PUBLIC HEALTH GOALS FOR CHEMICALS IN DRINKING WATER

CARBON TETRACHLORIDE

September 2000

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Public Health Goal for
CARBON TETRACHLORIDE
In Drinking Water

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PREFACE

Drinking Water Public Health Goals

Pesticide and Environmental Toxicology Section

Office of Environmental Health Hazard Assessment

California Environmental Protection Agency

This Public Health Goal (PHG) technical support document provides information on health effects from contaminants in drinking water. PHGs are developed for chemical contaminants based on the best available toxicological data in the scientific literature. These documents and the analyses contained in them provide estimates of the levels of contaminants in drinking water that would pose no significant health risk to individuals consuming the water on a daily basis over a lifetime.

The California Safe Drinking Water Act of 1996 (amended Health and Safety Code, Section 116365), amended 1999, requires the Office of Environmental Health Hazard Assessment (OEHHA) to perform risk assessments and adopt PHGs for contaminants in drinking water based exclusively on public health considerations. Section 116365 specifies that the PHG is to be based exclusively on public health considerations without regard to cost impacts. The Act requires that PHGs be set in accordance with the following criteria:

1. PHGs for acutely toxic substances shall be set at levels at which no known or anticipated adverse effects on health will occur, with an adequate margin of safety.
2. PHGs for carcinogens or other substances which can cause chronic disease shall be based upon currently available data and shall be set at levels which OEHHA has determined do not pose any significant risk to health.
3. To the extent the information is available, OEHHA shall consider possible synergistic effects resulting from exposure to two or more contaminants.
4. OEHHA shall consider the existence of groups in the population that are more susceptible to adverse effects of the contaminants than a normal healthy adult.
5. OEHHA shall consider the contaminant exposure and body burden levels that alter physiological function or structure in a manner that may significantly increase the risk of illness.
6. In cases of insufficient data to determine a level of no anticipated risk, OEHHA shall set the PHG at a level that is protective of public health with an adequate margin of safety.
7. In cases where scientific evidence demonstrates that a safe dose-response threshold for a contaminant exists, then the PHG should be set at that threshold.
8. The PHG may be set at zero if necessary to satisfy the requirements listed above.
9. OEHHA shall consider exposure to contaminants in media other than drinking water, including food and air and the resulting body burden.
10. PHGs adopted by OEHHA shall be reviewed every five years and revised as necessary based on the availability of new scientific data.

PHGs published by OEHHA are for use by the California Department of Health Services (DHS) in establishing primary drinking water standards (State Maximum Contaminant Levels, or

MCLs). Whereas PHGs are to be based solely on scientific and public health considerations without regard to economic cost considerations, drinking water standards adopted by DHS are to consider economic factors and technical feasibility. Each standard adopted shall be set at a level that is as close as feasible to the corresponding PHG, placing emphasis on the protection of public health. PHGs established by OEHHA are not regulatory in nature and represent only non-mandatory goals. By federal law, MCLs established by DHS must be at least as stringent as the federal MCL if one exists.

PHG documents are used to provide technical assistance to DHS, and they are also informative reference materials for federal, state and local public health officials and the public. While the PHGs are calculated for single chemicals only, they may, if the information is available, address hazards associated with the interactions of contaminants in mixtures. Further, PHGs are derived for drinking water only and are not to be utilized as target levels for the contamination of other environmental media.

Additional information on PHGs can be obtained at the OEHHA Web site at www.oehha.ca.gov.

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PUBLIC HEALTH GOAL FOR CARBON TETRACHLORIDE IN DRINKING WATER

SUMMARY

The Office of Environmental Health Hazard Assessment (OEHHA) has developed a public health goal (PHG) of 0.1 µg/L (or 0.1 ppb) for carbon tetrachloride in drinking water. The PHG is based on an increased incidence of hepatocellular carcinomas observed in male and female mice in a four-month oral gavage study conducted by Edwards *et al.* (1942). For the PHG calculation, a cancer potency of 1.8×10^{-1} (mg/kg-day)$^{-1}$ was estimated and a *de minimis* theoretical excess individual cancer risk level of one in 10^{-6} was used.

A level protective against noncancer health effects was calculated as 5 µg/L (ppb). The experimental no-observed-adverse-effect-level (NOAEL) selected for this calculation was derived from a subchronic gavage study conducted in adult Fisher rats over 12 weeks, reported by Bruckner *et al.* (1986). In this study, carbon tetrachloride exposure was associated with liver lesions as evidenced by mild hepatic centrilobular vacuolization and elevated serum enzyme levels. A NOAEL of 1 mg/kg-day and a lowest-observed-adverse-effect-level (LOAEL) of 10 mg/kg-day were identified. The NOAEL and LOAEL were further modified by a factor of 5/7 to account for five days per week dosing regimen. The calculation for the noncancer PHG employed an uncertainty factor of 1000 (10-fold for inter-species variation, 10-fold for human variability, and 10-fold to account for the use of a subchronic study for determining a lifetime value).

The current U.S. Environmental Protection Agency (U.S. EPA) maximum contaminant level (MCL) for carbon tetrachloride is 5 µg/L (U.S. EPA, 1998). In 1987, the California Department of Health Services (DHS) established a maximum contaminant level of 0.5 µg/L for carbon tetrachloride (DHS, 1988a) based on the limit of detection in water.

INTRODUCTION

Studies have shown that carbon tetrachloride exposures (both short and long term) produce liver and kidney damage in humans and animals. In animals, carbon tetrachloride ingestion increases the risk of cancer. U.S. EPA has classified carbon tetrachloride as a Group B2, probable human carcinogen (U.S. EPA, 2000).

The purpose of this document is to develop a PHG for carbon tetrachloride in drinking water. In this document, the available data on the toxicity of carbon tetrachloride are evaluated, with the primary focus on the literature related to oral exposures, which may be most appropriate for the establishment of a PHG for drinking water. The studies, which can be used to identify public health-protective levels, are reviewed and summarized. The results of this evaluation are described below.

CHEMICAL PROFILE

Carbon tetrachloride is the common name for tetrachloromethane, a nonflammable, volatile liquid that has a high vapor density. In liquid form it is clear, colorless, and has a characteristic odor (CPHF, 1988). The chemical identity of carbon tetrachloride is listed below in Table 1a, and the physical and chemical properties are listed in Table 1b. The structure of the compound is shown below in Figure 1.

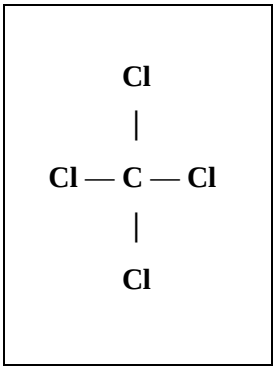
Chemical Identity

Table 1a. Chemical identity of carbon tetrachloride

Chemical Name	Carbon tetrachloride
Synonyms	Tetrachloromethane, perchloromethane carbona, carbon chloride,
Registered trade name	Freon® 10, Halon® 104, R 10, flukoids, necatorine
Chemical formula	CCl_4
Wiswesser line notation	GXGGG
CASRN	56-23-5

Chemical Structure

Figure 1. Chemical structure carbon tetrachloride



Tetrahedral structure with bond angles of 109.5^0

Table 1b. Physical and chemical properties of carbon tetrachloride

Property	Value or Information	References
Molecular weight	153.84	CPHF, 1988
Color	Colorless	NIOSH, 1994
Physical state	Liquid	NIOSH, 1994
Odor	Sweet, ether-like odor	NIOSH, 1994
Odor threshold (in water)	0.52 mg/L	U.S. EPA, 1998
Boiling point	76.7 ^{o}C	CPHF, 1988
Melting point	-23 ^{o}C	U.S. EPA, 1998
Flash point	NA (non-flammable)	NIOSH, 1994
Flammability limits	NA (non-flammable)	NIOSH, 1994
Autoignition temperature	NA (non-flammable)	NIOSH, 1994
Solubility		
Water	1160 mg/L at 25°C	CPHF, 1988
Water	800 mg/L at 20°C	CPHF, 1988
Specific gravity, density	1.59	NIOSH, 1994
Partition coefficients		
Log K_{ow}	2.64	CPHF, 1988
Soil sorption coefficient, K_{oc}	71, moves readily through soil	U.S. EPA, 1998
Bioconcentration factor	Log BCF = 1.24-1.48, not significant	U.S. EPA, 1998
Vapor pressure	90 Torr @ 20° C	CPHF, 1988
Conversion factor	1 ppm = 6.39 mg/m^{3}	NIOSH, 1994

Production and Uses

Carbon tetrachloride has been used as a dry cleaning agent and fire extinguishing material. It has also been used as a solvent for rubber cement as well as for cleaning equipment and machinery. Further uses include those of a refrigerant and as a feedstock chemical for fluorocarbon propellants (U.S. EPA, 1998). Prior to 1986, the largest source of carbon tetrachloride release was fumigation of grains and other substances; however, in 1986 fumigation by carbon tetrachloride was banned in the United States except for the preservation of museum artifacts (ATSDR, 1992). In 1988, the U.S. production of carbon tetrachloride was 761 million pounds. Its use has been declining since 1974 at a rate of 7.9 percent per year (U.S. EPA 1998). Only one company, Dow Chemical, produced carbon tetrachloride in California (CARB, 1987). The Dow

facility in Antioch, California, had a production capacity of 80 million pounds carbon tetrachloride per year, but the facility no longer produces the chemical as a saleable product (BAAQMD, 1998). Synthesis of the carbon tetrachloride chemical, use of the chemical as a feedstock in the production of chlorofluorocarbons, and agricultural use of carbon tetrachloride have stopped in California. There are no known significant industrial sources of this chemical within the state (BAAQMD, 1998).

