DRAFT

For Review Only

Public Health Goal for

1,1,2,2-TETRACHLOROETHANE

in Drinking Water

NOT A REGULATORY STANDARD

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PREFACE

Drinking Water Public Health Goals

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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 publish 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 published 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 1,1,2,2-TETRACHLOROETHANE IN DRINKING WATER

SUMMARY

The Office of Environmental Health Hazard Assessment (OEHHA) has developed a proposed Public Health Goal (PHG) of 0.0001 mg/L (0.1 µg/L or 0.1 ppb) for 1,1,2,2-tetrachloroethane (TCA) in drinking water. The current California MCL is 1 ppb for TCA in drinking water.

The proposed PHG is based on hepatocellular carcinoma observed in females in a carcinogenicity study in $B6C3F_1$ mice (50/dose) fed TCA in corn oil by gavage at an average daily dose of 88 or 175 mg/kg-day for 90 weeks. Using the LED_{10} approach and linear dose response extrapolation, an animal carcinogenic slope factor of 0.014 $(mg/kg\text{-}day)^{-1}$ is estimated. Upon applying an interspecies scaling factor and an adjustment factor for the less-than-lifetime exposure to the animal carcinogenic slope factor, a human carcinogenic slope factor of 0.15 $(mg/kg\text{-}day)^{-1}$ is estimated. The proposed PHG is calculated using the estimated human carcinogenic slope factor, a risk level of one in a million, and an assumed adult body weight of 70 kg. Water consumption rate is assumed equivalent to 4 L/day, based on direct consumption of 2 L/day and additional exposures by inhalation and dermal routes from other household uses of water.

In both male and female $B6C3F_1$ mice, significantly higher incidences of hepatocellular carcinomas were found in both dose groups, compared with the controls (NCI, 1978). The trends of tumor incidence are also statistically significant in both sexes. In the same study series, NCI (1978) reported that there was no evidence to indicate orally administered TCA was carcinogenic to male and female Osborne-Mendel rats. Since the evidence for carcinogenicity in animals is restricted to one species, and the information from humans was inconclusive, TCA has been classified as a Group C carcinogen, "possible human carcinogen," by the U.S. EPA (1994). The chemical has also been classified as a Group 3 carcinogen, "not classifiable as to its carcinogenicity to humans," by IARC (1999). However, the chemical is listed as "a chemical known to the State of California to cause cancer," and for this reason the PHG was developed based on its cancer potency.

The exact mechanism of liver tumor formation in male and female mice exposed to TCA is not known. Studies on the ability of TCA to induce gene mutations in prokaryotic systems, with and without metabolic activation, have yielded equivocal results. In mammalian cells, TCA added *in vitro* induced sister chromatid exchanges and caused transformation but not chromosomal aberrations, DNA repair, or unscheduled DNA synthesis (Galloway *et al*., 1987; Colacci *et al*., 1992). Following interperitoneal injection of TCA, free radicals and DNA adducts could be detected in the liver of the test animals (Paolini *et al*., 1992; Colacci *et al*., 1987).

The most sensitive endpoints for non-carcinogenic effects are increases in liver fat content, transient decreased body weight gains, and decreased pituitary ACTH activity in rats chronically exposed to TCA at 1.9 ppm in air (Schmidt *et al*., 1972). Assuming a

body weight of 0.4 kg, a net inhalation absorption of 50 percent, and an inhalation rate of 0.0167 m^3/hr (U.S. EPA, 1988), the lowest-observed-adverse-effect level (LOAEL) associated with these effects is estimated to be 1.1 mg/kg-day. Based on this level, the non-cancer health protective concentration is estimated to be 15 µg /L (15 ppb).

INTRODUCTION

In the past, TCA was used in the synthesis of trichloroethylene from acetylene. It was also used as a solvent for metal cleaning, as an oil extractant, and as an insecticide and herbicide. However, TCA is no longer widely used and has been replaced by alternative compounds (HSDB, 1999, ATSDR, 1996).

The current California MCL is 1 ppb for 1,1,2,2-tetrachloroethane (TCA) in drinking water. This level is based on the changes in liver fat content and transient body weight depression observed in rats exposed to TCA via inhalation (Schmidt *et al.*, 1972). TCA is also listed under Proposition 65 as a chemical that is known to the State of California to cause cancer (OEHHA, 2000). There is no federal MCL for TCA (U.S. EPA, 2002).

CHEMICAL PROFILE

Chemical Identity

The chemical formula for TCA and other chemical information is listed in Table 1.

Table 1. Chemical Identity of TCA (from HSDB, 1999)

Property	Value or information
Chemical name	1,1,2,2-tetrachloroethane
Synonyms	Acetylene tetrachloride;sym-tetrachloroethane; s-tetrachloroethane; tetrachloroethane; 1,1-dichloro-2,2-dichloroethane
Molecular formula	$C_2H_2Cl_4$
CAS registry number	79-34-5
RTECS registry number	NIOSH/KI8575000

Physical and Chemical Properties

TCA is a halogenated two-carbon alkane with four chlorine atoms. It is a colorless to pale-yellow liquid at room temperature. It is somewhat soluble in water (2,900 mg/L at 20°C), and is miscible with many organic solvents, including ethanol, methanol, ether, acetone, benzene, petroleum ether, carbon tetrachloride, chloroform, carbon disulfide, dimethylformamide, and oils (HSDB, 1999). Some of the physical and chemical

properties of TCA are listed in Table 2. It is considered to have the "highest solvent power" of the aliphatic chlorinated hydrocarbons (ACGIH, 1998).

Table 2. Physical and Chemical Properties of TCA (from HSDB, 1999)

Property	Value or Information
Molecular Weight	167.85
Color/Physical State	colorless to pale-yellow liquid
Odor	sweetish, suffocating, pungent, and chloroform-like
Odor Threshold	in water, 0.5 ppm (Amoore and Hautala, 1983; HSDB, 1999) in air, 1.5 ppm (Amoore and Hautala, 1983), 3 to 5 ppm (HSDB, 1999)
Conversion factors	1 ppm = 6.98 mg/m^3 at 20°C 1 mg/m^3 = 0.14 ppm at 20°C
Melting Point	-43.8°C
Boiling Point	145.1°C
Vapor Pressure	5.95 mm Hg at 25°C (Riddick *et al*., 1986) 9 mm Hg at 30°C (HSDB, 1999)
Flash Point	none, non-flammable
Solubility in water	2.86 g/L at 25°C (Merck, 1989) 2.87 g/L at 20°C (Riddick *et al*., 1986)
Log K_{OW}	2.39 (Hansch and Leo, 1985)
Henry's Law constant	4.55x10^{-4} atm-m^3/mole at 25°C (HSDB, 1999)
Density	1.594 g/ml at 20°C (Riddick *et al*., 1986)

Production and Uses

TCA can be produced by catalytic addition of chlorine to acetylene. It can also be produced by chlorination of either ethylene or 1,2-dichloroethane. Additionally, it can be produced by the catalytic chlorination of ethane. Production levels of TCA could not be obtained, but imports were estimated to be 134,000 pounds in 1985 (HSDB, 1999).

In the past, the major use of TCA was in the production of trichloroethylene, tetrachloroethylene, and dichloroethylene, but all large-scale commercial production of this solvent has now ceased (ATSDR, 1996). TCA was also used as a solvent in the cleaning and degreasing of metals, in paint removers, varnishes and lacquers, in the preparation of photographic films, and as an extractant for oils and fats. At one time, it was also used in soil as a fumigant, as a weed killer, as an insect repellent and as an

insecticide, but these uses are no longer registered and have been replaced by alternative compounds (HSDB, 1999, ATSDR, 1996).

ENVIRONMENTAL OCCURRENCE AND HUMAN EXPOSURE

The general population is potentially exposed to TCA from ambient air or from contaminated drinking water. The 1974 National Occupational Hazard Survey concluded that workers most likely to be exposed were those in industries such as toiletry preparations and electric service industries, the latter exposure stemming from the use of commercial solvent-based cleaners. A NIOSH survey, dated March 29, 1989, estimated that 4,143 workers were potentially exposed to TCA in the USA (HSDB, 1999).

