CHRONIC TOXICITY SUMMARY

AMMONIA

(Anhydrous ammonia; aqueous ammonia)

CAS Registry Number: 7664-41-7

I. Chronic Toxicity Summary

Inhalation reference exposure level	**100 μg/m^3** (U.S. EPA-RfC) This document summarizes the evaluation of non-cancer health effects by U.S. EPA for the RfC.
Critical effect(s)	Pulmonary function tests or subjective symptomatology in workers
Hazard index target(s)	Respiratory system

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

Description	Colorless liquid
Molecular formula	NH_3
Molecular weight	17.03 g/mol
Density	0.6818 g/cm^3 @ 20°C
Boiling point	-33.5° C
Vapor pressure	6460 mm Hg @ 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).

IV. Effects of Human Exposures

Comparisons were made between 52 workers and 31 control subjects 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), while controls were exposed to 0.3 ppm (0.21 mg/m^3). No differences in any endpoints were reported between the exposed and control groups.

Groups of human volunteers (4 per group) 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 volunteers was exposed to 50 ppm ammonia for 6 hours/day for 6 weeks. 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.

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. Exposure to 250 ppm ammonia alone resulted in nasal lesions (epithelial thickening and hyperplasia) unlike 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.

VI. Derivation of U.S. EPA RfC

Study	US EPA, 1995; Holness *et al.*, 1989; 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)
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
Human equivalent concentration	3 ppm for NOAEL group
LOAEL uncertainty factor	1
Subchronic uncertainty factor	1
Interspecies uncertainty factor	1
Intraspecies uncertainty factor	10
Modifying factor	3 (database deficiencies)
Cumulative uncertainty factor	30
Inhalation reference exposure level	0.1 ppm (100 ppb; 0.1 mg/m^3; 100 $\mu g/m^3$)

Significant strengths in the ammonia REL include (1) the availability of long-term human inhalation exposure data, and (2) the demonstration of consistent effects in experimentally exposed human volunteers following short-term exposures.

Major areas of uncertainty are (1) the lack of a NOAEL and LOAEL in a single study, (2) a lack of animal data with histopathological analyses, and (3) difficulties in estimated human occupational exposures.

VII. 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 μg/m^3**
Critical effect(s)	Lowered red and white blood cell counts in occupationally exposed humans
Hazard index target(s)	Circulatory system; teratogenicity; nervous system; immune system

II. Physical and Chemical Properties (HSDB, 1994)

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 mm Hg @ 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. 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).

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.

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

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).

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.

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.

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.

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).

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.

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 $\mu g/m^3$)

Tsai *et al.* (1983) 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 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. 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 or 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 results in an REL of 0.01 ppm.

Ward *et al*. (1996) determined a relationship between occupational exposures to benzene and decreased red and white cell counts. A modeled dose-response relationship that indicated a possibility for hematologic effects at concentrations below 5 ppm. However, no specific measures of the actual effects at these 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, the chronic inhalation study in mice by Baarson *et al.* (1984) showed that bone-marrow progenitor cells were markedly suppressed after intermittent exposures (6 hr/day, 5 days/week) to 10 ppm benzene. An extrapolation of this value to an equivalent continuous exposure resulted in a concentration of 1.8 ppm. Application of uncertainty factors of 10 each for inter- and intraspecies variability, and estimation of a NOAEL from the LOAEL would result in an REL of 2 ppb.

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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; immune system; reproductive system; teratogenicity; endocrine system; respiratory system; circulatory system

II. Physical and Chemical Properties (HSDB, 1995)

Description	white crystalline powder at 25° C
Molecular Formula	$C_{12}H_4Cl_4O_2$ (TCDD)
Molecular Weight	321.97 g/mol (TCDD)
Density	1.827 g/ml (estimated)
Boiling Point	412.2°C (estimated)
Vapor Pressure	1.52 x 10^{-9} mm Hg at 25°C
Solubility	In water: 7.91 ng/L at 20-22°C; 19.3 ng/L at 22°C
Log Kow	6.15-7.28
Log Koc	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.

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

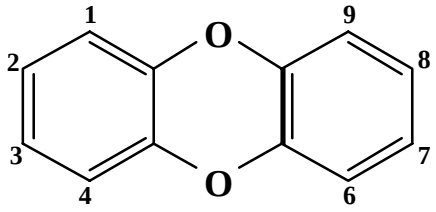
2,3,7,8-Tetrachlorodibenzo-p-dioxin is considered the most potent congener of the polychlorinated dibenzo-p-dioxins (PCDDs) and polychlorinated dibenzofurans (PCDFs) families of compounds. Potency of PCDD and PCDF congeners correlates with the binding affinity to the cytosolic Ah receptor. Structure 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.)