ENVIRONMENTAL OCCURRENCE AND HUMAN EXPOSURE

Air

Carbon tetrachloride is highly volatile and is relatively stable in the environment. Therefore, nearly all of the carbon tetrachloride produced is eventually emitted to the atmosphere, except that portion converted to other chemicals by manufacturing processes (Galbally, 1976; Simmonds *et al.*, 1983). Simmonds *et al.* (1983) calculated the average global concentration of carbon tetrachloride in the troposphere region of the atmosphere as 118 parts-per-trillion in 1981, with an annual increase of about 2 parts-per-trillion (1.8 percent). The estimated half-life of carbon tetrachloride in the atmosphere is 30-100 years and the primary removal mechanism from the air is purportedly the photolysis of carbon tetrachloride by shorter wavelength ultraviolet light in the stratosphere (ATSDR, 1992). The photolysis pathway is of concern as it produces atomic chlorine that reacts with and depletes ozone in the stratosphere (CARB, 1987). Some other processes that may contribute to the removal of carbon tetrachloride from the air are (1) absorption into the oceans, (2) reactions with OH radical, (3) biological degradation, and (4) photolysis after adsorption onto airborne particles (CARB, 1987).

As the major sources for atmospheric contamination of carbon tetrachloride have been discontinued, ambient air concentrations have steadily declined. Please see Figure 2.

Soil

Carbon tetrachloride moves readily through soil and adsorbs only slightly to sediment. Due to its low adsorption to soil particles, any releases or spills on soil would result in either rapid evaporation due to high vapor pressure or leaching to groundwater (U.S. EPA, 1998). No reports were found regarding background concentrations of carbon tetrachloride in soil (CPHF, 1988; Willis *et al.*, 1994).

Water

Carbon tetrachloride has been detected in the water of rivers, lakes, finished drinking water sources, manufacturing and sewage treatment plant effluents (Singh *et al.*, 1977; IARC, 1979). In the national Ground Water Supply Survey conducted in 1982, 3.2 percent of 466 samples contained carbon tetrachloride (CPHF, 1988). Carbon tetrachloride occurred at concentrations ranging from 0.5 to 30 µg/L in drinking water supplies according to data summarized from six national surveys in the United States (CPHF, 1988). In a 1985 survey of organic groundwater contaminants of large water systems (greater than 200 connections) in California, carbon tetrachloride was found in 39 wells at concentrations ranging from 0.2 to 29 µg/L. The mean

well concentration of carbon tetrachloride was 4.6 ± 5.6 (S.D.) µg/L and the median concentration was 2.2 µg/L (DHS, 1987; CPHF, 1988).

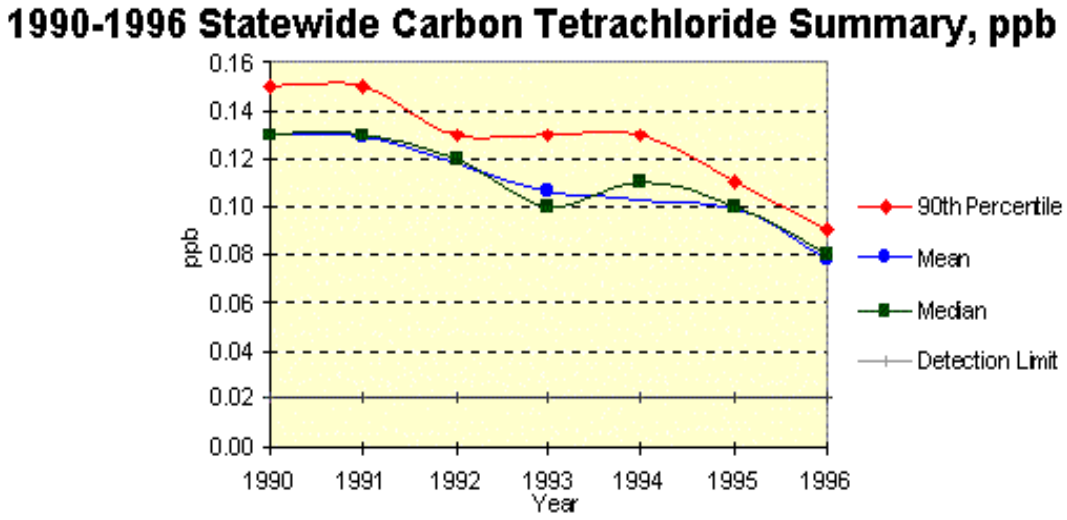
No information has been found on the breakdown of carbon tetrachloride within natural waters. Carbon tetrachloride is resistant to alteration in the environment and its removal from water occurs primarily through surface evaporation. Half-lives of 0.3-3 days in the Rhine river and 30-300 days in lakes have been reported for carbon tetrachloride (Zoetman *et al.*, 1980). These half-lives were determined for changes in carbon tetrachloride concentration caused by evaporation and other processes in natural waters (Zoetman *et al.*, 1980; CPHF, 1988).

In a laboratory study, a half-life of 29 ± 2.8 (S.D.) minutes was determined for the evaporative loss of carbon tetrachloride from stirred water (purified and deionized) that initially contained 1 mg/L (Dilling *et al.*, 1975; CPHF, 1988).

Evaporation appears to be the most important removal process of carbon tetrachloride in water systems although some limited data suggest that aerobic and anaerobic biodegradation may occur. The hydrolysis half-life in water is 7,000 years at 25° C (U.S. EPA, 1998).

Some atmospheric carbon tetrachloride is expected to partition into the ocean (U.S. EPA, 1998).

Figure 2. Ambient Air Concentration



Source: CARB (1999)

Note: Samples were collected every 12 days at 21 sites (20 sites before July 1995) throughout California.

Food

Carbon tetrachloride has a low potential to bioconcentrate. The logarithm of the bioconcentration factor in trout is 1.24 and in bluegill is 1.48 (U.S. EPA, 1998). Historically, most carbon tetrachloride in food was residual contamination from fumigation as a pesticide. The amount of carbon tetrachloride remaining as a residue in baked goods was generally dependent on fumigant dosage, storage conditions, length of aeration, and extent of processing (U.S. EPA, 1984).

METABOLISM AND PHARMACOKINETICS

Absorption

The absorption of carbon tetrachloride from different routes has been studied in many species, including humans. Due to its high lipid solubility, carbon tetrachloride is readily absorbed through inhalation, ingestion, and possibly by dermal contact with the liquid form (DHS, 1987; CPHF, 1988).

Oral

Carbon tetrachloride is readily absorbed from the gastrointestinal tract following oral administration. Robbins (1929) performed a series of experiments in which the amount of carbon tetrachloride absorbed from the gastrointestinal tract of dogs was determined by measuring the amount of exhaled carbon tetrachloride. The primary site of gastrointestinal absorption was the small intestine; much less was absorbed from the colon. Little, if any, carbon tetrachloride was absorbed from the stomach (Robbins, 1929). The oral absorption in rats was also studied by Recknagel and Litteria (1960) who determined that peak blood concentration occurred 1.5 hours following administration (Recknagel and Litteria, 1960). The rate of absorption of carbon tetrachloride through the gastrointestinal tract is rapid and greatly affected by diet. Fat or alcohol in the gut enhances carbon tetrachloride absorption (ATSDR, 1992).

Sanzgiri *et al.* (1997) found that deposition of carbon tetrachloride in all tissues was greater when delivered via oral bolus than by gastric infusion (over two hours) of carbon tetrachloride in the rat. The authors hypothesized that very rapid metabolic clearance of relatively small amounts of carbon tetrachloride (versus one large oral bolus) accounted for the lower levels in the tissues.

Inhalation

Inhalation absorption of carbon tetrachloride was studied by using rabbits, and it was observed that the absorption rate decreased from 34.7 to 4.7 percent, during a 3-hour exposure to 50 mg/L (~8000 ppm) (Lehmann and Hasegawa, 1910). Following exposure of dogs to 15,000 and 20,000 ppm carbon tetrachloride, blood carbon tetrachloride levels reached equilibrium in approximately five hours (Von Oettingen *et al.*, 1949; Von Oettingen *et al.*, 1950). However, exposure to such high concentrations decreased absorption due to severe toxicity that apparently decreased absorption (DHS, 1987).

McCollister *et al.* (1950, 1951) exposed three monkeys via inhalation to an average of 46 ppm of ^{14}C-labelled carbon tetrachloride for two to six hours. The absorption occurred at an average rate

of 1.34 mg/kg/hour, or 30 percent of the total weight of carbon tetrachloride inhaled. The authors observed that absorption of the material ranged from 26 to 37 percent. The highest absorption rate was obtained in the longest exposure. Equilibrium of carbon tetrachloride between the air and blood was not reached during the course of the experiment. Consequently, absorption following a longer exposure until equilibrium was reached would be expected to be above 37 percent (DHS, 1987).

In a study of carbon tetrachloride uptake in rats, Sanzgiri *et al.* (1997) found that uptake and distribution of inhaled carbon tetrachloride (1,000 ppm for two hours) was relatively high when compared with gastric infusion over two hours, and roughly comparable to the oral bolus route, although uptake was slower for the latter route. The vehicle for the orally delivered carbon tetrachloride was a polyethoxylated emulsion as opposed to corn oil, when gastrically infused.