Air

TCA may be released into the atmosphere as a result of its use as a metal degreasing agent, paint, varnish and rust remover, solvent, and as a chemical intermediate. TCA can be emitted from hazardous waste landfills. Based upon a vapor pressure of 6.0 mm Hg at 25°C, TCA is expected to exist mostly in the vapor phase in ambient air. TCA is practically inert in the troposphere with a half-life exceeding 800 days. As such, it can be transported long distances with some of it returning to earth in rain (HSDB, 1999).

One report of atmospheric levels of TCA revealed the following information: in urban/suburban areas, the median atmospheric concentration was 5.4 ppt for 853 U.S. sites; 25 percent of the samples exceeded 8.9 ppt, and the maximum concentration was 4,800 ppt. In this study, no TCA was detected in over 25 percent of the samples (HSDB, 1999). Also, in studies covering major U.S. cities, the concentrations of TCA in air ranged from trace amounts to 57 ppb. In industrial areas, 5 sites had a range from 0 to 0.25 ppb in air. In source-dominated areas, 25 percent of the air samples exceeded 27 ppt, while the maximum was 700 ppt (HSDB, 1999).

The Toxic Release Inventory (TRI, 1998) database reported that approximately 8,300 and 15,500 lbs of TCA were released into the air in the U.S. in 1995 and 1996 respectively. Of this, about 4,900 lbs were fugitive, and 3,400 lbs were stack emissions in 1995. In 1996, about 12,700 lbs were fugitive, and 2,900 lbs were stack emissions. In California, the last record of TCA release was in 1987, when approximately 22,000 lbs were estimated to have been released into the air via fugitive emissions.

Soil

A measured K_{oc} of 46 in a silt loam soil has been reported by Chiou *et al*. (1979). The value suggests that TCA is relatively mobile in soil and therefore can migrate from soil into groundwater. A calculated Henry's Law constant of 4.55×10^{-4} atm-m^3/mole at 25°C suggests volatilization of TCA from moist soils can occur (HSDB, 1999). Due to its moderately high vapor pressure, volatilization of TCA from dry soil will be fairly rapid.

Water

Laboratory measurements of the rate of evaporation of TCA from water gave a half-life of 32 to 56 minutes. In natural waters one would expect a half-life of volatilization on the order of days to weeks depending on mixing conditions. A Henry's Law Constant of 4.55×10^{-4} atm-m^3/mole indicates volatilization of TCA from environmental waters would occur (HSDB, 1999).

TCA was listed as a contaminant found in drinking water in a 1982 survey of U.S. locations including Pomona, Escondido, Lake Tahoe and Orange Co, CA, and Dallas, Washington, DC, Cincinnati, Philadelphia, Miami, New Orleans, Ottumwa, IA, and Seattle (HSDB, 1999). In surface water, traces to <1 ppb were measured in samples from the Ohio River; 1 ppb in the Detroit River, and traces to 1.9 ppb in the Schuylkill River at Philadelphia, PA. TCA was not detected in raw water in 30 Canadian potable drinking facilities in August through September and only one facility reported a detectable amount in November through December, a value of 12 ppb. Only 12 of 204 sites near heavily industrialized areas across the U.S. were positive, with values at positive sites ranging from 1 to 9 ppb. TCA is listed as a contaminant of Lake Erie, Lake Ontario, and the St. Lawrence River. In groundwater, 64 of 1,072 representative sources were positive in New Jersey. TCA was detected, but not quantified, in 10 most polluted wells from a survey of 408 urban wells in New Jersey. Groundwater samples from near the Hooker Chemical and Plastics Corporation disposal site at Love Canal, NY, were found to contain TCA. Six of seven groundwater sample from near the "Valley of Drums," KY, contained TCA at concentrations of 6.4, 18, 12, 5.7, 0.2 and 6.2 ppb (HSDB, 1999).

The Toxic Release Inventory database indicated that 2,200 and 130 lbs of TCA were released via water in 1995 and 1996, respectively, in the U.S. No release to water was noted for California.

Food

Unspecified levels of TCA were detected in volatile flavor constituents of broiled beef. In a separate analysis, the Food and Drug Administration's "market basket" collections were demarcated as fatty and non-fatty fractions at the 20 percent lipid point. The low, high and average TCA concentrations of the fatty and non-fatty food groups were 24, 85 and 58 ng/g, and 8, 89 and 57 ng/g, respectively (HSDB, 1999).

METABOLISM AND PHARMACOKINETICS

Absorption

TCA is readily absorbed after oral, respiratory or dermal exposures, as would be predicted from its relatively low molecular weight and a log octanol/water partition coefficient value of 2.39. Studies that quantify absorption of TCA after oral exposure in humans are not available. However, a number of case-reports of oral intoxications from TCA have been documented, indicating that this chemical readily crosses gastrointestinal

membranes. In studies in rats and mice, 70 to 100 percent of an oral dose was absorbed and excreted (ATSDR, 1996). Morgan *et al*. (1972) exposed human volunteers to [^{38}Cl]-labeled TCA and showed that 97 percent of a single breath of TCA was absorbed systemically. Net inhalation absorption of TCA in longer exposures can be expected to be about 50 percent, after equilibration of TCA in tissues (Raabe *et al*., 1996, 1998; Gargas and Anderson, 1989). TCA is absorbed through intact human skin and at least one human fatality has been attributed to dermal exposure (ACGIH, 1998). TCA, at a volume of up to 1 ml, was absorbed within 30 min after application to the skin of mice or guinea pigs (Jakobson *et al*., 1982).

Distribution

TCA is a two-carbon chlorinated aliphatic alkane. As a group, these chemicals are volatile, lipophilic, of small molecular size, readily cross biological membranes, and rapidly distribute throughout tissue compartments by passive diffusion processes. A common toxic action of this class of chemicals is central nervous system depression. From human experience, it is known that TCA has potent anesthetic activities. The mechanisms underlying anesthesia from organic solvents are attributed to occupancy of molecular sites within neuronal membranes that regulate neurotransmission. In studies on the excretion of halogenated aliphatics in the breath, the partition coefficients of eight chlorinated 2-carbon aliphatics (ethyl chloride, 1,1-dichloroethane, 1,2-dichloroethane, 1,1,1-trichloroethane, 1,1,2-trichloroethane, 1,1,2,2-TCA, 1,1,2-trichloroethylene, and TCA) in olive oil/air and serum/air were measured (Morgan *et al*., 1970). TCA had the highest affinities for olive oil and serum relative to the other compounds. The partition coefficients were directly correlated to anesthetic potencies (measured as ppm to produce loss of righting reflexes in rodents) and inversely correlated to rates of excretion via the breath. These experiments show that the potent anesthetic mechanism of action of TCA is determined by the physicochemical properties of the molecule that confer high affinity for lipophilic phases in the bio-environment. The high affinity for the bio-phase (versus the gaseous phase) also explains the slow excretion and long duration of anesthesia seen in clinical cases of acute poisoning.

Eriksson and Brittebo (1991) administered [^{14}C]-labeled TCA intravenously to mice and characterized tissue distribution by autoradiography. A selective uptake of non-volatile radioactivity occurred in the olfactory and tracheobronchial mucosa and in the mucosa of the oral cavity, tongue, nasopharynx, esophagus and cardiac region of the forestomach. Microautoradiography showed that the ^{14}C-labelling occurred in the epithelial cells lining these tissues. High levels of non-volatile radioactivity were also found in the liver, in contents of the biliary bladder, and in the inner zone of the adrenal cortex. The authors suggested that the tissue binding of TCA resulted from oxidative P-450 dependent processes in tissues that converted TCA to reactive intermediates.