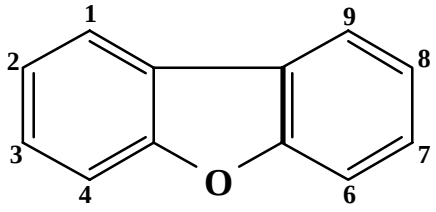
Compound[1,2]	I-TEF
Mono-, Di-, and Tri-CDDs and CDFs	0
TetraCDD	
2,3,7,8-substituted	1.0
Others	0
PentaCDD	
2,3,7,8-substituted	0.5
Others	0
HexaCDD	
2,3,7,8-substituted	0.1
Others	0
HeptaCDD	
2,3,7,8-substituted	0.01
Others	0
OctaCDD	0.001
TetraCDF	
2,3,7,8	0.1
Others	0
PentaCDF	
1,2,3,7,8-PentaCDF	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 of which 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 receiving diet with solvent vehicle alone 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 extraploation	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 has 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).

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	**0.06 µg/m^3**
Critical effect(s)	Hyperplasia in respiratory epithelium in female rats
Hazard index target(s)	Respiratory system

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

Description	Yellow/green gas
Molecular formula	Cl_2
Molecular weight	70.906 (Weast, 1989)
Density	2.9 g/L @ 25°C
Boiling point	-34.6° C
Vapor pressure	5 atm @ 10.3°C
Solubility	Slightly soluble in water (310 mL per 100 mL water at 10° C; 1.46 g per 100 mL water at 0 degrees C)
Conversion factor	1 ppm = 2.9 mg/m^3 @ 25° C

III. Major Uses and Sources

Chlorine is commonly used as a household cleaner and disinfectant (HSDB, 1995). In an industrial setting, chlorine is widely used as an oxidizing agent in water treatment and chemical processes. Chlorine is an integral part of the bleaching process of wood pulp in pulpmills.

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.

Chang-Yeung *et al.* (1994) conducted a clinical, functional and pathological study of three pulpmill workers who, after years of intermittent exposure to pulpmill "gassings," developed a cough, wheeze (chest tightness) and shortness of breath. The subjects were evaluated on the basis of lung function tests and nonspecific bronchial hyperresponsiveness. Previous "gassing" episodes caused immediate symptoms in the subjects, but did not cause persistent respiratory symptoms. However, the subjects were admitted for emergency hospital treatment after a severe exposure. Following that episode, the subjects were diagnosed with irritant-induced asthma and treated with steroid therapy. Changes were seen in the subjects' bronchial mucosa which were similar to those in allergic asthma and red-cedar induced asthma patients. A reduced level of T-lymphocytes was seen in the exposed subjects but was not observed in allergic asthma and red-cedar induced asthma patients. A variety of gasses can be emitted in a pulpmill setting including chlorine and chlorine dioxide (Kennedy *et al.*, 1991).

Courteau *et al.* (1994) evaluated 281 pulp mill construction workers, 257 of which were exposed to an average of 25 acute gassing episodes in addition to an average of 24 evacuations over a period of three to six months. The average age of the workers was 44 years. Twenty-four of the 281 workers were not exposed to chlorine gas at any time during their employment. Of the 257 exposed workers, 52 had left the construction site due to health problems caused by irritant gases. Workers (including the 52 that left) were evaluated on a retrospective basis, using health records and questionnaires to collect information on individual worker exposures. Smoking histories and pre-existing conditions were recorded.

Symptoms that were associated with each worker's most significant incident of chlorine gas exposure included eye and throat irritation and cough with a frequency of 67-78%. (Throat and cough symptoms had a mean duration of 8-11 days and eye irritation had a mean duration of 2 days.) Also prevalent were the symptoms of a flu-like syndrome, headache, nose and sinus congestion, cough, fatigue and shortness of breath, with a frequency of 53-63% (a mean duration of 7-14 days for the above symptoms except the flu). Additional symptoms included difficulty sleeping (37 %), nausea (36%), excessive sweating and distaste for smoking (30%) and abdominal pain (20%). Symptoms typically lasted 1-3 weeks. Researchers categorized the workers into low and high risk groups for developing chronic lung disease as a result of the repeated chlorine gas exposure, and those workers were enrolled in a prospective study (Bherer *et al.*, 1994).

Of the 438 air samples taken in the bleach plant, 36% were <0.5 ppm, 58% were 0.5-8 ppm and 6% were >8 ppm. Experts who examined the air sample data reported that the samples were taken after workers had been evacuated and that >65% of the samples were invalid due to technical flaws and errors.

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.

A case report by Donnelly and Fitzgerald (1990) described an incident of reactive airways dysfunction syndrome (RADS) in a thirty year old man following his exposure to chlorine gas. His symptomatic, clinical and physiological evidence of airway obstruction persisted after 6 years, suggesting that RADS patients can experience acquired persistent asthmatic symptoms.

