DRAFT

For Review Only

Public Health Goal for Trihalomethanes in Drinking Water

Prepared by

Office of Environmental Health Hazard Assessment
California Environmental Protection Agency

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PREFACE

Drinking Water Public Health Goals
Pesticide and Environmental Toxicology Branch
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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 (Health and Safety Code, Section 116365) 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. 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 that may cause chronic disease shall be based solely on health effects and shall be set at levels that 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 potential adverse effects on members of subgroups that comprise a meaningful proportion of the population, including but not limited to infants, children, pregnant women, the elderly, and individuals with a history of serious illness.
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. OEHHA shall consider additive effects of exposure to contaminants in media other than drinking water, including food and air, and the resulting body burden.
7. In risk assessments that involve infants and children, OEHHA shall specifically assess exposure patterns, special susceptibility, multiple contaminants with toxic mechanisms in common, and the interactions of such contaminants.

8. In cases of insufficient data for OEHHA to determine a level that creates no significant risk, OEHHA shall set the PHG at a level that is protective of public health with an adequate margin of safety.
9. 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.
10. The PHG may be set at zero if necessary to satisfy the requirements listed above in items seven and eight.
11. PHGs adopted by OEHHA shall be reviewed at least once every five years and revised as necessary based on the availability of new scientific data.

PHGs adopted by OEHHA are for use by the California Department of Public Health (DPH) in establishing primary drinking water standards (State Maximum Contaminant Levels, or MCLs).

PHGs are not regulatory requirements, but instead represent non-mandatory goals. Using the criteria described above, PHGs are developed solely for use by the California Department of Public Health (DPH) in establishing primary drinking water standards (State Maximum Contaminant Levels, or MCLs). Thus, PHGs are not developed as target levels for cleanup of ground or ambient surface water contamination, and may not be applicable for such purposes, given the regulatory mandates of other environmental programs.

Whereas PHGs are to be based solely on scientific and public health considerations, drinking water standards adopted by DPH are to consider economic factors and technical feasibility. Each primary drinking water standard adopted by DPH shall be set at a level that is as close as feasible to the corresponding PHG, with emphasis on the protection of public health. Each primary drinking standard adopted by DPH is required to be set at a level that is as close as feasible to the corresponding PHG, with emphasis on the protection of public health. MCLs established by DPH must be at least as stringent as the federal MCL, if one exists.

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 TRIHALOMETHANES IN DRINKING WATER

SUMMARY

This document provides a health risk assessment for each of the four major trihalomethanes (THMs) found in drinking water as a consequence of the chlorination disinfection process, and proposes four individual Public Health Goals (PHGs). The four THMs are chloroform ($CHCl_3$), bromoform ($CHBr_3$), bromodichloromethane ($CHBrCl_2$, or BDCM), and dibromochloromethane ($CHBr_2Cl$, or DBCM). The proposed PHG for chloroform is 0.001 milligram per liter (mg/L) or 1 microgram per liter (µg/L) or 1 part per billion (ppb); for bromoform 5 µg/L; for BDCM 0.4 µg/L; and for DBCM 0.7 µg/L. A proposed PHG is not developed for total THMs in drinking water, because California water utilities now commonly measure these disinfection byproducts individually. The proposed PHG values for the individual THMs are based on carcinogenicity, associated with a negligible lifetime theoretical cancer risk of one in a million (10^{-6}). Tumors are induced at a number of sites including liver, kidney, and large intestine by individual trihalomethane compounds in several rodent studies.

Trihalomethanes are ubiquitous contaminants in chlorinated water from drinking water supplies and swimming pools, some carbonated beverages, air, certain foods, and other sources. The pathways of human exposure include ingestion, inhalation, and dermal contact. Chronic exposure to chlorinated water has been associated with urinary bladder cancer in several epidemiological studies. Weaker associations have also been reported for other cancer sites such as colon and rectum, and for developmental and reproductive effects. The California Office of Environmental Health Hazard Assessment (OEHHA) concludes that the prudent public health protective assumption is that chloroform, bromoform, BDCM, and DBCM are capable of causing human tumors at the concentrations of these chemicals commonly found in drinking water after disinfection with chlorine, and that health-protective concentrations in water of these THMs should be protective against tumor occurrence.

The proposed PHGs are based on multiroute exposures to volatile THMs, which occur via showering, bathing, and other household uses. The proposed PHG for carcinogenic effects of each of the THMs is expected to protect the public against the potential noncancer effects as well, by providing an adequate margin of safety for potential adverse effects on the hepatic, renal, cardiac, hematopoietic, neurological, and reproductive and developmental systems.

The proposed PHG of 1 µg/L for chloroform in drinking water is based on several studies of carcinogenicity in experimental animals, calculated from the liver and kidney tumors (Jorgenson *et al*., 1985; NCI, 1976; Roe *et al*., 1979). Animal studies have amply demonstrated the ability of chloroform to produce tumors in the liver and kidney, while human epidemiological studies suggest an association of exposure to disinfection byproducts with tumors in humans. The estimated health protective level for noncancer effects of chloroform is 200 µg/L, based on hepatic effects (fatty lesions) in dogs exposed for over seven years.

However, the U.S. Environmental Protection Agency (U.S. EPA) has judged chloroform "not likely to be carcinogenic to humans by any route of exposure under exposure conditions that do not cause cytotoxicity and cell regeneration" (U.S. EPA, 2008c). Therefore U.S. EPA utilized a threshold approach for estimation of a safe dose of chloroform, rather than the default linear dose-response extrapolation of cancer risk. This results in a much lower estimate of chloroform hazards, compared to the approach used by OEHHA. U.S. EPA concluded that the high-dose cytotoxicity (cell damage) leads to increased cell division, and a secondary increase in the liver and kidney tumor rates. This implies that at lower doses (below the toxic effect threshold), there would be no increase in cell division, and no increase in tumors. Because there are no epidemiology data on chloroform alone and the genotoxicity data is weak, U.S. EPA concluded that the weight of evidence does not support the "standard" linear extrapolation for cancer risk estimation for chloroform.

OEHHA rejected the conclusion of a threshold mechanism for chloroform carcinogenicity, primarily because the increases in the biomarkers of cell damage do not correlate with development of tumors in the liver and kidney. Multiple toxic effects occur in the liver, kidney and other tissues, which lends considerable uncertainty to the assumption that the increases in tumors were all the result of the frank cell damage. Therefore OEHHA used the linear risk assessment approach, the standard method for carcinogen risk assessment.

The proposed PHG of 5 μg/L for bromoform in drinking water is based on carcinogenic effects in experimental animals. Evidence for carcinogenicity of bromoform was observed in male and female rats in a two-year oral exposure (gavage) study conducted by the National Toxicology Program (NTP, 1989a). Bromoform administered in corn oil by gavage resulted in statistically significant positive trends in the occurrence of historically rare tumors of the large intestine in male and female Fischer 344/N rats. The NTP (1989a) study of female rats provided clear evidence for the carcinogenicity of bromoform in rodents. This finding is supported by findings of genotoxicity in bacterial and mammalian systems both *in vivo* and *in vitro* and by results of testing in Strain A mice, which showed an increased incidence of lung tumors in animals injected intraperitoneally with bromoform. The estimated health protective level for noncancer effects of bromoform is 70 μg/L, based on liver effects (vacuolization) in a subchronic study in F344 rats.

The proposed PHG of 0.4 μg/L for BDCM in drinking water is based on carcinogenic effects in experimental animals. Evidence for carcinogenicity of BDCM has been observed in male and female rats and mice in two-year studies conducted by NTP (1987). Daily administration of BDCM by gavage in corn oil resulted in statistically significant increases in the incidence of historically uncommon tumors of the kidney and large intestine in male and female Fischer 344/N rats. Administration of BDCM by the same route to mice resulted in significant increases in the incidence of uncommon tumors of the kidney in male mice and liver tumors in female mice. These findings are considered clear evidence for the carcinogenicity of BDCM in rodents and are supported by evidence of genotoxicity in bacterial and mammalian test systems. The estimated health protective level for noncancer effects of BDCM in drinking water is 100 μg/L, based on hepatic lesions (fatty degeneration and granulomas) in males in a chronic study in Wistar rats.

The proposed PHG of 0.7 µg/L for DBCM in drinking water is based on carcinogenic effects in experimental animals. Evidence for carcinogenicity of DBCM has been observed in male and female $B6C3F_1$ mice in two-year studies conducted by NTP (1985). The proposed PHG is based on liver tumors in female mice (NTP, 1985). The estimated health protective level for noncancer effects of DBCM in drinking water is 30 µg/L, based on hepatic effects (fatty metamorphosis) in a 13-week subchronic study in F344 rats.

The current U.S. EPA (1979a,b, 2001b) Maximum Contaminant Level (MCL) for total THMs is 0.08 mg/L (80 µg/L, 80 ppb) as the annual average sum of the concentrations of chloroform, bromoform, BDCM, and DBCM. In 1998, the U.S. EPA (1998d,e,f,g,i,k) established a Maximum Contaminant Level Goal (MCLG) of zero for chloroform, zero for bromoform, zero for BDCM, and 0.06 mg/L for DBCM, but withdrew the zero MCLG for chloroform in 2000 (U.S. EPA, 2000e). The revised U.S. EPA (2001e) chloroform risk assessment concluded that chloroform poses no cancer hazard at low, non-cytotoxic concentrations and doses. The U.S. EPA has been in the process of developing MCLs for the individual trihalomethane and other disinfection byproducts for several years.

The California MCL for total THMs is 0.08 mg/L (80 µg/L) as the sum of the concentrations of chloroform, bromoform, BDCM, and DBCM in drinking water, established by the California Department of Public Health on June 17, 2006.

INTRODUCTION

The purpose of this document is to develop a proposed PHG for each of the four major trihalomethanes found in drinking water, including chloroform ($CHCl_3$), bromoform ($CHBr_3$), BDCM ($CHBrCl_2$), and DBCM ($CHBr_2Cl$). The proposed PHGs are based on a comprehensive analysis of information on the toxicology of the compounds. PHGs are based solely on protection of public health without regard to cost impacts or other factors. PHGs for carcinogens are set at a *de minimis* risk level of one in a million (10^{-6}).

Drinking water disinfection is a critically important process for control of microbial contamination of drinking water. Disinfection protects against cholera, typhoid fever, amoebic dysentery, giardiasis, and a number of other enteric diseases. Cholera and typhoid fever are frequently life-threatening. Other waterborne diseases may result mainly in diarrhea, and are most likely to have serious consequences in infants and the elderly (Frost *et al*., 2003; Craun *et al.*, 2003). More than 200 million people in the U.S. consume water that has been disinfected. However, disinfection itself leaves residual toxic byproducts in the drinking water. More than 250 different disinfection byproducts have been identified, and the THMs are one of the most important byproduct groups resulting from chlorination and other disinfection processes. Disinfection byproducts are formed when chlorine, chloramine, ozone, or chloride dioxide serving as oxidants react with natural organic and inorganic matter in water. Disinfection byproducts are halogenated organics and oxygenated halogens. Trihalomethane occurrence is influenced by chlorine dose, concentration and content of natural matter such as humic substances or bromide ions, chlorine contact time, water pH, water temperature, seasonal conditions,

reaction time of residual chlorine in water, spatio-temporal situations, and other factors (Charrois *et al*., 2004; Sadiq and Rodriguez, 2004; Rodriguez *et al*., 2004). Other disinfection byproducts include haloacetic acids (HAAs), including dichloroacetic acid, trichloroacetic acid, monochloroacetic acid, monobromoacetic acid, and dibromoacetic acid; haloacetonitriles; haloketones; aldehydes; chloropicrin; 3-chloro-4-(dichloromethyl)-5-hydroxy-2(5H)-furanone (MX or mutagen X); E-2-chloro-3-(dichloromethyl)-4-oxobutenoic acid (E-MX); chloral hydrate; cyanogen chloride; 2,4,6-trichlorophenol; chlorite; bromate; and other compounds (Woo *et al*., 2002).

Trihalomethanes have been detected in chlorinated water worldwide. Health Canada (1996) estimates that THMs comprise up to 50 percent by weight of all chlorination byproducts present in drinking water. Among the THMs, the most commonly occurring by concentration is chloroform, followed by BDCM, then DBCM, and bromoform (U.S. EPA, 1998c,i,j,k; WHO, 1994; Health Canada, 1996). A 1987 to 1988 survey found that chloroform represented over 73 percent of total THMs in 18 drinking water distribution systems supplying large municipalities in Quebec, Canada (Health Canada, 1996).

The U.S. government and the State of California have adopted MCLs for chemical contaminants that are created during drinking water disinfection. Because of the large population exposed, health risks associated with disinfection byproducts, even if small, need to be taken seriously. Since exposure to these substances is pervasive and the exposed population is large, these substances, which include suspected carcinogens and potential developmental and reproductive toxicants, have been regulated and the scientific evaluations of the toxicity have been updated.

Chloroform

Chloroform has been the subject of numerous reviews and risk assessments, including the U.S. EPA (1985a) Health Assessment Document for Chloroform, U.S. EPA (1980a) Ambient Water Quality Criteria for Chloroform, U.S. EPA (1994b) Drinking Water Criteria Document for Trihalomethanes, U.S. EPA (2001e) Toxicological Review Document for Chloroform and the corresponding U.S. EPA (2008c) IRIS entry, Agency for Toxic Substances and Disease Registry (ATSDR, 1997) Toxicological Profile for Chloroform, World Health Organization (WHO, 1994) Environmental Health Criteria Document, International Agency for Research on Cancer (IARC, 1999d) Monograph on Chloroform, International Life Sciences Institute (ILSI) Expert Panel evaluation of chloroform cancer risk (ILSI, 1997), Lawrence Livermore National Laboratory risk assessment of chloroform in California groundwater (Bogen *et al*., 1992b), and the California DHS (1990) cancer assessment.

Chloroform is a volatile chlorinated trihalomethane. It is produced for commerce and is formed as an inadvertent byproduct during disinfection of drinking water with chlorine. Chloroform is produced from chlorination of methane or methyl chloride, but is not produced commercially in California. The major use of manufactured chloroform is in production of the refrigerant, monochlorodifluoromethane HCFC-22, and as a raw material for polytetrafluoroethylene plastics (DeShon, 1979; HSDB, 2002b). Chloroform is also used as a solvent and reaction intermediate for organic compounds.

Chloroform is classified by the U.S. EPA (1994a, 2008c) as a Group B2, probable human carcinogen which is likely to be carcinogenic to humans by all routes of exposure under high-exposure conditions that lead to cytotoxicity and regenerative hyperplasia in susceptible tissues (U.S. EPA, 1998c,h). The IARC (1991b, 1999d, 2004) has concluded that there is sufficient evidence of carcinogenicity in animals and inadequate evidence in humans and has categorized chloroform as Group 2B, possibly carcinogen to humans. Chloroform is currently listed by the State of California under the Safe Drinking Water and Toxic Enforcement Act of 1986, also known as Proposition 65, as a chemical known to cause cancer (OEHHA, 2009).

Bromoform

The toxicological effects of bromoform in humans and experimental animals have been comprehensively reviewed by U.S. EPA (1980b, 1987b, 1994b, 2005) and ATSDR (1990). Additional information is available in U.S. EPA's IRIS (2008a) and in monographs prepared by IARC (1991b, 1999b).

Bromoform is a volatile brominated trihalomethane. It is produced in small amounts for commerce and is formed as an inadvertent byproduct during disinfection of drinking water with chlorine. Bromoform also occurs naturally in the environment as a result of biosynthesis by marine algae. Bromoform is not commercially produced in California. Bromoform is used as a laboratory reagent, in the vulcanization of rubber, as a solvent for liquid solvent extractions, for mineral ore separations, and for quality assurance purposes in the electronics industry. Bromoform is also used in the shipbuilding, aircraft, and aerospace industries. Bromoform is the major organohalide formed by the chlorination of seawater during desalination.

Bromoform is classified by the U.S. EPA (1994a, 2008a) as a Group B2, probable human carcinogen. The IARC (1991b, 1999b, 2004) has concluded that there is limited evidence of carcinogenicity in animals and inadequate evidence in humans and has categorized bromoform as Group 3, not classifiable as to carcinogenicity in humans. Bromoform is currently listed by the State of California as a chemical known to cause cancer under Proposition 65 (OEHHA, 2009). The U.S. EPA established an MCLG of zero, based on the classification of bromoform as a Group B2, probable human carcinogen.

Bromodichloromethane

The toxicological effects of BDCM in humans and experimental animals have been comprehensively reviewed in documents prepared by U.S. EPA (1980b, 1985b, 1987b, 1994b, 2005) and ATSDR (1989). Additional information is available in U.S. EPA's IRIS (2008b) and in monographs prepared by IARC (1991a, 1999a).

BDCM is a volatile brominated and chlorinated trihalomethane. BDCM is no longer manufactured commercially in the U.S., but is formed inadvertently during the chlorination of drinking water for disinfection. BDCM also occurs naturally in the environment as a result of biosynthesis by marine algae. It is currently used as a chemical reagent and intermediate in organic synthesis.

BDCM is classified by the U.S. EPA (1994a, 2008b) as a Group B2, probable human carcinogen. The IARC (1991a, 1999a, 2004) has concluded that there is sufficient evidence of carcinogenicity in animals and inadequate evidence in humans and has categorized BDCM as Group 2B, possibly carcinogenic in humans. BDCM is currently listed by the State of California as a chemical known to cause cancer under Proposition 65 (OEHHA, 2009). The U.S. EPA established an MCLG of zero, based on the classification of BDCM as a Group B2, probable human carcinogen.

Dibromochloromethane

The toxicological effects of DBCM in humans and experimental animals have been comprehensively reviewed in documents prepared by U.S. EPA (1980b, 1987b, 1994b, 2005) and ATSDR (1990). Additional information is available in U.S. EPA's IRIS (2008d) and in monographs prepared by IARC (1991c, 1999c).

DBCM is a volatile brominated and chlorinated trihalomethane. DBCM is no longer commercially produced in the U.S., but is formed as an inadvertent byproduct during drinking water disinfection with chlorine. It also occurs naturally in the environment due to biosynthesis by marine algae. DBCM is used as a laboratory reagent and intermediate in organic synthesis. DBCM was formerly used as a chemical intermediate in the production of fire extinguishing agents, aerosol propellants, refrigerants, and pesticides.

DBCM is classified by U.S. EPA (1994a, 2008d) as a Group C, possible human carcinogen. The IARC (1991c, 1999c, 2004) has concluded that there is limited evidence of carcinogenicity in animals and inadequate evidence in humans and has categorized DBCM as Group 3, not classifiable as to carcinogenicity in humans. DBCM is not currently listed by the State of California under Proposition 65. The U.S. EPA established an MCLG of 0.06 mg/L, based on classification of DBCM as a Group C, possible human carcinogen.

CHEMICAL PROFILE

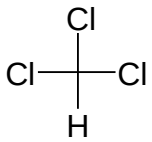
Chloroform

Chemical Identity

Chloroform is a chlorinated trihalomethane produced both synthetically and naturally. Chloroform is produced inadvertently during water disinfection by chlorination or intentionally for commerce. Chloroform also occurs naturally as a result of biosynthesis by marine algae in the oceans. The chemical formula, structure, synonyms, and several identification numbers of chloroform are listed in Table 1.

Table 1. Chemical Identity of Chloroform

Chemical name	Chloroform	Merck, 2001
Synonyms	trichloromethane	Merck, 2001
Chemical formula	$CHCl_3$	Merck, 2001

Chemical structure		ATSDR, 1997
Identification numbers CAS registry number NIOSH RTECS® number	 67-66-3 FS9100000	 Merck, 2001 RTECS, 2002d

Physical and Chemical Properties

Chloroform is a heavy, volatile liquid at room temperature, with a pleasant ethereal odor and sweet taste (Alexander *et al*., 1982). When stored, it decomposes to phosgene, hydrogen chloride, chlorine, carbon dioxide, and water; however, small amounts of ethyl alcohol are effective as a stabilizer (DeShon, 1979). Chloroform is an excellent solvent for organic chemicals. It is used to dissolve alkaloids, cellulose acetate and benzoate, ethyl cellulose, essential oils, fats, gutta-percha, halogens, methyl methacrylate, mineral oils, and many resins, rubbers, tars and vegetable oils (DeShon, 1979). Important physical and chemical properties of chloroform are provided in Table 2.

Table 2. Physical and Chemical Properties of Chloroform

Property	**Value or information**	**References**
Molecular weight	119.38 g/mol	Merck, 2001
Color	Colorless	Verschueren, 2001c
Physical state	Liquid	Merck, 2001
Odor	pleasant, etheric, nonirritating	HSDB, 2002b
Odor threshold	3.30 mg/L in air 2.4 mg/L in water at 25 °C	HSDB, 2002b Amoore and Hautala, 1983
Taste threshold	12 mg/L in water at 40 °C	Alexander *et al*., 1982
Melting point	−63.6 °C	Lide, 2000
Boiling point	61.7 °C at 760 mm Hg	Mackay and Shiu, 1981
Flammability limits	not flammable	ATSDR, 1997
Solubility in water at 20 °C in water at 25 °C Organic solvents	 8,100 mg/L 7,710 mg/L miscible with alcohol, benzene, and lipophilic solvents	 Mackay and Shiu, 1981 HSDB, 2002b Merck, 2001
Specific gravity at 20 °C	1.484	Merck, 2001
Partition coefficients Log K_{ow}	 1.97 at 20 °C	 Verschueren, 2001c

Property	Value or information	References
Log K_{oc}	2.0; 1.92;	Schwarzenbach and Westall, 1981
Vapor pressure at 20 °C at 25 °C	 160 mm Hg 192 mm Hg	 Verschueren, 2001c Mackay and Shiu, 1981
Henry's law constant	3.67×10^{-3} atm-m^3/mol 2.46×10^{-3} atm-m^3/mol at 17.5 °C	HSDB, 2002b Gossett, 1987
Conversion factors[1]	1 mg/m^3 = 0.20 ppm 1 ppm = 4.96 mg/m^3	Verschueren, 2001c Verschueren, 2001c

Chloroform is noncombustible under ordinary conditions (ATSDR, 1997; HSDB, 2002b). Mixtures of chloroform and methanol react explosively when combined with alkali bases. Chloroform is incompatible with chemically active metals, dinitrogen tetraoxide, triisopropylphosphene, and strong oxidizers. Oxidative breakdown of chloroform produces phosgene gas, hydrogen chloride, and chlorine gas (HSDB, 2002b).

Chloroform is moderately lipophilic and miscible with alcohol, benzene, ether, petroleum ether, carbon tetrachloride, carbon disulfide, and oils. The log of the octanol-water partition coefficient (log K_{ow}) of 1.97 indicates that there is at least 90 times more partitioning of chloroform into lipids than in an aqueous phase.

Production and Uses

Chloroform is a byproduct of drinking water chlorination disinfection processes. It is produced during chlorination of drinking water by reaction of the chlorine with natural organic substances (Wallace, 1997). Perwak *et al*. (1980) estimated that 3,466 metric tons of chloroform were produced in the U.S. during 1978 from chlorination of drinking water alone. The amount present in various drinking water supplies is dependent on factors such as organic content, temperature, salinity, pH, and type of chlorinating agent (Rickabaugh and Kinman, 1977; Perwak *et al*., 1980).

In the U.S., two manufacturers with four plants produce chloroform; none of the plants is located in California (Chemical Marketing Reporter, 2000). Large-scale production of chloroform is mainly by chlorination of methane (HSDB, 2002b). It is also produced by chlorination of methyl chloride (ATSDR, 1997). Annual U.S. production of chloroform was reported as 2.54×10^8 kg in 1994 by ATSDR (1997) and as 565 million pounds by NTP (2005b), citing a 1996 Chemical and Engineering News report. In 2000, the U.S. imported approximately 406,000 pounds of chloroform and exported over 220 million pounds (NTP, 2005b). More recent production information is not publicly available.

The majority of manufactured chloroform, approximately 96 to 98 percent produced in the U.S., has been used in production of the refrigerant monochlorodifluoromethane, or HCFC-22 (DeShon, 1979; NTP, 2005b). However, this use is expected to diminish due to the phase-out of chlorine-containing fluorocarbons in the U.S. by 2010 (HSDB, 2002b; NTP, 2005b). Chloroform is used as a raw material for polytetrafluoroethylene plastics

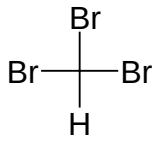
(HSDB, 2002b). Chloroform is also produced commercially for use as a solvent and reaction intermediate for organic compounds; a solvent for fats, oils, rubber, alkaloids, waxes, gutta-percha, and resins; a pharmaceuticals extraction and purification solvent; a fungicide for tobacco seedlings; a chemical intermediate for dyes and pesticides, and a cleansing and dry cleaning agent (Merck, 2001; HSDB, 2002b). Former uses of chloroform include: a sweetener, an anesthetic, and a component of cough syrups, toothpastes, liniments, and toothache compound (Verschueren, 2001c; HSDB, 2002b).

Bromoform

Chemical Identity

Bromoform is a brominated trihalomethane produced synthetically for commerce and inadvertently during water disinfection by chlorination. Bromoform also occurs naturally as a result of biosynthesis by marine algae in the oceans. The chemical formula, structure, synonyms, and identification numbers are listed in Table 3.

Table 3. Chemical Identity of Bromoform

Chemical name	Bromoform	Merck, 2001
Synonyms	tribromomethane	Merck, 2001
Chemical formula	$CHBr_3$	Merck, 2001
Chemical structure		ATSDR, 1990
Identification numbers CAS registry number NIOSH RTECS® number	 75-25-2 PB5600000	 Merck, 2001 RTECS, 2002c

Physical and Chemical Properties

Important physical and chemical properties of bromoform are provided in Table 4. Bromoform is nonflammable and noncombustible under ordinary conditions (HSDB, 2002d), but reacts explosively with crown ethers or potassium hydroxide and other strong caustics (HSDB, 2002d). It is incompatible with chemically active metals such as lithium, sodium, potassium, sodium/potassium alloys, calcium, powdered aluminum, zinc, and magnesium (HSDB, 2002d). When heated to decomposition it emits toxic fumes of hydrogen bromide (HSDB, 2002d). Air and light accelerate decomposition (Merck, 2001).

Table 4. Physical and Chemical Properties of Bromoform

Property	Value or information	References
Molecular weight	252.73 g/mol	Merck, 2001
Color	colorless to yellow liquid	HSDB, 2002d
Physical state	liquid, solid below 7.5 °C	HSDB, 2002d
Odor	chloroform odor, sweetish	Verschueren, 2001a
Odor threshold Water Air	 0.3 ppm 4.80×10^8 molecules/cm^3	 Verschueren, 2001a HSDB, 2002d
Melting point	8.0 °C, 8.3 °C	Lide, 2000; Weast, 1989
Boiling point	149.1 °C, 149.5 °C	Lide, 2000;Weast, 1989
Flammability limits	not flammable	ATSDR, 1990
Solubility in water at 20 °C in water at 25 °C Organic solvents	 3.0 g/L 3,100 mg/L miscible with alcohol, benzene, and lipophilic solvents	 Mabey *et al*., 1982 HSDB, 2002d Merck, 2001 Weast, 1989
Specific Gravity at 15 °C	2.899	Lide, 2000
Partition coefficients Log K_{ow} Log K_{oc}	 238, 2.40 2.06	 Mabey *et al*., 1982 ATSDR, 1990
Vapor pressure at 20 °C at 25 °C	 5 mm Hg 5.6 mm Hg	 Mabey *et al*., 1982 Verschueren, 2001a
Henry's law constant	5.35×10^{-4} atm-m^3/mol	Munz and Roberts, 1987
Conversion factors ppm (v/v) to mg/m^3 in air	 1 ppm = 10.34 mg/m^3	 Verschueren, 2001a
Relative vapor density	8.7 (air = 1)	Verschueren, 2001a
Stability	gradually decomposes in air, accelerated by light	Merck, 2001
Reactivity	reacts rapidly with strong caustics, alkali metals, powdered aluminum, zinc, magnesium	Sittig, 1985

Production and Uses

Bromoform is a byproduct of drinking water chlorination disinfection processes. It is produced during the chlorination of drinking water by reaction of the chlorine with natural organic substances in the presence of bromide ion (IARC, 1991b). Drinking

water chlorination disinfection is expected to be the predominant source of exposure of general populations to bromoform. Bromoform is the major organobromide produced by the chlorination of seawater during desalination (IARC, 1991b).

Bromoform is commercially produced in small quantities by reaction of acetone with sodium hypobromite, by treating chloroform with aluminum bromide, or by electrolysis of potassium bromide in ethyl alcohol (HSDB, 2002d). Two companies produce bromoform commercially in the U.S., neither of which have plants located in California (SRI, 2001). Production of bromoform in the U.S. was estimated to be between 50 and 500 tonnes in 1977. U.S. exports of bromoform were estimated to be 6 to 9 tonnes in 1988 (ATSDR, 1990). More recent production information is not publicly available.

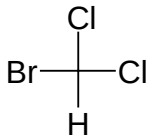
Bromoform has been used as an intermediate in organic synthesis, as an ingredient in fire-resistant chemicals, as a gauge fluid in geological assaying, as a solvent for waxes, greases and oils, and as a sedative and cough suppressant (ATSDR, 1990; IARC, 1991b; HSDB, 2002d). As a laboratory reagent, bromoform is used in nuclear magnetic resonance studies and as a catalyst, initiator, or sensitizer in polymer reactions. It is used in the vulcanization of rubber (ATSDR, 1990; HSDB, 2002d). Bromoform is used as an industrial solvent for liquid solvent extractions (HSDB, 2002d). In geological assaying, bromoform is used as a heavy liquid for mineral ore separations based on differences in specific gravity (Verschueren, 2001a). Bromoform is also used in the shipbuilding, aircraft, and aerospace industries (HSDB, 2002d).

Bromodichloromethane

Chemical Identity

BDCM is a brominated and chlorinated trihalomethane produced inadvertently during water disinfection by chlorination and naturally via biosynthesis by marine algae. The chemical formula, structure, synonyms, and identification numbers are listed in Table 5.

Table 5. Chemical Identity of Bromodichloromethane

Chemical name	bromodichloromethane	Merck, 2001
Synonyms	dichlorobromomethane, BDCM, dichloromonobromomethane, monobromodichloromethane	Merck, 2001 IARC, 1991a
Chemical formula	$CHBrCl_2$	Merck, 2001
Chemical structure		ATSDR, 1989
Identification numbers CAS registry number NIOSH RTECS® number	 75-27-4 PA5310000	 Merck, 2001 RTECS, 2002a

Physical and Chemical Properties

Important physical and chemical properties of BDCM are given in Table 6. It is nonflammable and noncombustible under ordinary conditions (HSDB, 2002a). When heated to decomposition BDCM emits toxic fumes of hydrogen bromide and hydrogen chloride (HSDB, 2002a). BDCM is moderately lipophilic. The log of the octanol-water partition coefficient (log K_{ow}) of 2.0 indicates that there is 100 times more partitioning of BDCM into lipids than in the aqueous phase.

Table 6. Physical and Chemical Properties of Bromodichloromethane

Property	Value or information	References
Molecular weight	163.83 g/mol	Merck, 2001
Color	colorless	Verschueren, 2001b
Physical state	liquid	Verschueren, 2001b
Odor threshold	1,680 mg/m^3	HSDB, 2002a
Melting point	−57 °C, −57.1 °C	Lide, 2000; Weast, 1989
Boiling point	90 °C, 90.1 °C	Lide, 2000; Weast, 1989
Flammability limits	not flammable	HSDB, 2002a
Solubility in water at 20 °C in water at 30 °C Organic solvents	 4,500 ppm 3,030 ppm, 3,968 ppm very soluble in ethanol, ether, and acetone	 Mabey *et al*., 1982 Yalkowsky and Dannenfelser, 1992 Lide, 2000
Specific Gravity at 20 °C	1.980	Lide, 2000
Partition coefficient Log K_{ow}	 2.0	 HSDB, 2002a
Vapor pressure at 20°C	50 mm Hg	Verschueren, 2001b
Henry's law constant at 25 °C	2.12×10^{-3} atm-m^3/mol	HSDB, 2002a
Conversion factors at 25 °C, 760 mm Hg	1 ppmv = 6.70 mg/m^3	IARC, 1991a

Production and Uses

BDCM is produced during the chlorination of drinking water by reaction of the chlorine with natural organic substances in the presence of bromide ion (IARC, 1991a). Drinking water chlorination disinfection is the predominant source of exposure of general populations to BDCM.

BDCM is no longer produced and is not used commercially in the U.S. with no import or export expected (HSDB, 2002a; NTP, 2005a). In the past, BDCM was manufactured by

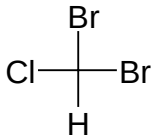
reaction of chlorine with anhydrous aluminum bromide or with hydrogen bromide in the presence of an aluminum halide catalyst (ATSDR, 1989; HSDB, 2002a). BDCM has been used as an intermediate in organic synthesis and as a standard in drinking water analysis (IARC, 1991a, Merck, 2001). Former uses of BDCM include a fire extinguisher fluid ingredient, a solvent for fats, waxes, resins, and a heavy liquid for mineral and salt separations (ATSDR, 1989; Verschueren, 2001b; NTP, 2005a).

Dibromochloromethane

Chemical Identity

DBCM is a brominated and chlorinated trihalomethane produced inadvertently during water disinfection by chlorination and naturally via biosynthesis by marine algae. The chemical formula, structure, synonyms, and identification numbers are listed in Table 7.

Table 7. Chemical Identity of Dibromochloromethane

Chemical name	dibromochloromethane	Merck, 2001
Synonyms	chlorodibromomethane, chlorobromoform, monochlorodibromomethane, dibromomonochloromethane, DBCM, CDBM	Merck, 2001 IARC, 1991c RTECS, 2002b
Chemical formula	$CHBr_2Cl$	Merck, 2001
Chemical structure		ATSDR, 1990
Identification numbers CAS registry number NIOSH RTECS® number	 124-48-1 PA6360000	 Merck, 2001 RTECS, 2002b

Physical and Chemical Properties

Important physical and chemical properties of DBCM are given in Table 8. DBCM is nonflammable under ordinary conditions (HSDB, 2002c). When heated to decomposition it emits toxic fumes of hydrogen bromide and hydrogen chloride (HSDB, 2002c).