Metabolism

The primary study on the metabolism of TCA was conducted by Yllner (1971) who administered [^{14}C]-TCA interperitoneally to mice at 210 to 320 mg/kg and monitored the

course of elimination of radioactivity over 3 days. Using paper chromatography and isotope dilution analysis, the principal pathways of degradation and excretion were mapped. The metabolism of TCA was found to be quite rapid and only 4 percent was expired unchanged. In 24 hr, 60 to 70 percent of the dose was excreted as various degradation products, mainly carbon dioxide. Metabolites occurring in the urine were found in these proportions: dichloroacetic acid, 27 percent; trichloroacetic acid, 4 percent; trichloroethanol, 10 percent; oxalic acid, 7 percent; glyoxylic acid, 0.9 percent; and urea, 2 percent. A similar rate of metabolism of TCA was found after oral dosages in rats and mice at 25 to 200 mg/kg (Dow, 1988; Mitoma *et al*., 1985 as cited in ATSDR, 1996). Sixty to eighty percent of the administered dose was metabolized and excreted within 48 to 72 hours.

The kinetic constants of TCA metabolism in rats exposed to 350 ppm of the chemical for 6 hours were determined in gas uptake studies (Gargas and Andersen, 1989). The rate of exhalation of TCA was measured and combined with previously published values for partition coefficients for blood/air, liver/blood, muscle/blood, and fat/blood. A kinetic model for TCA predicting a K_m and V_{max} of 4.77 μM and 12 mg/hr (scaled to a 1 kg rat) was reported.

Yllner (1971) proposed the metabolic pathways for TCA shown in Figure 1. TCA is mainly metabolized by (a) a sequential hydrolytic cleavage of carbon-chlorine bonds via dichloroacetic acid (b) oxidation to glyoxylic acid (c), which is further metabolized (l, m, n). An alternative route (d) is non-enzymatic dehydrochlorination to trichloroethylene, which is further metabolized (e, f, g) to trichloroacetic acid and trichloroethanol. Route (h) is oxidation to tetrachloroethylene. Route (i) is cytochrome P-450-dependent oxidation, followed by dehydrohalogenation, to form dichloroacetyl chloride. Route (j) is reductive dechlorination.

Yllner (1971) showed that TCA was converted by dechlorination by non-enzymatic pathways to form trichloroethylene and tetrachloroethylene. These chemical events were shown to occur in phosphate buffer at pH 7 and at 37°C. Others have found evidence that dechlorination can be facilitated by reductive enzymatic pathways utilizing cytochrome P-450 systems (Halpert, 1982). More recent data from an *in vitro* study using rat liver have shown the oxidative conversion of TCA to dichloroacetic acid is dependent on microsomal cytochrome P-450 systems and is the principal pathway for metabolic conversion of TCA to urinary metabolites (Halpert, 1982; Casciola and Ivanetich, 1984).

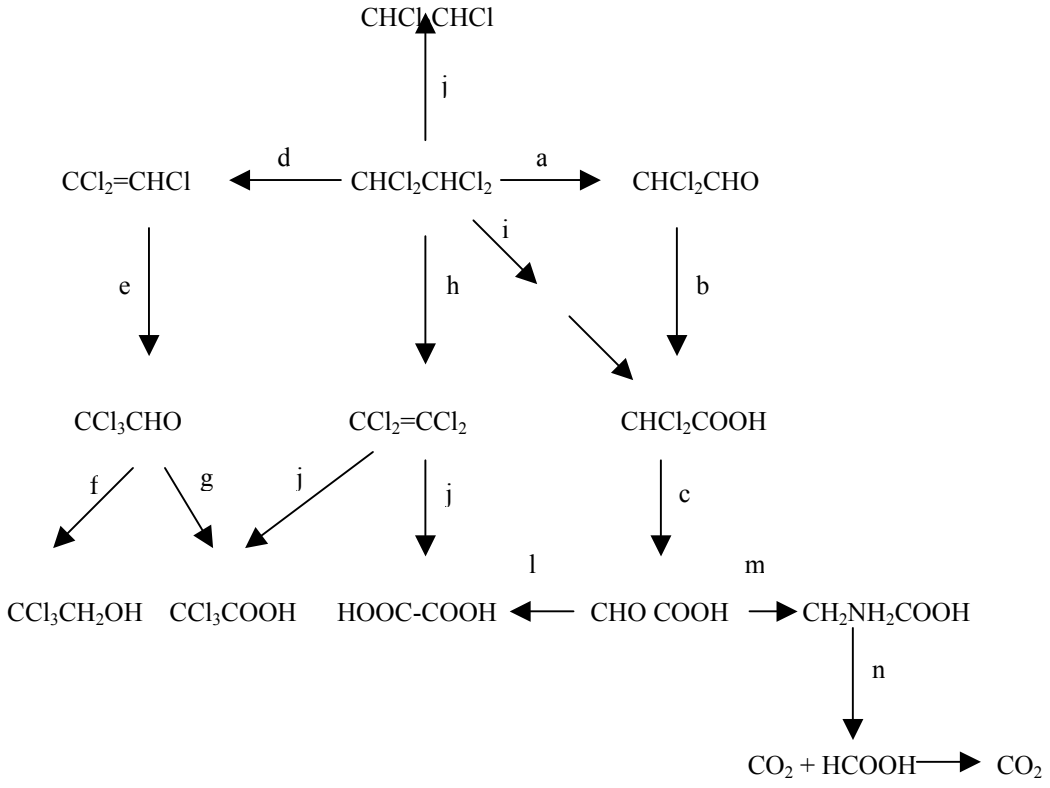

Figure 1. Metabolic Pathways of 1,1,2,2-Tetrachloroethane in Mice.

1,1,2,2-Tetrachloroethane is metabolized (a) by a sequential hydrolytic cleavage of carbon-chlorine bonds via dichloroacetic acid (b) to glyoxylic acid (c), which is further metabolized (l, m, n). An alternative route (d) is non-enzymatic dehydrochlorination to trichloroethylene, which is further metabolized (e, f, g) to trichloroacetic acid and trichloroethanol. Route (h) is oxidation to tetrachloroethylene. Route (i) is the P-450-dependent oxidation, followed by dehydrohalogenation to form dichloroacetyl chloride. Route (j) is reductive dechlorination (from ATSDR, 1996).

The mechanisms underlying the hepatotoxic effects of TCA are linked to the metabolic transformation of this chemical. Many two-carbon chlorinated aliphatics undergo metabolism by tissue enzymes before excretion. These reactions – reduction, oxidation, and conjugation – may generate intermediates that bind to tissue constituents such as proteins and DNA and cause toxicity. The mechanisms of activation and toxicity have been investigated for TCA.

Toxicity via Reductive Pathways. Yllner (1971) showed that TCA was converted by dehydrochlorination to form trichloroethylene and tetrachloroethylene by non-enzymatic pathways. Others have found evidence that dechlorination can be facilitated by reductive enzymatic pathways utilizing cytochrome P-450 systems (Salmon *et al*., 1981). Paolini *et al*. (1992) administered TCA interperitoneally at 300 or 600 mg/kg to mice and showed there was extensive disruption of enzyme functions in the liver. Using electron spin resonance spectroscopy, direct evidence was obtained for the presence of free radical metabolites of TCA, a reaction presumably mediated by cytochrome P-450. Additionally, there was evidence of lipid peroxidation as measured by the presence of diene conjugates. This mode of toxic action is similar to that of carbon tetrachloride, a hepatotoxin that forms free radical products and stimulates lipid peroxidation of liver cell membranes. The results of Paolini *et al*. (1992) provide evidence that reductive dehydrochlorination of TCA to free radicals is the primary mechanism of mouse hepatotoxicity.

Toxicity via Oxidative Pathways. The oxidative conversion of TCA to tri- and di-chloroacetic acid in rats is dependent on microsomal cytochrome P-450 systems and is the principal pathway for metabolic conversion of TCA to metabolites that appear in the urine (Casciola and Ivanetich, 1984). It is known that some reactive acyl chloride intermediates may be generated in these oxidative processes and subsequently bind to proteins and DNA to exert hepatotoxic effects (Halpert, 1982; Colacci *et al*., 1987).