Rea *et al.* (1989) exposed fifty individuals previously categorized as “chemically sensitive” to <0.33 ppm chlorine gas under double blind challenge conditions. The patients were between the ages of 21-61 and had a variety of vascular, asthmatic and arthritic conditions. Primary signs, symptoms and pulse rate were recorded before the exposure, immediately after and every 15 minutes for four hours after the exposure. Each patient was observed for specific symptoms connected with his/her sensitivity as well as other general signs. Of the 100 patients originally screened for this study, 50 of them were excused as they were too sensitive to withstand the 15

minutes of exposure that was required, thus leaving 50 moderately sensitive patients to be involved in the research. Four of the 50 patients experienced two of the three following responses to exposure to <0.33 ppm chlorine: an increase in pulse rate beyond three standard deviations over the baseline; appearance of primary signs and symptom response (unspecified) of ≥20% over the baseline; and no response to placebo measured by primary signs, symptoms or statistically significant increase in pulse rate. The LOAEL for this study was unquantified, but was below 0.33 ppm.

In a study by Gautrin *et al.* (1994) in which acute reversibility of reactive airways dysfunction syndrome (RADS) was compared to that of occupational asthma (OA) with a latency period, chlorine inhalation appeared to cause RADS in 12 of the 15 subjects evaluated. The subjects showed FEV_1 of <80% of the predicted value and had a history of acute symptoms which occurred minutes to hours after accidental chlorine exposure. Asthma symptoms persisted after the initial symptoms disappeared.

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	Respiratory epithelial lesions (see following table)
LOAEL	0.4 ppm
NOAEL	Not established
Exposure continuity	6 hours/day, 3 days/week (MWF)
Average experimental exposure	0.043 ppm for LOAEL group
Human equivalent concentration	0.0069 ppm for LOAEL group (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	10
Subchronic uncertainty factor	1
Interspecies uncertainty factor	3
Intraspecies uncertainty factor	10
Cumulative uncertainty factor	300
Inhalation reference exposure level	0.02 ppb (0.06 $\mu g/m^3$)

The Wolf *et al.* (1995) study 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 (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 accumulation	49/70 (70%)	60/70 (85%)	59/70 (84%)	65/70 (93%)
Glandular epithelium eosinophilic accumulation	16/70 (23%)	28/70 (40%)	52/70 (75%)	53/70 (76%)
Olfactory epithelium eosinophilic 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, 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 accumulation to derive the BMC_{05} resulted in a 3-fold lower value than the LOAEL observed above, or BMC_{05} = 0.14 ppm. A BMC_{05} or BMC_{10} has been considered to be similar to a NOAEL in estimating a concentration associated with a low level of risk.

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

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

VII. References

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CHRONIC TOXICITY SUMMARY

CHLOROFORM

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

CAS Registry Number: 67-66-3

I. Chronic Toxicity Summary

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

II. Chemical Property Summary (HSDB, 1995)

Description	Colorless liquid
Molecular formula	$CHCl_3$
Molecular weight	119.49 g/mol
Boiling point	61^{o}C
Vapor pressure	200 mm Hg 25 ^{o}C
Solubility	Soluble in water (8220 g/L); miscible in carbon tetrachloride, carbon disulfide alcohols, benzene, ethers and oils
Conversion factor	4.9 µg/m^3 per ppb at 25^{o}C

III. Major Uses and Sources

Chloroform ($CHCl_3$) is used in industry and laboratory settings as a solvent for adhesives, pesticides, fats, oils and rubbers. It is also used as a chemical intermediate in the synthesis of fluorocarbon 22, dyes, pesticides, and tribromomethane. Chloroform is produced as a byproduct of water, sewage, and wood pulp chlorination (HSDB, 1995).

IV. Effects of Human Exposure

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

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

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

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

More recently, Li *et al.*(1993) reported on chloroform exposed workers from a variety of production factories. Exposure levels varied widely, from 4.27 to 147.91 mg/m^3 (119 samples), with 45% of the samples below 20 mg/m^3. The authors' report that exposed workers displayed altered neurobehavioral function and liver damage (abnormal activities of serum enzymes).

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

V. Effects of Animal Exposure

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

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

VI. Derivation of Chronic Reference Exposure Level (REL)

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

Cumulative uncertainty factor	300
Inhalation reference exposure level	0.05 ppm (50 ppb; 0.30 mg/m^3; 300 µg/m^3)

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

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

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

VII. References

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CHRONIC TOXICITY SUMMARY

DI(2-ETHYLHEXYL)PHTHALATE

(DEHP; Bis-(2-ethylhexyl)phthalate: BEHP; 1,2-benzenedicarboxylic acid; bis(ethylhexyl)ester)

CAS Registry Number: 117-81-7

I. Chronic Toxicity Summary

Inhalation reference exposure level	**10 µg/m^3**
Critical effect(s)	Increased liver weight with the appearance of lung alveolar thickening and foam-cell proliferation in rats
Hazard index target(s)	Alimentary system; respiratory system