Table 8. Physical and Chemical Properties of Dibromochloromethane

Property	Value or information	References
Molecular weight	208.28 g/mol	Merck, 2001
Color	clear, colorless to pale yellow	Verschueren, 2001d
Physical state	liquid	Verschueren, 2001d
Melting point	−20 °C	Lide, 2000
Boiling point	120 °C	Lide, 2000
Flammability limits	not flammable	ATSDR, 1990
Solubility in water at 22 °C in organic solvents	 4,400 mg/L soluble in ethanol, diethyl ether, acetone, benzene	 Verschueren, 2001d Lide, 2000
Specific Gravity at 20 °C	2.451	Lide, 2000
Partition coefficients Log K_{ow}	 2.24	 Verschueren, 2001d
Hydrolysis constant at 25 °C, neutral pH, K	2.88×10^{-8} hour^{-1}	Mabey *et al*., 1982
Vapor pressure at 20 °C	76 mm Hg	Verschueren, 2001d
Henry's law constant at 20 °C	7.83×10^{-4} atm-m^3/mol	HSDB, 2002c
Conversion factors at 25 °C, 760 mm Hg	1 ppmv = 8.52 mg/m^3	IARC, 1991c

DBCM is moderately lipophilic; the log of the octanol-water partition coefficient (log K_{ow}) of 2.24 indicates that there is about 170 times more partitioning of DBCM into lipids than into an aqueous phase.

Production and Uses

DBCM is produced during chlorination of drinking water by reaction of the chlorine with natural organic substances in the presence of bromide ion (IARC, 1991c). Drinking water disinfection by chlorination is expected to be the predominant source of exposure of the general population to DBCM.

DBCM is no longer produced commercially in the U.S. (HSDB, 2002c). DBCM has been used as a chemical intermediate in the manufacture of fire extinguishing agents, aerosol propellants, refrigerants and pesticides (ATSDR, 1990; IARC, 1991c; Verschueren, 2001d). DBCM is used as a laboratory chemical reagent and intermediate in organic synthesis (HSDB, 2002c).

ENVIRONMENTAL OCCURRENCE AND HUMAN EXPOSURE

Trihalomethanes in Chlorinated Water

Trihalomethanes were first identified in finished drinking water in 1974 in the U.S. with links to the practice of water chlorination (Bellar *et al*., 1974; Rook, 1974). Krasner *et al*. (1989) reported THMs as the largest class of disinfection byproducts detected in chlorinated drinking water in a study of 35 water utilities across the U.S., including California. Similar findings were reported by McGuire and Meadow (1988) in the U.S., Chen and Weisel (1998) in New Jersey, Keegan (1998) in north England, Kuo *et al*. (1997) in Taiwan, and Shin *et al*. (1989) in South Korea. Cumming and Jolley (1993), Krasner (2001), and Singer (1993, 2001) summarized the formation, characterization, occurrence, and variability of disinfection byproducts in chlorinated drinking water and water distribution systems. American Water Works Association (AWWA, 1991) summarized THMs occurring in chlorinated drinking water in several national surveys.

A total THMs survey by the American Water Works Association Research Foundation of 727 U.S. cities representing over 67 percent of the population served by water utilities with more than 10,000 customers, collected from 1984 to 1986, gave a mean of 42 μg/L or a median of 39 μg/L, ranging from nondetected to 360 μg/L (McGuire and Meadow, 1988). A median total THM concentration of 37 μg/L was reported for municipal drinking water supplies collected by the U.S. EPA, AWWA, and California Department of Health Services (DHS) in 1987 from 25 national facilities and 10 California facilities (Krasner *et al*., 1989). A similar median of 39 μg/L was reported in a more recent study (Richardson *et al*., 1999). A survey by the U.S. EPA (1989b,c) of all disinfection byproducts at 25 drinking water facilities including 10 in California (DHS, 1989) found an annual median total THM concentration of 36 μg/L, with 25 and 75 percentiles of about 22 μg/L and 58 μg/L, respectively (U.S. EPA, 1998j). In all of these surveys the ranking of individual THM concentrations, from highest to lowest, was chloroform, BDCM, DBCM, and bromoform. In the U.S. EPA (1989b,c) survey, the values included chloroform 14 μg/L; BDCM 6.6 μg/L, DBCM 3.6 μg/L, and bromoform 0.57 μg/L.

Krasner *et al*. (1989) reported that the proportions of individual THMs could be significantly affected by the amount of bromide in the source waters. Sources of bromide include saltwater intrusion, connate water (ancient geologically trapped seawater), oil-field brines, and industrial and agricultural chemicals as reported by the American Public Health Association (APHA, 1995). Among the 35 utilities studied, influent bromide levels ranged from less than 10 to 3,000 μg/L. The utility with the highest bromide level of 2,800 to 3,000 μg/L gave essentially a reversed pattern of THM occurrence: chloroform 0.59 μg/L, BDCM 2.9 μg/L, DBCM 9.2 μg/L, and bromoform 40 μg/L. The total THM levels were similar to other utilities studied. Another utility with somewhat lower bromide of 400 to 800 μg/L showed significant seasonal variation in THM composition, with chloroform from one to six percent and bromoform from 36 to 67 percent of THMs by weight. Source waters with higher bromide levels are more likely to be found in California than other regions. Reports on six utilities in North Carolina over three seasons showed low bromide of 10 μg/L with chloroform dominant among the

THMs. Ten surface water utilities in Ontario, Canada over two seasons similarly had low bromide of less than 20 μg/L with chloroform dominant (Krasner, 1999).

The geometric mean concentration for 44 pool water samples collected from eight indoor swimming pools in London, England was 5,800 μg/L for total organic content, 132.4 μg/L for total THMs, and 113.3 μg/L for chloroform. There was a clear positive linear correlation between the number of people in the swimming pool and concentrations of total THMs and chloroform ($r = 0.7$, $p < 0.01$), and a good correlation between concentrations of total organic content and total THMs ($r = 0.5$, $p < 0.05$) and water temperature and concentrations of total THMs ($r = 0.5$, $p < 0.01$). The variation in concentrations of THMs was greater within pools than between pools (Chu and Nieuwenhuijsen, 2002). Aggazzotti *et al*. (1995) reported total THMs in swimming pool water in Italy of 9 to 179 μg/L. Fantuzzi *et al*. (2001) reported THMs in five indoor swimming pools in Modena, Italy of 17.8 to 70.8 μg/L with a mean of 39.8 ± 21.7 μg/L. Judd and Bullock (2003) measured total THM concentrations of 5 to 65 μg/L in a swimming pool in the United Kingdom.

The formation of chloroform and BDCM by chlorination of the materials of human origin such as hair, lotion, saliva, skin, and urine, in a swimming pool model system has been reported. Chlorination reactions took place with a sufficient supply of chlorine residuals (0.84 mg Cl_2/L < total chlorine < 6.0 mg Cl_2/L) in 300 mL glass bottles at 30 °C and pH 7.0, for 24 or 72 hours. The longer reaction period or a higher content of organic materials led to the increased formation of disinfection byproducts, and chloroform was a major compound found. The formation of chloroform and BDCM per unit total organic carbon concentration was suppressed when all types of materials of human origin were added to water that already contained disinfection byproduct precursors such as humic substances (Kim *et al*., 2002).

In the Ground Water Supply Survey, conducted by the U.S. EPA from December, 1980, to December, 1981 (Westrick *et al.*, 1984), samples were tested from 466 randomly chosen groundwater supply systems and 479 nonrandom systems were chosen on the basis of location near industrial, commercial, or waste disposal activities. For the randomly chosen systems servicing more than 10,000 people, in the 57 percent of the samples where the compound was detected, the median positive concentration of chloroform was 1.6 μg/L; bromoform was 3.8 μg/L with an occurrence rate of 31 percent; BDCM was 1.4 μg/L with an occurrence rate of 36 percent; and DBCM was 2.1 μg/L with an occurrence rate of 31 percent. For the randomly chosen systems servicing less than 10,000 people, the median concentration of chloroform in the 37.1 percent positive samples was 1.4 μg/L; bromoform was 2.4 μg/L with an occurrence rate of 16 percent; BDCM was 1.6 μg/L with an occurrence rate of 54 percent; and DBCM was 2.9 μg/L with an occurrence rate of 52 percent. The nonrandomly chosen systems had a median concentration of chloroform of 1.8 μg/L in the 53 percent positive samples; bromoform 4.2 μg/L in 31 percent; BDCM 2.1 μg/L in 51 percent; and DBCM 3.9 μg/L in 46 percent (Westrick *et al*., 1984).

In U.S. EPA surveys (1991b, 2001c,d), occurrence data from the Unregulated Contaminant Information System database (Round 1 monitoring) and the Safe Drinking Water Information System database (Round 2 monitoring) provided representative

national occurrence information for unregulated drinking water contaminants. For Round 1, occurrence data were selected from approximately 22,000 public water systems, 88 percent from ground water and 12 percent from surface water, from 24 states including California. The 99th percentile values for chloroform, bromoform, BDCM, and DBCM were 87.0 µg/L, 7.3 µg/L, 22.0 µg/L, and 12.7 µg/L, respectively. For Round 2, occurrence data were selected from approximately 27,000 public water systems, 89 percent from groundwater and 11 percent from surface water, from 22 states not including California. The 99th percentile values for chloroform, bromoform, BDCM, and DBCM were 110.0 µg/L, 6.5 µg/L, 18.8 µg/L, and 9.7 µg/L, respectively.

In the California Water Quality Monitoring Database for public drinking water systems that use either ground or surface water sources (Storm, 1994), chloroform was detected in 4.5 percent of samples (536/11,848); bromoform was detected in 1.2 percent of samples (145 of 11,765); BDCM was detected in 1.4 percent of samples (167/11,773); and DBCM was detected in 1.5 percent of samples (171/11,782). The mean concentration in samples with detected chloroform was 6.20 µg/L, with detected bromoform was 7.63 µg/L, with detected BDCM weas 6.99 µg/L, and with detected DBCM was 8.95 µg/L.

In another survey of finished drinking water samples tested for THMs in clearwell effluent collected during 1988 and 1989 from 35 U.S. water treatment plants including 10 California plants (Krasner *et al*., 1989), quarterly median concentrations of chloroform ranged from 9.6 to 15 µg/L; bromoform from 0.33 to 0.88 µg/L; BDCM from 4.1 to 10 µg/L; and DBCM from 2.6 to 4.5 µg/L.

Chloroform

Air

Chloroform is ubiquitous in the environment, and can be measured in low concentrations in the air in both urban and rural areas. Chloroform is expected to exist solely as a vapor in the atmosphere (HSDB, 2002b). Chloroform was found to be emitted from anthropogenic and natural sources including the oceans and terrestrial areas (U.S. EPA, 1985a). Chloroform is a major contributor to natural gaseous chlorine involved in various catalytic atmospheric reaction cycles, due to the wide use of chloroform in industrialized societies and its release into the environment. Chloroform emissions from anthropogenic sources were estimated to account for less than 10 percent of the total emissions. Marine and terrestrial environments were estimated to be equal contributors of global biogenic chloroform emission. Among terrestrial sources, emissions of chloroform to the air from northern temperate forests were estimated to contribute less than one percent to the annual global atmospheric input (Laturnus *et al*., 2002).

The median ambient chloroform concentration was 0.06 ppb in the 1987 update of the National Ambient Volatile Organic Compounds Database (ATSDR, 1997). The background ambient level for chloroform in the U.S. was estimated to be 0.04 ppb based on 4,368 samples from 117 populated locations throughout the nation (Kelly *et al*., 1994).

In California, ambient air levels of chloroform were measured at 22 locations in 1985 to 1987. The air levels ranged from 0.02 to 3.54 ppb. The mean concentrations ranged

from 0.026 to 0.36 ppb, with a median of 0.06 ppb and a population weighted average of 0.03 ppb. Chloroform was above the detection limit of 0.02 ppb in 96 percent of ambient air samples collected at several sites in Southern California in 1982 to 1983 (Shikiya *et al*., 1984). Chloroform concentrations can be higher in air samples taken near point sources (ATSDR, 1997). The maximum concentration of chloroform found in air around 20 California municipal landfills was 0.61 ppm (Wood and Porter, 1987).

The main source of chloroform in indoor air is evaporation from chlorinated water during activities such as showering, bathing, clothes washing, and dish washing (Wallace, 1997). Median overnight indoor and outdoor concentrations in air samples from communities in Los Angeles, California, during the winter of 1984 were 0.32 ppb and 0.13 ppb, respectively. Median overnight indoor and outdoor concentrations in air samples from communities in Los Angeles, California, during the summer of 1984 were 0.17 ppb and 0.01 ppb, respectively. Median overnight indoor and outdoor concentrations in air samples collected from the Antioch/West Pittsburg air sampling station in Contra Costa County, California during the summer of 1984 were 0.08 ppb and 0.12 ppb, respectively (Pellizzari *et al*., 1986).

Howard and Corsi (1998) reported formation of chloroform due to the use of chlorine bleach or chlorinated tap water in residential washing machines. Olson and Corsi (2004) reported chloroform formation due to the use of hypochlorite-containing detergents in dishwashers. Liquid concentrations of chloroform ranged from 1 to 41 mg/L. Background concentrations of chloroform in the water supply were generally between 0 and 10 μg/L; liquid chloroform levels in the wash cycle were typically at least 50 μg/L.

Chloroform is also detected in the air above swimming pools and hot tubs (Wallace, 1997). Air samples above indoor swimming pools showed mean THM levels from 16 to 853 $\mu g/m^3$ (Aggazzotti *et al*., 1990, 1998; Fantuzzi *et al*., 2001). In a study by Fantuzzi *et al*. (2001) evaluating occupational exposure to THMs in public indoor swimming pools in Modena, Italy, chloroform was the most prevalent THM in the air above the pool, accounting for 79.5 percent of the total THMs. BDCM accounted for 15 percent, and DBCM for 5.4 percent. Bromoform was found in the air above one out of the five indoor swimming pools. The mean levels of THMs in air were 25.6 ± 24.5 $\mu g/m^3$ in the engine room, 26.1 ± 24.3 $\mu g/m^3$ in the reception area and 58.0 ± 22.1 $\mu g/m^3$ at the poolside. The mean chloroform levels in air were 21.7 ± 22.7 $\mu g/m^3$ in the engine room, 21.8 ± 19.3 $\mu g/m^3$ in the reception area and 46.1 ± 18.6 $\mu g/m^3$ at the poolside (Fantuzzi *et al*., 2001).

Soil

Based on its physical properties, chloroform is expected to be mobile in soil and easily volatilized from moist soil surfaces (HSDB, 2002b). These properties together with moderate water solubility suggest that chloroform will partition to water and air more than it will partition to soil (ATSDR, 1997). Chloroform was detected in eight percent of 425 sediment samples from U.S. EPA's STORET database, with median concentrations less than 5 μg/kg (Staples *et al*., 1985).

Hoekstra *et al*. (1998) studied the natural formation of chloroform and brominated THMs from added $Na^{37}Cl$ in soil in two forests in the Netherlands. The percentage of

chloroform formed from $Na^{37}Cl$ had a maximum of 17 weight percent. The maximum concentration of chloroform was found at a depth of 20 cm and remained almost stable at 20 to 30 ng/L down to 160 cm. The concentration of chloroform in a spruce forest in Denmark increased linearly with depth, up to about 55 ng/L at 40 cm. The study suggests that chloroform is not formed in the top layer of soil, but in deeper soil layers.

Water

Chloroform is inadvertently produced when natural water is chlorinated. It is also formed in disinfection with chlorine dioxide and in the chloramination disinfection process, although usually at lower levels (IARC, 1991b, NRC, 1986). When chlorine is added to water as a disinfectant, it forms hypochlorous acid. Hypochlorous acid can then react with organic materials in the water to form chloroform (Wallace, 1997). Ozonation before chlorination can enhance formation of chloroform (AWWA, 1991).

Chloroform tends to be the most prevalent, with THMs as the largest class of disinfection byproducts detected in chlorinated drinking water; however, brominated THMs can occur at high levels when water with high bromide levels is chlorinated (McGuire and Meadow, 1988; Krasner *et al*., 1989; Chen and Weisel, 1998; Keegan, 1998; Shin *et al*., 1999; Nieuwenhuijsen *et al*., 2000b). Quarterly mean concentrations of chloroform in drinking water from 9.6 to 15 μg/L were reported in 35 U.S. utilities (Krasner *et al*., 1989); and at 14 or 33 μg/L for cold or warm season in the U.S. (Chen and Weisel, 1998).

Chloroform concentrations in California ground water were reported from a survey of utilities with more than 200 service connections (DHS, 1986). From January, 1984, through December, 1985, 2,947 out of 5,650 wells in 819 water systems were sampled for organic chemical contamination. Chloroform was found in 112 wells with a maximum concentration of 54 mg/L and a median of 1.27 mg/L. Generally, the most contaminated wells were found in the heavily urbanized areas of the state. Los Angeles County registered the greatest number (76) of wells with detectable chloroform.

Chloroform has been detected in swimming pool water and the surrounding air (Nieuwenhuijsen *et al*., 2000b). Weisel and Shepard (1994) reported a mean chloroform level of 85 μg/L, range 32 to 150 μg/L, in swimming pools in the U.S. Lindstrom *et al*. (1997) measured chloroform levels of 68 and 73 μg/L in swimming pools. The geometric mean concentration in 44 water samples from eight indoor swimming pools in London was 113.3 μg/L for chloroform (Chu and Nieuwenhuijsen, 2002). In the Netherlands, water chloroform concentration was 18.4 μg/L in indoor pools and 24.0 μg/L in outdoor pools (Aiking *et al*., 1994). The median chloroform concentration of 21 water samples from spas in public indoor pools in Germany was 3.8 μg/L with a maximum concentration of 6.4 μg/L (Erdinger *et al*., 1997). Chloroform concentration of the water was 7.1, 20.7, and 24.8 μg/L in three samples of an indoor swimming pool and 11.6 ± 1.2 μg/L as the mean of hourly monitoring over one day of the same pool (Erdinger *et al*., 2004), and ranged from 3.04 to 27.8 in μg/L in indoor swimming pools in Germany Cammann and Hubner, 1995). Several studies in northern Italy reported chloroform levels in swimming pool water of 14 to 198 μg/L (Aggazzotti *et al*., 1990, 1993, 1995, 1998) with means of 274.4 nmol/L (Aggazzotti *et al*., 1990), 312.9 nmol/L

(Aggazzotti *et al*., 1993), 32.7 μg/L (Aggazzotti *et al*., 1995), 33.7 μg/L (Aggazzotti *et al*., 1998), or 33.2 ± 24.6 μg/L (Fantuzzi *et al*., 2001).

Food

Chloroform and related THMs may occasionally occur in soft drink products as a result of superchlorination of process waters to control taste, odor, and microbial contamination (Brown *et al*., 1993). Commercially bottled drinks are expected to have the highest concentration of THMs among U.S. food sources, since they are often prepared directly from chlorinated water (McNeal *et al*., 1995). In a survey of soft drinks, juices, beers, bottled water, and canned foods purchased from markets in Washington, D.C. from 1991 to 1992, chloroform levels ranged from nondetected up to 94 μg/L in the 44 foods analyzed, at a detection limit of 0.05 μg/L (McNeal *et al*., 1995).

The U.S. Food and Drug Administration (U.S. FDA, 2000) has routinely tested foods for volatile organic compounds including chloroform since 1995. Chloroform concentration in a subset of 70 table-ready food items in 14 Market Baskets tested from 1995 to 1999 was reported to be 14 to 176 ppb in 12 sour cream samples, 3 to 107 ppb in 37 cheese samples, 35 to 83 ppb in 14 butter samples, 11 to 27 ppb in six cola samples, 0 to 27 ppb in five sherbet samples, and 7 to 14 ppb in three margarine samples (Fleming-Jones and Smith, 2003). Chloroform was found in dairy products, bakery products, meats, eggs, nut, avocado, orange, strawberry, peanut butter, popcorn, olive oil, safflower oil, beer, potato chips, and french fries.

Possible explanations for the occurrence of chloroform are migration of chloroform from packaging solvents, glues, and inks, use of chlorinated water to clean surfaces that come into contact with dairy products, use of hypochlorite disinfectants, or the exposure of dairy cows to chlorinated water or other chlorinated substances (Wallace, 1997).

Other Sources

Chloroform has occasionally been used in medicinal products as a solvent and in earlier times as an anesthetic (ATSDR, 1997). Chloroform is produced by tropical red algae *Asparagopsis armata* and red seaweed *Asparagopsis taxiformis*. The transfer of biogenic chloroform from tropical oceans into air has been estimated as 350×10^6 tonnes per year (Dewulf and Van Langenhove, 1997). Chloroform has been found at 1 to 2 ppm in peat bog samples from Lower St. Mary's Bog in New Brunswick, Canada (Silk *et al*., 1997).

Bromoform

Air

Bromoform is expected to exist solely as a vapor in the ambient atmosphere (HSDB, 2002d). Atmospheric concentrations of bromoform are expected to be small because bromoform is not produced or used in large quantities (ATSDR, 1990). Bromoform was found above the detection limit of 0.01 ppb in 31 percent of ambient air samples collected at several sites in Southern California in 1982 to 1983 (Shikiya *et al*., 1984).

Bromoform has been detected in the same situations as the other THMs. Median air concentration in four urban and suburban areas was 0.64 μg/m^3; swimming pool air in four German indoor pools ranged from 0.7 to 6 μg/m^3; air above five outdoor U.S. swimming pools had less than 0.1 μg/m^3; four indoor U.S. pools were from less than 0.1 to 20 μg/m^3; four U.S. hot tubs were from less than 0.1 to 62 μg/m^3 (IARC, 1991b). Air samples collected in 1984 to 1987 in Point Barrow, Alaska showed a mean of 6.3 ng/m^3 bromoform, while in Cape Kumukahi, Hawaii, the mean was 3.1 ng/m^3 (IARC, 1991b).

As with chloroform, the main source of bromoform in indoor air is evaporation from chlorinated water during household activities such as showering, bathing, clothes washing, and dish washing.

Soil

Soils may become contaminated with bromoform through spills, waste disposal in landfills, or the discharge of chlorinated water (ATSDR, 1990). Based on its physical properties, bromoform is expected to be very mobile in soil. Volatilization of bromoform from moist soil surfaces is also expected (HSDB, 2002d). These properties, together with moderate water solubility, suggest that bromoform will partition to water and air more than it will partition to soil (ATSDR, 1990). Bromoform was detected in atmospheric air at concentrations of 0.018 ± 0.006 ng/L, and was not detected in soil (U.S. EPA, 1985b). After the addition of KBr solution to soil of a Douglas fir forest, the formation of bromoform was observed at 0.14 ± 0.08 ng/L.

Water

Bromoform is inadvertently produced when natural water containing bromide ion is chlorinated (IARC, 1991b). When chlorine is added to water as a disinfectant, it forms hypochlorous acid which can then oxidize bromide ion to form hypobromous acid. Finally, hypobromous acid can react with organic materials to form bromoform, BDCM, or DBCM (Wallace, 1997). Bromoform is also produced during ozonation if bromide ion is present (Sadiq and Rodriguez, 2004). In addition, the decomposition of tribromoacetic acid in water at pH six to nine to form bromoform via a decarboxylation pathway has been reported (Zhang and Minear, 2002). The U.S. EPA's (1998c) survey of 25 drinking water treatment plants showed seasonal median bromoform concentrations ranging from 0.33 μg/L in the spring to 0.88 μg/L in the fall. Bromoform has been detected in swimming pool water (Aggazzotti *et al*., 1990, 1998; Fantuzzi *et al*., 2001). Fantuzzi *et al*. (2001) reported that bromoform was detected in four out of five water samples from five indoor swimming pools at 0.4 ± 0.5 μg/L in northern Italy.

Food

In a survey of soft drinks, juices, beers, bottled water, and canned foods purchased in Washington, D.C. from 1991 to 1992, bromoform was not found in any of the products at a detection limit of 0.2 μg/L (McNeal *et al*., 1995). The U.S. FDA (2000) has routinely tested foods for bromoform in the Total Diet Study since 1995. Bromoform concentration was analyzed in a subset of 70 food items and no detections were reported.

Because chloroform has been found in dairy products, it is possible that other THMs such as bromoform can also occur (Wallace, 1997). In a study conducted in Spain, bromoform was detected in fruit juice samples at 2.5 to 4.2 µg/L (Campillo *et al*., 2004).

Other Sources

Bromoform is produced naturally by marine algae (IARC, 1991b). One study measured a bromoform production rate of 83 ng/hour-g (wet weight) for coralline algae (*Corallinaceae*) from the Sea of Japan. Another study measured bromoform production rates of 5.7 to 12.7 (median 7.1) ng/hour-g fresh weight and 5.7 to 12.7 (median 10.7) ng/hour-g fresh weight for giant kelp (*Macrocystis pyrifera*) collected from the southern California coastal region (HSDB, 2002d).

Bromodichloromethane

Air

BDCM occurs mainly as a vapor in the ambient atmosphere (HSDB, 2002a). Atmospheric concentrations of BDCM are expected to be small because BDCM is not produced or used in large quantities (ATSDR, 1989). BDCM was above the detection limit of 0.3 ppb in only 35 percent of ambient air samples collected at several sites in Southern California in 1982 to1983 (Shikaya *et al*., 1984). BDCM was detected in 64 percent of Texas ambient air samples at a mean concentration of 1.23 $\mu g/m^3$ and in 17 percent of North Carolina ambient samples at a mean of 0.83 $\mu g/m^3$ (IARC, 1991a).

The main source of BDCM in indoor air is from evaporation of chlorinated water during activities such as showering, bathing, clothes washing, and dish washing (Wallace, 1997). BDCM has also been detected in air above swimming pools and hot tubs (Wallace, 1997). In a study evaluating occupational exposure to THMs in air at indoor swimming pools in northern Italy, the mean BDCM levels in ambient air were 2.9 to 8.7 $\mu g/m^3$ around the facilities (Fantuzzi *et al*., 2001).

Soil

Based on its physical properties, BDCM is expected to volatilize from moist soil surfaces and to be very mobile in soil (HSDB, 2002a). These properties together with moderate water solubility suggest that BDCM will partition to water and air more than to soil (ATSDR, 1989). The U.S. EPA (1985b) reported analysis of 581 sediment samples with a mean BDCM concentration of 10.8 µg/kg (range from nondetected to 55 µg/kg). BDCM was detected in nearly all the soil air samples, at concentrations from 0.03 to 0.31 ng/L, with higher levels at deeper depths.

Water

BDCM is inadvertently produced when water is chlorinated (IARC, 1991a). When chlorine is added to water as a disinfectant, it forms hypochlorous acid. Hypochlorous acid can then oxidize bromide ion to form hypobromous acid. Finally, hypobromous acid

can react with organic materials to form BDCM (Wallace, 1997). Decomposition of bromodichloroacetic acid in water at a pH in the range of six to nine to form BDCM via a decarboxylation pathway has also been reported (Zhang and Minear, 2002).

The STORET database reported a mean BDCM concentration of 11.14 μg/L based on 19,550 water samples ranging from nondedected to 10.1 mg/L (U.S. EPA, 1985b). U.S. EPA's (1998j) survey of 25 drinking water treatment facilities reported median seasonal BDCM concentrations from a low of 4.1 μg/L in winter to a high of 10 μg/L in summer. BDCM has been detected in swimming pool water (Aggazzotti *et al*., 1990, 1998; Fantuzzi *et al*., 2001). Fantuzzi *et al*. (2001) reported that BDCM was detected in all five water samples from five indoor swimming pools in northern Italy, at 4.2 ± 1.3 μg/L.

Food

In a survey of soft drinks, juices, beers, bottled water, and canned foods purchased from markets in Washington, D.C. from 1991 to 1992, BDCM was found at levels ranging from nondetected to 12 μg/L, at a detection limit of 0.1 μg/L (McNeal *et al*., 1995).

The U.S. FDA (2000) has analyzed for BDCM in the Total Diet Study since 1995. BDCM was assayed in 70 food items in 14 Market Baskets and detected in one sample each of bologna, fried eggs, canned pork and beans, peanut butter, homemade cornbread, oranges, canned pineapple, boiled collards, tomato, green pepper, and cooked hamburger from 1995 to 1999 (U.S. FDA, 2000). The highest concentration found was 37 ppb, in fast food hamburger (Fleming-Jones and Smith, 2003). Abdel-Rahman (1982) analyzed various U.S. soft drinks for BDCM. Cola beverages had mean ranges of 0.9 to 5.9 μg/L while other soft drink types without caramel coloring were somewhat lower, at 0.1 to 3.3 μg/L. Uhler and Diachenko (1987) analyzed process water and food products from 15 U.S. food-processing plants, and detected BDCM in seven process water samples at less than 1 to 14.1 μg/kg, in three soft drink samples from one plant at 1.2 to 2.3 μg/kg, and in three ice cream samples from one plant at 0.6 to 2.3 μg/kg. A later analysis found concentrations of 2.3, 3.4, and 3.8 μg/L in three cola drinks and 7 μg/kg in a butter sample (NTP, 2002a). In a study conducted in Spain, BDCM was detected in pineapple juice at 2.1 μg/L (Campillo *et al*., 2004).

Other Sources

BDCM is produced naturally by marine algae (IARC, 1991a). BDCM was found in intertidal macroalgae tissue from three sites around Cape Cod, Massachusetts, with mean concentrations of 7 to 22 ng/g dry weight. The algal species sampled were the brown algae (*Ascophyllum nodosum* and *Fucus vesiculosis*), the green algae (*Entermorpha linza* and *Ulva lacta*), and the red algae (*Gigartina stellata*) (Gschwend *et al*., 1985). Marine microalgae communities from McMurdo Sound, Antarctica, contained BDCM at concentrations ranging from 0.1 to 50 ng/cm^3 (Sturges *et al*., 1993).

Dibromochloromethane

Air

DBCM occurs as a vapor in the ambient atmosphere (HSDB, 2002c). Atmospheric concentrations of DBCM are expected to be small because DBCM is not produced or used in large quantities (ATSDR, 1990). DBCM was above the detection limit of 0.01 ppb in only 17 percent of ambient air samples collected at several sites in Southern California in 1982 to 1983 (Shikaya *et al*., 1984). In another study, DBCM was detected in ambient air at a median concentration of 120 ng/m^3 in 21 urban/suburban areas (Brodinsky and Singh, 1983). Levels of DBCM in air samples collected in 1982 to 1985 over the Atlantic Ocean were 0.06 to 10 ppt (0.5 to 85.2 ng/m^3). Levels of biogenic DBCM in air from marine algae were 0.1 to 0.5 ppt (0.9 to 4.3 ng/m^3) (IARC, 1991c).

The main source of DBCM in indoor air is volatilization from chlorinated water during activities such as showering, bathing, clothes washing, and dish washing (Wallace, 1997). DBCM has also been detected in air above outdoor swimming pools at 0.4 to 2 μg/m^3, indoor pools at 0.5 to 17 μg/m^3, and hot tubs at less than 0.1 to 31 μg/m^3 (Armstrong and Golden, 1986). DBCM has also been detected in the air above swimming pools and hot tubs (Wallace, 1997). In a study evaluating occupational exposure to THMs from indoor swimming pools in northern Italy, the DBCM levels in indoor ambient air around the facilities were 1.6 to 3.1 μg/m^3 (Fantuzzi *et al*., 2001).

Soil

Soils may become contaminated with DBCM through spills, waste disposal in landfills, or the discharge of chlorinated water (ATSDR, 1990). Based on its physical properties, DBCM is expected to be very mobile in soil and readily volatilized from moist soil surfaces (HSDB, 2002c). These properties together with moderate water solubility suggest that DBCM will partition to water and air more than to soil (ATSDR, 1990). DBCM was not detected either in atmosphere or soil air (U.S. EPA, 1985b). After addition of KBr solution to soil of a Douglas fir forest, formation of DBCM (at the detection limit of 0.005 ng/L) was observed.

Water

DBCM is inadvertently produced when natural water is chlorinated (IARC, 1991c). When chlorine is added to water as a disinfectant, it forms hypochlorous acid, which can then oxidize bromide ion to form hypobromous acid. Finally, hypobromous acid can react with organic materials to form DBCM (Wallace, 1997). In addition, the decomposition of dibromochloroacetic acid in water at a pH range of six to nine to form DBCM via a decarboxylation pathway was reported (Zhang and Minear, 2002).

The U.S. EPA STORET database lists DBCM concentrations in 130 surface water samples ranging from 0.1 to 1.0 μg/L in 80 percent of samples and 1 to 10 μg/L in 20 percent of samples (Perwak *et al*., 1980). U.S. EPA's (1998j) survey of 25 drinking water treatment facilities reported median seasonal DBCM concentrations from a low of 2.6 μg/L in the spring to a high of 4.5 μg/L in the summer.

DBCM has been detected in swimming pool water (Aggazzotti *et al*., 1990, 1998; Fantuzzi *et al*., 2001). Fantuzzi *et al*. (2001) reported that DBCM was detected in all five water samples from five indoor swimming pools in northern Italy at 1.9 ± 2.0 μg/L.

Food

Commercially bottled drinks are expected to have the highest concentration of THMs among U.S. food sources since they are often prepared directly from chlorinated water (McNeal *et al*., 1995). In a FDA survey of beverages and canned foods purchased from markets in Washington, D.C. from 1991 to 1992, DBCM was found at levels from nondetected (detection limit 0.1 μg/L) to 2 μg/L (McNeal *et al*., 1995). DBCM is not included among the 18 volatile hydrocarbons monitored in U.S. FDA's Total Diet Study (U.S. FDA, 2000). Bauer (1981) reported that the mean DBCM concentration in 12 milk products including ice cream, yogurt, curds, and buttermilk was 0.1 μg/kg fresh weight.