Dermal

Two Rhesus monkeys were exposed dermally to vapor concentrations of 485 and 1150 ppm for approximately four hours via a large exposure bag (McCollister *et al.*, 1951). Carbon tetrachloride blood levels were equivalent to 0.012 and 0.03 mg per 100 g blood, respectively (CPHF, 1988). No radioactivity from the labeled carbon tetrachloride was found in the blood or expired air (sampled via a mask) after 48 hours (McCollister *et al.*, 1951). Although absorption of vapor by the dermal route is slight, dermal absorption of liquid carbon tetrachloride can result in toxicity (DHS, 1987). Prolonged skin contact with liquid carbon tetrachloride can result in systemic effects (Fleming and Hodgson, 1992).

Distribution

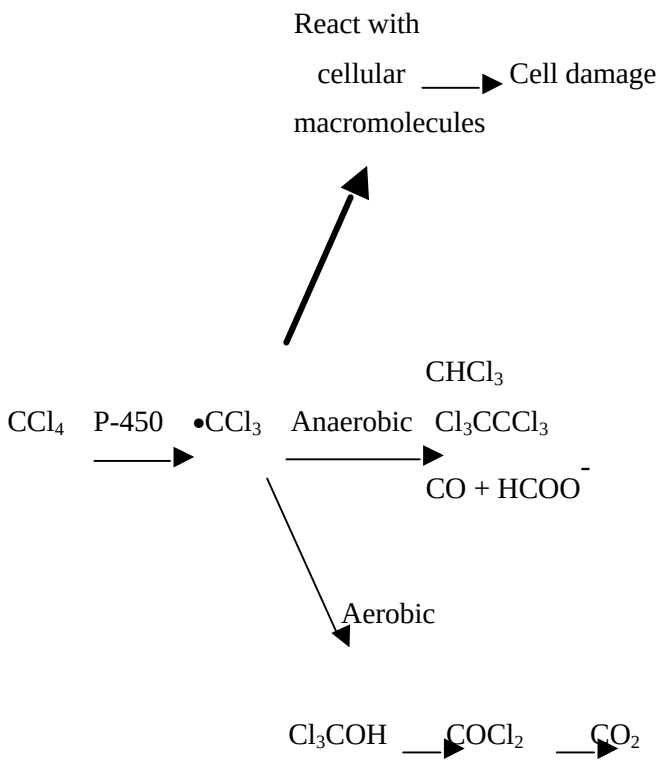
The distribution of carbon tetrachloride in animals varies with the route of administration, concentration, and the duration of exposure (Von Oettingen, 1964). Because of carbon tetrachloride's lipophilic solubility properties, most of the compound accumulates in tissues with high fat content, such as adipose tissue, liver, and bone marrow (Robbins, 1929; McCollister *et al.*, 1950; McCollister *et al.*, 1951). Fowler (1969) examined the distribution and metabolism of carbon tetrachloride in rabbits following oral administration of one ml/kg (presumably 1.59 g/kg). The highest concentrations of carbon tetrachloride were found in the fat, followed by the liver, kidneys, and muscle (Fowler, 1969). Carbon tetrachloride metabolites (such as chloroform and hexachloroethane) were also detected in fat, liver, kidney, and muscle (DHS, 1987). In a more recent study, performed on rats at a 179 mg/kg dose level via the inhalation, oral bolus, and gastric infusion routes of administration, carbon tetrachloride was most rapidly taken up by the brain, the kidney, and the liver. The carbon tetrachloride content diminished in these tissues while accumulating more slowly in the adipose tissues. For oral bolus dosing (the most quickly distributed of the three routes) the maximum concentrations (t_{max}) occurred at 1, 5, and 15 minutes following dosing in the liver, kidney, and brain, respectively. The t_{max} for fat was 120 minutes. Further, the elimination half-lives were 323, 278, and 313 minutes, for liver, kidney, and brain, respectively. These were much faster than the 780 minutes observed for carbon tetrachloride elimination from fat (Sanzgiri *et al.*, 1997).

Metabolism

The metabolism of carbon tetrachloride has been investigated in the rat, rabbit, dog, and human. Nearly half of the absorbed carbon tetrachloride is excreted unchanged, but the remainder is metabolized to the trichloromethyl free radical via the cytochrome P-450 enzyme system. The major human enzyme responsible for carbon tetrachloride bioactivation at lower, "environmentally relevant" levels is P450-2E1 (Zangar *et al.*, 2000).

Figure 3.

Products of Carbon Tetrachloride Metabolism



Source: ATSDR, 1992

The free radical may bind directly to microsomal lipids and other cellular macro-molecules contributing to the breakdown of membrane structure and disrupting cell energy processes and reactions. The trichloromethyl free radical can also undergo anaerobic reactions, which may result in the formation of such toxic compounds as chloroform, hexachloroethane, and carbon monoxide (Fleming and Hodgson, 1992; ATSDR, 1992). In addition to the binding of carbon tetrachloride metabolites to protein and lipid macromolecules, there is also evidence of binding to DNA which is discussed in the section on genotoxicity (DHS, 1987). The $\bullet CCl_3$ radical can yield trichloromethanol, a precursor to carbonyl chloride (phosgene), which is then hydrolyzed to form CO_2 (ATSDR, 1992). A generalized metabolic scheme has been postulated as shown in Figure 3 (DHS, 1987; ATSDR, 1992).

McCollister *et al.* (1950, 1951) exposed monkeys to radiolabeled carbon tetrachloride vapor by inhalation. An estimated 40 percent of the absorbed material was exhaled unchanged, while 11 percent was exhaled as carbon dioxide. In the blood, a number of unidentified radiolabeled materials were isolated and classified as "alkaline volatiles," "acid volatiles," or "non-volatiles." In the urine, some of the labeled carbon was in the form of urea and carbonate, but 95 percent was a nonvolatile, unidentified compound.

Excretion

Following inhalation, ingestion, or injection, carbon tetrachloride is predominantly excreted via the lungs (Robbins, 1929; McCollister *et al.*, 1951; ATSDR, 1992). Excretion of carbon tetrachloride is apparently biphasic and the second phase is relatively slow; thus accumulation of carbon tetrachloride with repeated exposure can result in chronic intoxication (DHS, 1987). After exposure ceases, carbon tetrachloride emerges from fat and is removed primarily from the lungs, unmetabolized. Most of the carbon tetrachloride that remains in the body forms adducts with proteins and other cellular macromolecules. These are degraded and excreted mainly in the urine and feces, with a half-life of about 24 hours (ATSDR, 1992).

TOXICOLOGY

Toxicological Effects in Animals and Plants

Acute Toxicity

Carbon tetrachloride produces acute systemic toxicity following ingestion or inhalation. The major effects are central nervous system depression, hepatic damage, and kidney damage (ATSDR, 1992; U.S. EPA, 1995). Symptoms of hepatic toxicity may appear after a delay of one to four days following acute exposure. Pulmonary toxicity has been reported (ATSDR, 1992). Hemolysis and other circulatory disturbances have been observed (Shulze and Kappus, 1980; Von Oettingen, 1964).

The acute oral toxicity of carbon tetrachloride is relatively low with a single dose median lethal dosage (LD_{50}) value of 13,000 mg/kg for mice (Hayes *et al.*, 1986). A rat study yielded an oral LD_{50} value of about 8,000 mg/kg (Thakore and Mehendale, 1991).

Recognizing the lack of oral dosing studies from which to derive a no-observed-adverse-effect level (NOAEL) and lowest-observed-adverse-effect-level (LOAEL) values for the noncarcinogenic toxic effects of carbon tetrachloride, Bruckner *et al.* (1986) undertook a comprehensive study to establish these oral values for acute and subchronic exposure durations. The authors found “little evidence of toxicity” in 300-350 g Sprague-Dawley rats following single doses of 20, 40, 80, or 160 mg/kg carbon tetrachloride. The authors described 80 mg/kg as the one-day LOAEL for acute oral effects due to hepatic centrilobular vacuolization and elevated serum enzyme levels. They further described 40 mg/kg as the NOAEL for the acute portion of the study (Bruckner *et al.*, 1986).

Subchronic Toxicity

Subchronic (and chronic) exposure studies yielded results similar to those found in acute studies (DHS, 1987). Subchronic and chronic exposures affect the same targets as acute exposure: the nervous system, the liver, and the kidney.

Hayes *et al.* (1986) reported dose-dependant increases in liver-body-weight ratios in mice that received 12, 120, 540 or 1200 mg/kg carbon tetrachloride by gavage for 90 days. Additionally, the authors observed a dose-dependant increase in the serum activity of glutamic-oxaloacetic

transaminase (GOT) glutamic-pyruvic transaminase (GPT) sorbitol dehydrogenase (SDH) and alkaline phosphatase (ALP) as well as increases in cholesterol and bilirubin levels (DHS, 1987).

Pendergrast *et al.* (1967) performed a subchronic inhalation study using the rat, guinea pig, rabbit, dog, and monkey all at (1, 10, and 80 ppm). Exposures were for 90 days except for the high dose group for which exposure was 42 days. In the high dose groups, mortality, weight loss, and liver and lung pathology were observed in all species except for the rat, which sustained no mortality. In the medium dose groups, all species were observed to depressed growth and enlarged livers or histological liver changes. In the low (1ppm) dose groups, growth depressions and histopathological changes described as "slight" were observed in all species except for the rat (Pendergrast *et al.*, 1967). On the basis of these "slight" effects, subchronic inhalation LOAELs are estimated as 1.6 mg/kg-day (monkey), 2.0 mg/kg-day (dog), and 2.4 mg/kg-day (rabbit). U.S. EPA (1984) reported that the studies performed by Pendergrast *et al.* (1967) have been criticized due to small sample size, inconsistent reporting (for example, over ten different descriptions of liver damage were mentioned) and vague information such that only the general conclusion that liver damage follows carbon tetrachloride inhalation can be made.