II. Physical and Chemical Properties (HSDB, 1995)

Description	colorless to light colored liquid
Molecular formula	$C_{24}H_{38}O_4$
Molecular weight	390.54 g/mol
Boiling point	230^{o}C at 5 mm Hg
Vapor pressure	1.32 mm Hg at 200^{o}C
Solubility	<0.01% in water; miscible with mineral oil and hexane
Conversion factor	1 ppm = 15.97 mg/m^3 at 25 ^{o}C

III. Major Uses and Sources

Di(2-ethylhexyl)phthalate (DEHP) is predominantly used as a plasticizer for polyvinyl chloride (PVC) and vinyl chloride resins. Plastics derived from these compounds may contain up to 40% DEHP. Plasticizers increase the flexibility of PVC for use in many items such as toys, vinyl upholstery, adhesives, coatings, and as components of paper or paperboard. Polyvinyl chloride is also used to produce disposable medical and surgical gloves, and the flexible tubing used for blood transfusions, hemodialysis, and parenteral solutions (HSDB, 1995).

IV. Effects of Human Exposure

No studies have investigated the toxic effects of DEHP in humans after chronic inhalation exposure. Limited medical case studies have discussed the potential for adverse effects due to DEHP exposure from respiratory tubing systems (Roth *et al.*, 1988) or hemodialysis equipment

(Ganning *et al.*, 1984; Woodward, 1990). In one case study, a dialysis patient had an increased number of liver peroxisomes after 1 year, but not after 1 month of treatment (Ganning *et al.*, 1984). Patients on long-term dialysis may be at risk for polycystic kidney disease, with DEHP postulated as a possible causative agent; however, there are insufficient data to confirm a causative role (Woodward, 1990).

V. Effects of Animal Exposure

Experimental data on the inhalation toxicity of DEHP is very limited. The majority of studies have focused on oral exposure to DEHP (reviewed in USDHHS, 1993). Oral exposure to DEHP causes liver enlargement and peroxisome proliferation in rodents at levels down to 10 mg/kg/day DEHP. Humans, other primates, and hamsters are considered more resistant to such oral DEHP exposure related hepatomegaly and peroxisome proliferation (Butterworth *et al.*, 1989; Ganning *et al.*, 1991; Rhodes *et al.*, 1986; Short *et al.*, 1987). Oral exposure to DEHP also produces adverse reproductive and developmental effects in rodents, including decreased maternal body weight, decreased fetal weight, increased fetotoxicity, and increased fetal malformation (Tyl *et al.*, 1988).

Schmezer *et al.* (1988) conducted a chronic inhalation study in Syrian Golden hamsters to evaluate the carcinogenic potential of DEHP (mortality and tumor incidence). No treatment related differences in survival or carcinogenicity were observed after 23 months exposure to 0.015 mg/m^3 DEHP (free-standing NOAEL). Extremely limited histopathological and systemic endpoints, and no clinical chemistry data were evaluated. The single DEHP inhalation dose evaluated (0.015 mg/m^3) corresponds to the highest concentration at which aerosol formation due to condensation is prohibited and to an approximate oral dose of 7 to 10 mg/kg/day (investigators' calculation).

One intermediate duration study of inhaled DEHP aerosols in rats identified a LOAEL of 1000 mg/m^3 (62.6 ppm) for increased liver weights (males and females), lung weights (males) and foam cell proliferation (males) after 4 weeks of exposure (Klimisch *et al.*, 1992). Animals were exposed (head and nose) to respirable particle size aerosol concentrations of 0, 10, 50 or 1000 mg/m^3 DEHP (mass median aerodynamic diameter < 1.2 μm) 6 hours/day for 4 weeks. All these treatment related effects appeared to reverse after an 8 week post-exposure period. Additionally, this study included a fertility assessment. DEHP exposure did not impact mating performance and male fertility after two matings of treated males with untreated females.

The only other inhalation study evaluated the developmental toxicity of DEHP in Wistar rats following an acute 10 day exposure (Merkle *et al.*, 1988). In an initial range finding experiment, dams exposed to 0, 200, 500 or 1000 mg/m^3 DEHP aerosol for 6 hours/day demonstrated an increasing trend in hepatic peroxisome proliferation. A second group of dams was then exposed to 0, 10, 50 or 300 mg/m^3 DEHP aerosol 6 hours/day for 10 days. No treatment related pre- or postnatal mortality or developmental effects were observed. The developmental study design limited the number of systemic endpoints evaluated.