Other Sources

DBCM is produced naturally by marine algae (IARC, 1991c). DBCM was found in intertidal macroalgae tissue from three sites around Cape Cod, Massachusetts, with mean concentrations ranging from 160 to 590 ng/g dry weight. The algal species sampled were the brown algae (*Ascophyllum nodosum* and *Fucus vesiculosis*), the green algae (*Entermorpha linza* and *Ulva lacta*), and the red algae (*Gigartina stellata*) (Gschwend *et al*., 1985). Marine microalgae communities from McMurdo Sound, Antarctica, contained DBCM at concentrations ranging from 10 to 25 ng/cm^3 (Sturges *et al*., 1993).

EXPOSURE ASSESSMENT

Concern about chronic exposure to disinfection byproducts, particularly the THMs, and human cancer has been a controversial subject for many years. Epidemiological studies on the relationship between exposure to contaminants in chlorinated surface water and adverse developmental and reproductive effects have increased the concern and controversy. As noted by U.S. EPA (1998c): "the assessment of public health risks from chlorination of drinking water currently relies on inherently difficult and incomplete empirical analysis. On one hand, epidemiological studies of the general population are hampered by difficulties of design, scope, and sensitivity. On the other hand, uncertainty is involved in using the results of high dose animal toxicological studies of a few of the numerous byproducts that occur in disinfected drinking water to estimate the risk to humans from chronic exposure to low doses of these and other byproducts."

Kerger *et al.* (2000, 2005) evaluated airborne concentrations of THMs in selected living spaces of three homes in an arid urban area, each having three bedrooms, a full bath, and approximately 1,000 square feet; two homes had standard refrigeration-type central air conditioning and the third had a central evaporative cooling system called a 'swamp cooler,' supplied with chlorinated tap water containing more than 85 ppb total THM. Trihalomethanes were concurrently measured on four test days in tap water and air in the bathroom, living room, the bedroom closest to the bathroom, and outside. No bromoform

was detected in any sample. Air samples were collected prior to, during, and after water use for 16 shower and seven bath events (Kerger *et al.*, 2000). The increase in average airborne concentration ± standard error during showers, expressed as $\mu g/m^3$ in the shower enclosure or bathroom air per µg/L in water, was 3.3 ± 0.4 for chloroform, 1.8 ± 0.3 for BDCM, and 0.5 ± 0.1 for DBCM (N = 12), and during baths was 1.2 ± 0.4 for chloroform, 0.59 ± 0.21 for BDCM, and 0.15 ± 0.05 for DBCM (N = 4). The relative contribution of each chemical to the airborne concentrations was consistent for all shower and bath events, and consistent with their relative concentration in tap water and their vapor pressures, with apparent release of chloroform greater than BDCM, followed by DBCM. When the shower findings for chloroform were normalized for water concentration, flow rate, shower volume, and duration, the average exposure concentrations in these urban residences were about 30 percent lower than those reported by other investigators using the same U.S. EPA analytical methods. This difference was likely attributable to greater air exchange rates in residential shower/bath stalls compared to more 'airtight' laboratory shower chambers. Relatively low airborne THM concentrations (similar to outdoors) were found in the living room and bedroom for the home with evaporative cooling, while the refrigeration-cooled homes showed significantly higher THM levels (three- to fourfold). This differential remained after normalizing air concentrations based on estimated THM throughput or water concentrations. The findings indicated that, despite higher throughput of THM-containing water in homes using evaporative coolers, the higher air exchange rates of these systems rapidly cleared THM to levels similar to ambient outdoor concentrations.

Backer *et al*. (2000) examined exposure of 31 adults to THMs through drinking 1 L of tap water during a 10-minute time period (N = 10), showering with tap water for 10 minutes (N = 11), or bathing for 10 minutes in a bathtub filled with tap water (N = 10), and concluded that bathing and showering are important routes for human exposure to THMs. The highest levels of THMs were found in the blood samples from people who took 10-minute showers, whereas the lowest levels were found in the blood samples from people who drank 1 L of water in 10 minutes.

Lynberg *et al*. (2001) and Miles *et al*. (2002), based on a field study conducted in Corpus Christi, Texas, and Cobb County, Georgia, on mothers living in either geographic area who had given birth to healthy infants from June 1998 through May 1999, documented elevated background levels of individual THMs in human blood. In the Corpus Christi water samples, brominated compounds accounted for 71 percent of the total THM concentration by weight; in Cobb County, chloroform accounted for 88 percent. Two 10-mL whole blood samples were collected from each participant before and immediately after her shower. Levels of chloroform, BDCM, DBCM, and bromoform were measured in whole blood at parts per trillion (ppt) and in water samples at ppb levels. Lynberg *et al*. (2001) also reported that blood levels of THM species vary substantially across populations depending on both water quality characteristics and water use activities. Miles *et al*. (2002), measured THM concentrations in blood and tap water for 50 women living in these two locations with different bromide concentrations and disinfectant types, and concluded that blood concentrations were not significantly correlated with tap water concentrations. This finding suggests that other factors, in addition to tap water concentrations, may be important in determining THM concentrations in the blood.

Blood samples were taken from each woman early in the morning prior to any major water-use activity and again immediately after showering. Each residence was sampled for THMs in tap water prior to the woman's shower. THMs in tap water and blood were compared using mole fraction speciation, extent of bromine incorporation, and correlation analysis. Results indicated that THMs in the blood rose significantly as a result of showering, that showering shifted the THM distribution in the blood toward that found in the corresponding tap water, and that THMs measured in the blood of women living in the two locations reflected species and concentration differences in their respective tap waters (Betts, 2002; Miles *et al*., 2002).

Disinfection byproduct concentrations in tap water in the city of Cherepovets, Russia, where heavy chlorination was used to disinfect organic-rich surface water were 205 ± 70 µg/L (mean ± standard deviation) of total THMs, 150 ± 30 µg/L of five haloacetic acids, and 160 ± 50 ng/L of 3-chloro-4-(dichloromethyl)-5-hydroxy-2(5H)-furanone or MX (Egorov *et al*., 2003). Concentrations of THMs and haloacetic acids exceeded the corresponding U.S. standards, while MX concentrations were the highest ever reported. Concentrations of chloroform in breathing zone air in bathrooms during showering were 330 ± 260 $\mu g/m^3$, shower room air at an industrial plant 2,600 ± 1,100 $\mu g/m^3$, and bedrooms of local residents 2 ± 2 $\mu g/m^3$. The mean concentration of chloroform was 3.2 $\mu g/m^3$ in exhaled air samples collected before showering and 110 $\mu g/m^3$ after showering.

Symanski *et al.* (2004) assessed spatial fluctuations, temporal variability, and errors due to sampling and analysis in levels of disinfection byproducts in tap water for an exposure assessment study. They concluded that the utility of routine monitoring data in assigning exposure in epidemiological studies was limited because of spatial variability in levels of disinfection byproducts across the distribution system. Measurement error contributed a relatively small proportion to the total variation in THM levels, which suggested that collecting more samples with fewer replicates at each sampling location would likely yield improved estimates of household exposure. THM levels were higher in the summer than in other seasons. Differences in the relative magnitude of the intra- and inter-household components of variation were observed between the two sets of THM measurements, with a greater proportion of the variation due to differences within seasons for the routine monitoring data and a greater proportion of the variation due to differences across locations for the exposure assessment study data.

King *et al*. (2004) used 360 Canadian household water samples in Eastern Ontario and Nova Scotia and interviewed questionnaire information on water-use behavior to examine several aspects of exposure assessment: (i) the distribution and correlation of specific THMs and haloacetic acids, (ii) spatial distribution system and temporal variation in disinfection byproduct levels, and (iii) the contribution of individual tap water ingestion and showering and bathing practices. In both provinces, total THMs correlated highly with chloroform (correlation coefficient $r > 0.95$) and less so with total haloacetic acids ($r = 0.74$ in Nova Scotia and $r = 0.52$ in Ontario). The correlation between total THMs and BDCM was higher in Nova Scotia ($r = 0.63$), but lower in Ontario ($r = 0.26$). The correlation between THM level in individual household samples and the mean THM level during the same time period from several distribution system samples was 0.63, while a higher correlation in THM level was observed for samples taken at the same location one

year apart (r = 0.87). A correlation of 0.73 was found between household THM level and a total exposure measure incorporating ingestion, showering, and bathing behaviors.

In a Korean study (Jo *et al.*, 2005), measurements of THM concentrations in tap water and indoor and outdoor air in households using municipal tap water treated with ozone-chlorine or chlorine were used to estimate the THM exposure from water ingestion, showering, and inhalation of indoor air. Chloroform was the most abundant THM in all three media. The winter median chloroform concentration in tap water treated with chlorine, at 22.1 µg/L, was significantly higher than that in the tap water treated with ozone-chlorine, at 16.8 µg/L. No bromoform was detected in any sample. However, the summer water median chloroform concentrations for the two treatments, at 26.9 µg/L and 27.5 µg/L, respectively, and the summer and winter water concentrations of BDCM and DBCM exhibited no significant differences between the chlorine and ozone-chlorine-treated water. The indoor air THM concentration trend was consistent with the water concentration trend. The THM exposure estimates from water ingestion, showering, and inhalation of indoor air when not in the shower suggested that residents' exposure to THMs in the home was mostly associated with their household water uses. The THM exposure estimates from tap water ingestion were similar to those from showering.

As with other volatile organic chemicals found in drinking water, tap water ingestion, inhalation of indoor air into which THMs have volatilized from tap water, and dermal absorption from direct water exposure (e.g., from washing and bathing) are expected to be the principal routes by which the general population may be exposed to THMs in tap water (Jo *et al*., 1990a,b, 2005; Nieuwenhuijsen *et al*., 2000a,b; Kerger *et al*., 2000, 2005; Wallace, 1997; Weisel and Jo, 1996). Boiling for one to five minutes can reduce THM concentrations from 64 to 98 percent in chlorinated water or 74 to 98 percent in chloraminated water (Krasner and Wright, 2005).

The principal source of human exposure to THMs is expected to be tap water from drinking water systems disinfected by chlorination (ATSDR, 1997). Evidence that other exposure sources are minor includes the small current uses and low detection in food and other consumer products (McNeal *et al*., 1995; ATSDR, 1997), and ambient air (Shikiya *et al*., 1984). Studies of human exposure to volatile organic chemicals including THMs in drinking water indicate that in addition to ingestion, inhalation exposure (from chemical volatilized from showering and other indoor activities) and dermal exposure can contribute significantly to daily intake (McKone, 1987; Jo *et al.*, 1990a,b; Weisel and Jo, 1996; Wallace, 1997; Weisel *et al*., 1999; Nieuwenhuijsen *et al*., 2000a,b; Xu *et al*., 2002).

Exposure Factors

Exposure assessment models, pharmacokinetic models, and experimental data on breath concentrations of chloroform associated with inhalation exposure and dermal absorption during showering in chlorinated water indicate that these exposure routes contribute significantly to overall exposure (McKone, 1987, 1989; Jo *et al*., 1990a,b). These studies indicated that the dose of volatile organic compounds associated with showering is similar to the dose resulting from ingesting 2 L of the same water. U.S. EPA (1991a)

suggested this in a guidance memorandum from its Risk Assessment Forum: "Exposure to volatile organic compounds in tap water during showering has been found to be approximately equivalent, within an order of magnitude (i.e., plus or minus a factor of three), to exposure from ingesting two liters per day of the same water."

Jo *et al*. (1990a,b) examined inhalation and dermal exposure to dilute aqueous chloroform in humans by measuring chloroform concentration in exhaled breath of volunteers, with and without rubber wetsuits, following showers using normal tap water at approximately 40 °C containing chloroform at about 5 to 35 ppb. Both respiratory and dermal uptakes of the aqueous chloroform were demonstrated. Chloroform levels in exhaled air were significantly higher in individuals exposed by inhalation and dermal route compared to an exposure restricted to the inhalation route. The dermal and inhalation routes were estimated to contribute the equivalent amount of chloroform to body burden during showering. Based on the ingestion of 2 L/day of drinking water per day and 50 percent absorption of chloroform by the inhalation route (the authors assumed 77 percent), 32 percent of the total exposure to chloroform in a domestic water supply is estimated to occur as the result of inhalation and dermal exposure in the shower.

The steady state permeation coefficients, Kp, of THMs in aqueous solution across human skin with *in vitro* diffusion chambers were estimated to be 0.16 cm/hour ± 4.8 percent for chloroform, 0.21 cm/hour ± 2.4 percent for bromoform, 0.18 cm/hour ± 4.3 percent for BDCM, and 0.20 cm/hour ± 2.5 percent for DBCM (Xu *et al*., 2002). Chloroform had the lowest Kp value and was the least permeable through the skin among the four THMs evaluated, while bromoform had the highest Kp value. The *in vivo* permeability of chloroform was estimated to be 0.015 cm/hour (Xu and Weisel, 2004).

Weisel and Jo (1996) measured separately the ingestion, inhalation, and dermal exposures to chloroform from tap water. Their experimental design involved six male and five female volunteers between 20 and 50 years of age, either wearing waterproof suits or breathing purified air during showering for 10 minutes or bathing for 30 minutes, to isolate the dermal or inhalation exposure routes. The chloroform concentrations in exhaled breath were elevated in each subject after both inhalation and dermal exposures during showering. Breath concentrations were also elevated after dermal exposure via bathing. The observations supported prior predictions of a PBPK model for chloroform exposures from tap water (Blancato and Chiu, 1993). They used a total ingestion of 10 μg/L of chloroform (0.5 L × 20 μg/L), which is similar to environmental levels. The amount of chloroform expired per μg chloroform/L of water was calculated from the expired breath profiles, assuming a respiration rate of 0.01 m^3/minute. The values ranged from 0.02 to 0.05 μg for inhalation-only exposure, from 0.02 to 0.13 μg for the dermal-only shower exposure, and from 0.33 to 0.56 μg for the dermal bathing exposure. The internal doses calculated from the air concentration, breathing rate, duration of shower exposure, and absorption efficiency of the lung ranged from 30 to 80 μg for chloroform. For typical drinking and showering activities, each exposure route contributes similar internal doses. However, the authors note that in contrast to ingestion exposure, after inhalation or dermal exposures, exhaled breath had elevated chloroform levels for extended time periods of a few hours, indicating extensive distribution throughout the body prior to metabolism. Thus depending on the site(s) and mode(s) of carcinogenic

action, dermal or inhalation exposures could differ significantly from ingestion exposures in assessed cancer risks.

A PBPK modeling study of human dermal absorption of chloroform from bath water at concentrations less than 100 ppb (Corley *et al*., 1999, 2000) indicated a lower contribution of 18 percent of total body burden, assuming 2 L/day via the ingestion route and a 30-minute bath at 40 °C. A significant decrease in the amount of chloroform exhaled was observed as exposure temperatures were decreased from 40 °C to 30 °C. The blood flows to the skin and effective skin permeability coefficient (Kp) were both varied to reflect the temperature-dependent changes in physiology and exhalation kinetics. At 40 °C there were no differences in the exhalation kinetics of chloroform between males and females; Kp was about 0.06 cm/hour at a skin blood flow rate of 18 percent of the cardiac output (Corley *et al*., 1999). At 30 and 35 °C, males exhaled more chloroform than females, indicating lower effective Kps for females. At these lower temperatures, the blood flow to the skin was also reduced. Total amounts of chloroform absorbed averaged 41.9 and 43.6 μg for males and 11.5 and 39.9 μg for females exposed at 35 and 40 °C, respectively. The total intake of chloroform would have ranged from 79 to 194 μg if the volunteers had consumed 2 L of water orally at the concentrations used in this study. Thus, the relative contribution of dermal uptake of chloroform to the total body burdens associated with bathing for 30 minutes and drinking 2 L of water was predicted to range from 1 to 28 percent, depending on the temperature of the bath, ignoring contributions from inhalation exposures (Corley *et al*., 2000) .

A subpopulation potentially at higher risk due to increased exposure to chloroform and other THMs is swimmers and staff in chlorinated swimming pools. Aggazzotti *et al*. (1990) evaluated swimmers and visitors who were exposed to chloroform at three indoor swimming pools in Modena, Italy. Chloroform was measured in blood plasma samples of 127 subjects at the pools and in 40 nonexposed subjects. Chloroform was present in all the pool subjects (median 7.5 nmol/L, range from 0.8 to 25.1 nmol/L). Concentrations of chloroform in pool waters ranged from 142 to 394 nmol/L (17 to 47 μg/L). Mean levels of chloroform in environmental air ranged from 553 to 5,445 nmol/m^3 (66 to 650 μg/m^3). Swimmers who trained for competitions (N = 102) showed a significantly higher mean value of plasma chloroform. Plasma chloroform levels were significantly correlated with chloroform water concentration, the number of swimmers in the pools, and the time spent swimming.

In a follow-up study, Aggazzotti *et al*. (1998) found that chloroform was the predominant THM in water and ambient air before and after swimming in chlorinated indoor pools. BDCM and DBCM were always present but at lower levels than chloroform, and bromoform was rarely present. Chloroform, BDCM, and DBCM were detected in all alveolar air samples collected inside the swimming pool. Before swimming, after one hour at the pool edge, the mean values of five swimmers were 29.4 ± 13.3, 2.7 ± 1.2 and 0.8 ± 0.8 μg/m^3, respectively, while after spending one hour swimming the levels were higher (76.5 ± 18.6, 6.5 ± 1.3 and 1.4 ± 0.9 μg/m^3, respectively). Only chloroform was detected in all blood samples (mean 1.4 ± 0.5 μg/L), while BDCM and DBCM were detected in only a few samples at a detection limit of 0.1 μg/L. The authors calculated THM absorption using the following relation:

$$U = (C_{amb} - C_{alv}) \times t \times V$$

In this equation U is uptake in μg/minute, C_{amb} is ambient air concentration (μg/m^3), C_{alv} is alveolar concentration (μg/m^3), V is pulmonary ventilation rate (L/minute), and t is exposure time in minutes. The uptake at rest was calculated using ventilation rates of 6.0 L/minute in women and 7.5 L/minute in men. After one hour at rest at an average air concentration of 100 μg/m^3, the THM uptake was about 30 μg/hour (26 μg/hour chloroform, trace bromoform, 3 μg/hour BDCM, and 1.5 μg/hour DBCM). After one hour swimming at an air concentration of 200 μg/m^3, the THM uptake was about seven times higher than at rest: 221μg/hour (177, 26, 18, respectively).

In another follow-up study, Fantuzzi *et al*. (2001) evaluated exposure to THMs of 16 male and 16 female workers at five indoor swimming pools in northern Italy, and observed statistically significant differences according to the main job activity. In pool attendants, THM levels in alveolar air were approximately double those in employees working in other areas of the swimming pools (25.1 ± 16.5 μg/m^3 versus 14.8 ± 12.3 μg/m^3, $p < 0.01$). THMs in alveolar air were significantly correlated with THM concentrations in ambient air ($r = 0.57$; $p < 0.001$).

Levesque *et al*. (1994) evaluated chloroform body burden in 11 male swimmers during a daily 55 minute exercise period in indoor swimming pools in Quebec City, Canada. From the first to the sixth period, the chloroform concentration in the water increased from 159 μg/L to 553 μg/L. Corresponding mean air chloroform concentrations ranged from 597 to 1,630 μg/m^3. To separate dermal exposure from inhalation, swimmers used scuba tanks during an additional exercise period. Chloroform concentrations were measured in alveolar air before and after each exercise period, as well as after 35 minutes of physical activity. Alveolar air chloroform concentration was correlated with both the air and water concentrations of chloroform. Intensity of exercise and physiological factors were also significantly correlated with alveolar air chloroform concentration. The authors estimate that approximately 24 percent of body burden resulted from dermal exposure. They estimated the chloroform dose resulting from a one-hour swim to be 65 μg/kg-day, assuming a breathing rate of 0.03 m^3/minute and a ratio of dermal to inhalation exposure of 0.3. These values are about 60-fold higher than the shower exposure estimates from Weisel and Jo (1996) described above, reflecting the much higher water and air chloroform concentrations and the more intensive exposure situation of the Lesveque *et al*. (1994) study.

In a study in three different pools (Levesque *et al*., 2000), mean water concentrations of chloroform varied from 18 to 80 μg/L, while those in air ranged from 78 to 329 μg/m^3. Analyses of alveolar air revealed that chloroform levels were correlated with ambient air and water levels, duration of exposure, and to a lesser degree with intensity of training. A third study by Levesque *et al*. (2002) of chloroform exposure in showering found mean chloroform concentrations in shower stall air and water, respectively, of 147 μg/m^3 (standard deviation (sd) 56.2 μg/m^3) and 20.1 μg/L (sd 9.0 μg/L). The mean increase in alveolar air chloroform concentration at the end of a 10-minute shower was 33 μg/m^3 (sd

14.7 μg/m^3). The increase in alveolar air chloroform was only associated with chloroform concentration in the shower stall air. These studies demonstrate that large chloroform exposures can occur through seemingly normal daily activities.

Lindstrom *et al*. (1997) measured alveolar breath to assess THM exposures during competitive swimming training. Breath samples were collected from one male and one female swimmer before, during, and after a typical two-hour training period in an indoor pool. The preexposure breath samples showed similar chloroform concentrations for both subjects (3.07 to 3.46 μg/m^3). These values are higher than reported in the U.S. EPA's Total Exposure Assessment Methodology (TEAM) studies, which give a geometric mean value of 1.3 μg/m^3 for 80,000 study subjects (Wallace, 1997), which may indicate a longer-term residual chloroform body burden. After two minutes of exposure, the male's chloroform in breath was 71.2 μg/m^3 and after eight minutes, 160 μg/m^3, which was higher than the pool air concentration of 148 μg/m^3. The subject's breath concentration continued to increase until it reached a peak of 371 μg/m^3 at 90 minutes. The concentration then fell to 257 μg/m^3, probably due to reduction of effort in the later stages of the workout. The female subject showed an essentially linear increase in chloroform in breath over the two-hour workout period, reaching a peak of 339 μg/m^3 at the end. Measurements of BDCM in breath in the two subjects showed similar concentration time patterns, with peak concentrations of about 4.5 μg/m^3 in the male and 5.5 μg/m^3 in the female. The breath concentrations observed in both subjects were about twice what could be accounted for by inhalation alone, essentially confirming the findings of Jo *et al*. (1990a,b) for showering exposure to chloroform, that the dermal and inhalation routes are of equal importance. The authors modeled the postexposure breath elimination of chloroform using the following triexponential model:

$$C_{alv} = \beta_1 e^{-k_1 t} + \beta_2 e^{-k_2 t} + \beta_3 e^{-k_3 t} + fC_{air}$$

In this equation, C_{alv} is the alveolar breath concentration at any time during elimination, the coefficients (βi) and rate constants (ki) are associated with the theoretical body compartments, *f* is the fraction of the parent compound exhaled at equilibrium, and C_{air} is the ambient air concentration of the compound. The model gave excellent fits to the elimination data, and the halflives of chloroform for the respective compartments were 1.36, 25.7 and 173 minutes for the male, and 0.95, 16.9 and 138 minutes for the female. For BDCM, a biexponential model was fit: male halflives were 6.08 and 346.6 minutes; female halflives were 13.86 and 346.6 minutes. The three compartments are generally thought to reflect blood, rapidly perfused, and slowly perfused tissues or blood and body tissues for a two-compartment model.

In a German study involving up to 17 subjects practicing in an indoor swimming pool with and without scuba tanks, as well as walking around the pool without swimming during a 60-minute exercising period, Erdinger *et al*. (2004) quantified the body burden resulting from exposure to three different concentrations of chloroform in water and air. The results indicate that chloroform was mainly taken up by the respiratory pathway, and about one third of the total burden was taken up by the skin. Chloroform concentration

of the water was 7.1, 20.7, and 24.8 μg/L. Corresponding air chloroform concentrations, measured at 20 cm and 150 cm above the water, ranged from to 85 to 235 $\mu g/m^3$. Blood chloroform concentration of participants with scuba tanks was 0.32 ± 0.26 μg/L, without scuba tanks 0.99 ± 0.47 μg/L, and for persons walking around the pool 0.31 ± 0.25 μg/L. Blood concentrations correlated better with air chloroform concentrations than with water concentrations using linear regression models.

Chloroform in ambient air was at a high concentration within one meter of the water surface and was correlated with the number of swimmers in the pool in an Italian indoor swimming pool study (Aggazzotti *et al*., 1990). Chloroform level in blood varied with its concentration in ambient air, intensity of exercise, duration of sport activities, and the number of swimmers in the pool. In a similar study, chloroform was detected in alveolar samples during sessions at the pool, but decreased quickly at the end of the swimming session and usually was no longer detectable within half an hour (Olivo *et al*., 1989). Human plasma and alveolar air samples from swimmers in 12 indoor pools showed that chloroform uptake in swimmers varied according to intensity of the physical activity and age. Elimination of chloroform in alveolar air in one subject showed a half-life of 20 to 27 minutes and complete clearance within 10 hours after exposure (Aggazzotti *et al*., 1995). In a British study, mothers and pregnant women who swam regularly received greater chloroform doses than did nonswimmers (Whitaker *et al*., 2003b).

The exposures described in Aggazzotti *et al*. (1990) and Levesque *et al*. (1994) were modeled using an adaptation of the Corley *et al*. (1990) PBPK model for simultaneous exposures via dermal, inhalation and ingestion routes (OEHHA, 1993; Brown *et al*., 1996). The model was formulated with the physiological parameters expressed as allometric functions of body weight. Adult body weights were then varied according to a normal distribution so that each PBPK model simulation represented a separate individual. For the low-exposure scenario the water concentration of chloroform was 350 nM, the indoor air concentration was 2.0 $\mu mol/m^3$, and ingestion for a one-hour swim was assumed to be 25 mL. The high-exposure scenario assumed 4.2 μM, 61 $\mu mol/m^3$, and 50 mL, respectively. For 50 simulations of a one-hour swim the low exposure gave metabolized dose estimates of 0.5 ± 0.06 μmol of chloroform equivalents in the liver and 3.5 ± 1.2 nmol in the kidney, for a total dose of 1.99×10^{-4} mg/kg-day. The high exposure simulations gave values of 2.4 ± 0.02 μmol and 16.3 ± 3.2 nmol in the liver and kidney, respectively, with a total dose of 4.1×10^{-3} mg/kg-day.

Exposure Estimates

Estimates of dermal absorbed dose due to exposure to THMs in domestic water were derived employing the permeation coefficients (Kp values) determined by Xu *et al*. (2002). Liter equivalent of dermal exposure for the various THMs (L_{eq}) are shown in Table 9. The applicable calculation is:

$$L_{eq} = Kp \times SA \times \tau$$

where

SA = surface area (20,000 square meters) (U.S. EPA, 1997f)

τ = time bathing (10 minutes) (U.S. EPA, 1997f)

Table 9. Multiroute Exposures to THMs in Domestic Drinking Water Supply

Exposure route	Chloroform	Bromoform	Bromodichloro-methane	Dibromochloro-methane
Ingestion, L/day	2.0	2.0	2.0	2.0
Inhalation*, L_{eq}/day	0.5	0.4	0.5	0.5
Dermal**, L_{eq}/day	0.5	0.7	0.6	0.7
Total, L_{eq}/day	3.0	3.1	3.1	3.2

*CalTOX multimedia environmental model (DTSC, 1994) prediction that inhalation exposures to all four THMs in drinking water supply were similar to chloroform.
**dermal exposure estimates based on 10 minute daily baths and Kps from Xu *et al*. (2002).

Based on the findings of Jo and associates (1990a,b), the dose absorbed by inhalation for chloroform is equivalent to the dose absorbed by the dermal route. The predicted absorbed dose in the shower of 1.0 L_{eq} is 33 percent of the total dose and is very similar to the exposure estimate associated with the shower that was reported by Jo and coworkers (1990a,b).

No comparable studies of the relative contributions of dermal and inhalation exposures in the shower for the other THMs were identified. Therefore the CalTOX multimedia exposure model (DTSC, 1994) was employed to determine if inhalation exposures to other THMs in the shower were similar to chloroform. CalTOX is a seven-compartment regional and dynamic fugacity model in a Microsoft Excel format. The chemical parameters for the respective THMs were loaded from the companion DatCal database. Human parameters were set for a 70 kg average human consuming 2.0 L/day of tap water (0.028 L/kg-day). The vadose zone soil compartment was loaded with various concentrations of individual THMs and the model was run to determine average inhouse exposures to the THMs in contaminated groundwater. Since the model predicts exposure rather than absorbed dose, the inhalation pathway values were adjusted to assume 50 percent absorption of the respective THMs into the blood at low concentrations in air, while ingested THMs were assumed to be 100 percent absorbed at low doses. CalTOX predicted that inhalation exposures to the various THMs are very similar. The exposure to bromoform was predicted to be slightly lower than the other THMs. Therefore, based on the CalTOX results, the inhalation exposure associated with DBCM and BDCM was established at the level observed for chloroform during showering (0.5 L_{eq}/day). Exposure to bromoform during showering was established at 0.4 L_{eq}/day (Table 9).

METABOLISM AND PHARMACOKINETICS

Metabolism

Overview of Metabolic Pathways

Chloroform is metabolized by two major pathways, oxidative, producing phosgene (CCl_2O); and reductive, producing the dichloromethyl free radical ($\bullet CHCl_2$); both pathways are catalyzed by cytochrome P450 enzymes in the presence of NADPH (Ahmed *et al.*, 1980. The majority of chloroform metabolism occurs in the liver, with substantial metabolism also occurring in the renal cortex and in the nasal turbinates (U.S. EPA, 1997b,d; ILSI, 1997). As cytochrome P450 enzymes are present in most tissues, metabolism of chloroform can occur throughout the body.

Brominated THMs are believed to be metabolized via at least two pathways, similar to chloroform (U.S. EPA, 1994a), plus one or more glutathione *S*-transferase theta-mediated conjugations. A generalized metabolic scheme for the "oxidative" and "reductive" pathways is shown in Figure 1. The oxidative pathway requires NADPH and oxygen, whereas the reductive pathway can utilize NADPH or NADH and is inhibited by high oxygen tension. Both reactions are believed to be mediated by cytochrome P450 isoforms. The initial, rate limiting reaction of oxidative metabolism is insertion of oxygen at the C–H bond of bromoform or other THMs to produce a trihalomethanol (CBr_3OH or CX_3OH), which spontaneously decomposes to yield a reactive dihalocarbonyl (CBr_2O or CX_2O), a structural analogue of phosgene. The dihalocarbonyl may form adducts with various cellular nucleophiles, hydrolyze to yield carbon dioxide, or undergo a glutathione-dependent reduction to yield carbon monoxide. When oxygen tension is low (reductive metabolism), the reaction products appear to be free radical species such as dihalomethyl radicals ($\bullet CHBr_2$ or $\bullet CHX_2$). These highly reactive radicals may also form covalent adducts with a variety of cellular macromolecules. Evidence supporting this metabolic scheme is presented below.

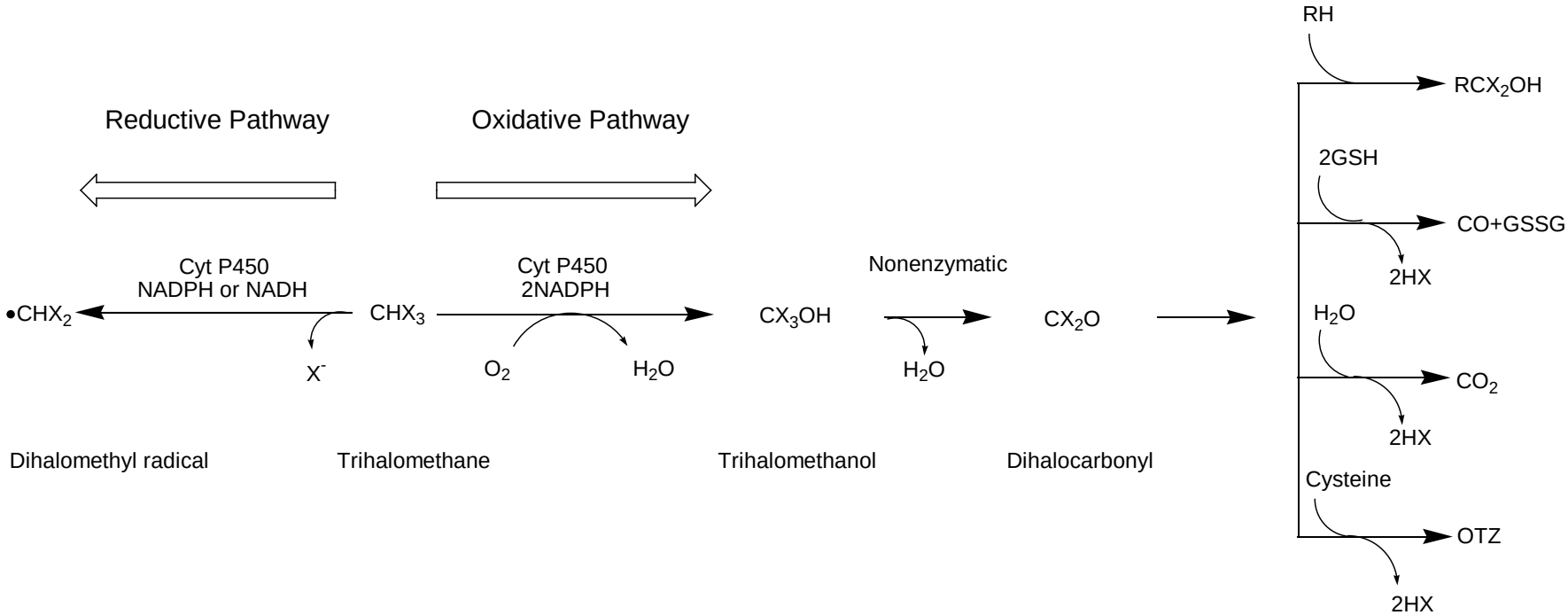


Figure 1. Proposed Metabolic Pathways for the Brominated Trihalomethanes

R = cellular nucleophile (protein, nucleic acid)
GSH = reduced glutathione
GSSG = oxidized glutathione
OTZ = oxothiazolidine carboxylic acid
Cyt P450 = cytochrome P450
adapted from Stevens and Anders (1981) and U.S. EPA (1994b, 2005d)

Oxidative Metabolism

Early studies of THM metabolism reviewed by Ahmed *et al*. (1980) established that chloroform is metabolized to carbon dioxide via a reactive dihalocarbonyl intermediate (phosgene). A subsequent series of metabolic studies using bromoform as a model THM (Ahmed *et al*., 1977; Anders *et al*., 1978; Stevens and Anders, 1979; 1981) was initiated following the observation that carbon monoxide and carboxyhemoglobin are elevated in the blood of rats administered THMs. These studies are relevant to each of the THMs because they are assumed to share common pathways for metabolism. Ahmed *et al*. (1977) established *in vitro* that bromoform is metabolized to carbon monoxide by a rat liver microsomal fraction requiring NADPH and molecular oxygen for maximal activity. Pretreatment of rats with cytochrome P450 inducers increased the rate of conversion by the microsomal fraction, while addition of cytochrome P450 inhibitors or storage at 4 °C reduced the rate of carbon monoxide formation. These data suggested that bromoform is metabolized to carbon monoxide via a cytochrome P450 dependent pathway.