Bruckner *et al.* (1986) conducted an investigation to characterize the subchronic potency of ingested carbon tetrachloride in rats. Two weight categories of Sprague-Dawley rats were gavaged with 0, 1, 10, or 33 mg/kg carbon tetrachloride five days per week for 12 weeks. A dose of 1 mg/kg had no apparent adverse effect; 10 mg/kg produced slight, but statistically significant increases in sorbitol dehydrogenase activity as well as mild hepatic centrilobular vacuolization. Thirty-three mg/kg caused marked hepatotoxicity. Microscopic examination of livers of the 33 mg/kg treated rats revealed cirrhosis, characterized by bile duct proliferation, fibrosis, lobular distortion, parenchymal regeneration, hyperplastic nodules, and single-cell necrosis. The fibrosis was not reversed within a 13-day recovery period. On the basis of this subchronic study, U.S. EPA selected 1 mg/kg (corrected by 5/7 to account for weekday dosing) as the NOAEL for the noncancer RfD calculation as the absence of mild hepatic centrilobular vacuolization (IRIS, 1998).

Chronic Toxicity

Chronic animal studies yielded results similar to subchronic and acute exposure durations. Rats and guinea pigs exposed to 10 ppm carbon tetrachloride in air, seven hours per day, five days per week, for six to nine months were observed to have increased liver weights and hepatic fatty degeneration (Adams *et al.*, 1952). Toxic effects have been reported following chronic inhalation exposure to 5 ppm or greater. The longest animal study reported lasted about 42 weeks. A NOAEL for a chronic exposure of the rat is 5 ppm (Adams *et al.*, 1952; DHS, 1987)

Adams *et al.* (1952) studied toxic effects of carbon tetrachloride via inhalation in rats, guinea pigs, rabbits, and monkeys. Groups of 15-25 rats and 5-9 guinea pigs for each sex were exposed to 0, 5, 10, 25, 50, 100, 200, or 400 ppm carbon tetrachloride in air for seven hours per day, five days per week, for six to seven months. Hepatotoxicity occurred in both the rats and guinea pigs at the 10 ppm level as evidenced by liver weight increases, total lipid increases, and elevated esterified cholesterol levels. Renal toxicity and growth retardation were evident at 50 ppm. The severity of the toxic effects continued to increase with dose level increases. For this study, a chronic, inhalation NOAEL of 5 ppm was established which corresponds to dose rates of 6, 6.5, 3.1, and 3.2 mg/kg-day for male and female rats and male and female guinea pigs, respectively (Adams *et al.*, 1952; DHS, 1987). The U.S. EPA (1984) reported that the studies performed by Adams *et al.* (1952) have been criticized due to incomplete presentation of data as well as confusing descriptions which limit the application of the study (U.S. EPA, 1994).

Alumot *et al.* (1976) conducted a two-year chronic feeding study in male and female rats, at 18 rats/sex/dose group. The animals were fed mash fumigated with either 80 or 200 ppm carbon tetrachloride, the latter level corresponding to14 mg/kg-day (DHS, 1987). With the exception of a slight but significant decrease in serum protein, all other measurements were normal, including evaluation of total liver fat, liver triglyceride phospholipids, glucose, cholesterol, GPT and GOT activity, and serum urea and uric acid. For this study, a dietary LOAEL range of 10 to 18 mg/kg-day can be determined on the basis of serum protein depression (Alumot *et al.*, 1976).

Genetic Toxicity

As mentioned previously, much of the carbon tetrachloride absorbed is exhaled unchanged; however, some is metabolized to form trichloromethyl radical. This reactive intermediate should bind to DNA. Adduct identification, however, is currently lacking (MacGregor and Lang, 1996). Radiolabeled ^{14}C-carbon tetrachloride as been shown to bind covalently to macromolecules, including DNA, *in vitro* and *in vivo*. Multiple studies have shown that it is first metabolized to the trichloromethyl radical, possibly at the nuclear membrane, prior to alkylation (DHS, 1987). An *in vitro* study indicated that the trichloromethyl radical interacted with all four DNA bases, but bound preferentially to guanine and adenine (Diaz Gomez and Castro, 1981). Consequently, carbon tetrachloride could produce a genotoxic response following metabolic activation (Diaz Gomez and Castro, 1981; DHS, 1987).

Almost all bacterial mutagenicity tests have been negative. Ames tests for reverse mutations using several strains of *Salmonella typhimurium* were negative both with and without exogenous metabolic activation (McCann *et al.*, 1975; Simmon *et al.*, 1977; MacGregor and Lang, 1996).

A weak positive genotoxic response was reported in yeast. Negative responses were reported in an *in vitro* study using a rat liver epithelial cell line. Negative or weak responses were observed in four studies examining unscheduled DNA synthesis. Based on these assays, carbon tetrachloride exhibits little mutagenicity, although its ability to bind to DNA suggests potential genotoxicity (DHS, 1987).

In their review of genetic testing performed with carbon tetrachloride, MacGregor and Lang (1996) found several reports of positive genotoxic results, including homozygosis by recombination or gene conversion, intrachromosomal recombination in histidine 3 and mitotic chromosome loss tests (all tested on *Saccharomyces cerevisiae*) and covalent binding to DNA in liver and kidney of Syrian hamster (*in vivo*). Additional, positive genotoxic results were reported for aneuploidy in Chinese hamster lung cells and anaphase anomalies on Chinese hamster ovary cells, both *in vitro* (MacGregor and Lang, 1996).

Amacher and Zelljadt (1983) tested carbon tetrachloride's ability to produce *in vitro* morphological transformation of Syrian hamster embryo (SHE) cells. Carbon tetrachloride produced a weakly positive response as indicated by the transformation of one to three of the test colonies. No transformed colonies were observed in the solvent controls. These results provide marginal support for other data suggesting that carbon tetrachloride is potentially genotoxic (Amacher and Zelljadt, 1983; DHS, 1987). Sina *et al.* (1983) developed an alkaline elution rat hepatocyte assay to measure DNA single-strand breaks (SSBs). In the test system, carbon tetrachloride produced a 3- to 5-fold greater number of SSBs than the controls; a positive response suggesting potential genotoxicity of carbon tetrachloride. The authors concluded that the test system correlates well (85 to 92 percent) with mutagenic and carcinogenic activity for the 91 compounds tested (Sina *et al.*, 1983). Carbon tetrachloride produced a positive genotoxic response in a test system of De Flora *et al.* (1984). In the study carbon tetrachloride was assayed

in a DNA-repair test with *E. coli* strains proficient and deficient in DNA repair. The genotoxic effect was ascertained by increased killing or growth-inhibition of bacteria lacking specific DNA-repair mechanisms, compared with the isogeneic repair-proficient strains (De Flora *et al.*, 1984; DHS, 1987).

Carbon tetrachloride appears to have genotoxic potential based on its ability to form reactive intermediates that can covalently bind to DNA, induce chromosomal rearrangements *in vitro*, cause SSBs and produce morphological transformation of SHE cells. Carbon tetrachloride has demonstrated very little, if any, mutagenic activity based on the standard bacterial mutagenic assays, a yeast assay, and determinations using unscheduled DNA synthesis. Further positive *in vitro* results were aneuploidy in Chinese hamster lung cells and anaphase anomalies in Chinese hamster ovary cells. Mutagenicity test results were generally negative (MacGregor and Lang, 1996; DHS, 1987).

Developmental and Reproductive Toxicity

Several studies demonstrated decreased weight in testes and accessory male reproductive organs. The most sensitive study that considered male reproductive effects was by Adams *et al.* (1952). Rats were exposed to 5, 10, 25, or 50 ppm (27-29 weeks) or 100, 200, or 400 ppm for 37 weeks via inhalation. Guinea pigs were exposed to carbon tetrachloride concentrations of 25 ppm for 26 weeks. All exposures were 7 hours/day for five days/week. In rats exposed to 200 ppm and above, the authors observed decreased weight of the testes compared to controls. Additionally, they observed moderate to marked degeneration of germinal elements of the testes, with some seminiferous tubules exhibiting complete atrophy of germinal elements. The guinea pigs showed no reproductive effect at this level. The NOAEL for reproductive effects caused in rats is 100 ppm (Adams *et al.*, 1952; DHS, 1987).

Carbon tetrachloride was shown in several studies to cross the placenta of pregnant rats and to produce fetotoxicity (DHS, 1987). Maternal toxicity was produced in these studies, and while it was associated with fetal toxicity, several investigators have shown that there does not appear to be a correlation between the severity of maternal toxicity and the severity of reproductive effects in the rat fetus for carbon tetrachloride intoxication (Sundareson, 1942; Bhattacharyya, 1965; Schwetz *et al.*, 1974; Wilson, 1954; DHS, 1987).

In a multigenerational study, Smyth *et al.* (1936) found that 200 and 400 ppm carbon tetrachloride diminished the number of litters and decreased the number of offspring per litter in rats, compared to controls. Up to three generations were observed for fertility following repeated 8 hours/day, five-days/week exposure of both sexes to 50, 100, 200, and 400 ppm carbon tetrachloride over 10.5 months. It was not clear if the decline in fertility resulted from effects of carbon tetrachloride on males, females, or both (Smyth *et al.*, 1936). The inhalation NOAEL from this study is 100 ppm or approximately 150 mg/kg-day.