VI. Derivation of Chronic Reference Exposure Level (REL)

Study	Klimisch *et al.* (1992)
Study population	Wistar rats (27 males & 17 females/group)
Exposure method	Discontinuous whole-body inhalation exposure (0, 10, 50, or 1000 mg/m^3 as aerosol)
Critical effects	Increased liver weight and lung weight with the appearance of lung alveolar thickening and foam-cell proliferation
LOAEL	1000 mg/m^3
NOAEL	50 mg/m^3
Exposure continuity	6 hrs/day, 5 days /week
Average experimental exposure	8.9 mg/m^3 for NOAEL group
Human equivalent concentration	3.4 mg/m^3 for NOAEL group (particulate with pulmonary respiratory effects, female rat RDDR = 0.38, based on MMAD = 1.0 µm, sigma g = 2.63, BW = 156 g, MV = 0.12 L/min, SA(ET) = 15 cm^2)
Exposure duration	4 weeks
LOAEL uncertainty factor	1
Subchronic uncertainty factor	10
Interspecies uncertainty factor	3
Intraspecies uncertainty factor	10
Cumulative uncertainty factor	300
Inhalation reference exposure level	0.01 mg/m^3 (10 µg/m^3)

Of the three DEHP inhalation studies available, only Klimisch *et al.* (1992) determined a NOAEL and LOAEL for the critical effects: increases in liver weight and lung weight with the appearance of lung alveolar thickening and foam-cell proliferation. Histopathological analysis was done at the end of DEHP exposure and in a smaller group after 8 weeks recovery post-exposure. The critical adverse liver effects identified (significant increases in absolute and relative liver weight) were similar to those seen in the more abundant oral DEHP rodent studies. However, this organ weight increase was not accompanied by histological effects, a pattern seen in oral DEHP studies (Woodward, 1988). Neither peroxisome proliferation nor alterations in plasma cholesterol levels were observed, even at the highest exposure level (1000 mg/m^3). The one other shorter term, 10 day, DEHP inhalation study in rats identified a NOAEL of 300 mg/m^3 for hepatic peroxisome proliferation (Merkle *et al.*, 1988), an exposure level falling between the principal study's NOAEL (50 mg/m^3) and LOAEL (1000 mg/m^3).

The adverse respiratory effects, increases in relative lung weight accompanied by foam cell proliferation and thickening of alveolar septa, have not been described in other DEHP studies following oral exposure (Klimisch *et al.*, 1992). However after intravenous administration, DEHP and its hydrolysis product mono(2-ethylhexyl)phthalate (MEHP) accumulated in the

lungs of rats. Pulmonary hemorrhage and inflammation were followed by death in this acute study (Schulz *et al.*, 1975). One medical study on three preterm infants identified pulmonary edema and bronchial asthma resembling hyaline membrane disease following artificial ventilation with DEHP-containing PVC respiratory tubes emphasizing the potential for adverse lung effects following inhalation of DEHP (Roth *et al.*, 1988).

Additionally, adverse renal effects including increases in kidney weight, focal cystic changes, decreased creatinine clearance and accumulation of lipofuchsin deposits in tubular cells (Rao *et al.*, 1990), although often identified in chronic oral DEHP studies, were not identified in this principal study (Klimisch *et al.*, 1992). The possible exposure to DEHP through kidney dialysis has been discussed as a potential negative effect on the human kidney (Woodward, 1990).

No epidemiological data exist relating DEHP exposure to any adverse inhalation or chronic systemic effects. The few medical case studies available describe the potential for adverse effects, but lack sufficient data to allow for correlation of dose and response, or any exposure parameters to reach conclusions concerning cause-and-effect.

The paucity of human (inhalation or oral) and animal data (inhalation) on the adverse effects of DEHP exposure lends a high degree of uncertainty to the chronic REL determination. The vast majority of animal studies have been conducted in rodent species using oral routes of exposure. Although the liver appears to be the critical target organ in rodents, with hepatomegaly and peroxisome proliferation being the two most common adverse endpoints, nonrodent species appear less susceptible to peroxide production by peroxisomes after exposure to DEHP (Butterworth *et al.*, 1989; Ganning *et al.*, 1991; Rhodes *et al.*, 1987; Short *et al.*, 1987).

The strengths of the inhalation REL include the availability of 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 reproductive and developmental toxicity studies, and the lack of chronic inhalation exposure studies. The apparent reversibility in adverse effects noted in the key study after an 8-week recuperative period may have implications in assessing risks from subchronic exposures, though not for the default scenario of persons exposed over a lifetime.

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CHRONIC TOXICITY SUMMARY

1,4-DIOXANE

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

CAS Registry Number: 123-91-1

I. Chronic Toxicity Summary

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

II. Chemical Property Summary (HSDB, 1995)

Description	Colorless liquid
Molecular formula	$C_4C_8O_2$
Molecular weight	88.10 g/mol
Boiling point	101.1°C
Vapor pressure	37 mm Hg @ 25°C
Solubility	Miscible with water, aromatic solvents, and oils
Kow	0.537
Conversion factor	3.60 μg/m^3 per ppb at 25°C

III. Major Uses and Sources

1,4-Dioxane (dioxane), a cyclic ether, is used as a degreasing agent, as a component of paint and varnish removers, and as a wetting and dispersion agent in the textile industry. Dioxane is used as a solvent in chemical synthesis, as a fluid for scintillation counting, and as a dehydrating agent in the preparation of tissue sections for histology (Grant and Grant, 1987; HSDB, 1995).