Reductive Metabolism

The reductive pathway of metabolism has been studied using various THMs, but remains less characterized than the oxidative pathway. Gao and Pegram (1992) reported that binding of reactive intermediates to rat hepatic microsomal lipid and protein under reductive (anaerobic) conditions was more than twice as high for BDCM as for chloroform). Tomasi *et al*. (1985) studied the anaerobic activation of bromoform to a free radical intermediate *in vitro* using rat hepatocytes isolated from phenobarbital induced male Wistar rats. The production of a free radical intermediate was measured by electron spin resonance spectroscopy. The intensity of the electron spin resonance signal was greatest for bromoform, when compared to other THMs. The largest electron spin resonance signal was detected when hepatocytes were incubated under anaerobic conditions. The signal was reduced by cytochrome P450 inhibitors such as SKF-525A, metyrapone, and carbon monoxide. These data were interpreted as evidence that free radical formation depends on cytochrome P450-mediated reductive metabolism. Comparison of electron spin resonance spectra obtained in the presence of deuterated chloroform and BDCM suggested that the free radical intermediate produced by chloroform metabolism was dichloromethyl radical ($\bullet CHCl_2$). The authors speculated that the brominated THMs are also metabolized by transfer of an electron directly from the cytochrome to the halocompound with successive formation of the dihalomethyl radical ($\bullet CHX_2$) and a halide ion (X^-).

Cytochrome P450 Isoforms Involved in Trihalomethane Metabolism

An important concern in characterizing hazards associated with THM exposure is which P450 isoforms are responsible for bioactivation of THMs to reactive metabolites. The identity of metabolizing enzymes is of concern because individuals or subpopulations with elevated levels of these enzymes may be at greater risk for adverse effects.

The identities of the cytochrome P450 isoforms responsible for THM metabolism have been investigated most intensively for chloroform. Studies of chloroform metabolism by

Nakajima *et al*. (1995) and Testai *et al*. (1996) indicate that concentration plays a critical role in determining the role of different cytochrome P450 isoforms and the associated effects of metabolic inducers. Nakajima *et al*. (1995) pretreated male Wistar rats with three inducers of specific P450 isoforms and subsequently administered a single dose of chloroform by gavage in corn oil. The inducers used were phenobarbital (CYP2B1/2), n-hexane (CYP2E1), and 2-hexanone (CYP2B1/2 and CYP2E1). Liver damage, as determined by serum enzyme activity and histopathology, was greatest at the middose in the hexane-treated animals. In contrast, rats pretreated with phenobarbital or 2-hexanone showed a dose- related increase of liver damage at all doses. The pattern of damage was consistent with the tissue distribution patterns of the induced cytochrome P450 isoform(s). The study authors concluded that CYP2E1 catalyzes chloroform metabolism at low doses and that CYP2B1/2 catalyzes chloroform metabolism at higher doses.

While experimental evidence indicates that CYP2E1 and CYPB1/2 catalyze the oxidative pathway for THM metabolism, the identities of the cytochrome P450 isoforms that catalyze the reductive pathway have not been established. CYP2E1 protein can catalyze reductive as well as oxidative reactions (Lieber, 1997) and this isoform has been implicated in the production of trichloromethyl radicals from carbon tetrachloride (Lieber, 1997). However, evidence for a dual role of either CYP2E1 or CYP2B1/2 in catalyzing the oxidative and reductive pathways for THM metabolism has been contradictory, perhaps as a result of the different concentrations of chloroform used in different experiments (summarized in Testai *et al*., 1996).

Glutathione *S*-Transferase

Studies in *Salmonella typhimurium* strains engineered to express the rat glutathione *S*-transferase theta (GSTT) gene (*GSTT1-1*) provide evidence for a third mechanism of bioactivation of brominated THMs via one or more glutathione *S*-transferase theta mediated conjugation pathways (DeMarini *et al*., 1997; Pegram *et al*., 1997).

Pegram *et al*. (1997) used the standard mutagenicity tester strain TA1535; TA1535 transfected with the rat *GSTT1-1* gene (+*GSTT1-1*); and TA1535 transfected with the same complementary DNA (cDNA) inserted in a nonfunctioning orientation (−*GSTT1-1*). These strains were tested in a standard plate incorporation mutation assay in a 24-hour exposure to BDCM under conditions that prevented loss of the compound by volatilization. Vapor concentrations tested ranged from 200 to 4,800 ppm and the assays were conducted without exogenous metabolic activation. BDCM exposure increased the number of revertant colonies in each of the three *Salmonella* strains. The frequency of the revertants in nontransfected TA1535 was significantly increased above background at the three highest concentrations (highest concentration 4,800 ppm; intermediate concentrations not explicitly stated), while mutation frequency in the −*GSTT1-1* transfected strain was increased only at the highest concentration. In contrast, expression of glutathione *S*-transferase theta protein in the +*GSTT1-1* transfected strain resulted in significantly increased numbers of revertants relative to control at each concentration of BDCM. An 18-fold increase in revertants was noted at the highest concentration (0.67 mM in agar after a 24-hour exposure to 4,800 ppm). Chloroform produced a positive response only at concentrations of 19,200 and 25,600 ppm. These results provide evidence that mutagenicity of BDCM is enhanced by glutathione *S*-transferase theta-

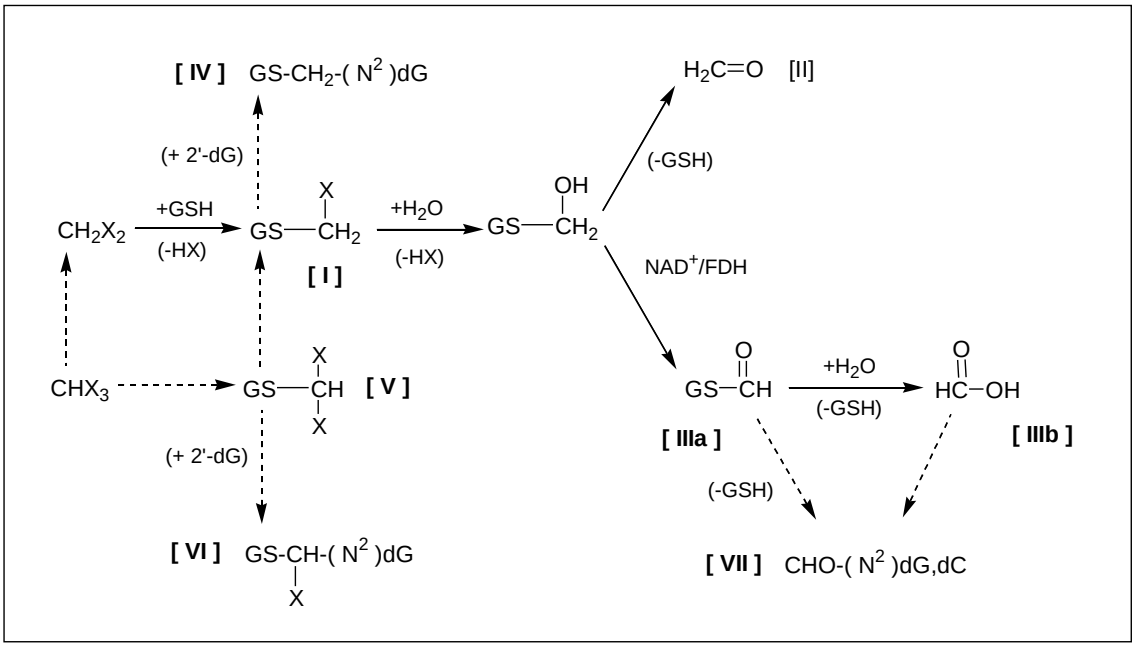
mediated conjugation with glutathione. The study authors noted that the comparatively low affinity of the glutathione *S*-transferase theta-mediated pathway for chloroform offers a possible mechanistic explanation for the differences observed in mutagenic potential of the brominated THMs compared to chloroform.

DeMarini *et al*. (1997) further elucidated the role of glutathione *S*-transferase theta in bioactivation of BDCM and other brominated THMs in additional experiments with *S. typhimurium*. The strains of *S. typhimurium* included RSJ 100[1] (engineered to express the *GSTT1-1* gene) and TPT 100, which has the *GSTT1-1* gene inserted in a nonfunctional orientation. BDCM at 3,200 ppm induced a 44-fold increase in revertant colonies in RSJ 100 compared to background revertant frequency. The spectrum of BDCM-induced mutations at the *his*G46 allele in strain RSJ 100 was analyzed by colony probe hybridization, finding predominately GC→AT transitions (99 percent) and a high degree of site specificity (middle C of a CCC sequence in target DNA). Mutagenicity of bromoform and DBCM was also markedly enhanced in the strain expressing *GSTT1-1*, in the order for mutagenic potency (number of revertants per ppm) of bromoform ≈ DBCM > BDCM. The mutational spectra for bromoform and DBCM were similar to that of BDCM, which suggests that a common reactive intermediate or class of intermediates is likely to mediate the mutagenicity of these brominated THMs. In contrast, chloroform mutagenicity was not enhanced in the strain expressing *GSTT1-1*, providing additional evidence that chloroform and the brominated THMs may be bioactivated via different mechanisms. These data may help to explain differences in the toxicity of chloroform and the brominated THMs, such as in the target organs for carcinogenicity.

Proposed routes for glutathione *S*-transferase theta mediated metabolic activation of the brominated THMs are illustrated in Figure 2.

[1] A TA1535 derived strain engineered to express the rat glutathione *S*-transferase theta 1-1 (*GSTT1-1*) gene

Figure 2. Proposed Routes for Glutathione *S*-Transferase Theta Mediated Metabolic Activation of the Brominated Trihalomethanes



Notes:
Solid arrows represent known pathways (modified from Gargas *et al*., 1986)
Dashed arrows represent proposed pathways lacking direct experimental evidence
GSH: glutathione
(I): *S*-(1-halomethyl)GSH
(II): formaldehyde
(IIIa): *S*-(1-formyl)GSH
(IIIb): formic acid
(IV), *S*-[1-(N^2-deoxyguanosinyl)methyl]GSH adduct (Thier *et al*., 1993)
(V): *S*-(1,1-dihalomethyl)GSH
(VI): *S*-[1-halo(N^2-deoxyguanosinyl)methyl]GSH adduct
(VII): *N*-formyl adduct on either G or C
Adapted from DeMarini *et al*. (1997) and U.S. EPA (2005d)

Chloroform

Absorption

Chloroform is readily absorbed through the lungs and gastrointestinal tract. Administration of chloroform by ingestion, inhalation, and intraperitoneal (ip) or subcutaneous (sc) injection has produced renal and hepatic toxicity in humans and laboratory animals. Although chloroform can be absorbed percutaneously, the rate and extent are relatively low compared with absorption by other routes.

Pulmonary Absorption

Chloroform was widely used as a surgical inhalation anesthetic until World War II (U.S. EPA, 1985a). Pulmonary uptake of chloroform depends on its concentration in air, duration of exposure, solubility of chloroform in blood and tissue, volume and rate of perfusion of tissues, rate of elimination, and rate of alveolar ventilation (i.e., that fraction of total respiratory ventilation from which volatile organic compounds may be cleared by absorption into alveolar capillary blood (Astrand, 1975; U.S. EPA, 1985a).

Uptake of inhaled vapors is driven by equilibration of the partial pressures in tissues with their partial pressure in air (U.S. EPA, 1994a). Partition coefficients describe the relative affinity of a chemical for one medium compared to another at steady state. The blood to air partition coefficient is an important determinant of pulmonary absorption of vapors. Blood to air partition coefficients of 7.4 to 10.7 have been reported for chloroform in humans (Batterman *et al*., 2002; Corley *et al*., 1990; Sato and Nakajima, 1979; Steward *et al*., 1973), indicating that this chemical readily passes from the air to the blood in the alveoli. The lipophilicity of chloroform, as expressed by an oil to air partition coefficient of 401, and adipose to blood partition coefficient of 35 (Sato and Nakajima, 1979; Steward *et al*., 1973) means that body weight and fat content will also influence the amount of pulmonary absorption of chloroform. Uptake and storage of chloroform in adipose tissue can be substantial. Therefore, chloroform retention would be expected to be higher in individuals with larger proportions of body fat (U.S. EPA, 1985a).

Under nonequilibrium conditions, the rate of pulmonary uptake of chloroform in humans is initially rapid, but decreases as the concentration of chloroform in tissues approaches equilibrium (Raabe, 1988). U.S. EPA (1985a) calculated 67 percent retention from the Smith *et al*. (1973) observations of patients anesthetized with chloroform. Human volunteers who inhaled 7 to 25 ppb chloroform for two hours through their nose or mouth retained 45.6 or 49.6 percent, respectively (Raabe, 1988). Assuming that alveolar ventilation represented 66.7 percent (U.S. EPA, 1988) or 70 percent (Raabe, 1988) of total respiratory volume, the Raabe (1988) data imply that the retained percentage of alveolarly respired chloroform ranges from 65 to 74 percent under the conditions tested. Physiologically based pharmacokinetic models (PBPK) discussed below provide a rigorous approach to quantifying respiratory uptake under different exposure conditions.

Yoshida *et al*. (1999) studied absorption of chloroform, BDCM, and DBCM in low-level inhalation exposures in rats. The compounds were injected into a closed chamber system containing one male Sprague-Dawley rat (270 to 330 g), and the air concentration of the substances was monitored. The pharmacokinetics of BDCM and DBCM were evaluated using a linear three compartment model, and chloroform by a nonlinear two compartment model. The rate constants in the metabolic and exhalation elimination process, k_e and k_2, were compared for each compound. For BDCM and DBCM, k_e was greater than k_2, 0.034/hour versus 0.012/hour and 0.049/hour versus 0.034/hour, respectively. At 1 ppb exposures, more chloroform (0.33 nmol/hour-kg) was absorbed by the rats than BDCM (0.11 nmol/hour-kg) or DBCM (0.072 nmol/hour-kg). The rate constants of the metabolic elimination process (Vmax ÷ (Km + Cr) = K) were calculated. The value of K exceeded k_2 at exposure concentrations less than about 950 ppm chloroform. Vmax was estimated to be 86 ppm/hour and Km, 107 ppm. The amounts metabolized at 1 ppb, 100 ppb and 10 ppm were estimated (in μmol/hour-kg) as: chloroform 3.3×10^{-4}, 3.3×10^{-2},

3.0; BDCM 7.2×10^{-5}, 7.2×10^{-3}, 7.2×10^{-1}; and DBCM 1.1×10^{-4}, 1.1×10^{-2}, 1.1. As indicated, the metabolism of all three THMs is linear below 10 ppm in air.

Oral Absorption

Animal mass balance studies with orally administered radiolabeled chloroform have typically recovered more than 90 percent of the radioactivity in expired air as carbon dioxide or unchanged parent compound. Relatively small amounts of radiolabel have also been recovered in the urine, feces, or carcass (Paul and Rubenstein, 1963; Fry *et al*., 1972; Taylor *et al*., 1974; Brown *et al*., 1974; Reynolds *et al*., 1984; Mink *et al*., 1986). Values are based on the assumption that the radiolabel recovered in urine, feces, carcass, and cage air represents material that has been absorbed through the stomach and intestines prior to elimination, which may not be strictly correct. For example, the chloroform measured in cage air, which presumably came from exhalation, may be derived in part from chloroform retained in material that passed through the digestive tract without systemic absorption. Mass balance studies of chloroform have typically recovered one to three percent, and as much as 13.6 percent, of the radiolabel in the carcass. These reports have not provided data on the specific disposition of the radioactivity in the carcass, i.e., whether or not radioactivity remained unabsorbed in the gastrointestinal tract. It is therefore possible that some carcass radioactivity is due to such unabsorbed material.

In mice and monkeys given 60 mg/kg of chloroform by gavage, and in humans who ingested 500 mg of chloroform, peak blood levels of chloroform were measured one hour after dosing (Fry *et al*., 1972; Brown *et al*., 1974; Taylor *et al*., 1974). However, in these experiments chloroform was administered in olive oil, which may slow the rate of gastrointestinal absorption of chloroform (see Withey *et al*., 1983). Administration of chloroform in drinking water or by gavage has produced a broad spectrum of adverse effects in laboratory animals within minutes of exposure. Rapid onset of toxicity has also been observed in humans after accidental ingestion of chloroform (U.S. EPA, 1985a).

Withey *et al*. (1983) showed that the vehicle may have pronounced effects on the rate and extent of gastrointestinal absorption of chloroform. Fasted male Wistar rats were given a single 75-mg/kg dose of chloroform by gavage, either in aqueous solution or in corn oil. Blood samples were taken approximately every two minutes for one hour, then every half hour until the concentration of chloroform became undetectable. When chloroform was administered in corn oil, the blood concentration profile approximated a relatively constant infusion of chloroform over a 30-minute period, then declined exponentially over 200 minutes, when it became undetectable. In contrast, when chloroform was given in water, its blood concentration reached an almost instantaneous peak, which was 6.5 times higher than with the corn oil vehicle. Eliminated was biexponential over the next five hours, and the area under the blood concentration-time curve was estimated to be 8.7 times greater than when chloroform was administered in corn oil. Withey *et al*. (1983) speculated that the corn oil might have sequestered the chloroform in immiscible globules, which did not come into immediate contact with the gastrointestinal tract.

Dermal Absorption

Several studies have demonstrated that chloroform can be absorbed through intact skin. Percutaneous absorption of pure, liquid chloroform occurs slowly, and is limited by the thickness of the epidermis and by the moderate lipophilicity of chloroform (U.S. EPA, 1985a). Chloroform can also be dermally absorbed from water or other matrices, and from the vapor phase (Brown *et al*., 1984).

Dick *et al*. (1995) measured absorption of chloroform through human skin. The ventral skin of the forearm of four volunteers was exposed to 50 µg of chloroform in water or 250 µg of chloroform in ethanol; the solution remained on the skin for eight hours. Absorption of chloroform, based on detection in the expired air and urine, was about eight percent with ethanol as the vehicle and about 1.6 percent with water as the solvent. Several studies of chloroform absorption in swimming pools by both dermal and inhalation routes are discussed above in the exposure section (Aggazzotti *et al*., 1990; Jo *et al*., 1990a,b; Levesque *et al*., 1994; Cammann and Huebner, 1995).

Tsuruta (1975) measured the *in vivo* rate of absorption of liquid chloroform (0.5 mL across the shaved skin of mice as 329 nmol/minute/cm^2. Over a 15-minute period of exposure, approximately 1,718 mg of chloroform were absorbed, or 0.2 percent of the applied dose (Tsuruta, 1975). Bogen *et al*. (1992a) measured the rate of percutaneous absorption of chloroform in a dilute aqueous solution in female hairless guinea pigs. Sedated animals were approximately 70 percent immersed in a glass chamber filled with water containing 10 to 100 ppb of ^{14}C-chloroform. The water was maintained at 32 °C to simulate bathing/showering temperatures. The dermal uptake chloroform was approximately constant over a 70-minute exposure period, with an average value (for five animals) of 0.13 ± 0.04 mL/cm^2-hour (mL of solution cleared of chloroform per square cm skin exposed per hour of exposure, equivalent to the unit of cm/hour). Bogen *et al*. (1992a) pointed out that the human dermal absorption estimate of Jo *et al*. (1990a,b) was not significantly different from the value they obtained for hairless guinea pigs.

Islam *et al*. (1996, 1999a,b) studied systemic uptake and clearance of chloroform by hairless rats following brief dermal exposures to aqueous solutions. Systemic absorption of chloroform was estimated by comparing the concentration × time curve (AUC) of blood concentration from dermal exposure to that obtained by iv infusion for 30 minutes. The systemic uptake was substantially higher than the predictions of several published exposure models. The authors concluded that the available physiologically based pharmacokinetic models have done a better job of predicting dermal uptake than the physical models (e.g., Chinery and Gleason, 1993; McKone, 1993).

Distribution

Chloroform readily distributes throughout the body (Lavigne and Marchand, 1974; Brown *et al*., 1974; Taylor *et al*., 1974; Lofberg and Tjalve, 1986). In both humans and experimental animals it has been found to concentrate most in adipose tissue (Chenoweth *et al*., 1962; Steward *et al*., 1973; Brown *et al*., 1974; Taylor *et al*., 1974; McConnell *et al*., 1975), and to a lesser extent, the liver and kidney. Within the latter two organs, radioactivity from ^{14}C-chloroform is typically concentrated in the region immediately surrounding the centrilobular vein of the liver and in the cortex of the kidney.

Sex-specific differences in chloroform-induced renal toxicity in mice have led to speculation that there may be sex-related differences in distribution of chloroform in this species. Taylor *et al*. (1974) observed distinct differences in the distribution of radioactivity between males and females of three mouse strains (LP, CBA, C57BL) after a single gavage dose of ^{14}C-chloroform. In males, chloroform was widely distributed, with radiolabel detectable in the stomach, intestine, bladder, liver, and kidney. Within the kidney, considerably more radioactivity was found in the cortex than the medulla. Females exhibited the same general distribution of chloroform, except that significantly less radioactivity was present in the kidney and there was little difference in radioactivity between the renal cortex and medulla. The ratio of renal radioactivity in males to that in females varied from 2.6 (C57BL) to 3.8 (CBA). Male CBA mice had significantly ($p < 0.0005$) more radioactivity in their kidneys than males from the other two strains. There were no significant differences by strain in the kidneys of female mice. No significant sex-dependent differences in radioactivity were found in other tissues (liver, fat, blood) in any of the strains. However, when data from all three strains was combined, the livers of females had significantly ($0.01 < p < 0.02$) more radioactivity than those of males.

Following oral administration of ^{14}C-chloroform to rats (16 μCi/kg) and mice (32 μCi/kg), Mink *et al*. (1986) noted an apparent species-specific difference in the percentage of radiolabel contained in the organs (bladder, brain, kidneys, liver, lungs, stomach, pancreas, thymus). However, the difference in average total organ content of ^{14}C (3.6 percent rats, 13.5 percent mice) may have been due simply to the relatively poor total recovery of ^{14}C in rats compared to mice (78.2 and 94.5 percent, respectively).

Chloroform crosses the placenta, and has been detected in fetal blood and tissue of mice, rats, and rabbits following maternal inhalation or ingestion of chloroform (Schwetz *et al*., 1974; Thompson *et al*., 1974, Dilley *et al*., 1977; Murray *et al*., 1979). McConnell *et al*. (1975) found chloroform in cow's milk from the United Kingdom. Danielsson *et al*. (1986) found that chloroform crosses the placenta of mice at all stages of gestation, with minor differences in distribution of radioactivity, depending on the fetal age. In "early gestation," metabolites of chloroform (i.e., nonvolatile radioactivity) accumulated in the brain. In "mid gestation," radioactivity was evenly distributed in fetal tissues, while by "late gestation" there was some evidence of accumulation in the liver, blood, respiratory tract, oral mucosa, and esophagus. Withey and Karpinski (1985) exposed rats on gestation day 17 to 250, 500, 1,000, 1,500, or 2,000 ppm chloroform in air for five hours. Maternal and fetal blood concentrations of chloroform were linearly related to air concentration of chloroform and fetal blood concentration was about 32 percent of that in maternal blood over the exposure range tested. The overall fetal uptake was low, which might be attributable to the generally low fat content of fetal tissues.

McConnell *et al*. (1975) analyzed postmortem tissues from eight United Kingdom residents. Although none of these persons had been occupationally exposed to chloroform, chloroform was detected in the liver (1 to 10 ppm), kidneys (2 to 5 ppm), brain (2 to 4 ppm), and adipose tissue (19 to 68 ppm). These data, and the partition coefficients for human tissue published by Steward *et al*. (1973) indicate that the relative concentrations of chloroform in humans would be: adipose tissue > brain > liver > kidney > blood. Dowty *et al*. (1975) reported that in humans, chloroform was present in placental cord blood in concentrations equal to or greater than that in maternal blood.

Metabolism

As discussed above, chloroform is rapidly and extensively metabolized by two major pathways, oxidative, producing phosgene, and reductive, producing the dichloromethyl free radical. Both pathways are catalyzed by cytochrome P450 enzymes in the presence of NADPH. The majority of chloroform metabolism occurs in the liver, with substantial metabolism also occurring in the renal cortex and in the nasal turbinates (Ahmed *et al.*, 1980; U.S. EPA, 1997b,d; ILSI, 1997).

Lofberg and Tjalve (1986) used a combination of whole body autoradiography and *in vivo* techniques to demonstrate that chloroform can be metabolized at a large number of extrahepatic sites, including the renal cortex, nasal olfactory and respiratory mucosa, cheek, esophagus, tongue, larynx, trachea, and lungs. There is no evidence that adipose tissue has any capacity to metabolize chloroform. Cohen and Hood (1969) showed that the radiolabel in this tissue was primarily volatile (i.e., unmetabolized chloroform). In contrast, radiolabel in liver was volatile shortly after exposure, but became largely nonvolatile (metabolites) over a two-hour postexposure period.

The cytochrome P450-dependent mixed-function oxygenase (MFO) pathway to phosgene, a highly reactive, electrophilic compound, is believed to go through trichloromethanol as an intermediate. However, its extremely short half-life has made trichloromethanol difficult to isolate (Ilett *et al*., 1973; Uehleke and Warner, 1975; Mansuy *et al*., 1977; Sipes *et al*., 1977; Uehleke *et al*., 1977a; Pohl *et al*., 1977, 1979, 1981). Phosgene readily forms covalent bonds with cellular macromolecules such as lipids and proteins (Brown *et al*., 1974; Hill *et al*., 1975; Uehleke and Werner, 1975; Docks and Krishna, 1976; Sipes *et al*., 1977). Phosgene can also undergo spontaneous hydrolysis to carbon dioxide, forming hydrogen and chloride ions (H^+ and $C1^-$) in the process (Fry *et al*., 1972; Brown *et al*., 1974; Taylor *et al*., 1974; Smith and Hook, 1983, 1984; Mink *et al*., 1986; Gram *et al*., 1986; Raabe, 1988). In rodents, a small percentage of this carbon dioxide is incorporated into the endogenous carbon pool, and is eliminated in the urine as bicarbonate, urea, and various amino acids (Brown *et al*., 1974). Glutathione-*S*-transferase mediates the formation of several separate phosgene-derived metabolic products, including carbon monoxide, oxidized glutathione-carbon monoxide (GSCOSG), N-(2-oxothiazolidine-4-carbonyl)-glycine (OTZG), and 2-oxothiazolidine-4-carboxylic acid (OTZ) (Wolf *et al*., 1977, Ahmed *et al*., 1977; Anders *et al*., 1978; Stevens and Anders, 1981; Pohl *et al*., 1981; Branchflower *et al*., 1984). There is some evidence that the dichloromethyl radical, $\bullet CHC1_2$, is formed by reductive dehalogenation of chloroform (Tomasi *et al*., 1985).

The role of cytochrome P450s such as CYP2E1 in chloroform bioactivation at typical low human exposures has been defined by integrating results from several experimental approaches: cDNA-expressed P450s, selective chemical inhibitors and specific antibodies, and correlation studies in a panel of phenotyped human liver microsomes (Gemma *et al*., 2003). Human liver microsomes bioactivated chloroform both oxidatively and reductively. Oxidative reaction was characterized by two components, suggesting multiple P450 involvement. The high-affinity process was catalyzed by CYP2E1, as indicated by kinetic studies, correlation with chlorzoxazone 6-hydroxylation ($r = 0.837$; $p < 0.001$), and inhibition by monoclonal antihuman CYP2E1 and 4-

methylpyrazole. The low-affinity phase was essentially catalyzed by CYP2A6. This conclusion was supported by the correlation with coumarin 7-hydroxylase ($r = 0.777$; $p < 0.01$), inhibition by coumarin and by the specific antibody, in addition to results with heterologously expressed P450s. Chloroform oxidation was poorly dependent on oxygen, whereas the reductive metabolism was highly inhibited by oxygen. Production of dichloromethyl radical was significant at a chloroform concentration greater than or equal to 1 mM, increasing linearly with substrate concentration. CYP2E1 was the primary enzyme involved in the reductive reaction, as indicated by all the different approaches. The reductive pathway seems to be scarcely relevant in the human liver, since it is active at high substrate concentrations, and in strictly anaerobic conditions.

In vitro and *in vivo* chloroform bioactivation in the liver and kidney was reported to be similar in Fischer 344 (F344) and Osborne Mendel rats in both metabolism and toxicokinetics (Gemma *et al*., 2004). Chloroform metabolism was fully saturated in the Osborne Mendel rat at doses of 90 and 180 mg/kg, working at a maximal rate of 40 and 50 µmol $^{14}CO_2$ expired/kg-hour. No acute hepatotoxicity, nephrotoxicity or consequent cell proliferation was evidenced at 180 mg/kg. In the rat liver, phosgene was confirmed as the major metabolite. Renal microsomes from both rat strains *in vitro* were unable to produce any oxidative metabolite, although adducts due to oxidative and reductive metabolites were detected *in vivo*. The results indicated the presence in the rat kidney of electrophilic metabolites other than phosgene, representing either oxidative metabolites formed elsewhere and sufficiently stable to be transported to the kidney or electrophilic metabolites secondary to the formation of reductive radicals.

Further data on the production of phosgene as an intermediate were provided by Pohl *et al*. (1977), who showed that when rat liver microsomes were incubated with chloroform in the presence of cysteine, the reaction product was identical to that formed by the reaction of phosgene and cysteine, 2-oxothiazolidine-4-carboxylic acid. Subsequently, Pohl *et al*. (1980b) demonstrated that covalent binding of ^{14}C to rat liver microsomes, and *in vitro* production of ^{14}C-carbon dioxide from ^{14}C-chloroform were markedly inhibited by cysteine. The reaction of phosgene with two molecules of glutathione leads to the formation of oxidized glutathione-carbon monoxide (Branchflower and Pohl, 1981; Pohl *et al*., 1981). Branchflower *et al*. (1984) speculated that oxidized glutathione-carbon monoxide is metabolized to N-(2-oxothiazolidine-4-carbonyl)-glycine via an intermediate, glutathionyl cysteinylglycinyl dithiocarbonate; N-(2-oxothiazolidine-4-carbonyl)-glycine is subsequently hydrolyzed to 2-oxothiazolidine-4-carboxylic acid.

Formation of phosgene from chloroform is mediated by the mixed function oxygenases, and is dependent on molecular oxygen and NADPH. Phosgene production was diminished by addition of the mixed function oxygenase inhibitors carbon monoxide and SKF 525A to rat liver microsomes incubated with chloroform (Mansuy *et al*., 1977; Pohl and Krishna, 1978).

Although the liver is the principal site of chloroform metabolism, and therefore the primary site of phosgene production, *in vitro* studies indicate that phosgene is also a metabolic intermediate in renal metabolism of chloroform in rodents. When Paul and Rubenstein (1963) and Smith *et al*. (1985) incubated rat kidney slices with chloroform, carbon dioxide, a metabolic product of phosgene, was produced. Renal homogenates from DBA/2J mice incubated with glutathione metabolized chloroform to phosgene, and

ultimately, to 2-oxothiazolidine-4-carboxylic acid (Branchflower *et al*., 1984). Kluwe and Hook (1981) showed that *in vivo*, chloroform caused depletion of renal glutathione in mice, presumably by the direct reaction of phosgene with glutathione to produce 2-oxothiazolidine-4-carboxylic acid. The kidneys of rabbits also metabolize chloroform to phosgene and N-(2-oxothiazolidine-4-carbonyl)-glycine. Renal metabolism of ^{14}C-chloroform was enhanced by pretreatment with phenobarbital, which produced a four-fold increase in metabolic rate.

To a limited extent, rat liver microsomes are capable of metabolizing chloroform to carbon monoxide (Ahmed *et al*., 1977; Wolf *et al*., 1977). Consistent with this finding are several reports of elevated carboxyhemoglobin levels in rats exposed to chloroform (1 to 4 mmol/kg, intraperitoneal) (Ahmed *et al*., 1977; Anders *et al*., 1978; Bellar *et al*., 1974; Stevens and Anders, 1981). *In vitro*, the production of carbon monoxide from chloroform required NADPH, was enhanced by molecular oxygen, but also took place under anaerobic conditions. Pretreatment of animals with phenobarbital stimulated production of carbon monoxide, while pretreatment with SKF 525A or phosgene inhibited the reaction (Ahmed *et al*., 1977). Anders *et al*. (1978) and Stevens and Anders (1979, 1981) provided evidence that carbon monoxide is formed *in vivo* from chloroform by the sequential reaction of phosgene with two molecules of glutathione to form oxidized glutathione-carbon monoxide, which is subsequently hydrolyzed to carbon monoxide, hydrogen and chloride ions. It has also been proposed that carbon monoxide is formed by the reductive dechlorination of chloroform via a carbene ($CC1_2$) intermediate (Wolf *et al*., 1977).