Alumot and coworkers (1976) performed a two-year study with carbon tetrachloride fed to rats at a level of 10 mg/kg-day in specially prepared mash. Growth, fertility, reproduction, and other biochemical parameters were observed in order to attempt to ascertain an acceptable daily intake. On the basis of fertility, litter size, and litter weight, 10 mg/kg-day was considered to be the NOAEL for chronic oral exposure (Alumot *et al.*, 1976; ATSDR, 1994).

Several studies have suggested that carbon tetrachloride is embryotoxic in rats. Administration of carbon tetrachloride to pregnant rats prior to the 12th day of gestation produced a failure to implant or increased intrauterine mortality (Sundareson, 1942). Carbon tetrachloride produced a decrease in the viability and in the number of pups per litter when compared to controls

(Gilman, 1971). In rabbits, carbon tetrachloride administered on days four and five of gestation produced cellular degeneration in the embryonic discs. The trophoblasts contained very large nuclei with prominent nucleoli, indicating that carbon tetrachloride can produce embryotoxicity prior to implantation (Adams *et al.*, 1961; Gilman, 1971; Sundareson, 1942; DHS, 1987).

Carbon tetrachloride is fetotoxic in rodents. When administered after the 12th day of gestation carbon tetrachloride exposure was associated with premature delivery, increased postnatal mortality, and liver degeneration and necrosis in the fetus (Sundareson, 1942; Bhattacharyya, 1965). Schwetz *et al.* (1974) reported that carbon tetrachloride inhalation by pregnant rats produced a significant decrease in fetal body weight and crown-rump length when compared to controls. Furthermore, carbon tetrachloride can diffuse into maternal milk and cause liver damage in the nursing neonate (Bhattacharyya, 1965; DHS, 1987).

Narotsky and Kavlock (1995) evaluated the effects of carbon tetrachloride (via gavage) on pregnant Fischer 344 rats. The rats were dosed daily on the 6th through 19th days following gestation at levels of 0, 112.5 and 150 mg/kg-day. Ten of 14 females resorbed their litters at the high dose level, resorption being the result of fetal death. Other indicators of developmental toxicity, such as kinky tail, microphthalmia, cleft palate, eye defects, and delayed parturition, were not observed for carbon tetrachloride treated dams (Narotsky and Kavlock, 1995; DHS, 1987).

The studies on embryo- and fetotoxicity suggest that, in rodents, carbon tetrachloride exposure exhibited a limited teratogenic potential.

Immunotoxicity

Subchronic exposure to carbon tetrachloride appears to suppress certain immune responses in mice.

Certain immune responses, such as those requiring helper T-cell cooperativity, T-cell dependent humoral responses, and some cell-mediated responses are sensitive to inhibition by carbon tetrachloride. Immunotoxic effects produced by carbon tetrachloride appear to be dependent on metabolic activation (Kaminski and Stevens, 1992). Delaney *et al.* (1994) observed that carbon tetrachloride suppresses T-cell-dependent immune responses by the induction of transforming growth factor β1. The authors illustrated that carbon tetrachloride exposure selectively inhibits T-cell activity by treating female B6C3F1 mice for seven consecutive days with 500 mg/kg in corn oil via gavage. The macrophages isolated from the spleens of the treated mice were purified, sensitized with sheep erythrocytes, then incubated for five days. The antibody-forming-response of the T-cells was less than half of that from either naïve or vehicle-treated mice (Delaney *et al.*, 1994).

In another study, A/PhJ mice were exposed i.p. to 1.7 mmol (260 mg)/kg carbon tetrachloride administered in olive oil for 2, 7, 14, or 23 days. The authors observed that acute exposure to carbon tetrachloride had a significant stimulative effect on phagocytosis and natural killer cell activity as well as lymphocyte response to nonspecific mitogens. Subchronic exposure at the same dose level, however, led to suppression of phagocytosis and natural killer cell activity as well as significant impairment of lymphocytic response to nonspecific mitogens (Jirova *et al.*, 1996).

Neurotoxicity

Pathological effects to the central nervous system were observed in rats after several weeks of carbon tetrachloride administration. Neuropathological changes were noted in the cerebellum, corpus striatum, and globus pallidus. Alteration of astrocytes with lobulated nuclei, enlarged nucleoli, and watery cytoplasm were prominent features of carbon tetrachloride-induced encephalopathy. Carbon tetrachloride neurotoxicity may be a direct result of hepatic dysfunction from carbon tetrachloride intoxication. The changes follow the onset of liver necrosis and are similar in nature to hepatic encephalopathy (Diemer, 1975; Diemer, 1976; Politis *et al.*, 1980).

Carcinogenicity

Carbon tetrachloride has been shown to produce liver tumors in mice, rats and hamsters by the oral and subcutaneous routes. These bioassays were either preliminary and qualitative in nature or carbon tetrachloride was administered to animals as a positive control. Furthermore, there was high noncancer mortality in most of the experiments. The International Agency for Research on Cancer's (IARC) evaluation of carcinogenicity concluded that there was sufficient evidence that carbon tetrachloride was carcinogenic in experimental animals and that carbon tetrachloride should be regarded as a potential human carcinogen (IARC, 1979).

Mouse Studies

Edwards (1941) and Edwards and Dalton (1942) administered carbon tetrachloride by gavage to different strains of male and female mice (Strains A, C, CH3 and Y) two to three times a week for 8 to 23 weeks. To assess the tumor-producing ability of carbon tetrachloride, animals were necropsied 12 to 21 weeks after the last treatment. For those animals exposed to approximately 2100 mg/kg-day of carbon tetrachloride, the incidence of hepatoma was 88.2 percent (strain CH3). Whether the carbon tetrachloride-induced hepatomas were malignant was not established histologically in the study. The animals were sacrificed starting at four months of age. Since tumor expression is a function of both dosage and the latency period, any risk assessment based on these studies, with their short observational periods, may underestimate the true carcinogenic risk. In another experiment Edwards and Dalton (1942) administered one, two, or three doses of carbon tetrachloride (260 to 2100 mg/kg) followed by long-term observation in Strain A mice. The doses were hepatotoxic, but when the animals were examined 12 months later no tumors were observed (Edwards, 1941; Edwards and Dalton, 1942).

Edwards *et al.* (1942) treated 56 male and 19 female L mice via oral gavage with 0.1 ml of 40 percent carbon tetrachloride two or three times/week over four months, for a total of 46 treatments. Animals were killed 3 to 3.5 months after the last treatment. The combined hepatoma incidence of treated male and female mice was 47 percent (34/73 vs. 2/152 in the untreated controls) (Edwards *et al.*, 1942). Weaknesses in this study include lack of vehicle controls (the control mice were untreated), the dosing schedule was short compared to lifespan, and the dosing schedule was irregular. (Please see Table 2.)

Eschenbrenner and Miller (1943, 1946) extensively examined carbon tetrachloride-induced tumor production in Strain A mice. In the first study they administered 30 doses via oral gavage of carbon tetrachloride in olive oil at intervals of one to five days (0, 160, 315, 625, 1250 and 2500 mg/kg). All animals were examined for tumors at 150 days following the first dose. Centrilobular liver necrosis was observed at all exposure levels. They reported that the incidence

of hepatomas was increased as the time interval between doses increased (Eschenbrenner and Miller, 1943).

In a second study Eschenbrenner and Miller (1946) administered the same total quantity of carbon tetrachloride via stomach tube either in 30 doses at four-day intervals or in 120 doses on consecutive days. This study was conducted to determine the effect of liver necrosis on tumor development. Dose levels were 1200, 2400, 4800, and 9600 mg/kg-day. They found that mice receiving the smaller daily dose over 120 days, which the authors referred to as a "non-necrotizing" dose, developed tumors at roughly the same or greater rate as those animals that received necrotizing doses (30 larger doses at four-day intervals). It appears that liver necrosis was not required for the production of tumors with carbon tetrachloride. This study showed that the total length of the exposure period (i.e., 120 versus 30 days), not the time between doses, may have been the major determining factor in the production of tumors (Eschenbrenner and Miller, 1946)

Three National Cancer Institute's (NCI) mouse bioassays used carbon tetrachloride as a positive control. Excess mortality was a severe problem encountered in the studies (NCI, 1976b; NCI, 1976a; DHS, 1987). Male and female B6C3F$_1$ mice were dosed by gavage (0, 1250, and 2500 mg/kg body weight) for five days/week for up to 78 weeks with a scheduled sacrifice at 92 weeks. However, only 14 percent of the animals survived to 78 weeks and less than 1 percent survived to 92 weeks, compared with 66 percent of the controls surviving the 92-week experiment. Hepatocellular carcinomas were found in almost every treated animal (Table 2). Carcinomas were observed as early as 16 weeks in the low-dose female group. The high mortality and tumor response rate limit the usefulness of this study for use in quantitative risk assessment.

Table 2. Carbon tetrachloride-induced liver tumor incidence in mice

Study	Strain	Dose (mg/kg-day)	Tumor incidence
Edwards *et al.*, 1942	strain L (male, female)	0	2/152 (2%)
		2100	34/73 (47%)
NCI, 1976	B6C3F$_1$ (male, female)	0	6/157 (4%)
		1250	89/89 (100%)
		2500	90/93 (97%)

Some additional perspective is gained in studies using mice that carry human genes. Hepatic tumors were generated in mice by repeated administration of carbon tetrachloride. Eight transgenic mice carrying a human c-H-ras proto-oncogene (rasH2 line) and 9 non- transgenic mice were killed and examined at 20 weeks. Transgenic mice developed more tumors than did non- transgenic littermates. Most tumors were neoplastic nodules, but one hepatocellular carcinoma was found in a transgenic mouse at 20 weeks. Three transgenic and 2 non- transgenic mice were kept without further administration of carbon tetrachloride. Two transgenic mice died at 30 weeks of hepatocellular carcinoma, and one transgenic mouse developed hepatocellular carcinoma with a mesenteric metastasis at 32 weeks. No hepatocellular carcinoma was found in two non- transgenic mice at 32 weeks. These results showed that the human c-H-ras gene

facilitates malignant transformation of hepatocytes under repeated administration of carbon tetrachloride (Tsunematsu *et al.*, 1994).