IV. Effects of Human Exposure

Dioxane is absorbed by all routes of administration (HSDB, 1995). In humans, the major metabolite of dioxane is β-hydroxyethoxyacetic acid (HEAA) and the kidney is the major route of excretion (Young *et al.*, 1976). The enzyme(s) responsible for HEAA formation has not been studied, but data from Young *et al.* (1977) indicate saturation does not occur up to an inhalation exposure of 50 ppm for 6 hours. Under these conditions the half-life for dioxane elimination is 59 min (plasma) and 48 min (urine). Although physiologically based pharmacokinetic (PBPK) modeling suggests HEAA is the ultimate toxicant in rodents exposed to dioxane by ingestion, the same modeling procedure does not permit such a distinction for humans exposed by inhalation (Reitz *et al.*, 1990).

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

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

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

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

V. Effects of Animal Exposure

In rats, the major metabolite of dioxane is HEAA which is excreted through the kidneys (Braun and Young, 1977). Exposure to dioxane by ingestion results in saturation of metabolism above 100 mg/kg given in single dose. Saturation of metabolism was also observed as low as 10 mg/kg if dioxane was administered in multiple doses. Dioxane itself is not cleared through the kidney.

A decrease in metabolic clearance with increasing dose (iv) has been interpreted as the saturation of metabolism at the higher doses (Young *et al.*, 1978).

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

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

Rats (Wistar) were exposed by inhalation to dioxane (111 ppm; 7 hours/day, 5 days/week) for 2 years (Torkelson *et al.*, 1974). Increased mortality and decreased body weight gains, compared to unexposed control rats, were not observed. Among the male rats, decreased blood urea nitrogen (kidney function), decreased alkaline phosphatase (cholestatic liver function), increased red blood cells, and decreased white blood cells were observed. According to the authors, exposure related non-cancerous tissue lesions were not observed during the 2-year period. In another inhalation study, rats were exposed to dioxane at levels of 0.15, 1.3, and 5.7 ppm (Pilipyuk *et al.*, 1978). Frequency was not specified, but the duration is given as “90 successive days”. At the end of the 3-month exposure, increased SGOT activity at the two highest doses and increased SGPT activity at all doses were measured in the sera of the exposed rats. Rats exposed to the highest dose also exhibited increased urinary protein and chloride levels, each of which returned to control levels during an unspecified recovery period. Pilipyuk *et al.* (1978) also report changes in the minimum time (ms) required for an electric stimulus to result in excitation of extensor and flexor muscles. Although Pilipyuk *et al.* (1978) consider the changes to be a reflection of adverse effects due to exposure to dioxane, Torkelson *et al.* (1974) do not consider the hematologic and clinical changes of toxicologic importance. In particular, toxic manifestations are usually associated with increased blood urea nitrogen and alkaline phosphatase levels, whereas these levels decreased in the Torkelson *et al.* (1974) investigation. The reason for the discrepancies between the two studies, in particular the extremely low dioxane exposure levels in the Pilipyuk *et al.* (1978) study, is unknown. One explanation could be the purity of the dioxane used, which was not described in the latter study, although such contamination would be unlikely to account for the large difference in exposure levels.

Kociba *et al.* (1974) exposed rats (Sherman) to dioxane by ingestion of drinking water for up to 2-years. The drinking water levels were 0, 0.01, 0.1, and 1.0 percent, which were converted to daily intake according to measured rates of water consumption during exposure. Exposure to the highest level resulted in decreased body weight gain and increased deaths. According to the

authors, exposure related hematologic changes did not occur. Histopathologic examination revealed evidence of regeneration of hepatic and kidney tissues in rats exposed to 1.0 or 0.1 percent, but not in rats exposed to 0.01 percent dioxane. On the assumption of total absorption of dioxane from the gastrointestinal tract, the exposure levels in female and male rats is as follows: 0.01%-18 ppm/F, 9.3 ppm/M; 0.1% -144 ppm/F, 91 ppm/M.

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

VI. Derivation of Chronic Reference Exposure Level (REL)

Study	Torkelson *et al.* (1974)
Study populations	Rats
Exposure method	Discontinuous inhalation
Critical effects	No effects on liver, kidney, or hematologic function were noted in this study. Such dysfunctions, however, were observed in rats exposed to dioxane by ingestion (Kociba *et al.* 1974) and humans (Theiss, *et al.*, 1976, described by NIOSH, 1977).
LOAEL	Not observed in inhalation studies
NOAEL	111 ppm
Exposure continuity	7 h/d x 5 days/wk
Average experimental exposure	23 ppm (111 x 7/24 x 5/7)
Human equivalent concentration	23 ppm (gas with systemic effects, based on RGDR = 1.0 using default assumption that lambda (a) = lambda (h))
Exposure duration	2 years
LOAEL uncertainty factor	1
Subchronic exposure	1
Interspecies uncertainty factor	3
Intraspecies uncertainty factor	10
Cumulative uncertainty factor	30
Inhalation reference exposure level	0.8 ppm (80 ppb; 3.8 mg/m^3; 3000 µg/m^3)