Excretion

The excretion of TCA was quantified for 72 hours after oral administration of 150 mg/kg TCA to rats and mice (Dow, 1988). Greater than 90 percent of the absorbed dose was metabolized in both species. In rats, 41 percent was excreted in breath, 23 percent in urine, and 4 percent in feces. In mice, 51 percent was excreted in breath, 22 percent in urine, and 6 percent in feces. In Yllner's study (1971), ^{14}C-labeled TCA was administered interperitoneally and after 72 hr, 4 percent of the radioactivity was expired unchanged in the breath, 50 percent was expired as CO_2, 28 percent was excreted in the urine, 1 percent was in the feces, and 16 percent remained in the carcass.

Rats and mice were exposed to 10 ppm ^{14}C-TCA in air for 6 hours and the excretion of radioactivity monitored for 72 hours (Dow, 1988). More than 90 percent of the absorbed dose was metabolized in both species. The percentage of the recovered radioactivity was: in rats, 33 percent in breath, 19 percent in urine, and 5 percent in feces; and in mice, 34 percent in breath, 26 percent in urine, and 6 percent in feces.

In a study on human volunteers, subjects wearing a nose-clip inhaled ^{38}Cl-labelled-TCA in a single breath and the rate of respiratory excretion was followed for one hour.

Radioactivity in the urine was also measured. Only 3.3 percent of the inspired dose was exhaled in one hour and the urinary excretion rate was estimated to be 0.015 percent of the absorbed dose per min (Morgan *et al*., 1972). These results showed TCA was readily retained and had a slower excretion rate than other more volatile chlorinated hydrocarbons.

In a study that estimated the rate of elimination of TCA after skin absorption, it was estimated half of an absorbed dose of TCA in guinea pigs was eliminated within 2 hr (Jakobson *et al*., 1982).

TOXICOLOGY

Toxicological Effects in Animals

Acute Toxicity

The median lethal dose of TCA administered by the oral route (single dose) to rats is about 250 to 330 mg/kg (ATSDR, 1996). The median lethal concentration of TCA in rats, after 4 to 6 hr of inhalation exposure, is estimated to be about 1,000 ppm (ATSDR, 1996). In mice and guinea pigs, the levels that cause death are approximately 5,000 to 6,000 ppm (30 minutes to 3 hour). In animals surviving more than a few days, fatty degeneration of the liver was seen at necropsy (Horiuchi *et al*., 1962).

The anesthetic action of TCA can be an immediate cause of mortality, but in surviving animals, the liver is also a major target organ for TCA toxicity. Rats that received a single oral dose of 100 mg/kg of TCA showed necrosis and fatty degeneration of the liver, but no changes in relative liver weight or body weight (Schmidt *et al*., 1980). Centrilobular swelling was observed in mice after a dose of 75 mg/kg-day of TCA for 4 days, but there were no effects in the livers of rats or mice at a dose of 25 mg/kg-day (Dow, 1988).

Mice exposed to 600 to 800 ppm TCA in air for 3 hours showed that 8 hr following exposure total lipid and triglycerides in the liver were increased by an average of 216 percent and 518 percent, respectively (Tomokuni, 1969, 1970).

Subchronic Toxicity

In a study by Truffert *et al*. (1977), 55 female Sprague-Dawley rats were exposed to 130 ppm TCA for 5 hours per day, 5 days per week, for 15 weeks. Liver, kidney, adrenal, genital, and lung pathologies were monitored and compared with controls. It was noted that this exposure resulted in increased liver to body weight ratios, and granulation and vacuolization in liver cells. Cellular changes regressed after 19 exposures and were no longer observed following the 39th exposure. Since these hepatic effects were reversible, were consistent with enzyme induction, and did not indicate a pre-neoplastic effect, they were considered by the author to be "minimal." No significant changes were noted in the other tissues monitored. The major limitation of this study was

that only one concentration (130 ppm or 907 mg/m^3) was examined. Assuming a body weight of 0.2 kg, an inhalation absorption efficiency of 50 percent, and an inhalation rate of 0.33 m^3/24 hr, the average dose is estimated to be 110 mg/kg-day.

In a study by NCI (1978), groups of 5 male and 5 female Osborne-Mendel rats were administered TCA by gavage in corn oil 5 days per week for 6 weeks at doses of 56**,** 100, 178, 316, and 562 mg/kg-day. A 24 percent reduction in body weight gain was seen at 100 mg/kg-day in the female rats, with no body weight effects noted at 56 mg/kg-day. In the male rats, a 38 percent reduction in body weight gain was noted at 178 mg/kg-day. Thus, a no-observed-adverse-effect level (NOAEL), based on weight gain in female rats in a 6-week study, is defined at oral doses of 40 mg/kg-day (after adjusted for the 5 days per week dosing schedule).

Chronic Toxicity

In a 9-month inhalation study by Schmidt *et al*. (1972), summarized elsewhere (BUA Report, 1989), male rats were exposed in 200 L chambers to a continuous flow of a TCA-air mixture. The average concentration in the experiment was 13.3 ± 0.24 mg/m^3 (1.9 ppm). In the 9-month chronic exposure experiments, 105 rats were exposed to TCA daily for 4 hours/day resulting in an estimated daily dose of 1.1 mg/kg-day (assuming a body weight of 0.4 kg, an inhalation rate of 0.0167 m^3/hr (U.S. EPA, 1988), and a net absorption of 50 percent [Raabe, 1986]). Groups of experimental and control rats were examined after 110, 265 and 325 days from the start of exposure. At the end of 110 days, the exposed rats weighed significantly less than the controls (415 ± 5.3 g versus 435 ± 4.9 g), while their white blood cell counts averaged 90 percent higher than the control values. After 265 days, there were wide variations in body weights and differences were no longer significant. No white blood cell data were provided, apparently meaning there were no further significant changes in this parameter. The ACTH content of the hypophysis was significantly decreased in the exposed rats at all three sampling times. The total fat content of the liver was about 34 percent higher in the treated than in control rats at 265 days, but results are not reported for the other two sampling times. There were no significant differences in mortality rates between the two groups (Schmidt *et al*., 1972). A LOAEL of 1.1 mg/kg-day can be estimated from this study based on the transient weight loss, the increased liver fat content, and the persistent decrease in hypophyseal ACTH.

In an NCI study (1978), groups of 50 male and 50 female Osborne-Mendel rats were administered TCA by gavage in corn oil 5 days per week for 78 weeks. Males received average doses of 44 or 88 mg/kg-day; females received average doses of 31 or 62 mg/kg-day (the doses were adjusted for the 5 days per week exposure schedule and the untreated periods). Deaths occurred in the high dose females as early as week 1, and by week 5 about 20 percent of the animals in this group had died from apparent compound toxicity.

Body weight gain was monitored, and gross and histological examination of the major organs and tissues was performed. A dose-related retardation in body weight gain was initially seen in TCA-treated rats of both sexes during the exposure period. However, body weights of dosed and control rats tended to converge during the post-treatment observation period. Some overt signs of toxicity noted in female rats at both dosages

were a hunched posture, red discharges from the eyes and urine stains on the abdominal fur. These signs diminished with time. All female and male rats exhibited labored respiration, wheezing, and nasal discharge during the first year, which increased as the animals aged. As the study approached termination, these signs were apparent in a significantly greater number of treated animals than controls.

Histological examination of tissues revealed no systemic effects in any organs examined, including the lungs and brain. The toxicological significance of the clinical respiratory effects (labored respiration, wheezing, and nasal discharge) is unclear since no significant histological effects were observed. However, since clinical respiratory effects were also reported in human case reports (Sherman, 1953), these effects appear to be sufficient for the definition of a LOAEL of 31 mg/kg-day for respiratory effects in female rats. No respiratory effects were noted in mice given TCA.

As noted below in the section on carcinogenicity bioassay, the primary finding in male and female $B6C3F_1$ mice given TCA at two doses for 78 weeks was an increased incidence of hepatocellular carcinoma (NCI, 1978). Body weight gains were not significantly affected by the treatment. Increased mortality occurred in male and female mice at the higher dose, and deaths of the high-dose females were attributed to hepatocellular carcinoma. Renal tubular necrosis was the cause of death in most high-dose males but many of these animals also had hepatocellular carcinomas.

