to the NOAEL. Use of the BMC_{05} results in a chronic REL of 7 $\mu g/m^3$.

VII. Data Strengths and Limitations for Development of the REL

The strengths of the inhalation REL for phosphoric acid include the availability of subchronic inhalation exposure data from a well-conducted study with histopathological analysis and the observation of a NOAEL. Major areas of uncertainty are the lack of adequate human exposure data, the lack of chronic inhalation exposure studies, and the discontinuous nature of exposures (only 2 1/4 hours per day).

The Aranyi *et al.* (1988a) study represents the most adequate study for the quantitative evaluation of the toxicity of phosphoric acid. It was conducted with a large number of animals with multiple doses, produced good dose-response data, and examined likely targets of toxicity (respiratory system) of smoke generated from the combustion of phosphorus and butyl rubber. Uncertainties associated with these data, however, include that (1) the study used combustion products of phosphorus rather than phosphoric acid itself, (2) the total exposure time was relatively short and discontinuous over the duration of the experiment, and (3) only one species/strain/sex was studied.

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CHRONIC TOXICITY SUMMARY

PROPYLENE GLYCOL MONOMETHYL ETHER

(1-Methoxy-2-propanol; 1-methoxypropanol; Propapsol solvent M)

CAS Registry Number: 107-98-2

I. Chronic Toxicity Summary

Inhalation reference exposure level	**7,000 µg/m^3** (2000 ppb) .
Critical effect(s)	Liver effects in rats
Hazard index target(s)	Alimentary system (liver)

II. Physical and Chemical Properties (HSDB, 1995)

Description	Colorless liquid
Molecular formula	$C_4H_{10}O_2$
Molecular weight	90.14
Density	0.962 g/cm^3 @ 20°C
Boiling point	118-118.5°C
Melting point	-96.7°C
Vapor pressure	11.8 torr @ 25°C
Solubility	Soluble in water, methanol, ether, and other organic solvents
Conversion factor	1 ppm = 3.69 mg/m^3 at 25°C

III. Major Uses or Sources

Propylene glycol monomethyl ether (PGME) is used as a solvent for cellulose, acrylics, dyes, inks and stains (HSDB, 1995). Thus, the primary use of PGME is in lacquers and paints. Use of PGME is anticipated to increase due to its low systemic toxicity. The annual specific statewide industrial emissions of PGME from facilities reporting under the Air Toxics Hot Spots Act in California, based on the most recent inventory, were estimated to be 205,769 pounds (CARB, 1999). (Many industries did not estimate emissions of specific glycol ethers so that in 1998 there were also emitted 2,922,744 pounds of the general category glycol ethers, some of which may be PGME.)

IV. Effects of Exposures on Humans

No reports or studies of human toxicity following chronic exposure to PGME were located in the literature. Slight eye irritation was reported by two of six human volunteers exposed to 100 ppm PGME for 2 hours (Stewart *et al.*, 1970). These subjects were exposed for a total of 3 1/2 hours during which no decrement in visual acuity, coordination, neurological responses or reaction

time was measured. The same experiment exposed 23 subjects to 250 ppm PGME. After 15 to 30 minutes of exposure, 8/23 reported eye irritation and 3/23 reported throat irritation; lacrimation was observed in 3/23 subjects. Three subjects each reported one of the following symptoms: irritation, headache, and nausea. While the subjects frequently reported the odor to be objectionable upon first entering the chamber, the odor was usually undetectable by the end of the exposure. Clinical chemistry and urinalysis completed following exposure was not altered as compared to pre-exposure measurements.

V. Effects of Exposures on Animals

Male and female rats (10 per sex per concentration) and rabbits (7 per sex per concentration) were exposed by inhalation to 300, 1000, or 3000 ppm PGME 5 hours per day, 5 days per week for 13 weeks (Landry *et al.*, 1983). Relative liver weights were statistically significantly higher than controls in both male and female rats exposed to 3000 ppm PGME. Hepatocellular hypertrophy was observed upon histopathologic examination of high dose females. The authors conclude that these effects are the result of physiologic adaptation rather than a manifestation of toxicity. The key observation in this study was sedation of rats and rabbits exposed to 3000 ppm PGME. The sedative effects were no longer apparent after 1-2 weeks of exposure.