Rat Studies

Several studies reported the production of malignant tumors in rats following subcutaneous injections and oral administration of carbon tetrachloride. Tumor production in rats has been demonstrated in at least four strains, and in both sexes.

Reuber and Glover (1967) injected different age groups of Buffalo rats subcutaneously twice per week for up to 12 weeks. Each group contained 10-14 rats of each sex. Control animals were given corn oil. The animals were 0.6, 4, 12, 24 or 52 weeks old at the beginning of the study. Newborn rats died in approximately eight days due to hepatic and renal necrosis. Animals were sacrificed and necropsied following exposure at 12 weeks. They reported hepatic hyperplasia, hyperplastic nodules and a few cases of hepatic carcinoma, with the number and size of the hyperplastic lesions being greater in the female rather than the male rat. The 24- and 52-week-old rats of both sexes developed more hyperplastic hepatic nodules, as well as some early carcinoma of the liver, than did rats of younger ages (Reuber and Glover, 1967). (Please see Table 3.)

In a later study, Reuber and Glover (1970) compared the carcinogenicity of carbon tetrachloride in 12-week-old male rats in different strains (Japanese, Osborne-Mendel, Wistar, Black, and Sprague-Dawley strains). Each group contained 12-17 male rats of each strain. The animals were subcutaneously injected (0, 2080 mg/kg-day body weight) twice per week for up to 105 weeks. Corn oil was administered to controls. All the Black and Sprague-Dawley strains died within 18 weeks. No carcinomas were observed. Hyperplastic nodules and hepatic carcinoma were reported in three other strains (Japanese, Osborne-Mendel and Wistar). (Please see Table 3.) Other lesions reported were hemangiomas (13 percent and 8 percent for Japanese and Osborne-Mendel rats, respectively), carcinomas of the thyroid gland (20 percent and 23 percent for Japanese and Osborne-Mendel rats, respectively), and subcutaneous leiomyosarcoma (7 percent in Japanese rats). Cirrhosis was reported in all animals. Due to the small group size, poor survival of several strains and the incomplete reporting of the total dosage, and most important, the route of exposure (subcutaneous injections), this study was not used in a quantitative risk assessment (Reuber and Glover, 1970).

As in the mouse studies, NCI used carbon tetrachloride as a positive control in rat bioassays of chloroform, 1,1,1-trichloroethane and trichloroethylene (NCI, 1976a,b). The Osborne-Mendel rats were administered a time-weighted average dose of carbon tetrachloride by gavage for 78 weeks (47, 97 and 80, 159 mg/kg body weight, respectively for males and females). Hepatic carcinomas were found at both doses in both sexes. (Please see Table 3.) A lower incidence was reported in the high-dose females, but this may have been a result of that group's high mortality rate prior to tumor development. Tumors in other tissues were not discussed (NCI, 1976b; NCI, 1976a).

Hamster study

Male and female Syrian golden hamsters were administered carbon tetrachloride in corn oil weekly by gavage (190 and 380 mg/kg of body weight, respectively) for 30 weeks (Della Porta *et al.*, 1961). Following treatment, the animals were kept for 25 weeks, terminated and examined. Only eight of the original 20 animals survived the full 55 weeks. Carcinomas were not observed in the animals that died prior to the 43rd week, but one or more liver cell carcinomas were reported in all the surviving animals, indicating that tumors may be produced at lower levels in this species. Liver tumor incidence in carbon tetrachloride-treated animals (males and females

combined) was 10/19 (53 percent) and 0/80 (0 percent) in controls (Della Porta *et al.*, 1961). High mortality rate and low number of animals tested were weaknesses in this study.

Table 3: Carbon tetrachloride-induced liver tumor incidence in rats

Study	Strain	Route	Dose (mg/kg-day)		Tumor incidence	
			M	F	M	F
Reuber and Glover (1967)	Buffalo	s.c. injection	0 2060	0 2060	0 12%[1]	0 40%[1]
Reuber and Glover (1970)	Japanese	s.c. injection	0 2080		0 20%[1] 80%[2]	
Reuber and Glover (1970)	Osborne-Mendel	s.c. injection	0 2080		0 31%[1] 62%[2]	
NCI (1976a,b)	Osborne-Mendel	Oral gavage	47 97	80 159	4%[2] 4%[2]	8%[2] 2%[2]

(Reuber and Glover, 1967; Reuber and Glover, 1970; NCI, 1976b; NCI, 1976a)

[1]Hyperplastic nodule
[2] Hepatic carcinoma

In summary, carbon tetrachloride has been shown to produce liver tumors in mice, rats, and hamsters by the oral and subcutaneous routes. Subcutaneous injection studies in rats also produce tumors at other sites (hemangiomas, leiomyosarcomas, and thyroid tumors). No inhalation cancer bioassays of carbon tetrachloride have been conducted.

Toxicological Effects in Humans

Acute Toxicity

The immediate effect of acute carbon tetrachloride exposure by all routes is central nervous system depression and frequently gastrointestinal effects such as nausea and vomiting. Individuals who survive carbon tetrachloride central nervous system (CNS) depression may yet become ill or die from hepatic or renal injury. Adverse effects to other organs are likely to be secondary to CNS, liver, or kidney damage (ATSDR, 1992).

Carbon tetrachloride is hepatotoxic in humans and the effects appear rapidly. In humans, alterations in lipid metabolism in the liver may be observed 30 minutes following administration. Histological changes may be observed within one hour. Centrilobular necrosis and hepatic steatosis (fatty degeneration) are characteristic toxic lesions. Hepatic effects usually manifest after CNS effects subside, occurring one to four days following acute exposure. Biological indicators of injury may include altered enzyme levels, such as increased serum glutamic oxaloacetic transaminase (SGOT), alkaline phosphatase, and aspartate amino transferase (DHS, 1987; ATSDR, 1992).

The kidney is also a major target of carbon tetrachloride toxicity. Characteristic renal injuries from carbon tetrachloride exposure are nephritis, nephrosis, and renal failure. Oliguria or anuria may develop a few days following exposure, accompanied by azotemia, proteinuria, hemoglobinuria, and glucosuria resulting in hypertension and acidosis and pulmonary edema. Renal failure closely follows hepatic damage and typically reaches a peak in the second week following exposure (ATSDR, 1992; DHS, 1987).

Fatal cases of carbon tetrachloride intoxication have been reported at oral exposure levels as low as 1.5 ml in adults and 0.18-0.92 ml in children (Lamson *et al.*, 1928). The histological characteristics of carbon tetrachloride liver injury in humans are similar to those observed in laboratory animals. These include centrilobular necrosis with hemorrhage. The initial necrosis is followed by the appearance of polymorphonucleocytes, then infiltration by macrophages and lymphocytes, and finally, regeneration of hepatic tissue from the surviving periportal cells (DHS, 1987).

There is an increased risk of harmful effects associated with acute carbon tetrachloride intoxication among those with a history of chronic, or recent, alcohol consumption or poorly-controlled diabetes (ATSDR, 1992). Death can occur two to 13 days following carbon tetrachloride ingestion, with symptoms including nausea, vomiting, jaundice, kidney failure, cyanosis, and pulmonary edema (Delic *et al.*, 1994).

Developmental and Reproductive Toxicity

Dowty *et al.* (1976) reported that carbon tetrachloride (as well as other halogenated hydrocarbons) could cross the human placenta and accumulate in the fetus. Blood samples were recovered from the umbilical cord and from paired maternal blood of 11 women after vaginal delivery. The authors indicated that exposure to carbon tetrachloride and other halogenated hydrocarbons may have occurred in drinking water. Carbon tetrachloride levels were higher in cord blood than in the maternal blood (Dowty *et al.*, 1976).

Bove *et al.* (1995) examined birth outcomes associated with carbon tetrachloride in drinking water supplied to 75 towns in northern New Jersey from 1985-1988. Odds ratios of greater than or equal to 1.50 were found for the association with the birth outcomes described in the following table. The authors stated, however, that the study could not, by itself, resolve whether the drinking water contaminants actually caused the adverse birth outcomes (Bove *et al.*, 1995).

Table 4. Positive associations of adverse birth outcomes with drinking water exposure to carbon tetrachloride (>1 ppb)

Birth outcome	Odds ratio	50% confidence interval
Low birth weight (term births)	2.26	1.92-2.66
Small for gestational age births	1.75	1.54-1.98
Very low birth weight	1.66	1.22-2.26
Fetal deaths	1.36	0.77-2.33
All surveillance birth defects	1.54	1.09-2.12
CNS defects	3.80	2.05-6.42
Neural tube defects	5.39	2.30-10.52

Table adapted from Bove *et al.*, 1995

Immunotoxicity

No information could be found regarding immunotoxicity of carbon tetrachloride to humans.

Neurotoxicity

Limited information exists on neurotoxic effects of carbon tetrachloride to humans. Carbon tetrachloride produces a rapid narcotic effect in the brain. Immediate fatalities can result from respiratory depression or cardiac arrhythmias. Autopsies have revealed nerve damage including focal areas of fatty degeneration and demyelination (ATSDR, 1992).