Genetic Toxicity

Studies on the ability of TCA to induce gene mutation in prokaryotic systems, with and without metabolic activation, have yielded equivocal results (see Table 3). Brem *et al*. (1974) first reported that TCA was mutagenic in *Salmonella typhimurium* strains TA1530 and TA1535 (strains which detect base-pair substitution) but not in strain TA1538 (a strain which detects frameshift mutations). Nestmann *et al*. (1980), however, was unable to find mutagenic activity of TCA in strains TA 1535, TA1537, TA1538, and TA98 and TA100, with and without conventional S-9 liver activation. Test concentrations were administered over a wide range, to the point of toxicity to the bacteria or to the limits of solubility. Haworth *et al*. (1983) and Milman *et al*. (1988) also reported negative results with TCA in strains TA98, TA100 and TA1535 and TA1537. Milman *et al*. (1988) used S-9 fractions prepared from Aroclor-1254-induced livers of Osborne-Mendel rats and of $B6C3F_1$ mice of both sexes. As noted elsewhere, these rodent strains were used for carcinogenicity bioassays in which TCA produced hepatocellular carcinoma in $B6C3F_1$ mice, but was not carcinogenic in the Osborne-Mendel rats. The positive and negative findings are further complicated by the results of Mersch-Sundermann *et al*. (1989) who reported that TCA was mutagenic in strains TA97 and TA98 (with metabolic activation), but not in strains TA100 and TA102. The technical details that contribute to the conflicting results have not been clarified.

In mammalian cells, TCA added *in vitro* induced sister chromatid exchanges but not chromosomal aberrations, DNA repair, or unscheduled synthesis of DNA (Galloway *et al*., 1987). Exposure to TCA at 349 mg/m^3 (7 hr/day) for 5 days did not induce dominant lethal mutations in rats, and results for chromosomal aberrations in rat bone marrow cells were equivocal (McGregor *et al*., 1980). TCA did not induce unscheduled

DNA synthesis in hepatocytes of mice exposed to doses of up to 1000 mg/kg by gavage (Mirsalis *et al*., 1989). TCA did not induce sex-linked recessive lethal mutations or mitotic recombination in *Drosophila melanogaster* (McGregor, *et al*., 1980; Woodruff *et at*., 1985; Vogel and Nivard, 1993). Colacci *et al*. (1990, 1992) demonstrated that TCA has initiating activity in mammalian cells. They reported that TCA induced sister chromatid exchanges and caused transformation in BALB/c3T3 cells *in vitro*.

Table 3. Genotoxicity of TCA *In Vitro*

Test System	Result	Reference
*Salmonella typhimurium**	positive for TA 1530 and TA 1535, negative in TA 1538	Brem *et al*., 1974
Salmonella typhimurium *	negative for TA 98, TA 100, TA 1535, TA 1537, TA 1538	Nestmann *et al*., 1980
Salmonella typhimurium *	negative for TA 98, TA 100, TA 1535, TA 1537	Haworth *et al*., 1983
Salmonella typhimurium *	negative for TA 98, TA 100, TA 1535, TA 1537	Milman *et al*., 1988
Salmonella typhimurium *	positive for TA 97 and TA 98 with metabolic activation, negative for TA 100 and TA 102	Mersch-Sundermann *et al*., 1989
Chinese hamster ovary cells, chromosomal aberrations	negative	Galloway *et al*., 1987
Chinese hamster ovary cells, sister chromatid exchange	positive	Galloway *et al*., 1987

* Histidine-dependent strains of *Salmonella typhimurium* designed by Prof. B.N. Ames for mutagenicity bioassays.

Genetic toxicity of TCA has been demonstrated *in vivo*. Colacci *et al*. (1987) injected radiolabeled TCA at 1.46 mg/kg (0.127 mCi/kg) to 6 male Wistar rats and 12 male BALB/c mice. Treated animals were killed 22 hr later. They reported that metabolites of TCA were covalently bound to DNA, RNA, and protein of liver, kidney, lung, and stomach of rat and mouse. Macromolecular specific activity was higher in liver than in other organs. Colacci *et al*. (1987) also found that the covalent binding indices (CBI) for liver DNA labeling of both rat and mouse for TCA (CBI: rat = 422, mouse = 543) are far higher than those measured for 1,1-dichloroethane (rat = 79, mouse = 65), 1,2-dichloroethane (rat = 47, mouse = 76), 1,1,1-trichloroethane (rat = 8, mouse = 16), and 1,1,2-trichloroethane (rat = 26, mouse = 73). CBI values of TCA are similar to those of 1,2-dibromoethane (rat = 515, mouse = 141) and of the same order of magnitude as those detected for carcinogens that are considered as moderate initiators.

Paolini *et al*. (1992) used an electron spin resonance spectroscopy spin-trapping method to characterize the free radical species involved in the observed liver damage associated with TCA exposure. They administered phenyl t-butylnitrone (PBN) (180 mg/kg) and TCA (600 mg/kg) by interperitoneal injection to male CD-1 mice. The animals were

killed after 30 min. A nitroxide radical was observed in all the five liver extracts from the mice treated with TCA. No electron spin resonance signal was observed in the control experiment.

Developmental and Reproductive Toxicity

In a chronic inhalation study, Schmidt *et al*. (1972) administered 13.3 mg/m^3 (1.92 ppm) TCA to male rats, 4 hr/day for 9 months. One week before the end of the exposure period, 7 male controls and 7 exposed males were mated to 5 untreated virgin females. During the mating period, the males continued to be exposed to 1.92 ppm TCA. Evaluation of the F1 generation did not reveal any significant differences in litter size, average body weight, sex ratio of newborn, or rearing mortality rate of young animals (BUA Report, 1989). Schmidt *et al*. (1972) also injected two strains of pregnant mice with 300, 400, or 700 mg/kg-day TCA, interperitoneally. Significant embryotoxic effects were observed and a low incidence of malformations (exencephaly, cleft palate, anophthalmia and fusion of ribs, vertebral arches and vertebral bodies) (BUA Report, 1989). The committee (BUA Report, 1989) that reviewed these studies by Schmidt *et al*. (1972) has noted, however, that the methods used did not conform to current techniques.

Carcinogenicity

The principal study on the potential carcinogenic properties of TCA was conducted by the National Cancer Institute (NCI, 1978). Technical grade (90 percent pure) TCA in corn oil was administered by gavage to Osborne-Mendel rats and to B6C3F$_1$ mice. There were two treatment groups of 50 animals and two control groups of 20 animals for each species and sex. One control group received the vehicle (corn oil), while the other remained untreated. The animals received the test substance in corn oil, by gavage, five days per week. Doses for both rats and mice varied during the study, as shown in Table 4. An observation period followed treatment, lasting 32 weeks for rats and 12 weeks for mice.

There is a statistically significant association between increased dosage and elevated mortality for both male and female mice. Thirty-three high dose male mice died in weeks 69 and 70, leaving only one high dose mouse that survived for the remainder of the study. Histopathologic examination of these animals revealed acute toxic tubular nephrosis as the apparent cause of death. Early mortality was also observed in the high dose female mice. Approximately 18 percent and 30 percent of the high-dose female mice died by week 60 and week 75, respectively. By comparison, 80 percent of the female in the vehicle control group and 75 percent of the female in the low-dose group survived by week 90.

The average daily dosages of TCA used in the cancer bioassays, after adjustments for the untreated periods, observation periods, and 5 days per week dosing routine, were estimated to be:

Mice (male and female)	88[1] and 175 mg/kg-day
Rats, male	32 and 55 mg/kg-day
Rats, female	22 and 39 mg/kg-day

Table 4. TCA Dosing Schedules for the NCI Cancer study (NCI, 1978)

	Dose (mg/kg-d)	Treated period (weeks)	Observation period (week)	Average daily dose during exposure	Average daily dose overall
Male Rat					
Untreated control	0		109	0	
Vehicle control	0	78	32	0	
Low dose	50	14			
	65	64		44	
	0		32		32[b]
High dose	100	14			
	130	18			
	130[a]	36	9	88	
	0		32		55[b]
Female Rat					
Untreated control	0		109	0	
Vehicle control	0	78	32	0	
Low dose	50	25			
	40	53		31	
	0		32		22[b]
High dose	100	25			
	80	7			
	80[a]	36	9	62	
	0		32		39[b]
Male and female mouse					
Untreated control	0		90	0	
Vehicle control	0	78	13	0	
Low dose	100	18			
	150	3			
	200	5			
	150	52		101	
	0		12		88[b]
High dose	200	18			
	300	3			
	400	5			
	300	52		202	
	0		12		175[b]

[a] These dosages were cyclically administered with a pattern of one dose-free week followed by 4 weeks (5 days per week) of treatment at the level indicated.
[b] Doses averaged over the treatment and observation periods.