Similar findings of mild CNS depression were observed by Hanley *et al.* (1984). Pregnant rats and rabbits were exposed to 500, 1500, or 3000 ppm PGME 6 hours per day either days 6-15 or days 6-18 of gestation, respectively. During the first 4-5 days of exposure, rats in the 3000 ppm PGME exposure group were lethargic and moderately ataxic. Statistically significant decreases in food consumption and maternal body weight gain were also observed during this period. A statistically significant increase in the incidence of delayed sternebral ossification was observed in the 3000 ppm exposure group. Rabbits exposed to 3000 ppm exhibited mild lethargy during the first 1-2 days of exposure with rapid post-exposure recovery. Overall maternal weight gain during the exposure (days 6-18 of gestation) was statistically significantly lower than controls.

No significant effect on fetal birth weight or on pup survival indices (e.g., proportion of pups surviving to day 3 post-delivery) was noted following exposure of pregnant rats to 200 or 600 ppm PGME 6 hours per day on days 6-17 of gestation (Doe *et al.*, 1983). Male rats were exposed to 200 or 600 ppm PGME 6 hours per day for 10 consecutive days. No significant effects on testicular weight or pathology were observed.

Increased liver and kidney weights were observed in male and female rats (10 per sex per concentration) following exposure to 6000 ppm for 7 hours per day, for 81 exposures over a 114-day period (Rowe *et al.*, 1954). No histopathological abnormalities were observed at necropsy.

Ciezlak et al. (1998) evaluated the potential chronic toxicity/oncogenicity and the response of liver and kidney tissue of Fischer 344 rats to propylene glycol monomethyl ether (PGME) at targeted vapor concentrations of 0, 300, 1000 or 3000 ppm. Groups of 50 male and female rats per sex were whole-body exposed under dynamic airflow conditions for 6 hours/day, 5 days/week for up to 2 years. Parameters evaluated included the general appearance and demeanor of animals, in-life body weights, survival, hematology, urinalysis and clinical

chemistry determinations, survival, selected organ weights, gross and microscopic pathologic changes and tumor incidence. (The metabolic and morphological bases for PGME-induced sedation, hepatic hypertrophy and renal toxicity were characterized in separate groups of male and female rats exposed to PGME for 6, 12 or 18 months. Hepatic enzyme induction and cellular proliferation, as well as renal cellular proliferation and accumulation of $alpha_{2u}$-globulin (males only) in the kidneys, were conducted in these separate groups of animals.)

PGME-induced sedation at 3000 ppm resolved in all animals during the second week of exposure in conjunction with the appearance of adaptive changes in the liver (cytochrome P450 induction and hepatocellular proliferation). Cytochrome P450 (pentoxyresorufin O-demethylase) activities dropped to near control concentrations by week 52, coinciding with a return of sedation at 3000 ppm PGME. In male rats, the loss of metabolic adaptation was followed by a dose-related increase in altered hepatocellular foci after two years of exposure to 1000 or 3000 ppm PGME. The kidney toxicity observed in male rats was confirmed immunohistochemically as an $alpha_{2u}$-globulin nephropathy. No statistically-identified increases in tumors were observed in any tissue. The authors established a NOEL of 300 ppm PGME for the study.

Ethylene glycol methyl ether (EGME), a structurally related compound, exerts considerable toxicity on the blood, thymus, testes, and developing fetus. The toxicity of EGME has been linked to its primary metabolite, methoxyacetic acid. Recent comparative toxicity and metabolism studies (Miller *et al.*, 1983, Miller *et al.*, 1984) indicate that the relatively low systemic toxicity exerted by PGME is due to its different metabolites. Following a single oral dose of PGME, the key urinary metabolites identified in rats were propylene glycol and the sulfate and glucuronide conjugate of PGME (Miller *et al.*, 1983).

VI. Derivation of Reference Exposure Level

Study	Ciezlak *et al.*, 1998
Study population	Fischer 344 rats (50/sex/concentration)
Exposure method	Discontinuous whole-body inhalation (0, 300, 1000, or 3000 ppm)
Critical effects	Increased eosinophilic foci of altered hepatocytes
LOAEL	1000 ppm
NOAEL	300 ppm
Exposure continuity	6 hours per day, 5 days per week
Average experimental exposure	54 ppm for NOAEL group (300 x 6/24 x 5/7)
Human equivalent concentration	54 ppm for NOAEL group (gas with systemic effects, based on RGDR = 1.0 using default assumption that lambda (a) = lambda (h))
Exposure duration	104 weeks
LOAEL factor	1
Subchronic uncertainty factor	1
Interspecies uncertainty factor	3
Intraspecies uncertainty factor	10
Cumulative uncertainty factor	30
Inhalation reference exposure level	2 ppm (2000 ppb, 7 mg/m^3, 7000 $\mu g/m^3$)

VII. Data Strengths and Limitations for Development of the REL

Strengths of the PGME RfC include the observation of a NOAEL and a LOAEL in the same study, and the availability of chronic exposure studies involving multiple concentrations. A major area of uncertainty is the lack of human data.