The metabolism of chloroform proceeds anaerobically as well as aerobically (Pohl *et al*., 1980b; Uehleke and Warner, 1975). Chloroform and other halogenated methanes compete with oxygen for electrons transferred through the mixed function oxygenases, and Tomasi *et al*. (1985) noted that this favors metabolic reduction of chloroform at low oxygen tension. Tomasi *et al*. (1985) demonstrated that a free radical metabolite of chloroform, possibly the dichloromethyl radical, $\bullet CHC1_2$, was produced in isolated rat hepatocytes incubated under anaerobic conditions. Similar incubations carried out in the presence of air also revealed much less evidence of free radical formation. The mixed function oxygenases inhibitors SKF 525A, metyrapone, and carbon monoxide decreased production of the free radical metabolite.

Testai and Vittozzi (1984) also examined the possibility that chloroform may be metabolized anaerobically to a reactive intermediate. When rat liver microsomes were incubated under aerobic conditions with chloroform, loss of 70 percent of the cytochrome P450 occurred. Destruction of cytochrome P450 was completely prevented by the mixed function oxygenases inhibitor SKF 525A, and, to a lesser extent, by cysteine or glutathione. Anaerobic incubation of microsomes resulted in a marked increase in the extent of cytochrome P450 loss, and addition of glutathione had no effect. Testai and Vittozzi (1986) and Testai *et al*. (1987, 1992) demonstrated that covalent binding of a chloroform metabolite to lipid and protein occurred during anaerobic incubations with microsomes from phenobarbital induced rats. However, substantially fewer adducts were formed anaerobically than in aerobic incubations. These observations indicate that both oxygen-dependent and independent metabolism may contribute to chloroform toxicity.

Lipscomb *et al*. (2004) demonstrated that differences in CYP2E1 content of microsomal protein among individuals and between species are largely responsible for observed differences in metabolism of chloroform and other low-molecular-weight halogenated compounds *in vitro*. Apparent maximum rate of metabolism (Vmax) values of 27.6 and 28.3 nmol/hour/mg microsomal protein and Michaelis-Menten rate constants for chloroform oxidation (Km) values of 1 and 0.15 µM in rats and human organ donors, respectively, were demonstrated *in vitro*. The specific activity of CYP2E1 toward chloroform in rats and humans was 5.29 and 5.24 pmol/min/pmol CYP2E1, respectively.

Constan *et al*. (1996, 1999) demonstrated that inhibition of CYP2E1, thereby eliminating metabolism of chloroform to phosgene, prevents the toxicity of chloroform in mice, providing evidence that oxidative metabolism of chloroform is of critical importance to its toxic effects. Under most conditions, the vast majority of chloroform metabolism occurs via the oxidative pathway, resulting in the formation of carbon dioxide. Microsomal studies indicate that the majority (about 75 percent) of covalent binding following treatment with chloroform is to phospholipids, which would be consistent with the actions of phosgene, and suggests that the major pathway for chloroform metabolism is the oxidative pathway (ILSI, 1997; U.S. EPA, 1998c). Furthermore, addition of glutathione to the test system completely negated binding to liver microsomes, as would be expected for the oxidative metabolism pathway. Reductive metabolism has only been documented *in vivo* following induction of P450 enzymes with phenobarbital, with negligible activity seen in uninduced animals (ILSI, 1997). At high exposure levels, the oxidative pathway may become saturated, leading to a smaller percentage of carbon dioxide formation in exposed animals (Corley *et al*., 1990; Plummer *et al*., 1990). However, data directly demonstrating an increased utilization of the reductive pathway at high doses are not available.

Species differences exist in the extent of chloroform metabolism (Brown *et al*., 1974, Taylor *et al*., 1974; Reynolds *et al*., 1984; Mink *et al*., 1986; Corley *et al*., 1990). Brown *et al*. (1974) reported that the mouse metabolized chloroform to carbon dioxide to the greatest extent (about 85 percent) and the rat to a lesser degree (67 percent); only a small amount (18 percent) of chloroform was metabolized by monkeys. Similarly, Corley *et al*. (1990) found that at high doses (about 360 mg/kg) of chloroform, $B6C3F_1$ mice metabolized a greater proportion of the total dose than did Osborne-Mendel rats. Vittozzi *et al*. (2000) also reported that $B63CF_1$ mice metabolized a greater proportion of an injected dose of chloroform to carbon dioxide than did Sprague-Dawley (SD) or Osborne-Mendel rats; the two strains of rats had similar levels of metabolism. In experimental animals, there is some indication that metabolism may be sex dependent as well (Taylor *et al*., 1974; Smith *et al*., 1984; Smith and Hook, 1983, 1984).

Excretion

Much of the absorbed chloroform is metabolized prior to its elimination; carbon dioxide is the predominant end product. Although a number of other metabolites of chloroform have been identified, quantification of metabolite production has largely been restricted to measurements of ^{14}C-carbon dioxide in expired breath after administration of radiolabeled chloroform. Even when urinary excretion of metabolites has been followed,

the identity of these metabolites has not been ascertained. A substantial amount of chloroform is eliminated through the lungs without undergoing metabolic transformation.

Fry *et al*. (1972) showed that humans given a single oral dose of ^{13}C-chloroform in an olive oil vehicle excreted chloroform and carbon dioxide in expired breath. The ^{13}C-labeled compounds were first detected 20 to 30 minutes after ingestion, presumably reflecting slow absorption from the olive oil vehicle. Peak concentrations in exhaled breath were found 40 minutes and two hours after administration. Although most of the administered dose was recovered within eight hours, Fry *et al*. (1972) noted that chloroform was detectable in the breath of some subjects up to 24 hours after dosing. An average of 44.3 percent of a 500 mg dose was recovered in exhaled air as unmetabolized chloroform in five male subjects, with substantial individual variability (17.8 to 66.6 percent). Females given 500 mg chloroform eliminated an average of 33.6 percent unmetabolized from the lungs, with a range of 25.6 to 40.4 percent. A single male given 1,000 mg of chloroform exhaled 64.7 percent through the lungs unchanged. Fry *et al*. (1972) also found that 48.5 to 50.6 percent of a 500 mg dose of chloroform was respired as carbon dioxide. No attempt was made to measure elimination of urinary metabolites.

Raabe (1988) exposed eight volunteers to 7 to 25 ppb of ^{14}C-chloroform in air for two hours; elimination of the radiolabel in expired air was followed during exposure and for 30 minutes thereafter. Among four individuals who inhaled chloroform through the mouth only, 38.2 percent of the retained dose was recovered as ^{14}C-carbon dioxide. Only 0.6 percent of the radiolabel was recovered as chloroform in expired breath or as urinary metabolites. Nearly identical values were obtained from four volunteers who inhaled chloroform through the nose. At the end of the recovery period, it was estimated that approximately 28.2 percent of the absorbed chloroform remained in the body. Consequently, the reported percentage of dose recovered as metabolites probably underestimates the actual extent of metabolism. The very large difference in percentage of exhaled intact chloroform, compared to tthe data of Fry *et al*. (1972) may relate to the great difference in doses in the two studies, with possible metabolic saturation in the study of Fry *et al*. (1972). Raabe (1988) used a one compartment model to calculate that the elimination half-life of chloroform and its metabolites in respired breath and urine were 3.5 hours and 5.2 hours, respectively.

Mice metabolize virtually all of small, orally administered doses of chloroform. Males of three mice strains eliminated 83.9 to 87.2 percent of a 60 mg/kg dose of ^{14}C-chloroform as ^{14}C-carbon dioxide over a 48 hour recovery period. An additional 4.0 to 5.0 percent of the dose was recovered in the urine, feces, and carcass. Small amounts (5.2 to 7.1 percent) were exhaled as the parent compound (Brown *et al*., 1974). Taylor *et al*. (1974) also distinguished the relative importance of urinary and fecal elimination of chloroform in mice. Of the 5.5 to 5.6 percent of a 60 mg/kg oral dose recovered in excretory products, 4.9 to 5.1 percent of the applied dose was recovered in the urine. Taylor *et al*. (1974) noted that the percentage of administered dose eliminated as parent compound was relatively small in both male and female mice (0.31 and 1.8 percent, respectively) and that the difference was statistically significant ($p < 0.001$). Corley *et al*. (1990) exposed male B6C3F$_1$ mice to ^{14}C-chloroform by inhalation at 10, 89, or 366 ppm for six hours; the percent of radiolabel recovered as metabolic products was 99 percent at 10 and 89 ppm, and 92 percent at 366 pm. ^{14}C-Carbon dioxide represented approximately 85

percent of recovered metabolites for all three exposure groups. Some metabolic saturation at the highest dose appears to be indicated by these data.

The data of Mink *et al*. (1986) are consistent with the hypothesis that metabolism of chloroform in mice is saturable at high doses (U.S. EPA, 1985a; Gargas *et al*., 1988). Although their results were compromised by the short recovery period (eight hours), 26 percent of a 150 mg/kg oral dose of chloroform was eliminated as unmetabolized parent compound, and only 50 percent of the dose was metabolized to carbon dioxide. A relatively large percentage (13.5 percent) of the applied dose was recovered in the carcass, perhaps because of the abbreviated recovery period.

Rats appear to metabolize a smaller percentage of an orally administered dose of chloroform than mice. Reynolds *et al*. (1984) and Brown *et al*. (1974) recovered 66.1 to 68.0 percent of a single oral dose of ^{14}C-chloroform to rats as ^{14}C-carbon dioxide in respired air. Reynolds *et al*. (1984) found that peak elimination of chloroform occurred within the first 30 minutes after oral dosing, while peak elimination of ^{14}C-carbon dioxide was found at 30 to 45 minutes after dosing with 11.9 mg/kg and at 60 to 105 minutes post-dosing with 35.8 mg/kg. Neither Reynolds *et al*. (1984) nor Brown *et al*. (1974) specified the quantity of radiolabel remaining in the carcass (if any), although Brown *et al*. (1974) recovered 7.6 percent of a 60 mg/kg dose in the urine and feces. Chloroform elimination through the lungs accounted for 5.2 to 6.0 percent of the dose in Brown *et al*. (1974) while Reynolds *et al*. (1984) found dose-dependent elimination of chloroform; five percent of the dose was eliminated as chloroform at 11.9 mg/kg, and 12 percent at 35.8 mg/kg. Utilizing a linear, two compartment model, Reynolds *et al*. (1984) estimated that in animals given 11.9 mg/kg of chloroform, the halftimes for oral absorption, pulmonary elimination of chloroform, and pulmonary elimination of carbon dioxide were 0.08, 3.83, and 2.1 hours, respectively. The corresponding values for animals given 35.8 mg/kg of chloroform were 0.13, 2.27, and 5.6 hours.

Bromoform

Absorption

Estimates of bromoform absorption from the gastrointestinal tract have been obtained from radioisotope studies in mice and rats. Estimates of dermal absorption of bromoform have been obtained *in vitro* using human skin (Xu *et al*., 2002). Estimates of bromoform absorption via inhalation are not available in the published literature.

Mink *et al*. (1986) administered single gavage doses of ^{14}C-bromoform in corn oil to fasted male SD rats (100 mg/kg, 16 μCi/kg) and male B6C3F$_1$ mice (150 mg/kg, 32 μCi/kg). The bromoform was rapidly absorbed by the rats and eliminated in expired air either as unchanged bromoform (67 percent of dose) or as ^{14}C-carbon dioxide (four percent of dose) in eight hours. The mice also rapidly absorbed and eliminated bromoform in expired air as unchanged bromoform (six percent of dose) and ^{14}C-carbon dioxide (40 percent of dose) in eight hours. Total recovery of label in breath (as carbon dioxide or parent compound), urine, and tissues after eight hours was 78.9 percent for rats and 62.2 percent for mice, indicating that bromoform is readily absorbed in the gastrointestinal tract of rodents. Radiolabeled carbon monoxide in exhaled air was not

quantified in this experiment, although this compound is a known catabolite of bromoform (Anders *et al*., 1978). Therefore these data of Mink *et al*. (1986) may underestimate total absorption. In another study, 2 to 10 mg/kg of bromoform given to rats by aqueous gavage was rapidly absorbed (Parra *et al*., 1986).

Distribution

Data on the distribution of brominated THMs in animals are available from experiments in rats and mice. Bromoform at 2 to 10 mg/kg given by aqueous gavage was rapidly absorbed and distributed to liver, brain, kidney, blood, and fat; the highest concentrations were found in fat 30 minutes after administration (Parra *et al*., 1986).

Mink *et al*. (1986) determined distribution ^{14}C-bromoform in fasted male SD rats and male B6C3F$_1$ mice following a gavage dose in corn oil of 100 mg/kg for rats and 150 mg/kg for mice. Tissue radioactivity levels (chemical form undetermined) were measured eight hours after dosing. In the rat, the total organ content of radioactivity was 2.1 percent of administered dose. Stomach (contents removed), liver, and kidneys contained higher levels than bladder, brain, lung, muscle, pancreas, and thymus. In mice, 4.6 percent of radioactivity was recovered in organs and an additional 10 percent was recovered in blood. The authors attributed this elevated recovery of label in blood to formation of carboxyhemoglobin, although they did not measure carboxyhemoglobin. Their conclusion was based on an earlier report by Anders *et al*. (1978) of elevated levels of carboxyhemoglobin in SD rats after ip administration of bromoform.

Metabolism

A series of studies on bromoform metabolism (Ahmed *et al*., 1977; Anders *et al*., 1978; Stevens and Anders, 1979, 1981) was initiated following the observation that carbon monoxide and carboxyhemoglobin are elevated in the blood of rats administered THMs. Ahmed *et al*. (1977) established *in vitro* that bromoform is metabolized to carbon monoxide via a cytochrome P450 dependent pathway by a rat liver microsomal fraction requiring NADPH and molecular oxygen for maximal activity. Anders *et al*. (1978) administered ^{13}C-bromoform to rats and found that the isotope was incorporated in carbon monoxide. Pretreatment of the animals with phenobarbital increased carbon monoxide production, and the cytochrome P450 inhibitor SKF-525-A significantly decreased production, thus confirming the role of cytochrome P450 metabolism. Administration of ^{2}H-bromoform resulted in lower production of carbon monoxide than administration of ^{1}H-bromoform, suggesting that breakage of the C–H bond for insertion of oxygen is the rate-limiting step in carbon monoxide formation.

Stevens and Anders (1979) found that formation of carbon monoxide from bromoform *in vitro* is markedly stimulated by glutathione. Formation of one mole of carbon monoxide resulted in loss of two moles of glutathione and production of one mole of oxidized glutathione. Incubation of isotope-labeled bromoform in the presence of ^{18}O-molecular oxygen or ^{18}O-water demonstrated that the oxygen atom in carbon monoxide produced from bromoform is derived from molecular oxygen, rather than water. Failure to detect ^{18}O-carbon monoxide following incubation with ^{18}O-water also excluded the possibility that carbon monoxide is formed via carbene formation at cytochrome P450 under

anaerobic conditions (Wolf *et al*., 1977). 2-Oxathiazolidine-4-carboxylic acid was detected when bromoform was incubated with cysteine. This finding indicates that dibromocarbonyl is formed as an intermediate, and is consistent with the metabolism of chloroform, indicating that bromoform and chloroform share at least one common metabolic pathway.

Stevens and Anders (1981) also confirmed in male SD rats *in vivo* the deuterium isotope effect for bromoform, and the inhibitory effect of glutathione depletion and cysteine treatment (to promote formation of 2-oxathiazolidine-4-carboxylic acid via an alternative metabolic pathway) on carbon monoxide production.

The reductive pathway of metabolism remains less well-characterized than the oxidative pathway. Tomasi *et al*. (1985) studied anaerobic activation of bromoform to a free radical intermediate *in vitro* using rat hepatocytes isolated from phenobarbital-induced male Wistar rats. Production of a free radical intermediate was measured by electron spin resonance (ESR) spectroscopy. Production was greatest when hepatocytes were incubated under anaerobic conditions, and decreased with addition of cytochrome P450 inhibitors such as SKF-525A, metyrapone, and carbon monoxide. These data were interpreted as evidence that free radical formation depends on cytochrome P450-mediated reductive metabolism. Free radical production appeared to be greatest for bromoform, compared to other THMs. Comparison of ESR spectra obtained with deuterated chloroform and bromoform suggested that the free radical intermediate produced by chloroform metabolism was $\bullet CHCl_2$. The authors speculated that the brominated THMs are also metabolized by transfer of an electron directly from the cytochrome to the brominated compound with successive formation of the dibromomethyl radical ($\bullet CHBr_2$) and a bromide ion (Br^-).

As discussed above, studies conducted in *S. typhimurium* strains engineered to express the rat glutathione *S*-transferase theta gene (*GSTT1-1*) provide evidence for a third mechanism of bromoform bioactivation via one or more glutathione *S*-transferase theta mediated conjugation pathways (DeMarini *et al*., 1997; Pegram *et al*., 1997). Proposed routes for glutathione *S*-transferase theta mediated metabolic activation of the bromoform are illustrated in Figure 2 above.

Excretion

Mink *et al*. (1986) examined excretion of bromoform in fasted male SD rats and male $B6C3F_1$ mice gavaged with 100 mg/kg or 150 mg/kg, respectively, of ^{14}C-bromoform in corn oil. Expired air was the principal excretion route in both species. In rats, four percent of the administered dose was expired as carbon dioxide and 67 percent as unmetabolized bromoform up to eight hours after administration. In mice, 40 percent of the dose was expired as carbon dioxide and six percent as unmetabolized bromoform. Carbon monoxide was not quantified. Small amounts of label (about 2.2 percent for rats and 4.6 percent for rats) were recovered in urine, but the chemical identity of the labeled compounds was not investigated. The half-life of bromoform was 0.8 hours for rats and eight hours for mice. No data are available on excretion following exposure via the inhalation or dermal routes.

Bromodichloromethane

Absorption

Estimates of BDCM absorption from the gastrointestinal tract have been obtained from radioisotope studies in mice and rats. Estimates of BDCM dermal absorption have been obtained using human skin *in vitro* (Xu *et al*., 2002). Estimates of BDCM absorption via inhalation are not available in the published literature.

Mink *et al*. (1986) administered single gavage doses of ^{14}C-BDCM in corn oil to fasted male SD rats (100 mg/kg, 16 μCi/kg) and male B6C3F$_1$ mice (150 mg/kg, 32 μCi/kg). In rats the majority of the dose was absorbed and expired in air as unchanged BDCM (42 percent) and ^{14}C-carbon dioxide (14 percent) within eight hours. In mice the dose was also rapidly absorbed and largely eliminated via expiration as unchanged DBCM (seven percent of dose) and ^{14}C-carbon dioxide (81 percent of dose) in eight hours. Total recovery of label in exhaled air (as carbon dioxide or parent compound), urine, and tissues after eight hours was 62.7 percent for rats and 92.7 percent for mice, indicating good gastrointestinal absorption of BDCM in rodents. Radiolabeled carbon monoxide in exhaled air was not studied, so these data may underestimate total absorption.

Mathews *et al*. (1990) gavaged male F344 rats with 1, 10, 32, or 100 mg/kg ^{14}C-BDCM in corn oil. Radioactivity was measured in exhaled air (volatiles, carbon dioxide, and carbon monoxide), urine, feces, and tissues. BDCM was well absorbed, with at least 87 percent of the dose recovered in tissues, expired air, and excreta within 24 hours.

Lilly *et al*. (1994, 1998) compared uptake of BDCM from corn oil or 10 percent aqueous Emulphor®[2] by F344 rats. Three animals/vehicle/time point received 400 mg/kg of BDCM by gavage in a constant volume of Emulphor® or corn oil (Lilly *et al*., 1994). The animals were sacrificed four, 16, or 64 minutes post-treatment and blood was collected for analysis. The highest blood levels of BDCM were observed four minutes after administration, indicating rapid BDCM absorption. The blood levels of BDCM were significantly greater (92 and 100 percent) at four and 16 minutes after administration in the aqueous Emulphor® vehicle than after the corn oil vehicle.

Lilly *et al*. (1998) administered BDCM by gavage at 0, 50, or 100 mg/kg in either corn oil or 10 percent Emulphor® to three male F344 rats/dose/vehicle. BDCM levels were monitored in blood and exhaled air. BDCM in blood and exhaled air reached maximal concentrations less than one hour after administration. Delivery of BDCM in 10 percent Emulphor® resulted in faster initial uptake, as inferred from higher blood, tissue and breath chamber concentrations, compared to administration in corn oil. Uptake after gavage appeared to be highly variable among individuals and pulsatile in nature. The study authors suggested that the pulsatile behavior results from discontinuous stomach emptying into the small intestine.

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[2] Emulphor® (EL-260, GAF Corp.) is a polyethoxylated oil nonionic surfactant used for suspending water insoluble agents for gavage administration to laboratory animals.

Distribution

No data were located on the distribution of BDCM in humans. Data on the distribution of BDCM in animals are available from oral exposure of rats and mice, but not from inhalation or dermal routes. Mink *et al*. (1986) determined the distribution of ^{14}C-BDCM in fasted male SD rats and male B6C3F$_1$ mice following a gavage dose of 100 mg/kg for rats and 150 mg/kg for mice in corn oil. Tissue levels of radioactivity were measured eight hours after dose administration. In the rat, the total organ content of radioactivity was 3.3 percent of the administered dose. The stomach (contents removed), liver, and kidneys contained the highest levels of residual radioactivity. In mice, 3.2 percent of the radioactivity was recovered in the organs.

Rats given BDCM (0.5 or 5.0 mg) in corn oil gavage once daily for 25 days exhibited average BDCM concentrations of 1 or 23 µg/L in serum and 51 and 1,800 ng/g in fat, respectively (Pfaffenberger *et al*., 1980). Three to five days after cessation of dosing the serum and fat concentrations were 1 µg/L and 3 to 4 ng/g fat for both dose levels.

Mathews *et al*. (1990) studied distribution of BDCM in male F344 rats given 1, 10, 32, or 100 mg/kg of ^{14}C-BDCM in corn oil by gavage. Three to four percent of the dose was detected in tissues after 24 hours, with the highest levels (one to three percent) found in liver. Liver, kidney, and stomach had the highest tissue to blood ratios of radiolabel. In another experiment, 10 or 100 mg/kg of BDCM was administered daily to male F344 rats for 10 consecutive days. Retention of the radiolabeled dose after 24 hours was 0.9 to 1.1 percent of the administered dose, with the greatest retention in the liver. The highest tissue to blood ratios were found in the liver, kidney, and stomach, similar to the results after a single dose. Thus, repeated dose administration did not result in altered disposition or bioaccumulation of BDCM.

Christian *et al*. (2001b) analyzed BDCM in parental tissues and fluids and F$_1$ generation tissues as part of a reproductive and developmental study in SD rats. BDCM was administered in drinking water at concentrations of 0, 50, 150, 450, or 1,350 ppm. Parental blood samples were collected at eight-hour intervals from three male and three female rats/dose level on study days one and 14, and from three female rats/dose on gestation day 20 and lactation day 15. Blood samples from weanling F$_1$ rats were collected on postpartum day 29. Pooled fetal blood, amniotic fluid, and placentas were collected from three litters/exposure group on gestation day 21. Milk samples were collected from lactating females on lactation day 15. Plasma collected from male and female rats prior to mating and from female rats during gestation and lactation did not contain quantifiable levels of BDCM (limit of quantification 0.22 µg/g). BDCM was detected at 0.38 µg/g in the milk from one female in the 1,350-ppm group. BDCM was not detected in placentas, amniotic fluid, or fetal tissue collected on gestation day 21 or in plasma from postpartum day 29 weanling pups. The lack of detectable BDCM in the majority of samples contrasts with the findings of other studies in rats (for example, Lilly *et al*., 1998). The study authors attributed the differing results to differences in strain, vehicle, and method of administration (i.e., consumption of small intermittent quantities in drinking water versus bolus gavage doses).

Christian *et al*. (2001b) also analyzed for BDCM in parental tissues and fluids and F$_1$ generation tissues as part of a reproductive and developmental study in New Zealand

White rabbits. Four rabbits/dose group were provided with drinking water containing 0, 50, 150, 450, or 1,350 ppm of BDCM. Maternal blood samples were collected on gestation days seven and 28. Amniotic fluid and placenta samples were collected on gestation day 29 and pooled by litter. Blood samples were collected from three fetuses per litter on day 29. BDCM was found at concentrations of 0.15 and 0.17 µg/g (limit of detection 0.11 µg/g) in placentas from two litters in the 1,350 ppm exposure group. BDCM was not quantifiable in maternal plasma, in placentas from does exposed up to 450 ppm, or in the amniotic fluid or fetuses from does exposed to up to 1,350 ppm. The authors concluded that these data indicate that BDCM can cross the placenta, but apparently does not accumulate in fetal tissues.

Metabolism

BDCM is largely metabolized to carbon dioxide as noted above. It is also metabolized to carbon monoxide *in vitro* (Ahmed *et al*., 1977) and *in vivo* (Anders *et al*., 1978). The studies focused on other THMs (Ahmed *et al*., 1977, 1980; Anders *et al*., 1978; Stevens and Anders, 1979, 1981) are relevant to BDCM because the brominated THMs are assumed to share common oxidative and reductive pathways for metabolism.

Gao and Pegram (1992) reported that binding of reactive intermediates to rat hepatic microsomal lipid and protein under reductive (anaerobic) conditions was more than twice as high for BDCM as for chloroform. Tomasi *et al*. (1985) studied the anaerobic activation of BDCM to a free radical intermediate *in vitro* using rat hepatocytes isolated from phenobarbital-induced male Wistar rats. Production of a free radical intermediate as measured by ESR spectroscopy was greater for BDCM than for chloroform. Greater production was indicated when hepatocytes were incubated under anaerobic conditions, with less production aerobically or in the presence of cytochrome P450 inhibitors such as SKF-525A, metyrapone, and carbon monoxide. These data were interpreted as evidence that free radical formation depends on cytochrome P450-mediated reductive metabolism.

Induction of CYP2E1 or CYP2B1/2 in F344 rats increases the acute hepatic toxicity of BDCM, and high doses of BDCM inhibit specific CYP isoform activities (Thornton-Manning *et al*., 1993).. The isoform-specific substrate results suggest that BDCM is a suicide inhibitor of CYP2B1/2, but not CYP2E1. Lilly *et al*. (1997a) obtained evidence in rats using an inhibitor of CYP2E1 (*trans*-dichloroethylene) that CYP2E1 is the major isoform involved in metabolism of BDCM when rats are exposed to 100 to 3,200 ppm by inhalation. Studies by Allis *et al*. (2001) suggest that BDCM may also be metabolized by CYP1A2 and that the pattern of isoform response is similar in male and female rats and after gavage or inhalation exposure.

Zhao and Allis (2002) observed that at a low concentration of 9.7 µM, the rate of BDCM metabolism was correlated with activity of CYP2E1 and not other P450 isoforms in microsomes prepared from eleven human liver samples. The ability to metabolize BDCM was demonstrated for CYP1A2 and CYP3A4 in addition to CYP2E1 in human liver microsomes and a recombinant P450 enzyme system (Allis and Zhao, 2002; Zhao and Allis, 2002). However, the Km and K_{cat} were much lower for the CYP2E1 isoform, indicating that metabolism is predominantly due to this isoform at the lower levels expected for environmental exposures. Zhao and Allis (2002) calculated the rate of

BDCM metabolism by human liver microsomes and in a recombinant system (Allis and Zhao, 2002). Their estimates of metabolism indicate that both CYP1A2 and CYP1A4 could contribute substantially to hepatic metabolism at high BDCM concentrations. Metabolism by the CYP1A2 isoform may be substantial at lower BDCM levels when the level of this enzyme is induced (which was demonstrated in rats administered 2,3,7,8-tetrachlorodibenzo-*p*-dioxin) (Allis *et al*., 2002).

Studies in *S. typhimurium* strains engineered to express the rat glutathione *S*-transferase theta gene (*GSTT1-1*) provide evidence for a third mechanism of BDCM bioactivation via one or more glutathione *S*-transferase theta mediated conjugation pathways (DeMarini *et al*., 1997; Pegram *et al*., 1997). The tested strains included TA1535, TA1535 transfected with the rat *GSTT1-1* gene (+*GSTT1-1*), and TA1535 transfected with the cDNA inserted in a nonfunctioning orientation (−*GSTT1-1*). These strains were tested in a standard plate incorporation mutation assay in a 24-hour exposure to BDCM under conditions that prevented volatilization loss, at vapor concentrations from 200 to 4,800 ppm, without exogenous metabolic activation. BDCM increased the number of revertant colonies in each *Salmonella* strain. Further details are discussed under chloroform metabolism. These results provide evidence that the mutagenicity of BDCM is enhanced by glutathione *S*-transferase theta-mediated conjugation with glutathione.

Further experiments of DeMarini *et al*. (1997) in *S. typhimurium* elucidated the role of glutathione *S*-transferase theta in the bioactivation of BDCM, as discussed earlier. Proposed routes for glutathione *S*-transferase theta mediated metabolic activation of BDCM are illustrated in Figure 2.

Excretion

Mink *et al*. (1986) studied BDCM excretion in fasted male SD rats and male $B6C3F_1$ mice gavaged with 100 and 150 mg/kg ^{14}C-BDCM in corn oil for rats and mice, respectively. Expired air was the principal route of excretion, although the proportions of carbon dioxide and unmetabolized BDCM differed by species. In rats, 14.2 percent of the dose was expired as carbon dioxide and 41.7 percent as unmetabolized BDCM by eight hours post-administration. In mice, 81.2 percent of the dose was expired as carbon dioxide and 7.2 percent as BDCM. Carbon monoxide exhalation was not measured. About 1.4 percent of the label for rats and 2.2 percent for mice was recovered in urine. The authors estimated a BDCM half-life of 1.5 hours for rats and 2.5 hours for mice.

Mathews *et al*. (1990) examined excretion of ^{14}C-BDCM in F344 rats given either a single oral dose of 1, 10, 32, or 100 mg/kg, or repeated doses of 10 or 100 mg/kg-day over a 10-day period, in a corn oil vehicle. After one dose 70 to 80 percent of the dose was exhaled as ^{14}C-carbon dioxide and three to five percent as ^{14}C-carbon monoxide within 24 hours. Approximately four percent of the radiolabel was present in urine and one to three percent in the feces. The daily excretion of carbon dioxide and carbon monoxide was unchanged in animals administered repeated doses of 10 mg/kg-day. At 100 mg/kg-day, the initial rate of carbon dioxide excretion during the first eight hours after dosing doubled between day one (30 percent of dose) and on days three and 10 (60 percent of dose). These results were interpreted by the study authors as evidence that BDCM can induce its own metabolism.

Narotsky *et al*. (1997) compared the blood levels and elimination kinetics of BDCM in pregnant rats. BDCM was administered by gavage in either an aqueous 10 percent Emulphor® solution or corn oil. Three to four inseminated females/vehicle/time point were given a dose of 75 mg/kg on gestation day six. BDCM concentrations were measured in whole blood collected at 30 minutes, 90 minutes, 4.5 hours, and 24 hours after dosing. The animals were sacrificed at 24 hours and pregnancy status was confirmed at necropsy. BDCM concentrations in blood decreased over time with both vehicles, but tended to be higher following corn oil administration. The vehicle-related difference in blood concentration reached statistical significance at the 4.5 and 24 hour time points. The elimination half-life of BDCM was estimated to be 3.6 hours when administered in corn oil and 2.7 hours when given in the aqueous vehicle.

No data are available on excretion following exposure via the inhalation or dermal routes.

Dibromochloromethane

Absorption

Estimates of DBCM absorption from the gastrointestinal tract have been obtained from radioisotope studies conducted in mice and rats. Estimates of DBCM dermal absorption have been obtained using human skin *in vitro* (Xu *et al*., 2002). Estimates of DBCM absorption via inhalation were not available.

Mink *et al*. (1986) administered single gavage doses of ^{14}C-DBCM in corn oil to fasted male SD rats (100 mg/kg, 16 μCi/kg) and male B6C3F$_1$ mice (150 mg/kg, 32 μCi/kg. Rats rapidly absorbed ^{14}C-DBCM, with 48 percent of the dose appearing unchanged and 18 percent as ^{14}C-carbon dioxide in expired air after eight hours. Mice showed 12 percent and 72 percent, respectively, in expired air after eight hours. Recovery of label in exhaled air (as carbon dioxide or parent compound), urine, and tissues after eight hours was 70.3 percent for rats and 91.63 percent for mice, indicating that DBCM is readily absorbed from the rodent gastrointestinal tract. Carbon monoxide was not quantified in exhaled air, and the data may therefore underestimate total absorption.

Distribution

No comprehensive data on distribution of DBCM in humans were identified in the published literature. Pellizzari *et al*. (1982) detected DBCM in one of 42 samples of breast milk from women in urban areas, indicating that DBCM can distribute to breast milk. Neither the level measured nor the detection limit was reported for this study.

Mink *et al*. (1986) determined the distribution ^{14}C-labeled DBCM in fasted male SD rats and male B6C3F$_1$ mice following gavage doses in corn oil of 100 mg/kg for rats and 150 mg/kg for mice. Tissue radioactivity levels were measured eight hours later. The chemical form of radioactivity was not determined. In the rat, the total organ content of radioactivity was 1.4 percent of the administered dose. The stomach (without contents), liver, and kidneys contained higher levels than bladder, brain, lung, muscle, pancreas, and thymus. In mice, 5.02 percent of the radioactivity was recovered in the organs.

Metabolism

The metabolism of DBCM is believed to share common oxidative and reductive pathways with the other brominated THMs, as discussed above (U.S. EPA, 1994a, 2005d) and shown in Figure 2. The studies discussed above on cytochrome P450 isoforms involved in metabolism of THMs are also relevant to DBCM. DBCM is largely metabolized to carbon dioxide as noted above. It is also metabolized to carbon monoxide *in vitro* (Ahmed *et al*., 1977) and *in vivo* (Anders *et al*., 1978).