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[1] Example calculation for the low dose mouse group:

Average daily dose = (100×18 + 150×3 + 200×5 + 150×52)/(18+3+5+52+12) × (5/7)

= 87.7 mg/kg-day (rounded to 88 mg/kg-day)

The primary findings were an increased incidence of hepatocellular carcinoma in male and female mice. Body weight gains were not significantly affected by treatment. Dose-related increased mortality occurred in male and female mice at the higher dose, and deaths of the high-dose females were attributed to hepatocellular carcinoma. Renal tubular necrosis was the cause of death in most high-dose males but many of these animals also had hepatocellular carcinomas. The results are shown in Table 5. The occurrence of hepatocellular carcinoma was dose-dependent and significant in mice of both sexes (NCI, 1978). The treatment was not associated with histopathological effects, other than those seen with neoplasms.

Table 5. Number of Mice with Hepatocellular Carcinoma (Percent).

	Untreated	**Vehicle control**	**Low dose (88 mg/kg-day)**	**High dose (175 mg/kg-day)**
Male	2/16 (13)	1/18 (6)	13/50 (26)	44/49 (90)
Female	0/18 (0)	0/20 (0)	30/48 (63)	43/47 (91)

Modified from NCI, 1978.

The incidence of neoplastic lesions in Osborne-Mendel rats exposed to TCA was not different from controls. However, two rats with hepatocellular carcinomas and one with a neoplastic nodule in the liver were observed in the high-dose males. These are considered rare tumors in rats. A dose-related retardation in body weight gain was initially seen in TCA-treated rats of both sexes during the exposure period. However, body weights of dosed and control rats tended to converge during the post-treatment observation period. Survival of male rats was not affected by treatment, but female rats in the higher dose had lowered survival because of the deaths of 10 animals in the first 5 weeks of the study. No treatment-related histopathological lesions were observed in rats. The authors of the NCI study (1978) concluded that orally-administered TCA was a liver carcinogen in $B6C3F_1$ mice of both sexes, but the results did not provide evidence for carcinogenicity in Osborne-Mendel rats.

TCA was also evaluated for tumor-initiating activity in strain A mice (Theiss *et al*., 1977). In this bioassay, the number of pulmonary adenoma on the surface of the lungs is counted after multiple interperitoneal injections of the test chemical. Three groups of 20 mice each were given multiple doses of TCA for a total dose of 400, 3,600 and 6,400 mg/kg over a period of about 2 to 6 weeks. The mice were sacrificed at 24 weeks and the number of lung nodules counted under a dissecting microscope. TCA at these doses was lethal to some mice, the number surviving after 24-week being 10/20, 15/20 and 5/20 for the 400, 3,600 and 6,400 mg/kg doses, respectively. The number of lung tumors per each surviving mouse (0.3, 0.5, 1.0) of all three groups was not increased significantly above vehicle control values (P value of 0.27).

In a study designed to clarify the initiating and promoting actions of chlorinated aliphatics on liver tumors, Milman *et al*. (1988) examined the effects of TCA on the rat liver foci assay, using gamma-glutamyltranspeptidase (GGT) as the putative pre-neoplastic indicator. In the first protocol for measuring GGT-initiation, TCA at 100 mg/kg (the estimated maximum tolerated dose) was administered by gavage to 10 rats, 24 hours after 2/3 partial hepatectomy. Six days after the partial hepatectomies, the rats were treated with phenobarbital (0.05 percent, w/w) in the diet for 8 weeks, after which they were killed and the livers examined. Milman *et al*. (1988) reported that this treatment did not produce initiating activity in the presence and absence of phenobarbital induction. In the second protocol for measuring GGT-promotion, 10 rats were initiated with 30 mg/kg diethylnitrosamine by interperitoneal injection 24 hours after 2/3 partial hepatectomy. Six days later the rats began receiving TCA in corn oil by gavage at 100 mg/kg-day, 5 days per week for 7 weeks. TCA was found to be active in promoting GGT-foci, with and without pretreatment by diethylnitrosamine as the initiator. These results showed that TCA was a promoter of liver tumor growth in the presence of an initiator. Furthermore, TCA may have some activity as a direct genotoxic agent *in vivo*, as it increased the number of foci in the promotion protocol without an initiator being administered.

Since the evidence for carcinogenicity in animals is restricted to one species, and the information from humans was inconclusive, TCA has been classified in Group C, as a "possible human carcinogen" by the U.S. EPA (1994) and as Group 3 "not classifiable as to its carcinogenicity to humans" by IARC (1999).

Toxicological Effects in Humans

Acute Effects in Humans

A number of fatalities have resulted from oral, inhaled or dermal exposures to TCA. Hunter (1968) summarized the early history of human experience with TCA as an industrial poison, which began in 1914. The first cases of acute toxicity were described in workers employed in spraying linen airplane wings with varnishes containing TCA as a solvent. Fourteen cases with four fatalities were described by Willcox (1915), the primary clinical manifestations of toxicity being acute liver failure. Preventive measures such as improved ventilation were taken in industry and safer alternative solvents were used. Nevertheless, von Oettigen (1955) noted 125 case-reports of toxic exposures up to 1937, with 26 deaths.

The use of TCA as a cleaning solvent has also resulted in fatalities from accidental or deliberate ingestion. Hepple (1927) and Forbes (1943) described case-reports of ingestion that resulted in deaths within 24 hr from central respiratory paralysis. The fatal acute dose in humans could not be precisely estimated, but generally a dose of 5 ml (approximately 110 mg/kg, assuming a body weight of 70 kg) was considered to be lethal (Elliott, 1933). Ingestion of a single dose of 3 ml of TCA, given by mistake to eight Africans as an anti-helminthic, did not produce lethal effects, although central nervous system depression (drowsiness and loss of consciousness) was observed in all subjects (Sherman, 1953).

Coyer (1944) described seven cases of poisoning from TCA, one of which was fatal. This report was unusual in that the fatal case had "admittedly used a rag and mop with bare hands to clean up spilled chemical from the floor." From the studies of Jakobson *et al*. (1982), it is known that TCA is absorbed through the skin of experimental animals such as guinea pigs. The TCA in this case of poisoning is likely to have been inhaled as well as absorbed through the skin. The signs and symptoms of toxicity that developed within six days were jaundice, fatigue, anorexia, headache, nausea, vomiting, and sensitivity to pain in the vicinity of the stomach and liver. The patient died on day 20 after the TCA exposure. Necropsy findings were liver cirrhosis and inflammation, cardiac hypertrophy, ascites, and hemorrhagic diathesis with bleeding in the gastrointestinal tract.

Lehmann and Schmidt-Kehl (1936) described experiments by scientists who inhaled eight different concentrations of TCA, from 0.02 to 2.3 mg/L (2.7 to 308 ppm), for up to 30 min. The principal results were summarized in the BUA Report (1989). During a 10-min exposure with up to 0.09 mg/L (12 ppm) the test subjects noticed no reaction at all, although the odor of TCA was perceptible even at the lowest concentration. This odor, which was stronger at first, was no longer noticeable after 10 min. A 20-min exposure to 0.8 mg/L (107 ppm) caused slight nausea and dizziness. With a 30-min exposure to 1 mg/L (134 ppm) dizziness occurred after 10 min, mucous membrane irritation after 12 min, and fatigue after 20 min. A 10-min exposure to 1.8 mg/L (241 ppm) caused fatigue and mucous membrane irritation in the mouth, nose and eye. A vile, bitter-sweet taste was perceived, which disappeared after 5 min. The 308 ppm dose exposure produced the above-described symptoms, but with an earlier onset. After 10 min, when the experiment was terminated, it was noted that there was a weakening of the strength of the knees, but no sign of unconsciousness in the test subjects.