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CHRONIC TOXICITY SUMMARY

PROPYLENE OXIDE

(1-,2-propylene oxide; methyl ethylene oxide; propene oxide)

CAS Registry Number: 75-56-9

I. Chronic Toxicity Summary

Inhalation reference exposure level	**30 µg/m^3 (9 ppb)**
Critical effect(s)	Degenerative and hyperplastic changes in the respiratory epithelium of rats
Hazard index target(s)	Respiratory system

II. Physical and Chemical Properties (HSDB, 1994)

Description	Colorless liquid
Molecular formula	C_3H_6O
Molecular weight	58.08
Density	0.83 g/cm^3 @ 20° C
Boiling point	34.23° C
Melting point	-112.13° C
Vapor pressure	445 torr @ 20° C
Solubility	Soluble in water, miscible in acetone, benzene, carbon tetrachloride, methanol, ether
Conversion factor	2.38 mg/m^3 per ppm at 25° C

III. Major Uses or Sources

Propylene oxide is used as a fumigant such as in the sterilization of packaged foods. It is also used as a chemical intermediate in the production of propylene glycol and glycol ethers and as a solvent. Propylene oxide is used in the preparation of surfactants and oil demulsifiers (HSDB, 1994). The annual statewide emissions from facilities reporting under the Air Toxics Hot Spots Act in California, based on the most recent inventory, were estimated to be 619,494 pounds of propylene oxide.

IV. Effects of Human Exposures

Conclusive data regarding the effects of occupational exposure to propylene oxide were not located.

An epidemiological study examining mortality among workers with exposure to asbestos and several chemicals, including propylene oxide, identified three deaths due to mesothelioma, a rare cancer associated with asbestos exposure, and a statistically significant increase in the number of deaths attributed to forms of heart disease other than ischemia and hypertension (Egedahl *et al.*, 1989). The latter finding was explained by the authors to be the result of differences in diagnostic accuracy between rural and urban, and primary and tertiary medical care settings. A statistically significant decrease in observed deaths was found for all respiratory cancers, cancer of the bronchus and lung, circulatory disease, digestive diseases, cirrhosis and other liver disease, and death due to accidents, poisonings, and violence. These observations may be partially attributed to a "healthy worker effect".

V. Effects of Animal Exposures

Male and female rats were exposed for 124 or 123 weeks (respectively) to 30, 100 or 300 ppm propylene oxide 6 hours per day, 5 days per week (Kuper *et al.*, 1988). Interim sacrifices were performed at 12, 18, and 24 months. Cumulative mortality was statistically significantly different from controls at 115 weeks in rats of both sexes exposed to 300 ppm propylene oxide. Cumulative mortality was also significantly different from controls at 119 weeks in female rats exposed to 100 ppm. However, a contributing factor to the increased mortality in female rats was the presence of mammary tumors. Atrophy of the olfactory epithelium and degenerative changes in the respiratory epithelium were observed in both male and female rats following 28 months of exposure to 30, 100, or 300 ppm propylene oxide. Severe hyperplastic changes in the olfactory epithelium were observed in male and female rats following 28 months exposure to 300 ppm propylene oxide. Mild hyperplastic changes were observed in the olfactory epithelium of female rats exposed to 100 ppm propylene oxide.

Rats and mice were exposed to 200 and 400 ppm propylene oxide 6 hours per day, 5 days per week for 103 weeks (NTP, 1985). Survival in mice was adversely affected in all groups exposed to propylene oxide; a statistically significant decrease in survival was observed in male and female mice exposed to 400 ppm propylene oxide. Survival in rats was not adversely affected by propylene oxide exposure. Rats exhibited exposure-related increases in suppurative inflammation of the nasal cavity, epithelial hyperplasia and squamous metaplasia.

Rats were exposed to 1500 ppm propylene oxide 6 hours per day, 5 days per week for 7 weeks (Ohnishi *et al.*, 1988). After 3-4 weeks of exposure the rats exhibited an awkward gait; the rats were ataxic by the seventh week. Histopathological examination revealed axonal degeneration of myelinated fibers of the hindleg nerve and fasciculus gracilis indicating central-peripheral distal axonopathy.