Juntunen *et al.* (1980) reported neurotoxic effects in two humans exposed accidentally to "high" concentrations of carbon tetrachloride for three days, as well as having "low grade" exposure for several years. Neuropathy was described as slight in one patient and clinically apparent in the other. Much of the apparent neurotoxicity of carbon tetrachloride may be related to its hepatotoxic properties (Juntunen *et al.*, 1980).

Carcinogenicity

Human case reports have not provided sufficient information to infer any conclusions about a causal association between carbon tetrachloride exposure and cancer in humans. Epidemiological studies have provided some suggestive associations, however. Accordingly, carbon tetrachloride is considered by the IARC to be an animal carcinogen and a potential human carcinogen (IARC, 1979).

Four excess cases of lymphoma were reported in a study on the residents in a rural valley polluted by vapors from a solvent recovery plant for about ten years. Chloroform, benzene, methyl isobutyl ketone, trichloroethylene, and 26 other organic agents were detected in the air in addition to carbon tetrachloride (Capurro, 1973; Capurro, 1979). Attributing these cancer cases to carbon tetrachloride alone would be inappropriate due to the potential confounding effects from the exposure to the other airborne contaminants.

In a preliminary study of 330 laundry and dry cleaning workers, Blair *et al.* (1979) examined occupational exposure to carbon tetrachloride and other dry cleaning agents. Information from death certificates indicated an excess of deaths from lung, cervical and liver cancer, and

leukemia. Katz and Jowett (1981) studied female laundry and dry cleaning workers in Wisconsin. Their results failed to show an overall increase in malignant neoplasms, but they did report an elevated risk for unspecified cancers of the kidney and genitals along with smaller excesses of bladder and skin cancer and lymphosarcoma. Quantitative data on exposure to carbon tetrachloride were not presented in these studies (Blair *et al.*, 1979; Katz and Jowett, 1981). Carbon tetrachloride is no longer used in dry cleaning (U.S. EPA, 1995).

Wilcosky *et al.* (1984) examined cancer mortality in rubber industry workers. The authors ascertained age-adjusted odds ratios of lymphatic leukemia (15.3, $p<0.001$) and lymphosarcoma (4.2, $p<0.05$) related to carbon tetrachloride exposure. These associations were derived from a 6678 member cohort case control analysis of tire plant workers. From this cohort, a 20 percent age stratified sample was taken for the examination of histories of those who died between 1964-1973 and comparison to control histories.

Heineman *et al.* (1994) applied a job exposure matrix for six chlorinated solvents, including carbon tetrachloride, to a case-control study of astrocytic brain cancer in 741 white men from petroleum refining and industrial production industries. An equal number of controls were randomly selected from white male residents who died of causes other than brain tumor, cerebrovascular disease, epilepsy, suicide, or homicide. The authors observed an association of astrocytic brain cancer with likely occupation exposure (at medium probability) to carbon tetrachloride. At medium exposure probability, odds ratios were 2.1 (0.4-11.0, 95 percent confidence interval) and 7.5 (0.9-16.9, 95 percent confidence interval) for respective exposure durations of three to 20 years and 21+ years. The risks increased significantly with increasing duration and cumulative exposure. The authors caution that the positive association of carbon tetrachloride exposure to brain cancer may have been confounded by simultaneous exposure to other carcinogenic chemicals.

Cantor *et al.* (1995) examined occupational exposures to several industrial chemicals, including carbon tetrachloride, and resulting influence on female breast cancer mortality. In the case control study, which excluded homemakers, the authors used a job exposure matrix to estimate probability and level of workplace exposure to carbon tetrachloride, in addition to other chemicals. The authors evaluated 33,509 cases and 117,794 controls using mortality records from 24 states for the years 1984-1989. After adjusting for socioeconomic status, suggestive associations for probability and level of exposure were found for carbon tetrachloride occupational exposures as well as for the other industrial chemicals. In order to investigate if breast cancer results differed by race, the authors performed separate analyses for data on black and white women. Estimated exposures to carbon tetrachloride were suggestively associated with risk of breast cancer mortality in several analyses. Exposure levels were determined in the job exposure matrix based on professional judgement. At the medium exposure level, odds ratios (with 95 percent confidence intervals not included) for all probability levels were 1.15 (white) and 1.32 (black). For white women, excluding women with low exposure probability, the odds ratios (with 95 percent confidence intervals not included) was 1.16. At the high exposure level, for white women, odds ratios in were 1.21 and 1.23 (for all probability v. low probability excluded groups, respectively), while the 95 percent odds ratio confidence interval included 1.0 for black women.

Although the carcinogenicity of carbon tetrachloride in laboratory animals is well established, the human data reveal at most, a suggestive relationship between carbon tetrachloride exposure and the occurrence of cancer in humans. Data from case reports of human exposure as well as epidemiological studies in occupational settings are largely inadequate due to the concurrent exposure to other chemicals (CPHF, 1988). IARC found that the case reports of liver cancer in humans are “suggestive”; and along with the sufficient evidence for carcinogenicity in

experimental animals, it is reasonable to regard carbon tetrachloride as if it presents a carcinogenic risk to humans (IARC, 1979). U.S. EPA has classified carbon tetrachloride as a group B2, probable human carcinogen of low carcinogenic hazard, based largely on studies in animals relating ingestion of carbon tetrachloride to liver cancer (U.S. EPA, 1985; IRIS, 1998).

DOSE-RESPONSE ASSESSMENT

Noncarcinogenic Effects

Numerous reports have identified adverse noncancer effects in humans resulting from exposure to carbon tetrachloride; however, these reports do not provide adequate quantitative exposure estimates to establish a dose-response relationship.

Of the studies conducted on experimental animals, the most relevant for the purpose of calculating a health protective concentration in drinking water is the study by Bruckner *et al.* (1986). In this report, the authors studied acute and subchronic toxicity of carbon tetrachloride in rats. In the subchronic portion of the study, male rats were given daily doses of carbon tetrachloride (five days per week for 12 weeks) via gavage at dosage levels of 0, 1, 10, and 33 mg/kg-day. The animals were observed to produce slight, but statistically significant, increases in sorbitol dehydrogenase activity as well as mild hepatic centrilobular vacuolization at the 10 mg/kg level which is the LOAEL for the study, but produced no apparent adverse effects at the 1 mg/kg dose (NOAEL) level. Importantly, observed effects occurred in the liver which is the target organ for carbon tetrachloride. On the basis of this subchronic study, 1 mg/kg is selected as the NOAEL for the calculation of the noncancer health-protective value. The LOAEL and NOAEL values were further modified by a factor of 5/7 to account for a dosing regimen of five days per week. There was no suitable chronic study on which to base a LOAEL or NOAEL.

Carcinogenic Effects

Carbon tetrachloride has been observed to induce liver tumors in male and female hamsters, mice, and rats, as described previously. U.S. EPA (1984) used the linearized multistage model in deriving risk estimates from the bioassay by Della Porta *et al.* (1961) in hamsters, the Edwards *et al.* (1942) study in mice and the NCI (1967a, b) in mice and rats. Carbon tetrachloride was administered by the oral route in all four of these studies. The upper 95 percent confidence bounds on cancer potencies ranged from 0.011 $(mg/kg\text{-}day)^{-1}$ to 1.2 $(mg/kg\text{-}day)^{-1}$, with a geometric mean value of 0.13 $(mg/kg\text{-}day)^{-1}$. Due to the deficiencies in these studies, U.S. EPA chose to combine the results as the geometric mean value for the oral slope factor of carbon tetrachloride (U.S. EPA, 1984).

The California Department of Health Services (DHS) used the same methods to estimate the cancer potencies as did U.S. EPA, but differed in their assumptions and source data to evaluate inhalation potency. DHS did not use the NCI rat study since it did not produce an unequivocally positive response once the tumor incidence data were adjusted for early mortality (DHS, 1987). DHS also considered the Della Porta *et al.* data inappropriate for dose response assessment due to the high mortality among the treated animals, 100 percent tumor incidence and small numbers of animals. In contrast to U.S. EPA, DHS (1987) assumed 50 percent rather than 40 percent absorption of inhaled carbon tetrachloride. DHS (1987) recommended a range of cancer potencies of 0.001 to 0.042 $(mg/m^3)^{-1}$ rather than a single figure or a mean value.

Using the same techniques, the California Public Health Foundation (CPHF) (1988) under contract with DHS re-analyzed all the studies considered by U.S. EPA. Because the study by Edwards *et al.* (1942) was relatively short, a correction factor was applied to the estimate of lifetime potency from oral exposure. The 95 percent upper confidence limits for cancer potency calculated by CPHF (1988) are shown in Table 5.

Table 5. Upper 95 percent confidence level estimates of the animal oral cancer potency of carbon tetrachloride*

Study	Sex	Tumors	Oral Potency $(mg/kg\text{-}day)^{-1}$
Edwards *et al.*, 1942	m/f mouse	Hepatoma	0.18
Della Porta *et al.*, 1962	m/f hamster	Hepatoma	8.85
NCI, 1976	m rat	Hepatocellular carcinoma	0.010
	m rat	Liver combined	0.016
	f rat	Hepatocellular carcinoma	0.008
	f rat	Liver combined	0.011
NCI, 1976	m mouse	Hepatocellular carcinoma	0.16[a]
	f mouse	Hepatocellular carcinoma	0.16[a]

[a] Highest dose group removed due to lack of fit. The model cannot be fitted due to 100 percent response at the remaining low dose group.
* A linearized multistage model was used for high-to-low dose extrapolation.
Source: CPHF (1988)

As described earlier, Edwards *et al.* (1942) treated 54 male and 19 female L mice with 0.1 ml of 40 percent carbon tetrachloride solution in corn oil via intubation two or three times per week over four months, for a total of 46 treatments. The time-weighted average dose over the average experimental duration of 7.25 months was calculated to be 447 mg/kg-day. Animals were killed 3 to 3.5 months after the last treatment. The combined hepatoma incidence of treated male and female mice was 47 percent (34/73 vs. 2/152 in the untreated controls). The authors described the appearance of the hepatomas as typically multiple, appearing as gray or grayish-yellow bulging nodules ranging from 2 to 15 millimeters in diameter. Microscopically, the tumors were not encapsulated, but not invasive, with adjacent hepatic tissue compressed by their rounded borders. There were no metastases (Edwards *et al.*, 1942).