Subchronic Effects in Humans

Humans exposed to TCA in the workplace manifest signs and symptoms of poisoning related to the gastric and nervous systems. Parmenter (1921) gives a clear description of the early signs of toxicity. The gastric effects include loss of appetite, feeling of nausea, fullness of stomach, with gas and frequent eructations. More advanced cases included abdominal pain, vomiting, diarrhea or constipation. The nervous effects include headache, dizziness, drowsiness, insomnia with nightmares, fatigue and a state of anxiety (Parmenter, 1921). Some of these gastric and nervous system disturbances were also seen in some 380 Indian workers who used TCA to make bracelets (Lobo-Mendonca, 1963). Exposure levels in the Indian study ranged from 9 to 98 ppm in air for work durations of one year or less, with dermal contact also being a source of exposure.

As noted earlier, the liver is a target organ of TCA toxicity. The development of jaundice, accompanied by liver failure and autopsy findings of liver inflammation and cirrhosis was characteristic of fatal cases in the workplace (Willcox, 1915). However, in a large-scale study of workers in the army during World War II who were exposed to TCA vapor in the manufacture of uniforms resistant to mustard gas, no significant increases in deaths from liver cirrhosis were found (Norman *et al*., 1981). The duration of solvent exposure ranged from 5 weeks to 1 year and the follow-up period for this

epidemiological study was 31 years (1946-1976). In this study, the relative risk for overall cancer mortality of 1,099 white males with TCA exposure was 1.26 times that of 1,319 men not involved in the TCA process. The risks for leukemia, lymphoma, and cancer of the genital organs were moderately elevated, but the numbers were small and no significant excesses were observed. Norman *et al*. (1981) stated that no definitive conclusions of a relationship between exposure to TCA and subsequent increased risk of cancer in humans could be drawn from this study.

Chronic Effects in Humans

No studies were found.

DOSE-RESPONSE ASSESSMENT

Noncancer

Inhalation Exposure

In a 9-month inhalation study (Schmidt *et al*., 1972), male rats were exposed to an average concentration of 13.3 ± 0.24 mg/m^3 (1.9 ppm) TCA. At the end of 110 days, the exposed rats weighed significantly less than the controls (415 ± 5.3 g versus 435 ± 4.9 g), while their white blood cell counts averaged 90 percent higher than the control values. After 265 days, there was wide variation in body weights and differences were no longer significant, although the total fat content of the liver was about 34 percent higher in the treated than in control rats at this time. The ACTH content of the hypophysis was significantly increased in the exposed rats at both sampling times during exposure, as well as at 325 days after the beginning of the study. This study showed that 13.3 mg/m^3 (1.9 ppm) was the LOAEL in rats exposed for 4 hr/day for 9 months. The average daily dose administered to the rats through inhalation exposure is estimated to be 1.1 mg/kg-day, assuming a body weight of 0.4 kg, an inhalation absorption efficiency of 50 percent, and an inhalation rate of 0.0167 m^3/hr (U.S. EPA, 1988).

Oral Exposure

In the NCI study (1978), TCA was administered by gavage to Osborne-Mendel rats for 78 weeks. Male rats exhibited labored respiration, wheezing, and nasal discharge at 44 mg/kg-day, while females showed the same symptoms at 31 mg/kg-day. No histopathological effects were found in the organs. The toxicological significance of the clinical respiratory effects (labored respiration, wheezing, and nasal discharge) is unclear. These effects appear to be sufficient for the definition of a LOAEL of 31 mg/kg-day in female rats.

For estimating a non-cancer PHG for TCA, a LOAEL of 1.1 mg/kg-day is identified. It is derived from a rat inhalation study where a decrease in weight gain, an increase in

white blood cell counts, and an increase in liver fat content were observed in rats exposed to 1.9 ppm TCA. This LOAEL is lower than those identified in oral toxicity studies.

Cancer

OEHHA generally follows the U.S. EPA draft guidelines for carcinogenic risk assessment (U.S. EPA, 1996, 1999) to calculate cancer risk. The type of high-to-low-dose extrapolation employed for a given chemical carcinogen is based on the data supporting linearity or non-linearity or a biologically based or case-specific model. When insufficient data are available to support a particular approach, the default is to use a linear extrapolation.

For the linear approach, either the 95 percent upper confidence limit on the linear term (q_1*) value or a carcinogenic slope factor (CSF) can be used for estimating cancer potency. The CSF can be calculated by dividing 0.1 by the LED_{10}. LED_{10} is defined as the 95 percent lower confidence limit on a dose associated with 10 percent extra risk.

Estimation of Cancer Potency and LED_{10}

The most relevant data for estimating the cancer potency are based on a dose-related increased incidences of hepatocellular carcinoma in $B6C3F_1$ mice given TCA in corn oil by gavage (NCI, 1978). The LED_{10} was calculated using a curve-fitting method in the Benchmark Dose Software (version 1.2.1) provided by U.S. EPA with a good fit criterion of $p>0.05$ for the Chi-square test. The animal CSF was calculated by linear extrapolation below the LED_{10} dose (the lower confidence limit on a dose associated with 10 percent extra risk). The q_1* cancer potencies or the 95 percent upper bound on the linear slope at low dose were calculated by the Tox_Risk program (v. 3.1, KS Crump Division, Clement International Corp., Ruston LA). The q_1*, LED_{10} and animal CSF estimates are given in Table 6.

Table 6. Mouse Potency Values for Q_1*, LED_{10} and Cancer Slope Factor (CSF) for TCA, from the $B6C3F_1$ Mice Data (NCI, 1978)

Sex	Tumor site and type	q_1* $(mg/kg\text{-}day)^{-1}$	LED_{10} mg/kg-day	CSF $(mg/kg\text{-}day)^{-1}$
Male	hepatocellular carcinoma	0.00180	37.76	0.00265
Female	hepatocellular carcinoma	0.0150	7.04	0.0142

Based on the liver tumor data of female mice reported by NTP, OEHHA estimated a q_1* of 0.0150 $(mg/kg\text{-}day)^{-1}$ and an animal CSF of 0.0142 $(mg/kg\text{-}day)^{-1}$ for TCA. As shown in Table 6, cancer potencies derived from the female data are higher than those derived from the male data, and for this reason, the female data set was selected for the determination of a cancer potency of TCA.

As the q_1* and the animal CSF derived from the liver tumor data of female mice are very close, an animal cancer potency of 0.014 was chosen for the determination of a human CSF for TCA. An interspecies scaling factor was applied base on assumed body weights of 30 g for mouse and 70 kg for humans, and a [body weight of human/body weight of mouse]$^{1/4}$ adjustment. An adjustment factor of $(104/90)^3$ was applied to account for the less-than-lifetime exposure. The human CSF was thus estimated to be 0.15 $(mg/kg\text{-}day)^{-1}$.

CALCULATION OF PHG

Noncarcinogenic Effects

Based on a rat study reported by Schmidt *et al*. (1972), a LOAEL of 1.1 mg/kg-day was selected for the calculation of a PHG for noncarcinogenic effects of TCA. Calculation of a public health-protective concentration (C, in µg/L) for TCA in drinking water for noncarcinogenic endpoints follows the general equation:

$$C = \frac{LOAEL \times BW \times RSC}{UF \times L_{eqs}/day}$$

$$= \frac{1.1\ mg/kg\text{-}day \times 70\ kg \times 0.8}{1{,}000 \times 4\ L_{eqs}/day} = 15.4\ \mu g/L\ (or\ 15\ ppb)$$

where:

LOAEL	=	lowest-observed-adverse-effect level of 1.1 mg/kg-day for transient weight gain depression and increased liver fat content;
BW	=	adult body weight, a default of 70 kg;
RSC	=	relative source contribution (a default of 20 percent to 80 percent), 80 percent is used as exposures from air and food are expected to be low;
UF	=	an overall uncertainty factor of 1,000 is used (10 to convert LOAEL to NOAEL, 10 to account for the uncertainty in inter-species extrapolation, and 10 for intra-species variability); and
L_{eqs}/day	=	a tap water exposure rate of 4 L_{eqs}/day is used to account for 2 L/day of drinking water consumption as well as 2 L_{eqs}/day for inhalation and dermal exposures to TCA from the use of contaminated tap water.