Pankow *et al*. (1997) studied DBCM metabolism to bromide and carbon monoxide in rats. After oral gavage of DBCM in olive oil at 0.4, 0.8, 1.6, and 3.1 mmol/kg, the mean bromide levels rose to 0.03, 0.16, 0.49, and 0.63 mmol/L at 180 minutes. The DBCM concentrations in blood and in fat tissue six hours after the last of seven daily doses of 0.8 mmol/kg were significantly lower than six hours after a single dose. After one dose of DBCM the glutathione disulfide level in liver was significantly increased. The oxidative metabolism of DBCM was influenced by glutathione concentration in the liver. The rate of carboxyhemoglobin (COHb) and bromide formation was decreased after glutathione depletion due to pretreatment with buthionine sulfoximine (BSO) and increased after pretreatment with butylated hydroxyanisole (BHA). DBCM is a substrate for cytochrome CYP2E1 as demonstrated by the relative decrease of bromide and carboxyhemoglobin after concurrent administration of the CYP2E1 inhibitor diethyldithiocarbamate. Increased bromide formation after DBCM administration in phenobarbital-pretreated rats indicated that CYP2B1 and CYP2B2 also play a role in DBCM metabolism. Oxidation of DBCM to carbonyl halogenides, which as electrophilic and very unstable intermediates readily react with nucleophiles in tissues, is a key step in its toxic action.

DeMarini *et al*. (1997) conducted experiments in *S. typhimurium* to elucidate the role of glutathione *S*-transferase theta in bioactivation of DBCM and other brominated THMs as discussed earlier. Proposed routes for glutathione *S*-transferase theta-mediated metabolic activation of DBCM are illustrated in Figure 2.

Excretion

Mink *et al*. (1986) studied excretion of DBCM in fasted male SD rats and male $B6C3F_1$ mice gavaged with ^{14}C-DBCM in corn oil by gavage at doses of 100 and 150 mg/kg for rats and mice, respectively. Expired air was the principal route of DBCM excretion in both species, although the proportion expired as carbon dioxide or unmetabolized parent compound differed by species. In rats, 18.2 percent of the dose was expired as carbon dioxide and 48.1 percent was expired as unmetabolized DBCM by eight hours after administration. In mice, 71.58 percent of the dose was expired as carbon dioxide and 12.31 percent was expired as unmetabolized DBCM. Carbon monoxide was not quantified. Approximately one percent of the dose for rats and two percent for mice was recovered in urine, but the identity of the labeled compounds was not investigated. The halflife of DBCM was 1.2 hours for rats and 2.5 hours for mice.

No data are available on excretion following exposure via the inhalation or dermal routes.

PHYSIOLOGICALLY BASED PHARMACOKINETIC (PBPK) MODELS

Physiologically based pharmacokinetic (PBPK) models mathematically describe the rate of uptake, distribution, metabolism, and elimination of xenobiotics in humans and experimental animals. These multicompartment models are based on physiologically realistic descriptions of tissue volume, cardiac output, lung capacity, and metabolism, and are based as much as possible on actual experimental measurements.

Feingold and Holaday (1977) first applied pharmacokinetic models to study the metabolism and elimination of chloroform in humans, under exposure conditions designed to mimic clinical use as an anesthetic. In their models, the body was divided into five compartments: vessel-rich, vessel-poor, muscle, fat, and liver (the compartment responsible for metabolism). One of their models was based on the assumption that human metabolism of chloroform is a linear, first order process, and that a constant fraction of the chloroform delivered to the liver (the hepatic fraction) is metabolized. Feingold and Holaday (1977) also tested a model that assumed that there is a specific, but nonlinear relation between the hepatic arterial concentration of chloroform and the metabolized. The percentage of dose metabolized (defined as the ratio of the amount metabolized to the amount of chloroform taken up) predicted by this model depended on the hepatic fraction value and on the exposure duration, i.e., as the period of exposure and the hepatic fraction increased, so did the amount metabolized. A similar relation was apparent when the data were expressed as mmol of chloroform metabolized. The nonlinear model predicted that the percentage of chloroform metabolized would not be substantially affected by the duration of exposure, varying from 33 percent after a one-hour exposure to 29 percent following an eight-hour exposure. This represented a significant precursor to the later models developed for industrial or environmental exposures, which are described in more detail below.

The NRC (1986) evaluated a PBPK model developed by Ramsey and Andersen (1984), which describes the uptake, metabolism, and excretion of styrene in rats and humans. This type of model has been applied to the study and prediction of animal and human pharmacokinetics for several volatile organic compounds (VOCs) including benzene, trichloroethylene, tetrachloroethylene, methylene chloride, 1,1,1-trichloroethane, and chloroform (NRC, 1986; Gargas *et al*., 1986; Andersen *et al*., 1987; Reitz and Nolan, 1986; Hattis *et al*., 1987; Bogen *et al*., 1987; Ward *et al*., 1988; Bogen, 1988; Bogen and McKone, 1988; Bogen and Hall, 1989; Andersen, 1999).

Chloroform - Bogen et al. (1992b) PBPK Model

Bogen *et al*. (1992b) developed a Ramsey and Andersen (1984) PBPK model for chloroform based on physiological and pharmacokinetic information on uptake, distribution, metabolism, and (respiratory) excretion in rodents and humans. Assuming that the values for physiological parameters and Km that they initially selected were good ones, they then adjusted the values of Vmax for mice, rats, and humans so that in the PBPK models the amounts of chloroform metabolism matched the observations in the studies selected for data extrapolation. Bogen *et al*. (1992b) used the latter "Vmax

validated" PBPK models to estimate (a) the metabolized (toxicologically effective) doses received by animals in rodent bioassays to estimate chloroform's carcinogenic potency, and (b) the potential effective doses to humans environmentally exposed to chloroform.

The rat PBPK model for chloroform used the blood to air and tissue to air partition coefficients of Gargas *et al*. (1989), derived from *in vitro* studies with F344 rat tissues. It also initially assumed values for Vmax (7.0 mg/hour) and Km (0.25 mg/L) determined for F344 rats, with Vmax scaled for a reference 1.0 kg rat (Gargas *et al*., 1988). Values for the physiological parameters Qa and Qb were based on a reference 0.25 kg rat (U.S. EPA, 1988) as shown in Table 10. The mass balance studies of Brown *et al*. (1974), Reynolds *et al*. (1984) and Mink *et al*. (1986) provided estimates of chloroform metabolized by rats dosed by gavage with a corn oil vehicle. To compare the modeled results to these data, tissue volumes were scaled directly to the relevant experimental rat body weight, flowrate parameter values (in units of L/hour) were scaled to the 0.74 power of body weight, and Vmax was scaled to the 0.70 power of body weight (Ramsey and Andersen, 1984; Gargas *et al*., 1986; Paustenbach *et al*., 1988). Oral absorption kinetics of chloroform were presumed to be similar to those observed by Withey *et al*. (1983). In that study, 400 g Wistar rats were given chloroform in 10 mL/kg of corn oil, which yielded a roughly constant, 30 minute absorptive pulse equivalent to chloroform clearance from the vehicle at about 0.33 mL vehicle/kg-minute. It was assumed that this clearance rate scaled to a power of body weight equal to $(0.74 - 1) = -0.26$, in accordance with the assumption regarding flowrate scaling made above. Ingested doses were assumed to be introduced directly into the liver compartment.

Comparing model predictions to the data of Mink *et al*. (1986) and one of the data sets (the 11.9 mg/kg dose group) of Reynolds *et al*. (1984), the model provided reasonable predictions of the amount of chloroform metabolized; the ratio of actual to predicted values ranged from 0.94 to 1.0. However, the Mink *et al*. (1986) data showed poor recovery of radiolabel (78.2 percent) and were based on a short recovery period (eight hour); both factors could underestimate the amount of chloroform metabolized. At higher doses in the Reynolds *et al*. (1984) and Brown *et al*. (1974) studies, the corresponding experimentally determined metabolized doses differed from the values predicted by the PBPK model using the rat Vmax estimate of Gargas *et al*. (1988). At the highest dose (60 mg/kg) of Brown *et al*. (1974), the model significantly underestimated the amount of chloroform metabolized. The data of Brown *et al*. (1974) represented the most comprehensive estimate of chloroform metabolism in rats, including measurements of respiratory elimination of the metabolite carbon dioxide and urinary and fecal elimination of metabolites over a 72 to 96 hr recovery period, that were then available. These data were therefore used to derive an "improved" estimate of Vmax (37.2 mg/hour for a 1.0 kg rat), which yielded an estimate of chloroform metabolism in accordance with the best experimental data (73.7 percent of a 60 mg/kg dose was metabolized).

Bogen *et al*. (1992b) then assessed how well the PBPK model predicted chloroform metabolism in mice observed in the studies by Brown *et al*. (1974) and Taylor *et al*. (1974), in which chloroform was administered by gavage in corn oil. The results of Brown *et al*. (1974) and Taylor *et al*. (1974) were averaged, because production of chloroform metabolites was nearly identical in each of the five data sets. The tissue to air partition coefficients for chloroform in mice were assumed to be identical to the Gargas

et al. (1989) rat values. Mouse metabolic constants were obtained by setting the mouse Km value equal to that of the rat and scaling the rat Vmax value to the 0.7 power of body weight, as described above. Other physiological parameters for mice were taken from U.S. EPA (1988), and scaled as described above. The rate of vehicle clearance from corn oil for mice was based on Withey *et al*. (1983) rat data scaled to the −0.26 power of body weight as described above. Compared to the averaged results of Brown *et al*. (1974) and Taylor *et al*. (1974), the PBPK model underestimated the amount of chloroform metabolized by mice by a factor of 2.2. Therefore, they again used a process of iterative optimization to calculate a "corrected" value of Vmax for mice.

Data on the metabolism and elimination of chloroform in humans is largely limited to that of Fry *et al*. (1972), who observed that an average of 49.5 percent of a single 500 mg oral dose of ^{13}C-chloroform administered to two humans was metabolized to ^{13}C-carbon dioxide within eight hours postexposure. Volunteers in the Fry *et al*. (1972) study received chloroform in one mL of olive oil, a quantity expected to be cleared in approximately 0.005 hour based on the data of Withey *et al*. (1983) for rats scaled as described above. The Michaelis constant, Km, was assumed to be equal to that used for rats. The value of Vmax for chloroform required to allow the PBPK model for humans to predict the extent of chloroform metabolism observed by Fry *et al*. (1972) was estimated by iterative numerical optimization to be 560 mg/hour for a reference 70 kg human. This value is approximately four times greater than that obtained by scaling the rat Vmax of Gargas *et al*. (1988) to the 0.7 power of body weight. Fry *et al*. (1972) also reported data on pulmonary excretion of chloroform from volunteers given a single oral chloroform dose of 250 to 1,000 mg. The exhaled amounts ranged from 12.4 to 66.6 percent of the doses (17.8 to 66.6 percent for eight volunteers exposed to 500 mg chloroform). If it can be assumed that all unexpired chloroform was actually metabolized in the latter study, then these data would suggest that as much as approximately 82 percent of an ingested 500 mg dose may be metabolized by humans. Therefore the Vmax estimate for humans may be too low (by a factor of roughly 1.7) to represent the largest value consistent with all available human data on chloroform metabolism.

Using the human physiological and metabolic parameters listed in Table 10, the metabolized fraction of the chloroform potentially available for absorption and metabolism can be predicted. With ingestion exposure, the quantity of interest is the fraction, fmo, of the maximum plausible metabolic rate, i.e., the metabolic clearance fraction given a continuous rate of ingestive absorption. With respiratory exposure to a constant concentration, the corresponding quantity is defined as the fraction fmr. The limiting values, f*mo and f*mr, as the ingested dose or respired concentration of chloroform approaches zero, are important in the context of environmental risk management. Using the appropriate equations to calculate these latter quantities presented in Bogen (1988), the PBPK parameter values for humans listed in Table 10 imply that, for a reference 70 kg human, f*mo =0.986 and f*mr = 0.662.

Table 10. Values Used in the Bogen *et al*. (1992b) Chloroform PBPK Model

Parameter, unit	Mouse	Rat	Human
Body weight, kg	0.025	1.0	70.0
Alveolar ventilation, L/hour	1.50	19.6	378.0
Cardiac output, L/hour	1.02	13.9	372.0
Tissue flows, fraction of cardiac output			
Liver	0.25	0.25	0.26
Fat	0.09	0.09	0.05
Richly perfused tissues	0.51	0.51	0.44
Poorly perfused tissues	0.15	0.15	0.25
Tissue volumes, fraction of body weight, L			
Liver	0.055	0.04	0.026
Fat	0.1	0.07	0.19
Richly perfused tissues	0.05	0.05	0.05
Poorly perfused tissues	0.70	0.75	0.62
Tissue/blood partition coefficients, unitless			
Liver	1.01	1.01	2.1
Fat	9.76	9.76	35.0
Richly perfused tissues	1.01	1.01	1.9
Poorly perfused tissues	0.668	0.668	1.5
Metabolic parameters for liver compartment			
Vmax, mg/hour	4.7	37.2	560
Km, mg/L	0.25	0.25	0.25

Chloroform - Corley et al. (1990) PBPK Model

Corley *et al*. (1990) developed a five compartment PBPK model for chloroform in mice, rats, and humans based on uptake and metabolism data available from the literature and generated as part of their PBPK analysis. The model includes a kidney compartment as a component of the richly perfused tissue compartment, which was assumed also to metabolize chloroform with Michaelis-Menten kinetics. The kidney compartment was assumed for mice, rats, and humans to have volumes equal to 29, 28.1, and 14 percent, respectively, of the corresponding liver volumes and to have rates of blood perfusion approximately equal to those of richly perfused tissue. The specific tissue volumes, blood flow rates, and PBPK model structure were otherwise similar to those shown in Table 11 (but scaled for a 28.5 g mouse and 230 g rat), except that partially recoverable enzymatic self-inhibition (governed by two additional rate parameters) was incorporated into the Michaelis-Menten model for chloroform metabolism in mice. The Km values for mice and rats (0.352 and 0.543 mg/L, respectively) were determined from gas uptake experiments on those species; the value for humans was taken as the average of those obtained for mice and rats. The corresponding Vmax values for mice and rats were 1.89 mg/hour for a 28.5 g mouse (equivalent to 1.72 mg/hour for a 25 g mouse) and 2.42

mg/hour for a 230 g rat (equivalent to 6.8 mg/hour for a 1.0 kg rat), which are somewhat lower than the values in Table 10 obtained using a four compartment PBPK model.

Table 11. Values Used in the Corley *et al*. (1990) Chloroform PBPK Model

Parameter, units	Mouse	Rat	Human
Body weight, kg	0.0285	0.23	70.0
Alveolar ventilation, L/hour	2.01	5.06	347.9
Cardiac output, L/hour	2.01	5.06	347.9
Tissue flows, fraction of cardiac output			
Liver	0.25	0.25	0.25
Kidney	0.25	0.25	0.25
Fat	0.02	0.05	0.05
Richly perfused tissues	0.29	0.26	0.26
Poorly perfused tissues	0.19	0.19	0.19
Tissue volumes, fraction of body weight			
Liver	0.0586	0.0253	0.0314
Kidney	0.0170	0.0071	0.0044
Fat	0.06	0.063	0.2310
Richly perfused tissues	0.033	0.0439	0.0327
Poorly perfused tissues	0.7414	0.7707	0.6105
Blood/air partition coefficient, unitless	21.3	20.8	7.43
Tissue/blood partition coefficients, unitless			
Liver	0.897	1.014	2.288
Kidney	0.516	0.529	1.480
Fat	11.36	9.760	37.685
Richly perfused tissues	0.896	1.014	2.288
Poorly perfused tissues	0.610	0.668	1.615
Metabolic and macromolecular binding constants			
Vmax, mg/hour-kg	22.8	6.8	15.7
Km, mg/L	0.352	0.543	0.448
k_{loss}, L/mg	0.000572	0	0
k_{resyn}, /hour	0.125	0	0
A (kidney/liver)	0.153	0.052	0.033
fMMB /hour, liver	0.003	0.00104	0.00202
fMMB /hour, kidney	0.010	0.0086	0.00931
Uptake constants			
K_{aS} /hour, corn oil vehicle	0.6	0.6	0.6
K_{aS} /hour, water	5.0	5.0	5.0

The Vmax value used for reference 70 kg humans was estimated by Corley *et al*. (1990) from a comparison of the *in vivo* metabolic data for mice and rats with corresponding data obtained *in vitro* using microsomes from livers and kidneys of mice, rats, and

humans, given the measured (or assumed) values of Km used in the analysis. This resulted in a Vmax estimate for 70 kg humans of 307 mg/hour (somewhat below the corresponding value in Table 10). In deriving an average value for human liver enzyme activity to obtain this Vmax estimate, Corley *et al*. (1990) excluded one of the nine measured enzyme activities, which was about seven times higher than the mean of the other eight values. Corley *et al*. (1990) stated that the high measure was associated with an "abnormal distribution of P450 isozymes which are inducible by ethanol and barbiturates." No estimate was offered of the extent to which such high level enzyme activities, perhaps associated with "abnormal" isozyme distributions, may be present in a human subpopulation.

The Vmax values were apparently used to represent metabolism within the liver compartment only, despite the fact that they were based on analysis of gas uptake data pertaining to total (modeled as liver + kidney) metabolism in exposed animals. To obtain a value of Vmax for kidney in each species, Corley *et al*. (1990) multiplied the Vmax for liver by a factor proportional to the value of the kidney to liver ratio, A, of the velocity to substrate ratio, R, determined for these tissues *in vitro*; i.e., as:

$$\text{Vmax[kidney]} = \text{Vmax[liver]} \times A \times \text{Volume[kidney]} \div \text{Volume[liver]}$$

where $A = R[\text{kidney}] \div R[\text{liver}]$. Using this procedure, the values of Vmax[kidney] for the mouse, rat and human were 4.4, 1.5 and 0.46 percent, respectively, of the corresponding values of Vmax[liver]. Because these percentages are relatively small, little error was likely introduced into the model by its failure to use the estimators:

$$\text{Vmax[liver]} = \text{Vmax} \{\text{Volume[liver]} \div (\text{Volume[kidney]} + \text{Volume[liver]} \div A)\}$$

$$\text{Vmax[kidney]} = \text{Vmax} \{\text{Volume[kidney]} \div (\text{Volume[kidney]} + \text{Volume[liver]} \div A)\},$$

which appear to be more consistent with the experimentally determined quantities.

In this model, the metric for tissue specific effective dose was taken to be the daily, time weighted average amount of chloroform metabolite(s) binding to macromolecules within the target tissue (kidney or liver). Such macromolecular binding was modeled as a first order accumulation within these tissues, and the corresponding rate constants were estimated based on a fit of the model to data on tissue specific binding measured six hours postexposure in rats and mice exposed to chloroform by inhalation at three different air concentrations. The fitted liver binding fraction for mice was approximately three times higher than that for rats, while the kidney binding fraction for mice was approximately 16 percent higher than that for rats; values for humans for each tissue were apparently set equal to the average of the corresponding mouse and rat values. Finally, to fit the five compartment PBPK model to data involving exposure by gavage or injection, Corley *et al*. (1990) assumed first order kinetics for gastric uptake from corn oil (rate constant = 0.6/hour), gastric uptake from water (rate constant = 5.0/hour), and uptake by ip injection (rate constant = 1.0/hour).

Predictions of the five compartment PBPK model were compared to data on chloroform metabolism gathered in the study and with literature data. The model predicted, within a factor of approximately two, total metabolism, body burden, total exhaled parent

compound, and macromolecular binding in kidney and liver for male B6C3F$_1$ mice and male Osborne-Mendel rats exposed to chloroform by inhalation at three different concentrations (Corley *et al*., 1990). The model also provided a similar or better ability to predict metabolic data for mice, rats and humans from the mass balance metabolism studies of Brown *et al*. (1974), Ilett *et al*. (1973), and Fry *et al*. (1972).

Smith and Evans (1995) evaluated the uncertainty in fitted estimates of apparent *in vivo* metabolic constants for chloroform. Joint confidence regions for Vmax and Km from each experiment, generated using maximum likelihood techniques, were used to evaluate three data sets (Corley *et al*., 1990; Withey and Collins, 1980). The Vmax and Km estimates obtained from these data sets differed by more than could be explained by a limited number of observations, measurement error, or stochastic error. The authors suggest misspecification of the model for metabolic kinetics. If there are two Michaelis-Menten kinetic processes, a low affinity high Km and a high affinity low Km, then the estimated Km will be dose-dependent. Testai *et al*. (1990) reported evidence for both low and high affinity oxidative processes for chloroform. Alternative explanations are possible. For example, an ordered bireactant system with chloroform and molecular oxygen as substrates may be a more realistic representation of chloroform oxidation. Simplification of a bireactant process by Michaelis-Menten kinetics should in principle result in an apparent Km that exhibits some dose-dependency.

The model of Corley *et al*. (1990) was further extended by Reitz *et al*. (1990), who added a component modeling a pharmacodynamic endpoint, specifically cytotoxicity, in the livers of exposed animals. Risk of cell death was modeled as a concentration-dependent, normal distribution function. Due to the higher dose rate, gavage exposure was assumed to cause more cell damage than an equivalent daily dose administered in the drinking water. The model of Gearhart *et al*. (1993) further refined this approach by incorporating the effects of decreased core body temperature resulting from high chloroform doses on the metabolic components. A similar approach was described by Delic *et al*. (2000), with loss and recovery of metabolism added to the mouse and human models of Corley *et al*. (1990) and used to predict metabolic rates of chloroform in rats and humans.

Blancato and Chiu (1994) added an input component representing dermal absorption to the Corley *et al*. (1990) model. They applied it to several human exposure scenarios where chloroform exhalation data were available, and reported that the model fit the data well. Smith *et al*. (1995) adapted the Corley *et al*. (1990) model to evaluate several dose surrogates as predictors of rodent tumorigenicity data; the model correlated well with the rodent hepatotoxicity data, but not with the renal data. Both Chinery and Gleason (1993) and McKone (1993) have also reported human PBPK models incorporating a dermal absorption component.

Bromodichloromethane PBPK Model

Lilly *et al*. (1997a, 1998) examined the effects of oral administration vehicle on the pharmacokinetics of BDCM in rats. The data were used to develop and validate a PBPK model to describe BDCM absorption, tissue dosimetry, and rates of metabolism for both oil and 10 percent Emulphor® vehicles. Estimates of oral absorption rate constants were determined by fitting blood and exhaled breath chamber concentration time curves

following gavage of male F344 rats with 50 or 100 mg/kg BDCM in corn oil or 10 percent Emulphor®, using the multicompartmental gastrointestinal tract submodel of Salmino *et al*. (1997) linked to the PBPK model. Independently estimated oral uptake and metabolic rate constants accurately described kidney BDCM concentrations and plasma bromide ion levels. Liver BDCM concentrations were simulated with less accuracy than the kidney dosimetry simulations.

Lilly *et al*. (1997a) also developed a PBPK model for inhalation toxicokinetics of BDCM in male rats. The model consisted of five compartments (liver, kidney, fat, and slowly and rapidly perfused tissues) linked by the arterial blood supply, with BDCM entering via the lungs. Air partition coefficients for blood and key tissues were determined using a vial equilibration technique. BDCM metabolism rates were estimated using data for bromide ion production during four hour constant concentration (50 to 3,200 ppm) inhalation exposures to BDCM and data from closed chamber gas uptake experiments. Bromide ion production appeared to be saturable. Metabolism was described using a single saturable pathway representing a high capacity, high affinity process and rate constants of 12.8 mg/hour-kg for V_{max} and 0.5 mg/L for K_m. The rate constants from bromide ion production adequately described data from the gas uptake experiments. After addition of an intermediate compartment for metabolite storage to the model, these rate constants also adequately fit the data of Mathews *et al*. (1990) for exhalation of carbon monoxide and carbon dioxide after gavage doses of 1 or 10 mg/kg BDCM.

Lilly *et al*. (1998) refined their PBPK model by adding a multicompartmental gastrointestinal absorption approach and parameterization using blood and exhaled breath chamber concentration-time data. The model was evaluated by measuring BDCM tissue concentrations and plasma bromide ions in F344 rats after gavage doses of 50 or 100 mg/kg BDCM in corn oil or 10 percent aqueous Emulphor®. Oral absorption rate constants were estimated by fitting blood and exhaled breath chamber concentration-time curves. Estimated oral uptake and metabolic rate constants described BDCM concentrations in kidney and bromide concentrations in plasma without adjustment. However, poor agreement was observed between measured liver concentrations and model simulations. The model simulations overestimated the liver concentrations for both vehicles and were only slightly improved by adjustment for a short period of continued metabolism following tissue excision.

No PBPK models for human exposure to BDCM were identified in the literature.

Dibromochloromethane and Bromoform PBPK Model

Da Silva *et al*. (1999a) described five-compartment PBPK models for each of the four THMs. The models for chloroform and BDCM were based largely on those described above by Corley *et al*. (1990) and Lilly *et al*. (1998). The partition coefficients for BDCM and bromoform were measured by the vial equilibration method of Gargas *et al* (1989). The metabolic constants for each compound were obtained by optimization. No metabolic parameters for DBCM or bromoform kidney metabolism were included. The authors report that it was not possible to adequately describe the oral uptake of the THMs using a single first order rate constant and that oral absorption is likely to be dose-dependent at the doses employed in the study (0.25 and 0.5 mMol/kg). Since the model

parameters (Vmax, Km, and Ka) were obtained by fitting simulations to the data, the models cannot be said to be validated by independent data. However, for the authors' purpose of simulating the consequences of hypothetical competitive inhibition of liver metabolism among the THMs, the PBPK models appear to be useful.

TOXICOLOGY

Chloroform

The toxicological effects of chloroform have been comprehensively reviewed in documents prepared by DHS (1990), U.S. EPA (1985a, 1994b, 2001e, 2005c), ATSDR (1997), IARC (1999d), and WHO (1994). The present document is based in part on information obtained from these existing reviews.

Toxicological Effects in Animals

Acute Toxicity

Acute lethal levels have been identified for chloroform in laboratory animals. Oral LD_{50} values in rats and mice are summarized in Table 12. By inhalation exposure, LC_{50} values of 1,260 ppm in mice (six hour exposure in adult female OF_1 mice) and 9,770 ppm in rats (four hour exposure in female SD rats) have been determined (Gradiski *et al*., 1978; Lundberg *et al*., 1986). Oral LD_{50} values range from 446 to 2,180 mg/kg in rats (Kimura *et al*., 1971; Torkelson *et al*., 1976; Chu *et al*., 1982a,b) and 36 to 1,400 mg/kg in mice (Jones *et al*., 1958; Bowman *et al*., 1978; Hill, 1978; Pericin and Thomann, 1979).

Table 12. Acute LD_{50} Values for Chloroform

Species	Route	Sex	Age	LD_{50} (mg/kg)	Reference
Rat	Oral	M M M	14 day young adult old adult	446 1,337 1188	Kimura *et al*., 1971
Rat	Oral	M, F	Adult	908 - 2,180	Torkelson *et al*., 1976 Chu *et al*., 1982a,b
Mouse	Oral	M F	Adult	36 - 460 353 - 1,366	Pericin and Thomann, 1979
Mouse	Oral	M, F	Adult	36 – 1,400	Jones *et al*., 1958 Bowman *et al*., 1978 Hill, 1978 Pericin and Thomann, 1979

The wide range of LD_{50} values in mice reflects, in part, variability among strains. Pericin and Thomann (1979) tested male and female mice of six different strains (C3H/tif, DBA/2/j, Tif:MAGf, A/J, Tif:MF2f, and C57BL/6J) under identical conditions. The LD_{50}s ranged from 36 to 460 mg/kg in males of different strains and 353 to 1,366 mg/kg

in the females. The most sensitive strain was C3H/tif for both sexes. For each strain, the female LD_{50} was two to 10-fold higher than the male LD_{50}, suggesting that males are more sensitive to chloroform lethality than females. Other researchers have reported similar findings in mice (Hill, 1978; Bowman *et al*., 1978).

The age at which animals were tested can also contribute to variability in LD_{50} results. Kimura *et al*. (1971) studied 14 day old, young adult, and old adult male rats, finding the 14 day old rats to be most sensitive (LD_{50} = 446 mg/kg), followed by the old adults (LD_{50} = 1,188 mg/kg). The young adults were least sensitive (LD_{50} = 1,337 mg/kg). Much of the variability in the rat LD_{50} values is due to the increased sensitivity of the 14 day old rats; the remaining values range over a factor of only about two (908 to 2,180 mg/kg). There are limited data in rats to suggest increased sensitivity of males versus females (Chu *et al*., 1982a,b), and no direct comparisons of lethality in different strains.

Prominent effects of acute chloroform toxicity are central nervous system and respiratory depression, cardiac arrhythmia, and liver and kidney damage, all of which are potential causes of lethality in exposed animals. The central nervous system depression produced by chloroform is well known because of its long history of use as an inhalation anesthetic. Animal studies of inhaled chloroform with exposures up to two hours reported symptoms of central nervous system depression at concentrations of about 3,000 ppm and above (Whitaker and Jones, 1965; Smith *et al*., 1973). By oral exposure, single doses of 300 mg/kg and above have been reported to produce symptoms of central nervous system depression in rodents (Kimura *et al*., 1971; Bowman *et al*., 1978; Balster and Borzelleca, 1982). Exposure to anesthetic levels by inhalation or ingestion can result in depression of respiration and cardiovascular effects, including reduced heart rate, altered blood pressure and cardiac arrhythmias (Taylor *et al*., 1976; Mueller *et al*., 1997; Uchigasaki *et al*., 1999). A study in dogs exposed to very high airborne levels of chloroform found that reduction in blood pressure started only 10 seconds after inhalation (Uchigasaki *et al*., 1999). Transient cardiac arrhythmias were observed in some dogs at the start of the decrease in blood pressure. Decreases in diastolic pressure, stroke volume, and cardiac output, and increased peripheral vascular resistance were seen in rabbits exposed for one minute to 50,000 ppm of chloroform vapor (Taylor *et al*., 1976). No effects on blood oxygen or carbon dioxide tension or pH were found, suggesting that the cardiac effects were not secondary to respiratory depression. In an oral study, a single dose of 149 mg/kg produced arrhythmogenic changes and decreases in heart rate, heart muscle contractility and conduction velocity in cardiac muscle fibers in conscious and urethane anesthetized rats (Mueller *et al*., 1997). Contrary to the inhalation results, blood pressure was increased. Evidence suggests that the cardiovascular effects of chloroform result from several mechanisms: a direct depressive effect on the heart, depressed function secondary to depression of the central nervous system, and sensitization of the heart to endogenous catecholamines, such as epinephrine (Mueller *et al*., 1997; Dutta *et al*., 1968).

Chloroform produces tissue damage in the liver and kidney following acute exposures. Characteristic pathological changes in the liver include congestion, enlargement, fatty infiltration, and centrilobular necrosis (Chu *et al*., 1980; Bowman *et al*., 1978; Lundberg *et al*., 1986; Kylin *et al*., 1963; Larson *et al*., 1994a,b,c, 1995a,b;). The severity of hepatotoxicity increases with dose. By inhalation, the threshold for production of liver

effects in rats appears to be between 100 and 150 ppm for a single exposure. A series of studies by Wang *et al*., (1994, 1995, 1997a) showed increases in serum alanine aminotransferase (ALT, formerly called SGPT) and aspartate aminotransferase (AST, formerly called SGOT) indicative of hepatotoxicity, and depletion of hepatic glutathione in male Wistar rats exposed for six hours to chloroform at 500 ppm, but not at 100 ppm or lower. Lundberg *et al*. (1986) observed increases in serum sorbitol dehydrogenase (SDH) at 153 ppm, but not 76 ppm in female SD rats exposed for four hours. Four hour exposure of male SD rats produced evidence of hepatotoxicity (increased sorbitol dehydrogenase and glutamyl lactate dehydrogenase (GLDH)) at 292 ppm and above, but not at 137 ppm (Brondeau *et al*., 1983). Similar findings were reported after exposures over a number of days. Larson *et al*. (1994a) observed fatty change in the livers of male F344 rats exposed to 271 ppm six hours/day for seven days, but found no hepatic effects at 100 ppm. Templin *et al*. (1996a) observed liver lesions at concentrations of 10 to 90 ppm in male F344 rats after four days, six hours/day, but the lesions were minimal and there was no clear relation to dose.

Short-Term Toxicity

In mice, more limited single inhalation exposure data showed hepatic effects at 100 ppm and above (fatty change, elevated serum enzyme biomarkers), but did not find a NOAEL (Kylin *et al*., 1963; Culliford and Hewitt, 1957; Gehring, 1968). Studies in which mice were repeatedly exposed to chloroform over a number of days identify a threshold in the range of 5 to 10 ppm for liver effects. Incidence and severity of hepatic lesions were increased at 10 ppm and above in female $B6C3F_1$ mice exposed six hours/day for four days (Larson *et al*., 1996). Exposure of female $B6C3F_1$ mice for seven days, six hours/day, produced severe fatty lesions and necrosis at about 100 ppm, mild to moderate fatty lesions at 10 ppm, and no effects at 3 ppm (Larson *et al*., 1994a). In BDF1 mice exposed for four days, six hours/day, 90 ppm produced increased labeling index and liver lesions in both sexes (more severe in males), 30 ppm produced a significant increase in hepatic labeling index in males only, and 5 ppm produced no hepatic effects (Templin *et al*., 1996b). Experiments in CBA and WH mice found no evidence for any sex-related differences in susceptibility to hepatic effects of chloroform (Culliford and Hewitt, 1957).

Degenerative or necrotic lesions in the kidney tubules are characteristic of chloroform toxicity in acute and short-term animal studies. Acute inhalation studies have found renal effects at concentrations down to 30 ppm. Larson *et al*. (1994a) reported a significant increase in renal labeling index in male F344 rats exposed to 30 ppm for seven days, six hours/day, and no effect at 10 ppm. Templin *et al*. (1996a) found minimal lesions in some male F344 rats exposed to 30 or 90 ppm for four days, six hours/day, but no significant difference from controls below 300 ppm, which produced minimal vacuolation of proximal tubule in all tested rats and significantly increased labeling index. In mice, Templin *et al*. (1996b) observed necrosis of the proximal convoluted tubule, tubule dilation, accumulation of hyaline casts, focal mineralization and significantly increased labeling index in the kidneys of male BDF1 mice exposed to 30 or 90 ppm of chloroform six hours/day for four days. Severity of lesions was graded mild to moderate at 30 ppm and moderate to severe at 90 ppm. At either concentration, extension of the exposure period to two weeks resulted in severe kidney toxicity and, in

many cases, death. There were no renal effects in male mice exposed to 5 ppm or below, or female mice at any concentration.