The lifetime rat inhalation study of Torkelson *et al.* (1974) is the only detailed inhalation study available in the literature. The Pilipyuk *et al.* (1977) study contains useful and consistent data,

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

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

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

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CHRONIC TOXICITY SUMMARY

ETHYL CHLORIDE

(Chloroethane; monochloroethane; ether hydrochloric)

CAS Registry Number: 75-00-3

I. Chronic Toxicity Summary

Inhalation reference exposure level	**10,000 µg/m^3** (U.S. EPA RfC) This document summarizes the evaluation of non-cancer health effects by U.S. EPA for the RfC
Critical effect(s)	Delayed fetal ossification in mice
Hazard index target(s)	Teratogenicity; alimentary system

II. Physical and Chemical Properties (HSDB, 1995)

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

III. Major Uses or Sources

Ethyl chloride is used as a starting point in the production of tetraethyl lead and as a refrigerant, solvent and alkylating agent (HSDB, 1995). It is also used as a topical anesthetic (Clayton and Clayton, 1994).

IV. Effects of Human Exposure

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

V. Effects of Animal Exposure

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

No significant adverse effects were observed in rats and mice exposed to 0 or 15,000 ppm ethyl chloride for 6 hours per day, 5 days per week for 102 weeks (rats) or 100 weeks (mice) (NTP, 1989). At necropsy, a complete histopathologic examination failed to identify evidence of toxicity. The same study also exposed rats and mice to 2500, 5000, 10,000 or 19,000 ppm ethyl chloride 6 hours per day, 5 days per week for 13 weeks. No exposure-related clinical signs of toxicity or histological changes were observed in exposed animals.

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

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

Cardiac sensitization to epinephrine in dogs resulting from acute exposure to anesthetic concentrations of ethyl chloride has been reported (Haid *et al.*, 1954; Morris *et al.*, 1953).

VI. Derivation of U.S. EPA RfC

Study	Scortichini *et al.*, 1986; U.S. EPA, 1995
Study population	Mice
Exposure method	Discontinuous whole-body inhalation (on days 6-15 of gestation)
Critical effects	Delayed ossification of skull foramina
LOAEL	13,000 mg/m^3
NOAEL	4,000 mg/m^3
Exposure continuity	6 hours per day
Exposure duration	Days 6-15 of gestation
Average experimental exposure	1,000 mg/m^3 for NOAEL group
Human equivalent concentration	1,000 mg/m^3 for NOAEL group (gas with systemic effects, based on RGDR = 1.0 using default assumption that lambda (a) = lambda (h))
LOAEL uncertainty factor	1
Subchronic uncertainty factor	1
Interspecies uncertainty factor	3
Intraspecies uncertainty factor	10
Modifying factor	10
Cumulative uncertainty factor	300
Inhalation reference exposure level	10 mg/m^3 (10,000 $\mu g/m^3$; 30 ppm; 30,000 ppb)

The RfC is based on a subacute developmental toxicity study. In accordance with U.S. EPA methodology, a time-weighted average concentration for the discontinuous exposure experiment was not used since the key effect was developmental toxicity. The database deficiencies leading U.S. EPA to employ a modifying factor include the lack of a multigenerational reproductive study.

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

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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	**1000 µg/m^3** (U.S. EPA-RfC) This document summarizes the evaluation of non-cancer health effects by U.S. EPA for the RfC.
Critical effect(s)	Developmental toxicity in rabbits and rats
Hazard index target(s)	Teratogenicity; alimentary system; kidney

II. Physical and Chemical Properties (HSDB, 1994)

Description	colorless gas
Molecular formula	C_8H_{10}
Molecular weight	106.16 g/mol
Boiling point	136.2°C
Vapor pressure	10 mm Hg @ 25.9°C
Density	0.867 g/cm^3 @ 20°C
Solubility	soluble in ethanol and ether, partially soluble in water
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).

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. Male rats had dose-dependently increased liver weights 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 100 ppm, respectively.

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). 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 U.S. EPA RfC

Study	U.S. EPA, 1994; Andrew *et al.*, 1981; Hardin *et al.*, 1981
Study population	Rats (78-107 per group) and rabbits (29-30 per group)
Exposure method	Discontinuous inhalation
Critical effects	Skeletal abnormalities in offspring, maternal hepatomegaly and enlarged kidney and spleen (rats) Reduced number of live kits (rabbits)
LOAEL	1,000 ppm
NOAEL	100 ppm
Exposure continuity	6 or 7 hours/day, 5 days/week
Exposure duration	days 1-19 of gestation (rats); 1-24 (rabbits)
Average experimental exposure	100 ppm for NOAEL group (per daily exposure period considered by U.S. EPA)
Human equivalent concentration	100 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
Modifying factor	10 (database deficiencies)
Cumulative uncertainty factor	300
Inhalation reference exposure level	0.3 ppm (300 ppb; 1 mg/m^3; 1,000 µg/m^3)

The RfC is based on a subacute developmental toxicity study. The NOAEL in the study was 100 ppm, and the LOAEL was 1000 ppm. Other studies discussed above (e.g. NTP, 1988, 1989, 1990) identify higher concentrations as NOAELs, but do not measure developmental toxicity. 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 a loss of confidence in its results.