Thus, the health protective concentration for TCA in drinking water based on noncarcinogenic effects is proposed to be 15 µg/L or 15 ppb.

Carcinogenic Effects

The following general equation can be used to calculate the public health-protective concentration (C) for TCA in drinking water (in mg/L) based on carcinogenicity:

$$C = \frac{BW \times R}{CSF \times L_{eqs}/day} = mg/L$$

where:

BW	=	adult body weight (a default of 70 kg);
R	=	d*e minimis* level for lifetime excess individual cancer risk (a default of 10^{-6});
CSF	=	potency derived from an animal cancer bioassay data set, using the lower 95 percent confidence limit on the 10 percent tumor dose (LED_{10}), converted to human equivalent; and
L_{eqs}/day	=	a tap water exposure rate of 4 L_{eqs}/day is used to account for 2 L/day of drinking water consumption as well as 2 L_{eqs}/day for inhalation and dermal exposures to TCA from the use of contaminated tap water.

For TCA, a human CSF of 0.15 $(mg/kg\text{-}day)^{-1}$ was derived from the liver tumor data in female $B6C3F_1$ mice (NCI, 1978). An RSC is not included in the calculations for carcinogenic health protective concentrations; it is assumed that the use of the low dose extrapolation is adequately health protective.

$$C = \frac{1 \times 10^{-6} \times 70\ kg}{0.15\ (mg/kg\text{-}day)^{-1} \times 4\ L/day} = 0.00012\ mg/L = 0.1\ ppb$$

Thus the health protective concentration for TCA in drinking water based on carcinogenic effects is proposed to be 0.0001 mg/L or 0.1 ppb.

RISK CHARACTERIZATION

The commercial use of TCA as a solvent and as an intermediate in chemical synthesis has declined in the past 10 years, with virtual termination of its use and production in California. Thus, large-scale contamination of drinking water with this chemical is not likely to occur except, perhaps, near sites of previous commercial use or disposal, and its concentration in air is likely to be low to non-detectable.

TCA is recognized as a potent toxic substance to the liver and to the central nervous system of humans. In occupational settings, the use of TCA has produced fatalities

(acute liver failure) and nervous system disorders (loss of appetite, fatigue). Accidental or deliberate ingestion of TCA can result in death from the anesthetic and hepatotoxic actions of this chemical. It is estimated that the fatal acute dose in humans is approximately 110 mg/kg (Elliott, 1933).

Rodent studies on the mechanisms of liver toxicity of TCA indicate that reductive generation of free radicals, leading to lipid peroxidation and disruption of intracellular membranes, is the most likely pathway of cell damage.

In most prokaryotic and eukaryotic assays, TCA does not manifest overt genotoxic effects, but the results have been conflicting in some instances. In mammalian cells, TCA added *in vitro* induced sister chromatid exchanges and caused transformation but not chromosomal aberrations, DNA repair, or unscheduled synthesis of DNA (Galloway *et al*., 1987; Colacci *et al*., 1992). Following interperitoneal injection of TCA, free radicals and liver DNA adducts could be detected in the test animals (Paolini *et al*., 1992; Colacci *et al*., 1987).

TCA is designated by the U.S. EPA (1994) as Group 3 and as "not classifiable as to carcinogenicity in humans" by IARC (1999). In a 78-week feeding study conducted by the National Cancer Institute, TCA produced dose-dependent hepatocellular carcinoma in $B6C3F_1$ mice of both sexes but not in Osborne-Mendel rats. TCA is listed under Proposition 65 as a chemical that is known to the State of California to cause cancer (OEHHA, 2000).

In developing a PHG based on the carcinogenic effect of TCA, OEHHA used the liver tumor data in female mice (NCI, 1978). A linearized multistage model was used for fitting the tumor incidence data in the observed range and to determine the 95 percent lower bound LED_{10}. From the $LED_{10,}$ a model-free linear low dose extrapolation was made. In the absence of specific data on scaling between rats and humans, i.e., relative concentrations or activities of the active material at the target site, it was converted to human equivalent based on body weight to the 3/4 power scaling.

The proposed TCA PHG of 0.1 ppb is based on a cancer risk level of 10^{-6}. The concentrations in water corresponding to cancer risk levels of 10^{-5} and 10^{-4} are 1 and 10 ppb, respectively. These values may be an over or underestimation depending on the assumptions used in the body weight scaling and the high-to-low dose extrapolation.

The most sensitive endpoints for non-carcinogenic effects are transient decreased body weight gains and increases in liver fat content in rats exposed to TCA at 1.9 ppm in air (Schmidt *et al*., 1972). Assuming a body weight of 0.4 kg, an inhalation absorption efficiency of 50 percent, and an inhalation rate of 0.0167 m^3/hr, the LOAEL associated with these effects is estimated to be 1.1 mg/kg-day. Based on this level, the non-cancer health protective concentration is estimated to be 15 µg /L or 15 ppb. The lower health-protective value based on the one in one million cancer risk value will therefore protect against all non-cancer effects. The proposed PHG of 0.1 ppb also appears to be adequate to protect infants, children, and other potentially sensitive populations against adverse effects.

OTHER REGULATORY STANDARDS

NIOSH has recommended that TCA be treated as a potential human carcinogen. NIOSH usually recommends that occupational exposures to carcinogens be limited to the lowest feasible concentration. A 10 hr Time-Weighted Average of 1 ppm (7 mg/m^3) has been recommended by the American Conference of Governmental Industrial Hygienists (ACGIH, 1998).

There is no federal MCL for TCA (U.S. EPA, 2002). The current California MCL is 1 ppb for TCA in drinking water, based on a LOAEL of 1.4 mg/kg-day derived from an inhalation study by Schmidt (1972). The California MCL was calculated by assuming an adult body weight of 70 kg, a relative source contribution of 20 percent, and a water consumption rate of 2 L/day. An overall uncertainty factor of 10,000 was applied (10 for extrapolating from LOAEL to NOAEL, 10 for inter-species extrapolation, 10 for intra-species variability, and 10 to account for the possible carcinogenic potential of TCA) (CDHS, 1988).

Subsequent to the development of the California MCL, TCA was listed under Proposition 65 as a chemical that is known to the State of California to cause cancer (OEHHA, 2002). Under this program, a "No Significant Risk Level" of 3 µg/day has been established for TCA (OEHHA, 2002). The level is based on an estimated human cancer potency of 0.27 $(mg/kg\text{-}day)^{-1}$ and a risk level of 1 in a hundred thousand.

Using the female mice data reported by NCI (1978) and a linearized multistage modeling procedure, U. S. EPA (2002) estimated a human oral slope factor of 0.2 $(mg/kg\text{-}day)^{-1}$ and a drinking water TCA concentration of 0.2 ppb for one in a million extra cancer risk. Using the same carcinogenicity data set and a benchmark dose modeling procedure, OEHHA has developed a human cancer slope factor of 0.15 $(mg/kg\text{-}day)^{-1}$ for the oral exposure to TCA. By using an exposure model and exposure assumptions similar to those of U. S. EPA, OEHHA estimated that a drinking water TCA concentration of 0.1 ppb is associated with one in a million extra cancer risk. The difference in the estimated drinking water concentrations is due to the use of different drinking water consumption rates and rounding to one significant figure.

Table 7 shows regulatory standards of TCA in drinking water utilized by various other states.

Table 7. State Standards for TCA In Drinking Water (from ATSDR, 1996)

State	Standard (μg/L)
LA	0.16
AZ	0.17
MO	0.17
SD	0.17
MI	0.18
FL	0.2
WI	0.2
VT	0.7
CA	1
NJ	1
KS	1.7
MN	2
MA	2
RI	2
NY	0 to 2.5
TX	4.26

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