Other studies in mice have also found that males are more sensitive than females to the renal effects of chloroform. No renal effects were seen in female CBA, WH or C3H mice at vapor concentrations that produced extensive tubular necrosis in males (246 to 692 ppm for one to three hours) (Culliford and Hewitt, 1957). Manipulation of hormone status showed that the nephrotoxicity in male mice is related to the presence of androgens (Culliford and Hewitt, 1957). Androgen treatment made adult females susceptible to renal necrosis. Conversely, treatment with estrogens reduced susceptibility of males, which was eliminated entirely by castration and/or adrenalectomy. Renal necrosis was not produced in male mice under 11 days old even after massive doses of androgen, but could be induced in those between the ages of 11 and 30 days by addition of androgens, and occurred spontaneously in those older than 30 days. Although less sensitive than males, renal effects can occur in females as well. Mild renal lesions (proximal tubules lined with regenerating epithelium) were observed in female $B6C3F_1$ mice exposed for seven days to 300 ppm, but not for four or seven days to 90 ppm or below (Larson *et al*., 1994a, 1996).

Whereas liver toxicity is similar between the sexes, male rats and mice are more sensitive than females to develop renal toxicity after chloroform exposure as a result of differences in renal cytochrome P450-mediated reactive intermediate formation because chloroform requires biotransformation to produce nephrotoxicity (Van Vleet and Schnellmann, 2003). Castration before exposure decreased cytochrome P450 levels and afforded resistence to male mice, and pretreatment of female mice with testosterone increased both general cytochrome P450 content and renal susceptibility to chloroform. Cytochrome P450 isoform CYP2E1 is implicated in the activation of chloroform in the liver and kidney of mice. Chloroform appears to be metabolized through CYP2E1 to phosgene which is thought to initiate renal cell injury by protein binding. Nephrotoxicity symptoms included increased kidney weight, fatty degeneration, swelling of the tubular epithelium, formation of tubular casts, and marked necrosis of the proximal tubules which is characterized by proteinuria, glucosuria, and increased blood urea nitrogen.

Effects on the liver and kidney by oral exposure are similar to those observed by inhalation exposure. Oral doses as low as 35 mg/kg have been reported to produce mild liver fatty changes, while more severe necrotic lesions are associated with doses in the range of 140 to 400 mg/kg in rats and mice (Larson *et al*., 1993, 1994b; Wang *et al*., 1994, 1995, 1997b; Moore *et al*., 1982; Munson *et al*., 1982; Pereira, 1994). Severe renal lesions were reported at 35 to 200 mg/kg in these species (Gemma *et al*., 1996; Larson *et al*., 1993, 1994b, 1995a,b; Moore *et al*., 1982). Males were more sensitive to nephrotoxicity than females. Larson *et al*. (1995a) reported degeneration of renal proximal tubules at 34 mg/kg and above in male F344 rats treated by gavage in oil for four days, whereas renal effects were reported only at 200 mg/kg and above in females (Larson *et al*., 1995b).

Strain-related differences have also been reported after oral exposure. Templin *et al*. (1996c) found that male F344 rats treated by a single gavage dose in corn oil had mild liver (fatty change) and kidney (vacuolation in epithelial cells of proximal tubule) lesions at 477 mg/kg, increased liver labeling index at 477 mg/kg, and increased kidney labeling

index at or above 180 mg/kg. Identically treated Osborne-Mendel rats had no liver or kidney lesions and no increase in liver labeling index at any dose, but did have an increased kidney labeling index at all doses tested (equal to or greater than 10 mg/kg). In mice, Hill (1978) found no clear difference in hepatotoxicity between male mice of the DBA/2J, B6D2F1/J, and C57BL/6J strains treated with a single dose of chloroform by gavage in oil, but found that renal toxicity, as indicated by glucosuria or proteinuria, differed notably among the three strains (ED_{50} = 89, 119, and 163 mg/kg, respectively). The researchers noted that serum testosterone levels in the strains were correlated with susceptibility to renal effects (males in the most sensitive strain tended to have the highest testosterone levels), and hypothesized that testosterone might act by sensitizing renal proximal convoluted tubules to chloroform through a testosterone receptor mechanism. The correlation between testosterone levels and nephrotoxicity is consistent with data regarding the differences in renal susceptibility between male and female rodents (Culliford and Hewitt, 1957).

Oral administration vehicle appeared to influence the hepatic and renal toxicity of chloroform. Moore *et al*. (1982) found that chloroform administered by gavage in corn oil produced no toxic effects in mice at 17 mg/kg, increased kidney weight and renal thymidine uptake at 66 mg/kg, and both kidney (increased serum urea and thymidine uptake, necrosis) and liver (increased serum alanine aminotransferase and thymidine uptake, centrilobular hepatocellular enlargement) effects at 273 mg/kg. When administered in toothpaste, chloroform produced no effects at 18 or 59 mg/kg, although effects at 199 mg/kg were similar to the comparable oil gavage dose.

In male rats or mice treated by gavage in corn oil, renal effects were more severe than hepatic effects at the same doses (Larson *et al.*, 1993, 1994b), suggesting that the kidney is a more sensitive target than the liver under these conditions. However, Lilly *et al*. (1997b) reported hepatotoxicity reflected by increased serum sorbitol dehydrogenase, alanine aminotransferase, or aspartate aminotransferase at all doses, and nephrotoxicity reflected by increased urinary lactate dehydrogenase (LDH), N-acetylglucosaminidase (NAG) or aspartate aminotransferase, or serum blood urea nitrogen (BUN) only at the three highest doses in male F344 rats treated with 0, 89, 119, 179, 239, or 358 mg/kg of chloroform by gavage in aqueous 10 percent Emulphor® solution. A followup study to determine the threshold for hepatotoxicity by this exposure regimen found hepatic effects at doses down to 60 mg/kg, but not at 30 mg/kg or below (Keegan *et al*., 1998). There is some evidence that the greater renal toxicity of chloroform when administered in corn oil in male rodents is due to an interaction between chloroform and corn oil. Male SD rats treated with chloroform by gavage in corn oil had enhanced nephrotoxicity (assessed by *in vitro* accumulation of *para*-aminohippuric acid in renal cortical slices) compared to rats treated with chloroform in aqueous five percent Emulphor® solution (Raymond and Plaa, 1997). Significant dose-related reductions in *para*-aminohippuric acid incorporation were found at all doses tested in the corn oil experiment (143 to 525 mg/kg), but only at 298 mg/kg and above in the Emulphor® experiment. At the highest doses, the decrease produced with corn oil vehicle was significantly greater than the decrease produced with aqueous vehicle.

Several studies have compared the effects of chloroform administered by gavage in oil and in drinking water. Larson *et al*. (1995a) treated male F344 rats with several doses of

chloroform by gavage in oil or in drinking water over a four day period. When given by gavage in corn oil, no liver effects were found at 10 mg/kg or below. Mild to moderate degeneration of centrilobular hepatocytes was observed at 34 mg/kg and above, with focal necrosis at the higher doses (90 and 180 mg/kg). In the kidney, mild to moderate dose-related degeneration of the proximal convoluted tubule was noted in rats treated with 34 mg/kg and above. When administered in the drinking water, the only effect in the liver was mild hepatocyte vacuolation at the two high doses of 57 and 68 mg/kg, with no effects at 33 mg/kg or below. No effects were seen in the kidney after drinking water exposure to 68 mg/kg or below for four days. Larson *et al*. (1994c) conducted a similar study in female $B6C3F_1$ mice. Administration in corn oil produced no liver effects at 90 mg/kg or below, moderate centrilobular vacuolar degeneration at 238 mg/kg and severe centrilobular necrosis at 477 mg/kg. Administration in drinking water at 16 to 105 mg/kg produced no evidence of necrosis or proliferation at any dose. A subtle effect was indicated by pale cytoplasmic eosinophilic staining of centrilobular hepatocytes at 53 mg/kg-day and above, but not 26 mg/kg-day or below.

The studies by Larson *et al*. (1994c, 1995a) observed lower toxicity when chloroform was administered in drinking water, but the animals were exposed to lower doses by this route. Coffin *et al*. (2000) reported a similar experiment, also in female $B6C3F_1$ mice, that showed that severity of hepatic lesions produced by chloroform (graded on a semi-quantitative scale from a low of one to a high of four) was lower in mice treated at a reported dose of 338 mg/kg-day via drinking water (rated 1.0) than in mice treated with 130 (1.6) or 260 mg/kg-day (3.2) by gavage in corn oil. However, the reported dose (based on measured water consumption of the mice) does not appear to reflect any decrease in the animal's drinking water intake, which is at odds with the findings of other investigators (e.g., Larson and coworkers (1994c) reported a dose of 105 mg/kg/day for the first four days in mice exposed to the same concentration of chloroform in drinking water). Coffin *et al*. (2000) proposed that the slower rate of delivery by drinking water exposure, compared to gavage, increases the opportunity for detoxification and might explain the relatively weak toxicity by this mode of administration.

Kidney effects have also been found after acute dermal exposure to chloroform. Doses of 1,000, 2,000 or 4,000 mg/kg were applied for 24 hours under an impermeable cuff to the bellies of rabbits (Torkelson *et al*., 1976). Dose-related degenerative changes in the kidney tubules were seen at all dose levels when the rabbits were sacrificed two weeks later. No effects were observed in the liver.

Acute studies have also found the nasal epithelium to be a target for chloroform by inhalation or oral exposure. Inhalation studies found dose-related increases in the incidence and/or severity of nasal lesions and labeling index in the nasal turbinates in male F344 rats and female $B6C3F_1$ mice at chloroform concentrations of 10 ppm and above in four day studies, with no effect at 2 ppm in either species (Templin *et al*., 1996a; Larson *et al*., 1996). The nasal lesions can be characterized as primarily a proliferative response in the ethmoid portion of the nose. Specific changes observed were thickening of the bone due to periosteal hyperplasia and new bone growth, and edema and degeneration of Bowman's glands in the adjacent lamina propria (Templin *et al*., 1996a; Larson *et al*., 1994a, 1996; Mery *et al*., 1994). Focal atrophy of the olfactory epithelium and hypertrophy of goblet cells in the respiratory epithelium were observed in male rats

at high concentrations (at or above 90 ppm). By oral exposure, similar lesions were reported to have occurred in a dose-related fashion in female F344 rats treated with 34 to 400 mg/kg-day chloroform by gavage in corn oil for four days (Larson *et al*., 1995b). Other oral studies did not report nasal lesions, but may not have examined this tissue for histopathological effects.

Subchronic Toxicity

The primary target organs for chloroform with subchronic exposure are the liver, kidney, and nasal epithelium. The lowest-dose effects are summarized by study in Table 13.

Table 13. Summary of Significant Low-dose Subchronic Effects of Chloroform

Species, strain	Route	Sex	Duration	Doses	Effects	Reference
Rats, rabbits, guinea pigs, dogs	Inhalation	M, F	7 hours/day, 5 days/week for 6 months	25 - 85 ppm	liver and kidney toxicity, greatest in rats, least severe in guinea pigs and dogs	Torkelson *et al*., 1976
Rat F344	Inhalation	M, F	6 hours/day, 7 days/week for 3, 6, or 13 weeks	0, 2 10, 30, 90, or 300 ppm	hepatic lesions in M/F ≥ 90 ppm, renal lesions ≥ 2 ppm in F, 30 ppm in M, nasal lesions ≥ 2 ppm in M	Templin *et al*., 1996a
Mouse $B6C3F_1$	Inhalation	M, F	6 hours/day, 7 days/week for 3, 6, or 13 weeks	0, 0.3, 2 10, 30, or 90 ppm	hepatic lesions in M/F ≥ 30 ppm, renal lesions ≥ 30 ppm in M, nasal lesions transient at ≥ 2 ppm in F	Larson *et al*., 1996
Mouse BDF_1	Inhalation	M, F	6 hours/day, 5 days/week for up to 13 weeks	0, 1,5, 30, 90 ppm	mild hepatic effect at 30 ppm, lesions at 90 ppm in M/F, renal toxicity in M ≥ 30 ppm	Templin *et al*., 1998
Rat Wistar	Inhalation	M	6 hours/day, 5 days/week or contin. for 4 weeks	275 ppm intermittent and 50 ppm continuous	liver effects in both groups, more severe in the continuous exposure group	Plummer *et al*., 1990
Dog Beagle	Oral, capsules	unk*	7 days/week for 12 - 18 weeks	30 - 120 mg/kg-day	dose-related liver and kidney toxicity with LOAEL 30 mg/kg, weight loss 60 mg/kg	Heywood *et al*., 1979
Rat Sprague-Dawley	Oral, gavage in toothpaste	M, F	7 days/week for 13 weeks	< 30 - 410 mg/kg-day	increased liver and kidney weight at 150 mg/kg, with hepatic necrosis	Palmer *et al*., 1979

Species, strain	Route	Sex	Duration	Doses	Effects	Reference
Mouse Swiss	Oral, gavage in toothpaste	M, F	6 days/week for 6 weeks	60 - 425 mg/kg-day	decreased weight gain at 60 mg/kg	Palmer *et al*., 1979
Rat Osborne-Mendel	Oral, in drinking water	M	7 days/week for 30, 60, or 90 days	0, 20, 38, 57, 81, 160 mg/kg-day	dose-related decreased water consumption, decreased weight gain at highest dose	Jorgenson and Rushbrook, 1980
Rat Sprague-Dawley	Oral, in drinking water	M	28 day, 7 days/week ?	"up to 41 mg/kg-day"	no treatment related effects	Chu *et al*., 1982a
Rat Sprague-Dawley	Oral, in drinking water	M, F	7 days/week for 90 days	0.6, 45, 150 (M) or 142 (F) mg/kg-day	decreased food intake and body weight gain at highest dose, increased mortality	Chu *et al*., 1982b
Mouse CD1	Oral, gavage in oil	M	14 days	0, 37, 74, 148 mg/kg-day	dose-related liver and kidney toxicity with LOAEL 37 mg/kg	Condie *et al*., 1983
Mouse B6C3F1	Oral, gavage in corn oil or in aqueous Emulphor®	M, F	7 days/week for 90 days	0, 60, 130, 270 mg/kg with each vehicle	in corn oil, liver damage at ≥60 mg/kg; in Emulphor, minimal liver lesions; increased liver weights ≥ 60 mg/kg, both vehicles	Bull *et al*., 1986
Mouse $B6C3F_1$	Oral, gavage in corn oil	F	5 days/week for 33 or 159 days	0, 263 mg/kg-day	increased relative liver weight, other liver changes at both time points	Pereira, 1994
Mouse $B6C3F_1$	Oral in drinking water	F	5 days/week for 33 or 159 days	363 or 438 mg/kg-day	no effects at 33 day, only increased relative liver weight at 159 day	Pereira, 1994
Mouse $B6C3F_1$	Oral, gavage in corn oil	M	5 days/week for 3 weeks	0, 34, 90, 138, 277 mg/kg-day	dose-related liver and kidney toxicity, hepatic LOAEL 90 mg/kg, renal LOAEL 34 mg/kg	Larson *et al*., 1994b
Mouse $B6C3F_1$	Oral, gavage in corn oil	F	5 days/week for 3 weeks	0, 3, 10, 34, 90, 238, 477 mg/kg-day	dose-related liver toxicity, LOAEL 34 mg/kg; no renal effects	Larson *et al*., 1994c
Mouse $B6C3F_1$	Oral, in drinking water	F	5 days/week for 3 weeks	0, 16, 43, 82, 184, 329 mg/kg-day	liver weight increased at 82 mg/kg, no frank toxicity, LOAEL 34 mg/kg; no renal effects	Larson *et al*., 1994c
Rat	Oral,	M	5 days/week	0, 3, 10, 34,	dose-related liver and	Larson *et al*.,

Species, strain	Route	Sex	Duration	Doses	Effects	Reference
F344	gavage in corn oil		for 3 weeks	90, 180 mg/kg-day	kidney toxicity, hepatic NOAEL 30 mg/kg, kidney NOAEL 90 mg/kg	1995a
Rat F344	Oral, in drinking water	M	5 days/week for 3 weeks	0, 6, 17, 62, 106 mg/kg-day	possible kidney changes > 6 mg/kg, mild hepatocyte vacuolation at 106 mg/kg, NOAEL 62 mg/kg	Larson *et al*., 1995b
Rat F344	Oral, gavage in corn oil	F	5 days/week for 3 weeks	0, 34, 100, 200, 400 mg/kg-day	dose-related liver and kidney toxicity; NOAELs: liver 100, kidney 34 mg/kg	Larson *et al*., 1995b
Mouse $B6C3F_1$	Oral, in drinking water	F	unk. days/week for 33 days	"up to 475 mg/kg"	"no histopathological lesions in liver"	Periera and Grothaus, 1997
Rat	Oral, gavage	unk.	unk. days/week for 3 weeks	400 mg/kg-day	nasal lesions	Dorman *et al*., 1997
Mouse $B6C3F_1$	Oral, gavage in oil	F	5 days/week for 3 weeks	unk.	dose-related hepatic toxicity ≥ 55 mg/kg	Melnick *et al*., 1998

*unk. = unknown (not stated).

Several studies have investigated chloroform toxicity in animals exposed to by inhalation. Considerable variation in sensitivity to chloroform vapor was found, depending on species and sex, with a threshold for production of the most sensitive subchronic effects in the range of 2 to 10 ppm.

In the earliest of these studies, Torkelson *et al*. (1976) established that the liver and kidney were targets of inhaled chloroform in several species following subchronic exposure to relatively low concentrations. They observed dose-related hepatotoxicity (mild to marked centrilobular degeneration, foamy vacuolation, focal necrosis, and fibrosis) and nephrotoxicity (cloudy swelling of the tubular epithelium and nephritis) in male and female rats, rabbits, and guinea pigs exposed to 25 to 85 ppm of chloroform vapor (seven hours/day, five days/week) for six months. The lesions were most severe in male rats and least severe in guinea pigs. Despite the observed liver and kidney lesions, associated clinical chemistry parameters (measured only in rats and rabbits) were not different from controls. No lesions were seen in rats exposed only one, two, or four hours/day to 25 ppm for six months. Dogs exposed seven hours/day, five days/week to 25 ppm had no liver effects, but renal pathology was noted in the females.

Subsequent studies in rats and mice utilized a broader range of exposure concentrations to identify threshold levels for hepatic, renal, and nasal lesions, and also included evaluation at several time points to assess progression of the lesions (Templin *et al*., 1996a). In the rat study, male and female F344 rats were exposed to 0, 2, 10, 30, 90, or 300 ppm of chloroform six hours/day, seven days/week for three, six, or 13 weeks. There were clear increases in incidence and severity of hepatic lesions (ranging from vacuolation to degeneration and necrosis) in males at 300 ppm after three to six weeks and ≥ 90 ppm after 13 weeks, and in females at ≥ 90 ppm after three to 13 weeks. Hepatic labeling index was increased at 300 ppm at all time points in both males and females. Renal lesions (ranging from tubular vacuolation to enlarged pyknotic nuclei and necrosis) were clearly increased in incidence and severity in males ≥ 30 ppm after three to six weeks, but only ≥ 90 ppm after 13 weeks, indicating regression of the lesions and development of tolerance at the 30 ppm exposure. In females, renal lesions appeared to be increased in all groups (2 ppm and above) after three to13 weeks. Kidney labeling index was significantly increased ≥ 30 ppm in males and females at all time points. Nasal lesions ranging from edema and loss of Bowman's glands to olfactory metaplasia, basal lamina mineralization, and atrophy of the ethmoid turbinates were found in almost all exposed male rats (not quantified in females), increasing in severity with dose. The difference from controls was clear ≥ 10 ppm after three weeks and ≥ 2 ppm after six or 13 weeks. Labeling index in the turbinates (measured only in males) was significantly increased ≥ 10 ppm at all time points.

Larson *et al*. (1996) performed an almost identical study in $B6C3F_1$ mice at exposure levels of 0.3, 2, 10, 30, and 90 ppm. Hepatic lesions were increased in incidence and severity ≥ 30 ppm in male and female mice at three to 13 weeks. Hepatocyte labeling index was significantly increased in females ≥ 30 ppm, but only at 90 ppm in males. Renal lesions were increased in male mice ≥ 30 ppm at three to 13 weeks, and labeling index was significantly increased at these same levels. There was no effect on renal pathology or cell proliferation in female mice at any level tested. Nasal lesions (quantified only for female mice) were not notably different in exposed mice than in controls at three to 13 weeks, as mild lesions seen after four days (see acute toxicity section above) had resolved by this time. Labeling index in the nasal turbinates (measured in females only) was slightly increased at ≥2 ppm after three weeks, but only at 90 ppm after six weeks, and not at any level after 13 weeks.

Templin *et al*. (1998) tested BDF1 mice using a similar protocol, with results almost the same as for $B6C3F_1$ mice. Male BDF1 mice were exposed to the vapor for up to 13 weeks at six hours/day, five days/week. Renal tubular regeneration was observed at chloroform concentrations of 30 and 90 ppm, but not 1 or 5 ppm. Renal labeling index was increased at the same levels. Female mice had no renal lesions and no increase in renal labeling index at any concentration. In the liver, there were no lesions at 1 or 5 ppm, centrilobular swelling was observed at 30 ppm, and centrilobular to midzonal vacuolation and degeneration were observed at 90 ppm in both male and female mice. Hepatic labeling index was increased only at 90 ppm in both sexes.

All of the studies described above employed an intermittent exposure protocol. Plummer *et al*. (1990) conducted a four week study to compare hepatic effects in male Wistar rats exposed to 275 ppm of chloroform intermittently (six hours/day, five days/week) with

rats exposed to the equivalent continuous exposure concentration of 50 ppm. Minor liver injury was observed in both groups, but the incidence and severity of lesions were greater in the continuous exposure group. The researchers suggested that saturation of chloroform metabolism may have led to reduced hepatotoxicity in the intermittent exposure group. Kinetic studies performed by the researchers suggested a threshold of about 100 ppm for saturation of chloroform metabolism in the rats.

In oral subchronic studies, doses as low as 30 to 45 mg/kg-day have been reported to produce toxic effects on the liver and kidney. The lowest effect level identified in a subchronic study was for hepatic effects in dogs (Heywood *et al*., 1979). Beagle dogs treated with 30 to 120 mg/kg-day of chloroform in toothpaste by capsule seven days/week for 12 to 18 weeks had dose-related liver effects ranging from occasional increases in serum alanine aminotransferase at 30 mg/kg-day to increased liver weight and slight fatty change at 45 mg/kg-day, and hepatocellular hypertrophy, fatty degeneration, and increases in several serum enzyme indicators of hepatotoxicity ≥ 60 mg/kg-day. Marked body weight losses occurred in dogs ≥ 60 mg/kg-day and jaundice and loss of general condition were found in dogs treated with 120 mg/kg-day.

Rats and mice were also tested in studies in which chloroform was administered in toothpaste. Palmer *et al*. (1979) treated male and female SD rats with chloroform in toothpaste via gavage daily for 13 weeks. No effects were observed at up to 30 mg/kg-day, but slight increases in liver and kidney weight were found at 150 mg/kg-day, and a definite toxic response at 410 mg/kg-day, as indicated by increased organ weight with fatty change and necrosis in the liver, atrophy of the gonads (both sexes), and increased cellular proliferation in bone marrow. Roe *et al*. (1979) treated outbred Swiss mice with chloroform by gavage in toothpaste for six weeks (six days/week). This study found moderate decreases in body weight gain in males and females at 60 mg/kg-day, and markedly reduced body weight gain and increased mortality at 150 or 425 mg/kg-day.

Condie *et al*. (1983) treated male CD-1 mice with 0, 37, 74, or 148 mg/kg-day of chloroform by gavage in oil for 14 days, finding effects on the liver and kidney at all doses, including histopathological lesions (centrilobular pallor, mitotic figures, and focal inflammation in the liver, and intratubular mineralization, epithelial hyperplasia, and cytomegaly in the kidney, all increasing in incidence and severity with increased doses), progressively decreased uptake of *para*-aminohippurate in renal cortical slices at 74 and 148 mg/kg-day, and significantly increased BUN and serum alanine aminotransferase at 148 mg/kg-day. Body weight was significantly decreased at 148 mg/kg-day.

Melnick *et al*. (1998) gave chloroform by gavage in corn oil to female $B6C3F_1$ mice five days/week for three weeks, finding dose-related hepatotoxicity ≥ 55 mg/kg-day, as indicated by increases in incidence and severity of fatty degeneration in the liver, increased hepatic labeling index, increased serum sorbitol dehydrogenase and alanine aminotransferase activity, and increased relative liver weight.

Larson *et al*. (1994b,c, 1995a,b) conducted a series of studies to identify critical effect levels in male and female rats and mice by corn oil gavage and drinking water exposure. Male F344 rats treated with 0, 3, 10, 34, 90, or 180 mg/kg-day of chloroform by gavage in oil five days/week for three weeks exhibited dose-related increases in serum enzymes indicative of hepatotoxicity and hepatic lesions at the high dose of 180 mg/kg-day

(Larson *et al*., 1995a). Relative liver weight was increased ≥ 90 mg/kg-day, but no other liver effects were seen at this dose, and no liver effects at all at 34 mg/kg-day and below. In the kidney, there were no effects at 90 mg/kg-day and progressive degeneration of the proximal tubule at 180 mg/kg-day. A three week drinking water study reported in the same paper found only mild hepatocyte vacuolation at the high dose of 106 mg/kg-day, and no liver effects at 62 mg/kg-day or below. Renal tubular regeneration and cell proliferation were slightly increased at drinking water doses of 17 to 106 mg/kg-day with no effect at 6 mg/kg-day, but did not increase with dose, and may not have been a treatment-related effect. Female F344 rats treated with chloroform by gavage in corn oil for three weeks exhibited slight diffuse vacuolation and focal centrilobular degeneration in the liver at 400 mg/kg-day, slight centrilobular vacuolation at 200 mg/kg-day, and no hepatic effects at 100 mg/kg-day or lower (Larson *et al*., 1995b). Regenerating tubular epithelium, tubular dilation, and mineralization were observed in the kidneys of the female rats at ≥ 100 mg/kg-day. There were no renal effects at 34 mg/kg-day.

Male B6C3F$_1$ mice treated with 0, 34, 90, 138, or 277 mg/kg-day of chloroform by gavage in corn oil five days/week for three weeks showed dose-related increases in regeneration in the kidney proximal convoluted tubules at doses of 34 to 138 mg/kg-day, and severe nephropathy characterized by degeneration, necrosis, and regeneration of the proximal tubules at 277 mg/kg-day (Larson *et al*., 1994b). In the liver, there were no effects at 34 mg/kg-day, swelling of centrilobular hepatocytes and loss of eosinophilia at 90 mg/kg-day, and hepatocellular degeneration and necrosis at 138 mg/kg-day.

Female B6C3F$_1$ mice were given chloroform in oil by gavage (0, 3, 10, 34, 90, 238, or 477 mg/kg-day) or in their drinking water (0, 60, 200, 400, 900, or 1,800 ppm) for four days or for three weeks (five days/week) (Larson *et al*., 1994c). In animals treated with chloroform in corn oil, hepatotoxicity was reported to be more severe at three weeks. At three weeks the hepatotoxicity was dose-related and increased from mild vacuolation and serum enzyme changes at 34 mg/kg-day to severe necrosis and markedly increased cell proliferation at 238 and 477 mg/kg-day. Drinking water consumption was markedly reduced after four days (yielding average daily doses of 0, 16, 26, 54, 81, and 105 mg/kg, as calculated by the study authors), but substantially recovered by three weeks (yielding average daily doses of 16, 43, 82, 184, or 329 mg/kg). After three weeks of exposure, no hepatic lesions were observed at average daily doses up to 329 mg/kg (1,800 ppm), although liver weight was significantly increased at or above 82 mg/kg-day (no effects at 16 or 43 mg/kg-day). Because the mice given chloroform in water initially received a lower dose due to reduced water consumption, some protection may have been provided against subsequent higher doses of chloroform as water consumption increased. In addition, animals given chloroform in corn oil received a higher dose longer, perhaps accounting for the more severe hepatotoxicity with the corn oil vehicle. No renal effects were found at any dose by either mode of administration in the female mice.

Pereira (1994) observed increased relative liver weight and focal areas of swollen and necrotic hepatocytes in female B6C3F$_1$ mice treated with 263 mg/kg-day of chloroform by gavage in corn oil for 33 or 159 days (five days/week). Exposure to 1800 ppm of chloroform in drinking water for the same durations produced no effects at an average dose of 438 mg/kg-day after 33 days, and only an increase in relative liver weight at a time-averaged dose of 363 mg/kg-day after 159 days.

Chu *et al*. (1982a) found no treatment related effects on body weight, food consumption, mortality, hematology, clinical chemistry, organ weights, or histopathology in weanling male SD rats treated with up to approximately 41 mg/kg-day of chloroform in the drinking water for 28 days. No histopathological lesions were observed in the livers of female $B6C3F_1$ mice exposed to chloroform in the drinking water for 33 days at concentrations leading to doses up to about 475 mg/kg-day (Periera and Grothaus, 1997). Furthermore, pretreatment of the mice with chloroform in drinking water for 30 days reduced the hepatotoxicity of subsequent gavage doses of chloroform in corn oil.

Jorgenson and Rushbrook (1980) exposed male Osborne-Mendel rats to estimated doses of 0, 20, 38, 57, 81 or 160 mg/kg-day of chloroform in the drinking water for up to 90 days. There were dose-related signs of central nervous system depression during the first week of the study, dose-related reductions in water intake, decreased weight gain in the high dose group, and an increased incidence of fatty degeneration in the liver (not clearly related to dose) after 30 and 60 days, but not 90 days Similar results were seen in female $B6C3F_1$ mice at doses of 0, 32, 64, 97, 145, or 290 mg/kg-day in the same study. In the mice, fatty change in the liver was seen at ≥ 64 mg/kg-day at 30 days, but only at ≥ 145 mg/kg-day at 60 and 90 days. Fat content of the liver was increased at all time points in the 290 mg/kg-day group.

Chu *et al*. (1982b) observed decreased food consumption and body weight gain, and increased mortality that they attributed to starvation, in male and female SD rats exposed to 2,500 ppm (150 mg/kg-day in males, 142 mg/kg-day in females) of chloroform in the drinking water for 90 days. These effects were not seen at 5 to 500 ppm (0.6 to 45 mg/kg-day). Mild lesions were noted in the liver (males and females) and thyroid (males only) of exposed rats, but it is not clear to what extent these lesions were treatment-related as differences from controls were not statistically significant, increases did not necessarily correspond with dose, and findings were similar in groups allowed to recover for 90 days before sacrifice.

Drinking water exposure differs from corn oil gavage both in terms of vehicle (water versus corn oil) and intensity (bolus versus continuous). Bull *et al*. (1986) conducted gavage experiments to investigate the specific influence of vehicle on chloroform toxicity in a 90 day study in $B6C3F_1$ mice. They found that chloroform given by gavage in corn oil produced a suite of hepatic effects at ≥ 60 mg/kg-day in male and female mice, including dose-related increases in absolute and relative liver weights, dose-related histopathology increasing from extensive vacuolation to cytomegaly and early cirrhosis, and at 270 mg/kg-day, increased serum aspartate aminotransferase and decreased serum triglycerides. Body weight was decreased at 270 mg/kg-day in males only. When chloroform was administered by gavage in aqueous Emulphor®, histopathology was limited to minimal focal necrosis in a few individuals at 130 to 270 mg/kg-day. No hepatic fatty changes were observed at any dose and there were no effects on serum triglycerides or aspartate aminotransferase. Absolute and relative liver weights were increased in females ≥ 60 mg/kg-day and relative weights were increased in males at 130 and 270 mg/kg-day. Final body weight was decreased in males at 270 mg/kg-day. These results suggest that the enhanced toxicity of chloroform by gavage exposure in corn oil is not simply a result of bolus dosing, and could be at least partially attributable to an interaction between chloroform and the corn oil vehicle.

Chloroform was administered by Sehata *et al*. (2002) to $CB6F_1$ *ras*H2-Tg male transgenic mice at doses of 28, 90, or 140 mg/kg, to *ras*H2-Tg females at 24, 90, or 240 mg/kg by gavage for 26 weeks, and to wild type non-Tg $CB6F_1$male mice at 140 mg/kg and females at 240 mg/kg. No increases were seen in incidence of neoplastic lesions although the incidence of hepatocellular foci in the *ras*H2- and non-Tg females receiving 240 mg/kg was increased. Swelling or vacuolation of hepatocytes, a toxic change induced by chloroform, occurred in both the *ras*H2-Tg and non-Tg mice. The authors suggested that the *ras*H2-Tg mouse model, a hemizygous carrying a human prototype c-Ha-*ras* gene, might not be appropriate for evaluating carcinogenic potential of chloroform because the c-Ha-*ras* gene might not be involved in the chloroform carcinogenesis.

Although most oral subchronic studies reported only hepatic or renal effects associated with chloroform ingestion, there is some evidence for effects on other tissues. Nasal lesions, such as have been found by inhalation exposure and acute oral exposure, have also been found in rats following gavage exposure to 400 mg/kg-day for three weeks (Dorman *et al*., 1997). This study further demonstrated that the nasal lesions, which include degeneration and regenerative hyperplasia of the olfactory epithelium in the ethmoid turbinates, are not associated with a functional olfactory deficit in the rats.

Miklashevskii *et al*. (1966) reported effects on the heart, stomach, and central nervous system, in addition to the liver, in rats treated with 125 mg/kg-day of chloroform for up to five months (not further described). Effects included fatty infiltration, necrosis, and cirrhosis of the liver, lipoid degeneration and proliferation of interstitial cells in the myocardium, edema of the submucosal and muscular layers of the stomach, and decreased performance in a conditioned reflex test. These effects were also reported in guinea pigs treated with 35 mg/kg-day, along with an increase in blood globulin, decrease in albumin, depression of blood catalase activity, and reduction in leukocyte phagocytic activity. No adverse effects were seen at 0.4 mg/kg-day in either species. Mueller *et al*. (1997) observed no cardiac arrhythmias in male Wistar rats treated with 37 mg/kg-day of chloroform by gavage in olive oil five times/week for four weeks. However, rats that received the same treatment but were anesthetized with urethane after the last dose showed slight decreases in heart rate, heart muscle contractility and conduction velocity in cardiac muscle fibers, and sensitization of the heart to injected catecholamines.