In accordance with U.S. EPA methodology, a time-weighted average concentration for the discontinuous exposure experiment was not used since the key effect was developmental toxicity. The database deficiencies leading U.S. EPA to employ a modifying factor include the lack of a multigenerational reproductive study.

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

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CHRONIC TOXICOLOGY SUMMARY

ETHYLENE GLYCOL

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

CAS Registry Number: 107-21-1

I. Chronic Toxicity Summary

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

II. Physical and Chemical Properties (HSDB, 1996)

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

III. Major Uses and Sources

Ethylene glycol is used as an antifreeze agent in cooling and heating systems (HSDB, 1996). It is used in hydraulic brake systems; as an ingredient in electrolytic condensers; as a solvent in the paint and plastics industries; and in inks for ball-point pens and printer's inks. It is used in the manufacture of some synthetic fibers (Terylene and Dacron), and in synthetic waxes. It is a vehicle for some pharmaceutical preparations. It is used in some skin lotions and flavoring essences. Also, it is used in asphalt emulsion plants, in wood stains and adhesives, and in leather dyeing. It has been used as a de-icing fluid for airport runways.

IV. Effects of Human Exposure

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

In a study of 20 volunteer male prisoners, 20 hour/day exposure to ethylene glycol concentrations of up to 20 ppm (49 mg/m^3) for 30 days was without effect (Wills *et al.*, 1974). Respiratory irritation was noted after 15 minutes at an exposure concentration of 75 ppm (188 mg/m^3), and became quickly intolerable at 123 ppm (308 mg/m^3). No effects were observed in clinical serum enzyme levels for liver and kidney toxicity, hematotoxicity, or psychological responses. The irritation resolved soon after exposure with no long term effects noted after a 6-week follow-up period.

V. Effects of Animal Exposure

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

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

In mice, reduced body weight and gravid uterine weight during and after the exposure were observed at the 888 and 2090 mg/m^3 concentrations. Increased nonviable implants per litter and

reduced fetal body weights were also observed in groups exposed to 888 or 2090 mg/m^3. External, visceral, skeletal, and total malformations were increased in the 888 and 2090 mg/m^3 groups. The NOAEL for these effects in mice was 119 mg/m^3.

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

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

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

VI. Derivation of Chronic Reference Exposure Level

Study	Wills *et al.* (1974)
Study population	Human volunteer prisoners
Exposure method	Discontinuous whole-body inhalation
Critical effects	Respiratory tract irritation
LOAEL	75 ppm
NOAEL	20 ppm
Exposure continuity	20 hours/day
Exposure duration	30 days
Average exposure	16.7 ppm for NOAEL group (20 x 20/24)
Human equivalent concentration	16.7 ppm
LOAEL uncertainty factor	1
Subchronic uncertainty factor	10
Interspecies factor	1
Intraspecies factor	10

Cumulative uncertainty factor	100
Inhalation reference exposure level	0.2 ppm (200 ppb; 0.4 mg/m^3; 400 μg/m^3)

The subchronic study by Wills *et al.* (1974) represents the only human inhalation data for ethylene glycol toxicity. The experiment showed a concentration-response relationship, with onset of irritation occurring at 188 mg/m^3 and intense and intolerable irritation occurring at 308 mg/m^3. The volunteers were followed for 6 months without any apparent long-term effects from the exposures. Although the irritation experienced in the human subjects appears to be an acute phenomenon and not a cumulative lasting effect, the subchronic uncertainty factor was retained to protect against other systemic effects which may occur over a long-term exposure.

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

The strengths of the inhalation REL include the use of human exposure data, the use of controlled inhalation exposures, and the observation of a NOAEL. A major area of uncertainty is the lack of chronic inhalation exposure studies.

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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	**200 µg/m^3** (US EPA RfC) This document summarizes the evaluation of non-cancer health effects by US EPA for the RfC.
Critical effect(s)	Testicular degeneration and decreased hemoglobin in rabbits
Hazard index target(s)	Reproductive system; circulatory system

II. Chemical Property Summary (from HSDB, 1996)

Description	Colorless liquid
Molecular formula	$C_4H_{10}O_2$
Molecular weight	90.12
Boiling point	135°C
Vapor pressure	3.8 mm Hg @ 20° 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.

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.

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 U.S. EPA Reference Concentration (RfC)

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	3
Intraspecies factor	10
Cumulative uncertainty factor	300
Inhalation reference exposure level	0.06 ppm (60 ppb, 0.2 mg/m^3 , 200 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.

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 for the development of the reference concentration (RfC). 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.

The strengths of the inhalation REL 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 and the lack of chronic inhalation exposure studies.