Eldridge *et al*. (1995) exposed male F344 rats to 0, 10, 20, 50, 150, or 525 ppm propylene oxide vapor for up to 4 weeks (with up to 4 weeks of recovery). Histopathology showed that the incidence and severity of respiratory epithelial hyperplasia increased with exposure time and regressed after termination of exposure, with complete recovery after 4 weeks. Cell proliferation (determined by bromodeoxyuridine incorporation) was elevated following 1 and 4 weeks of exposure, but decreased to control values after 1 week of recovery. Degeneration of the

olfactory epithelium was found after 4 weeks of exposure with a decrease in incidence and severity after termination of exposure. Proliferation of olfactory epithelium was elevated during the 4-week exposure period and 1 week post-exposure and returned to control values after 4 weeks of recovery. The authors report a 4-week NOAEL for propylene oxide effects in nasal epithelium of 50 ppm.

Artificially inseminated rabbits were exposed to 500 ppm propylene oxide on days 1-19 or 7-19 of gestation (Hardin *et al.*, 1983). Maternal toxicity as indicated by a significant reduction in food intake and a significant decrease in maternal body weight gain was observed in both exposed groups. An increased number of resorptions per litter, with no change in total resorptions, was observed in rabbits exposed on days 1-19 of gestation. Sternebral and limb anomalies (considered minor by U.S. EPA and the authors) were significantly increased in the offspring of rabbits exposed on days 1-19 of gestation.

The same study also reported similar findings in sperm-positive rats exposed to 500 ppm propylene oxide on either days 1-16 or 7-16 of gestation or daily for 3 weeks prior to mating and then daily on days 1-16 of gestation. Reproductive capacity was impaired in rats exposed prior to breeding; the number of corpora lutea, implantation sites, and live fetuses were reduced. Those dams exposed pregestationally to propylene oxide also exhibited more resorptions. Maternal toxicity as indicated by decreased food intake and decreased body weight gain was observed in all exposed rats. Significant reductions in fetal body weight and fetal crown-rump length were observed in all exposed groups. An increased incidence of wavy ribs and reduced ossification were observed in the offspring of rats exposed from days 1-16 of gestation.

Harris *et al*. (1989) evaluated the developmental toxicity potential of propylene oxide in Fischer 344 rats. Four groups of 25 mated female rats were exposed to 0, 100, 300, and 500 ppm for 6 hours per day on gestation days 6 through 15. Cesarean sections were performed on all females on gestation day 20 and the fetuses were removed for morphological evaluation. Exposure to propylene oxide did not adversely affect survival, appearance, or behavior at any level. Maternal body weight gain and food consumption were reduced significantly at the 500 ppm level during exposure. Only one exposure-related effect was noted with respect to maternal water consumption, organ weights, cesarean section, or fetal morphological observations: increased frequency of seventh cervical ribs in fetuses at the maternally toxic exposure level of 500 ppm. Thus 300 ppm was considered the NOAEL.

VI. Derivation of Chronic REL (U.S. EPA Reference Concentration (IRIS, 1995))

Study	Kuper *et al.*, 1988
Study population	Rats (male and female)
Exposure method	Inhalation (0, 30, 100 or 300 ppm)
LOAEL	30 ppm
Critical effects	Degenerative and hyperplastic changes in the respiratory epithelium
NOAEL	Not observed
Exposure continuity	6 hr/day for 5 days/week
Exposure duration	124 weeks
Average experimental exposure	5.4 ppm for LOAEL group (30 x 6/24 x 5/7)
Human equivalent concentration	1.2 ppm for LOAEL group (gas with extrathoracic respiratory effects, RGDR = 0.23, based on MV = 0.3 m^3/day, SA(ET) = 11.6 cm^2)
LOAEL uncertainty factor	3 (mild effects only observed during last 4 months of exposure)
Subchronic uncertainty factor	1
Interspecies uncertainty factor	3
Intraspecies uncertainty factor	10
Cumulative uncertainty factor	100
Inhalation reference exposure level	0.009 ppm (9 ppb, 0.03 mg/m^3, 30 µg/m^3)

VII. Data Strengths and Limitations for Development of the REL

The chronic REL is equivalent to the US EPA RfC. The major strength of the REL for propylene oxide is the use of a well-conducted, long-term, multi-concentration study with adequate histopathological analyses. Weaknesses include the lack of adquate human data and the lack of a chronic NOAEL observation.

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