CALCULATION OF PHG

Calculations of concentrations of chemical contaminants in drinking water associated with negligible risks for carcinogens or noncancer toxicants must take into account the toxicity of the chemical itself, as well as the potential exposure of individuals using the water. Tap water is used directly as drinking water, and for preparing foods and beverages. It is also used for bathing or showering, and in washing, flushing toilets and other household uses resulting in potential dermal and inhalation exposures.

Noncarcinogenic Effects

The calculation of the public health-protective concentration (C, in mg/L) for carbon tetrachloride follows a general formula for noncancer endpoints:

$$C = \frac{\text{NOAEL x RSC x BW}}{\text{UF x L/day}} = \text{C in mg/L}$$

where,

NOAEL	=	no-observed-adverse-effect-level (1 mg/kg-day x 5/7) as the absence of mild hepatic centrilobular vacuolization,
RSC	=	relative source contribution of 40 percent (0.4). The range is 0.2-0.8, however volatile chemicals such as carbon tetrachloride are less likely to be found in food and soil,
BW	=	body weight for an adult male (70 kg),
UF	=	uncertainty factor of 1000 (10-fold for inter-species variation, 10-fold for human variability, 10-fold to account for the use of a subchronic study for determining a lifetime value), and
L/day	=	volume of drinking water consumed by an adult (4 L/day). The default is 2 L/d, however the higher value accounts for additional inhalation exposure from various uses of drinking water, such as bathing.

Therefore,

$$C = \frac{\text{5/7 mg/kg-day x 0.4 x 70 kg}}{\text{1000 x 4 L/day}} = 0.005 \text{ mg/L} = 5.0 \ \mu\text{g/L} = 5 \text{ ppb}$$

The health-protective concentration of carbon tetrachloride for noncancer health effect in water is 5.0 µg/L (5 ppb).

Carcinogenic Effects

$$C = \frac{R \times BW}{q_1^* \times L/day} = \text{PHG in mg/L}$$

where,

BW	=	body weight for an adult male (70 kg),
R	=	*de minimis* level for lifetime excess individual cancer risk (a default of 10^{-6}),
q_1*	=	q_1* is the upper 95 percent confidence limit on the cancer potency slope calculated by the linearized multistage model. This is the oral potency slope. The q_1* for carbon tetrachloride is 1.8×10^{-1}(mg/kg-day)$^{-1}$, and

L/day = volume of drinking water consumed by an adult (4 L/day). The default is 2 L/day, however the higher value accounts for additional inhalation exposure from various uses of drinking water such as bathing.

Therefore,

$$C = \frac{(1 \times 10^{-6}) \times 70 \text{ kg}}{(1.8 \times 10^{-1}) \times 4 \text{ L/day}} = 0.1 \ \mu\text{g/L} = 0.1 \text{ ppb}$$

The calculation for a health protective concentration for carbon tetrachloride based on its carcinogenicity uses the cancer slope factor adopted by DHS. In 1988, DHS determined that the appropriate study upon which to derive the *de minimis* drinking water concentration was that of Edwards *et al.* (1942), although the reference was indirect (DHS, 1988a). Also in 1988, DHS recommended the potency value of 0.18 $(\text{mg/kg-day})^{-1}$, from the analysis of Edwards *et al.* (1942) data performed by CPHF (1988) to establish a risk-specific intake level for carbon tetrachloride ingestion exposure for Proposition 65 (DHS, 1988b). In a review of the carcinogenic potency for the Standards and Criteria Workgroup, the Reproductive and Cancer Hazard Assessment Section (RCHAS) of DHS (now in OEHHA) concurred that the potency value of 0.18 $(\text{mg/kg-day})^{-1}$ derived by CPHF (1988) from the analysis of Edwards *et al.* (1942), was the appropriate potency value for the ingestion route of exposure (RCHAS, 1991). Calculation of a PHG from this potency results in the same value of 0.1 ppb (rounded to one significant figure) for a 10^{-6} risk level as would result from use of the U.S. EPA geometric mean potency value (U.S. EPA, 1989).

Based on all the above factors, and considering both noncancer and cancer endpoints, the PHG value for carbon tetrachloride is 0.1 ppb.

RISK CHARACTERIZATION

The primary sources of uncertainty regarding the development of the PHG for carbon tetrachloride are the limitations of the experimental animal bioassays that form the basis of the risk calculation. Della Porta *et al.* (1961) and Edwards *et al.* (1942) only tested one dose level, and Della Porta *et al.* (1961) did not report concurrent control incidence. In the NCI mouse (1976a,b) studies, tumor incidence was nearly 100 percent, precluding the goodness-of-fit criteria for the multistage model. In the NCI rat study, tumor incidence was highest in the low dose group (IRIS, 1998).

To compare approaches in risk assessment using data derived from limited studies, the U.S. EPA Carcinogen Assessment Group used data from the Edwards *et al.* (1942) (mouse), Della Porta *et al.* (1961) (hamster), and NCI (1976) (rat and mouse) studies for their risk assessment. U.S. EPA recognized that the studies used were each deficient in some respect for dose-response assessment purposes, precluding the choice of any one study as most appropriate. Accordingly, U.S. EPA calculated the geometric mean of the upper limit unit risk estimates (3.7×10^{-6}) from four data sets, and selected this mean as the unit risk corresponding to drinking water containing 1 µg/L. U.S. EPA assumed a human water consumption of 2 L/d and a human body weight of 70 kg to derive a slope factor of 1.3×10^{-1} $(\text{mg/kg-d})^{-1}$ from the above unit risk (U.S. EPA, 1984; U.S. EPA, 1989).

The use of the geometric mean of the unit risks in this case was not adopted by DHS (DHS, 1987). DHS selected the study by Edwards *et al.* (1942) (mouse) as the principal study upon which to base the state maximum contaminant level (MCL), although the information supporting the rationale used for selection is limited (DHS, 1988a). We have concurred with this decision.

The PHG of 0.1 ppb is based on a carcinogenic potency of 1.8×10^{-1} (mg/kg-day)$^{-1}$. Using the U.S. EPA potency value of 1.3×10^{-1} (mg/kg-day)$^{-1}$ would result in the same potency value (rounded to one significant figure). In calculating the proposed PHG, a *de minimis* theoretical excess individual cancer risk of 1×10^{-6} was used. The corresponding drinking water concentrations for cancer risk levels of 1×10^{-5} or 1×10^{-4} are 1 ppb and 10 ppb, respectively.

OTHER REGULATORY STANDARDS

California's current drinking water standard for carbon tetrachloride is 0.5 ppb. DHS adopted this standard, referred to as the state maximum contaminant level (MCL), in 1988. The current U.S. EPA MCL for carbon tetrachloride is 5 µg/L (U.S. EPA, 1998). The state and federal MCLs are based on the carcinogenic potential of carbon tetrachloride. The U.S. EPA combined the upper limit risk estimates from several studies (each considered separately insufficient) to arrive at a geometric mean upper limit risk estimate (U.S. EPA, 1984; U.S. EPA, 1989). DHS considered the mouse study of Edwards *et al.* (1942) to be the most appropriate study upon which to base the MCL (DHS, 1988a). OEHHA also used the Edwards (1942) study in the calculation of the PHG for carbon tetrachloride. More detailed information is provided above, as described under Risk Characterization.

U.S. EPA has classified carbon tetrachloride as a group B2 (probable human) carcinogen. In California, carbon tetrachloride is listed under the Safe Drinking Water and Toxic Enforcement Act of 1986 (Proposition 65) as a chemical known to the state to cause cancer. The following table includes selected national and state regulations and guidelines for comparison to the recommended PHG.

Table 6. Selected Guidelines and Regulations for Carbon Tetrachloride

Agency	Standard or Criterion	Level	Comment
DHS	MCL (maximum contaminant level)	0.5 µg/L	based on limit of detection
ATSDR	oral MRL (minimum risk level), intermediate duration	0.007 mg/kg-d	based on absence of observed hepatic effects
NIOSH	REL (recommended exposure level) in air	2 ppm	recommended occupational inhalation level (60 min)
OSHA	PEL (permissible exposure limit) in air	10 ppm	occupational inhalation level
U.S. EPA	MCL (maximum contaminant level)	5 µg/L	national drinking water standard
U.S. EPA	MCLG (maximum contaminant level goal)	0 mg/L	drinking water goal, includes safety margin
U.S. EPA	DWEL (drinking water equivalent level)	2.5×10^{-2} mg/L	noncancer, lifetime exposure
U.S. EPA	Lifetime Health Advisory	2.5 µg/L	lifetime, in drinking water
ACGIH	TLV-TWA (threshold limit value-time weighted average)	5 ppm	occupational inhalation
OEHHA	Proposition 65, no significant risk level	5 µg/d	Prop-65 levels are set to a *de minimis* risk level of 1×10^{-5}

Table adapted from (Faroon *et al.*, 1994; NIOSH, 1994; DHS, 1988a)

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