Genetic Toxicity

Extensive genotoxicity tests have been conducted on chloroform, with the majority of the results negative. Because chloroform is volatile and activated by metabolism to a highly reactive intermediate, phosgene, the most relevant studies would be conducted in a closed system to prevent loss of the chemical and would include activation, preferably by an endogenous system where the metabolites are formed inside the test organism. Highly reactive products of exogenous activation outside the test organism might never reach the genetic material inside to generate genotoxicity. The chloroform genotoxicity studies have met these conditions to varying degrees, but there is no pattern to indicate that negative results can be attributed to inadequate test systems. The positive results that have been found have typically been weak positive results occurring at high and cytotoxic concentrations. The apparent genotoxic effect in such cases may be secondary

to the toxic damage (Brusick, 1986). There is also the possibility that false positive results may have occurred in some cases as a result of reaction between phosgene (formed from chloroform) and ethanol (used as a solvent or preservative for chloroform) to form ethyl or diethyl carbonate, which are potent alkylating agents (U.S. EPA, 2001e).

The overall weight of evidence indicates that chloroform is not a potent genetic toxicant. The absence of a strong pattern in the types of assays that produced positive responses suggests that these responses may have had more to do with experimental conditions than with chloroform. All of these lines of evidence support the conclusion that chloroform has low genetic toxicity.

In Vitro Assays

Gene Mutation in Bacteria

Assays for mutagenicity of chloroform in *S. typhimurium* and *Escherichia coli* bacteria were almost universally negative, with or without metabolic activation or sealed conditions (Greim *et al*., 1977; Simmon *et al*., 1977; Uehleke *et al*., 1977b; San Agustin and Lim-Sylianco, 1978; Nestmann *et al*., 1980; Rapson *et al*., 1980; De Serres and Ashby, 1981; Gocke *et al*., 1981; Kirkland *et al*., 1981; Van Abbe *et al*., 1982; Varma *et al*., 1988; Roldan-Arjona *et al*., 1991; Roldan-Arjona and Pueyo, 1993; LeCurieux *et al*., 1995; Pegram *et al*., 1997; Sasaki *et al*., 1998; Kargalioglu *et al*., 2002). Uehleke *et al*. (1977b) used a suspension protocol to test the mutagenicity of 5 mM of chloroform to *S. typhimurium* strains TA1535 and TA1538 (with exogenous microsomal S9). At this concentration, there was little evidence of toxicity, and no evidence of mutagenicity. Simmon *et al*. (1977) also evaluated the mutagenic activity of chloroform to *S. typhimurium* in a suspension test. Strains TA1535, TA1537, TA1538, TA98, and TA100 were incubated with 1.24 M chloroform in the presence of S9, but with negative results. When exposures were carried out in a sealed desiccator to prevent escape of chloroform, Simmon *et al*. (1977) still found no evidence of mutagenic activity.

Van Abbe *et al*. (1982) compared the mutagenicity of chloroform over a range of concentrations (0 to 10 mg/plate) in five strains of *S. typhimurium* (TA1537, TA1538, TA1535, TA98, TA100). Tests were conducted in suspension with and without S9 fraction obtained from rat kidney, rat liver, or mouse liver. An additional series of experiments were conducted in which strains TA1535 and TA1538 were exposed in a sealed jar to a stream of chloroform vapor in the presence and absence of S9. All results were negative. Gocke *et al*. (1981) tested the same strains of *S. typhimurium* in a plate incorporation assay in a sealed desiccator, with chloroform at 0 to 3.6 mg/plate. Chloroform was not mutagenic, regardless of the presence or absence of S9.

A positive response of *S. typhimurium* to chloroform was reported by San Agustin and Lim-Sylianco (1978). They evaluated chloroform in a host-mediated assay in which strains TA1535 and TA1537 were injected into the peritoneal cavity of male and female mice. Chloroform induced mutations only in strain TA1537 recovered from male mice. However, discrepancies in the data reported for TA1535, and omission of the exposure concentration(s) and other procedural details make these results difficult to evaluate. Kirkland *et al*. (1981) used both plate incorporation and preincubation protocols to study the effects of chloroform on *E. coli* strains WP2p and WP2uvr-Ap with and without rat

liver S9. Negative results were obtained over a broad range of chloroform concentrations (0.1, 1.0, 10.0, 100, 1,000, and 10,000 mg/plate) .

Pegram *et al*. (1997) tested chloroform and BDCM for mutagenicity in a strain of *S. typhimurium* TA1535 that was transfected with rat theta class glutathione *S*-transferase T1-1 (+GST). The +GST and its nontransfected parent strain (−GST) were used in a plate incorporation assay at vapor concentrations of each THM up to 25,600 ppm in sealed Tedlar® bags. At 4,800 ppm BDCM (calculated concentration in the agar medium of 0.67 mM), there were 419 ± 75 revertants/plate versus 23 ± 5 in the control background. Chloroform produced a doubling of revertants only at the two highest concentrations tested of 19,200 and 25,600 ppm. The results indicate that bromination of THMs confers the capability for theta class glutathione-mediated metabolism to mutagenic products. Further, the apparent low affinity of the glutathione-dependent pathway for chloroform demonstrates that different modes of action may be operating in the carcinogenicity of individual THMs.

Araki *et al*. (2004) reported that chloroform was not mutagenic in *S. typhimurium* TA98, TA100, TA1535, TA1537, or *E. coli* WP2uvrA/pKM101 with or without S9 mix, and was not mutagenic in TA98, TA100, TA1535, TA1537, or WP2uvrA/pKM101 in the presence of glutathione-supplemented S9 mix using a gas exposure method. However, chloroform at concentrations of 0.5 to 2 percent was mutagenic in the excision-repair proficient strain WP2/pKM101 in the presence of glutathione-supplemented S9 mix using a gas exposure method (Araki *et al*., 2004). Positive results in other studies with *S. typhimurium* (San Agustin and Lim-Sylianco, 1978; Varma *et al*., 1988), as well as assays for mutagenicity in *Photobacterium phosphoreum* (Wecher and Scher, 1982) and DNA damage in *Bacillus subtilis* (San Agustin and Lim-Sylianco, 1978), were questionable due to poor reporting, absence of dose response, or coexposure with ethanol.

Gene Mutation in Yeasts

Tests in lower eukaryotes have also been mostly negative. In the yeast *Saccharomyces cerevisiae*, two studies obtained positive results for mutation and gene conversion, albeit only at very high, toxic levels (Callen *et al*., 1980; Brennan and Schiestl, 1998). Chloroform caused genetic alterations in *S. cerevisiae* D7 when cultured in suspension. Callen *et al*. (1980) measured mitotic gene conversion, mitotic crossing over, and reversion in yeast exposed to 21, 41, or 54 mM chloroform in a closed test tube. At the two lower concentrations, Callen *et al*. (1980) observed a dose-related increase in the number of convertants, and a nine-fold increase in the number of genetically altered colonies (due to gene conversion and mitotic crossing over). Although the survival of colonies exposed to 54 mM was extremely poor (six percent), there was a small increase in convertants and a 19-fold increase in the ratio of convertants per 10^5 survivors compared to untreated controls.

Other studies in *S. cerevisiae* were negative (Sharp and Parry, 1981; Zimmermann and Scheel, 1981; Mehta and von Bortsel, 1981; Kassinova *et al*., 1981; Jagannath *et al*., 1981). Aneuploidy was induced by high, toxic concentrations of chloroform in the yeast *Aspergillus nidulans* (Crebelli *et al*., 1988), but no effects were found in *Aspergillus nidulans* exposed to lower concentrations (Gualandi, 1984; Crebelli *et al*., 1988).

Gene Mutation in Mammalian Cells

Largely negative results have also been demonstrated in mammalian cells *in vitro*, including assays for mutation in Chinese hamster lung fibroblasts (Sturrock, 1977), unscheduled DNA synthesis or DNA damage in rat and mouse hepatocytes (Sina *et al*., 1983, Althaus *et al*., 1982; Larson *et al*., 1994d) and human lymphocytes (Perocco and Prodi, 1981; Butterworth *et al*., 1989), sister chromatid exchange in Chinese hamster ovary cells (White *et al*., 1979; Perry and Thomson, 1981) and human lymphocytes (Kirkland *et al*., 1981), and chromosome aberrations in human lymphocytes (Kirkland *et al*., 1981). A few assays reported positive results for mutation in mouse lymphoma cells (Mitchell *et al*., 1988) and sister chromatid exchange in rat leukemia cells (Fujie *et al*., 1993) and human lymphocytes (Morimoto and Koizumi, 1983; Sobti, 1984). However, the positive results in these studies tended to be weak, associated with cell toxicity, or possibly related to use of ethanol as a solvent.

Sturrock (1977) exposed cultured Chinese hamster lung fibroblasts to 1.0 or 2.5 percent chloroform for 24 hours to examine the ability of chloroform to induce mutations at the hypoxanthine-guanine phosphoribosyl transferase (HGPRT) locus. No statistically significant increase in the frequency of mutants was observed. An exogenous source of metabolism was not used in these experiments; consequently, although the results indicate that chloroform is not directly mutagenic in this assay, they provide no information on the potential indirect mutagenic activity of chloroform.

DNA Damage

Mirsalis *et al*. (1982) modified the standard *in vitro* unscheduled DNA synthesis assay so that animals, rather than isolated hepatocytes, were treated with the test chemical. They dosed F344 rats with 40 or 400 mg/kg chloroform by gavage; hepatocytes were isolated from perfused livers and incubated with ^{3}H-thymidine two or 12 hours after treatment. They found no evidence that chloroform induced unscheduled DNA synthesis. Geter *et al*. (2004) reported negative results of *in vitro* DNA strand breaks in CCRF-CEM human lymphoblastic leukemia cell lines exposed to chloroform at 5 or 10 mM for two hours or in primary rat hepatocytes exposed to chloroform at 5 or 10 mM for four hours.

DiRenzo *et al*. (1982) measured covalent binding of chloroform to DNA *in vitro*. Radiolabeled chloroform (1 mM) was incubated with phenobarbital-induced rat hepatic microsomes and calf thymus DNA for one hour. Previous experiments had established the exposure concentration and incubation time necessary to maximize covalent binding. To minimize protein contamination, the calf thymus DNA was purified prior to addition. As a further precaution, DNA was also isolated following incubation, and analyzed a second time for protein. The authors noted that no protein was detected with a limit of detection of less than 10 μg/mg DNA. DiRenzo *et al*. (1982) found that chloroform bound to DNA at 0.46 ± 0.13 nmol/mg DNA/hour (mean of six experiments).

Sister Chromatid Exchange

White *et al*. (1979) found no evidence that chloroform induced sister chromatid exchanges in Chinese hamster ovary cells exposed to 0.88 mM chloroform for one hour. However, this experiment employed only one concentration of chloroform, and the extremely brief exposure period may have been inadequate for metabolic activation.

Kirkland *et al*. (1981) reported that cultured human lymphocytes exposed to chloroform for two hours at 50, 100, 200, or 400 μg/mL showed no statistically significant increase in chromosome breakage, compared to controls. Separately, Kirkland *et al*. (1981) found that cultured human lymphocytes exposed to 25, 50, 75, 100, 200, or 400 μg/mL of chloroform for two hours exhibited a small increase (compared to controls) in the number of sister chromatid exchanges (SCEs) at 50 μg/mL. This increase was not statistically significant, and there was no evidence of a dose-dependent relationship.

Morimoto and Koizumi (1983) found that a 72-hour exposure to chloroform (2, 10, or 50 mM) induced a concentration-dependent increase in the number of sister chromatid exchanges per cell in cultured human lymphocytes. This increase became statistically significant ($p < 0.05$) at equal to or greater than 10 mM. In parallel *in vivo* experiments, mice were fed 0 to 200 mg/kg-day of chloroform for four days. Bone marrow cells from these animals exhibited a dose-dependent increase in the number of sister chromatid exchanges per cell. The sister chromatid exchange frequency was significantly ($p < 0.05$) higher than in control animals at doses ≥ 50 mg/kg-day.

In Vivo Assays

Gene Mutation

A measure of induced eukaryotic mutation that has been used to experimentally assess genotoxic potential *in vivo* is the ability of a compound to initiate hepatocellular foci in rats. Pereira *et al*. (1982) found that GTPase-positive foci were not induced in either intact or partially hepatectomized rats given 244 mg/kg of chloroform in a tricaprylin vehicle (a smaller dose of the known mutagen, diethylnitrosamine, did induce such foci). Similarly, Deml and Oesterle (1985, 1987) found no evidence that chloroform had initiating activity, as administration of chloroform (25 to 400 mg/kg twice weekly for 11 weeks) did not significantly increase the number of ATPase-deficient foci, gamma glutamyl transferase GGT-positive foci, or glycogen-positive foci in the liver of rats.

DNA Damage

In vivo assays in vertebrates have found no evidence for DNA damage or repair induced by chloroform (Larson *et al*., 1994d; Potter *et al*., 1996; Reitz *et al*., 1982; Mirsalis *et al*., 1982; Petzold and Swenberg, 1978; Kitchin and Brown, 1989; Geter *et al*., 2004). Studies of DNA binding have generally shown negative results (Diaz Gomez and Castro, 1980a,b; Reitz *et al*., 1982; Pereira *et al*., 1982). Positive results in one study (Colacci *et al*., 1991) may not have been due to chloroform since binding was the same in the liver and kidney as in other tissues and did not increase with induction of metabolism. Chloroform did not induce mutations in B6C3F$_1$ *lac*I transgenic mice exposed to 90 ppm for up to 180 days (Butterworth *et al*., 1989), a concentration that has been shown to produce tumors (Nagano *et al*., 1998).

More mixed results have been reported in clastogenicity studies, although again positive results were reported primarily at overtly toxic doses. Micronucleus tests reported both positive (San Agustin and Lim-Sylianco, 1978; Robbiano *et al*., 1998; Sasaki *et al*., 1998; Shelby and Witt, 1995) and negative (Gocke *et al*., 1981; Salamone *et al*., 1981; Tsuchimoto and Matter, 1981; LeCurieux *et al*., 1995) results. Doses in the positive studies were high and associated with liver and kidney toxicity. Assays for chromosomal

aberrations (Fujie *et al*., 1990) and sister chromatid exchange (Morimoto and Koizumi, 1983) were positive at high toxic doses.

DNA damage followed by DNA repair in the form of unscheduled DNA synthesis (UDS) can be measured by quantifying the uptake of hydroxyurea-resistant ^{3}H-thymidine in isolated hepatocytes. Reitz *et al*. (1980) reported that oral administration of 240 mg/kg chloroform to mice did not induce unscheduled DNA synthesis. No additional details of the experiment were provided. Low levels of DNA alkylation were measured in the liver (0.0003 mol percent) or kidney (0.0001 mol percent) of male mice given an oral dose of 240 mg/kg ^{14}C-chloroform (Reitz *et al*., 1980).

Micronuclei Induction

Two studies examined the ability of chloroform to induce formation of micronuclei in polychromatic erythrocytes, an *in vivo* assay that measures production of chromosome fragments (i.e., micronuclei) in bone marrow erythrocytes following a chemical treatment. San Agustin and Lim-Sylianco (1978) gave mice ip injections of 10, 100, 200, 400, 600, 700, 800, or 900 mg/kg of chloroform. The timing of the chloroform doses was not specified. In general, treatment with chloroform was associated with an increase in the number of micronuclei. However, this increase was not clearly dose-dependent, and was not statistically significant. When Gocke *et al*. (1981) gave mice two ip injections of chloroform (238, 476, or 952 mg/kg) at 0 and 24 hour, they found that chloroform caused a slight but nonsignificant increase in the number of micronuclei.

Sister Chromatid Exchange

Morimoto and Koizumi (1983) fed mice 0 to 200 mg/kg-day of chloroform for four days. Bone marrow cells from the mice exhibited a dose-dependent increase in sister chromatid exchanges per cell. The sister chromatid exchange frequency was significantly ($p < 0.05$) higher than in control animals at doses equal to or greater than 50 mg/kg-day.

Recessive Lethality

Sex linked recessive lethal assays in *Drosophila* were negative (Gocke *et al*., 1981; Vogel *et al*., 1981).

Sperm Abnormalities

The induction of sperm head abnormalities has been used as a test for identification of compounds that induce transmissible genetic damage in mammals. Sperm head abnormalities are thought to occur as a result of deletion or point mutations, which ultimately interfere with the differentiation of developing sperm (Wyrobek and Bruce, 1978). Topham (1980) tested 54 compounds, including chloroform, for their ability to induce morphological abnormalities in the sperm of mice. No effects of chloroform were reported at 371 mg/kg ip (U.S. EPA, 2001e).

Mice exposed to chloroform by inhalation at 0.04 or 0.08 percent in air for four hours/per day over five days had a significantly increased ($p < 0.01$) incidence of sperm head abnormalities compared to controls (Land *et al*., 1981). However, Rosenthal (1987) questioned the validity of the statistical tests used to determine significance, and concluded that because of the need for additional statistical analysis, the conclusions of Land *et al*. (1981) were not clear evidence of a positive response to chloroform.

In Vivo / In Vitro Tests

Diaz Gomez and Castro (1980a) studied *in vivo* and *in vitro* binding of ^{14}C-chloroform to DNA. In the first phase of these experiments, mice were pretreated with phenobarbital daily for four days or 3-methylcholanthrene twice a week for two weeks. Six hours after injection of 5 mg/kg of the radiolabeled chloroform, animals were killed and their livers were analyzed for DNA and/or RNA adducts. For the *in vitro* experiments, radiolabeled chloroform was incubated with microsomes and mouse liver DNA for 30 minutes. No appreciable binding was observed in either experiment.

Diaz Gomez and Castro (1980b) examined whether rat liver nuclei were able to metabolically activate chloroform to a substance capable of binding to nuclear protein or lipid. When ^{14}C-chloroform was incubated for 30 minutes with an NADPH-generating system and either nuclei or microsomes, both reaction mixtures revealed evidence of binding of the radiolabel to protein. The microsome mixture bound approximately twice as much radiolabel as the mixture containing rat liver nuclei.

These results can be contrasted with the study of DiRenzo *et al*. (1982), who incubated radiolabeled chloroform (1 mM) with phenobarbital-induced rat hepatic microsomes and calf thymus DNA for one hour, and found that chloroform bound to DNA at 0.46 ± 0.13 nmol/mg DNA/hour.

Rosenthal (1987) attributed the disparate results of these three studies to the use of less than optimal conditions by Diaz Gomez and Castro (1980a,b). She interpreted the results of DiRenzo *et al*. (1982) to be evidence that metabolically activated chloroform can bind to DNA *in vitro* at very low levels. However, because the reaction mixture of DiRenzo *et al*. (1982) did not include glutathione, direct extrapolation of these results to *in vivo* conditions may be inappropriate.

Mirsalis *et al*. (1989) observed that chloroform, while inactive in the *in vivo/in vitro* hepatocyte DNA repair assay, did cause a response in S-phase synthesis. Bis(2-chloro-1-methylethyl)ether, chloroform, polybrominated biphenyls, 1,1,2-trichloroethane, and trichloroethylene all induced increases in S-phase synthesis in mouse hepatocytes. These increases reflected the observed hepatocarcinogenicity of these compounds in the mouse.

Larson *et al*. (1994d) evaluated chloroform-induced DNA repair *in vitro* and *in vivo* in female mouse hepatocytes. In the *in vitro* assay, cultured hepatocytes from female $B6C3F_1$ mice were incubated with 0.01 to 10 mM chloroform and ^{3}H-thymidine. Unscheduled DNA synthesis was measured by quantitative autoradiography. No induction of DNA repair was observed at any chloroform concentration. In the *in vivo* assay, the animals were treated with 238 or 477 mg/kg chloroform in corn oil. Primary hepatocyte cultures were prepared at two and 12 hours post-treatment, incubated with ^{3}H-thymidine, and evaluated for induction of unscheduled DNA synthesis as above. No DNA repair activity was seen at either dose and time point.

Developmental and Reproductive Toxicity

Major developmental and reproductive toxicity studies in laboratory animals exposed to chloroform by both inhalation and oral routes are summarized in Table 14.

Table 14. Summary of Significant Developmental and Reproductive Toxicity Studies on Chloroform

Species, strain	Route	Sex	Duration	Doses	Effects	Reference
Rat Sprague-Dawley	Inhalation	F	7 h/d on gestation day 6–15	0, 30, 100, or 300 ppm	↓ food consumption and body weight gain, ↓ pregnancies, live fetuses/litter, and pup size at 300 ppm, ↑ anomalies at 30 and 100 ppm	Schwetz *et al*., 1974
Rat Wistar	Inhalation	F	7 h/d on gestation day 7–16	0, 30, 100, or 300 ppm	dose-related ↓ in food consumption and body weight, ↓ live fetuses and fetal length at all doses, and fetal body weight at 300 ppm	Baeder and Hoffman, 1988
Rat Wistar	Inhalation	F	7 h/d on gestation day 7–16	0, 3, 10, or 30 ppm	dose-related ↓ in food consumption and body weight, ↓ ossification at 3 and 10 ppm, ↓ fetal size at 30 ppm	Baeder and Hoffman, 1991
Rat Sprague-Dawley	Inhalation	F	1 h/d on gestation day 7–14	0, 942, 2,232, or 4,117 ppm	↑ resorptions and ↓ fetal body weight at 4,117 ppm	Newell and Dilley, 1978
Mouse CF-1	Inhalation	F	7 h/d on gestation day 1-7, 6-15, or 8-15	0, 100 ppm	pregnancies ↓ in the early exposures, ↓ food consumption and body weight gain in all exposures, ↓ fetal body weight and size	Murray *et al*., 1979
Mouse strain not specified	Inhalation	M	4 h/d for 5 days	0, 400, or 800 ppm	↑ abnormal sperm, 28 day after exposure	Land *et al*., 1981
Rat Sprague-Dawley	Oral, gavage in corn oil	F	gestation day 6-15	0, 20, 50, 126 mg/kg-day, in 2 divided doses	maternal ↓ food consumption and body weight gain, fatty liver at two higher doses; ↓ fetal body weight at 126 mg/kg	Thompson *et al*., 1974
Rabbit Dutch-belted	Oral, gavage in corn oil	F	gestation day 6-18	0, 20, 35, or 50 mg/kg-day	4/15 does died of hepatic toxicity, ↓ body weight gain at 50 mg/kg, ↓ fetal body weight all doses	Thompson *et al*., 1974
Rat Sprague-Dawley	Oral, gavage in corn oil	F	gestation day 6-15	0, 100, 200, or 400 mg/kg-day	3/15 dams died at 400 mg/kg; ↓ body weight gain, hemoglobin ↓, ↑ relative liver weight at all doses; fetal ↓ body weight and ↑ variations at 400 mg/kg	Ruddick *et al*., 1983

Species, strain	Route	Sex	Duration	Doses	Effects	Reference
Mouse ICR	Oral, gavage in vegetable oil/ Emulphor®/saline	M, F	21 days before mating to weaning; offspring treated postnatal day 7-22	0 or 31 mg/kg-day	no effects on reproductive parameters, pup body weight ↓ postnatal day 7 - 22, ↓ pup forelimb placement response postnatal day 5 and 7 but not 9	Burkhalter and Balster, 1979
Mouse CD-1	Oral, gavage in corn oil	M, F	7 d/wk multi-generation, from before mating through F_2 birth	0, 6.6, 16, or 41 mg/kg-day	no effects on reproductive performance, ↑epididymis weight and epithelial degeneration in F_1 males, ↑ liver weight and liver lesions in F_1 females	NTP, 1988
Mouse ICR	Oral, drinking water	M, F	7 d/wk multi-generation, from 5 wk before F_0 mating through sacrifice of F_{2b} pups	est. 0, 19, 190, 950 mg/kg-day	several deaths and ↓ body weight gain in F_0 at 950 mg/kg; ↓ body weight in F_{1b} females at 190 mg/kg; dose-related liver toxicity; ↓ reproductive performance in F_1 and F_2	Borzelleca and Carchman, 1982

Chloroform inhalation studies in rats and mice found evidence for developmental effects. In one rat study, groups of presumed pregnant female SD rats were exposed to chloroform vapor concentrations of 0 (N = 77), 30 (N = 31), 100 (N = 28), or 300 (N = 20) ppm seven hours/day on days six to 15 of gestation and the fetuses examined on day 21 (Schwetz *et al*., 1974). A second control group was pair-fed with the 300 ppm group (N = 8) because a preliminary study had observed marked anorexia in dams at 300 ppm. Food consumption was dramatically reduced throughout exposure in the 300 ppm dams, and was also significantly reduced at 100 ppm. Dam body weights during exposure were significantly decreased by about 40 percent in the 300 ppm group (30 percent in pair-fed controls) and 10 percent in the 30 and 100 ppm groups. In the 300 ppm group, there were marked significant decreases in the number of rats pregnant at sacrifice (15 percent versus 88 percent of controls), live fetuses per litter (4 ± 7 versus 10 ± 4), fetal body weight (3.42 ± 0.02 versus 5.69 ± 0.36 g), and fetal crown rump length (36.9 ± 0.2 versus 43.5 ± 1.1 mm), and increases in percent of implantations resorbed (61 percent versus eight percent) and percent litters with resorptions (100 percent versus 57 percent). The pair-fed group had results similar to controls, indicating that the effects observed in the 300 ppm group are due to chloroform, and not just secondary to the decrease in maternal body weight. There were no effects on these parameters in the 30 and 100 ppm groups, although there were significant increases in litters with anomalies or variations in both groups (100 ppm: acaudia, short tail, imperforate anus, subcutaneous edema, missing ribs, delayed ossification of sternebrae; 30 ppm: wavy ribs, delayed ossification of skull). Similar anomalies and variations were seen at 300 ppm, but were not significantly increased due to the small number of litters (N = 3) in this group.

Rats were exposed to higher concentrations of chloroform for only one hour/day by Newell and Dilley (1978). Presumed pregnant female SD rats were exposed to 0, 942, 2,232, or 4,117 ppm of chloroform for one hour/day on gestation days 7 to 14. Effects were similar to those observed in the other rat inhalation studies. Increased resorptions and decreased fetal body weight were observed in the 4,117 ppm group. No effects were noted in the lower exposed groups.

Developmental effects in mice were studied following inhalation exposures by Murray *et al*. (1979). Groups of 34 to 40 CF-1 presumed pregnant female mice were exposed to 0 or 100 ppm of chloroform vapor 7 hours/day on gestation days 1 to 7, 6 to 15, or 8 to 15, and sacrificed on day 18. Food consumption and body weight gain were significantly reduced in the treated dams from all three exposure regimens. The number of pregnancies was reduced in treated rats in the gestation days 1 to 7 group (44 percent) and gestation days 6 to 15 group (43 percent), compared to controls (74 and 91 percent, respectively), but not in the group exposed only later in gestation. The number of resorptions was also significantly increased in the gestation days 1 to 7 group. Fetal body weight and crown rump length were significantly decreased, and the incidence of delayed skeletal ossification increased, in both the gestation days 1 to 7 and gestation days 8 to 15 groups. The incidence of cleft palate was significantly increased in the gestation days 8 to 15 group. Cleft palate was typically found in fetuses with low body weight, and the researchers speculated that it may have resulted from an indirect embryotoxic effect rather than a direct teratogenic effect.

Mice exposed to 400 or 800 ppm of chloroform vapor 4 hours/day for 5 days showed a significant increase in the incidence of abnormal sperm, measured 28 days after exposure (Land *et al*., 1981). The abnormalities observed indicated that chloroform affected the first stage of sperm development in the exposed mice.

Embryotoxic and fetotoxic effects of chloroform similar to those following inhalation exposure have been reported after oral exposures. Thompson *et al*. (1974) conducted developmental toxicity studies of chloroform in rats and rabbits. In the rat study, groups of 25 mated female SD rats were gavaged with 0, 20, 50, or 126 mg/kg-day of chloroform in corn oil (in two divided doses) on days 6 to 15 of gestation, and sacrificed one or two days prior to expected parturition. Maternal toxicity was noted in dams treated with 50 or 126 mg/kg-day, including reductions in food consumption and body weight gain, and mild fatty changes in the liver. Fetal effects (significantly decreased fetal body weight and increased frequency of extra ribs) were observed only at 126 mg/kg-day. In the rabbit study, groups of 15 pregant female Dutch belted rabbits were gavaged once daily with 0, 20, 35, or 50 mg/kg of chloroform in corn oil on days 6 to 18 of gestation, and sacrificed on day 29. Four does in the 50 mg/kg-day group died due to acute hepatotoxicity. Body weight gain was significantly decreased at this dose as well. Fetal effects were limited to decreases in fetal weight in all groups (statistically significant only at 20 and 50 mg/kg-day).

Ruddick *et al*. (1983) treated groups of 15 presumed pregnant female SD rats with chloroform doses of 0, 100, 200, or 400 mg/kg-day by gavage in corn oil on gestation days 6 to 15, and sacrificed them on day 22. Three dams died at 400 mg/kg-day, and there were decreases in body weight gain, hemoglobin, and hematocrit, and increases in relative liver weight of dams at all dose levels. Fetal effects occurred only at 400 mg/kg-

day, consisting of a significant reduction in fetal body weight and increases in the incidence of sternebral variations and interparietal malformations.

Baeder and Hoffman (1988) exposed groups of 20 presumed pregnant Wistar rats to chloroform concentrations of 0, 30, 100, or 300 ppm in air seven hours/day on gestation days 7 to 16, and sacrificed them on gestation day 21. Food consumption and body weight were decreased in all treated groups in a dose-related manner throughout gestation. There were significant decreases in number of live fetuses and fetal crown rump length in all treated groups and fetal body weight in the 300 ppm group. No effect on fetal skeletal development was found. Baeder and Hoffman (1991) conducted another study using the same methodology, employing lower exposure concentrations (0, 3, 10, or 30 ppm). Maternal food consumption was decreased significantly in all treated groups, while maternal body weight was significantly decreased only in the 10 and 30 ppm groups. Fetal effects in the 30 ppm group were significant decreases in fetal crown-rump length and fetal body weight, and increases in number of small fetuses (3 g or less) and number of fetuses with little or no ossification of individual skull bones. Fetal effects at 3 and 10 ppm were significant delays in ossification of vertebrae and sternebrae.

Offspring of female Wistar rats (N = 12 for each of three groups) given chloroform at 0 or 75 µg/L in drinking water for two weeks prior to mating until parturition (*in utero* exposure) or until weaning (*in utero* and lactational exposure from birth to postnatal day 21) had physiological changes resulting in significantly impaired postnatal growth at weaning and 26 weeks of age (Lim *et al*., 2004). Pups of dams exposed to chloroform had significantly higher serum glucose levels and lower insulin levels at postnatal day 1; this effect was not due to β-cell depletion in the neonatal pancreas. Glucose homeostasis in response to a glucose challenge was not changed by chloroform treatment. Chloroform exposure did not affect birth weight. Fetal and neonatal exposure to chloroform did not elicit physiological changes associated with onset of type 2 diabetes.

The effect of chloroform on behavior of developing mice was studied by Burkhalter and Balster (1979). Intended as a screening study, the experiment started with only five mice of each sex and used only one dose level. The groups of five ICR mice of each sex were treated with 0 or 31 mg/kg-day of chloroform by daily gavage in a vehicle of mixed vegetable oil, Emulphor®, and saline starting 21 days prior to mating and continuing through weaning. Offspring were treated with the same doses as their parents on postnatal days 7 to 22. Three pups/litter were tested for various aspects of neurobehavioral performance during the postnatal period. No effect on reproductive performance was found. Pup body weights in the treated group were slightly reduced during pup treatment on postnatal days 7 to 22. The only significant effect in the behavioral testing was a decrease in forelimb placement response on postnatal days 5 and 7, but not 9. Recovery on day 9 indicates that the effect did not represent a serious delay in behavioral response.

Multigeneration studies of reproductive performance are available for mice exposed orally to chloroform. CD-1 mice (20 group) were treated with 6.6, 16, or 41 mg/kg-day of chloroform by daily gavage in corn oil starting prior to mating and continuing through weaning (NTP, 1988). A vehicle control group included 40 mice of each sex. F_1 mice (20/sex/group) from the control and high dose groups were continued at the same dose as their parents starting on postnatal day 22 and continuing through production of the F_2

generation. No effects on reproductive performance were found in the F_0 or F_1 generations. Histopathological changes were observed in F_1 animals in the 41 mg/kg-day group, when examined as adults. Increased epididymis weight and degeneration of the epididymal epithelium were observed in adult F_1 males, although there were no accompanying effects on sperm. Increased liver weight and degenerative liver lesions were observed in adult F_1 females.

In contrast to these results, significant effects on mouse reproductive performance were reported in a drinking water study (Borzelleca and Carchman, 1982). Chloroform in 0.1 percent Emulphor® was added to the drinking water (in closed bottles) of groups of 10 male and 30 female ICR mice at 0, 100, 1,000, or 5,000 ppm (estimated doses of 0, 19, 190, or 950 mg/kg-day, using U.S. EPA (1987a) reference values for water consumption of 0.0057 L/day and body weight of 0.03 kg) starting five weeks before mating in the F_0 generation and continuing through sacrifice of F_{2b} pups. Several deaths occurred among F_0 males and females in the 950 mg/kg-day group, and body weight gain in this group was depressed. Body weight was also reduced in the F_{1b} females exposed to 190 mg/kg-day. F_0 and F_{1b} mice showed dose-related hepatotoxicity, ranging from yellow discoloration of the liver at 19 mg/kg-day to black discoloration and nodules in the liver at 950 mg/kg-day. Impairment of reproductive function was shown by decreases in fertility, litter size, gestation index, and viability index in all F_1 and F_2 generations (statistically significant only in the 950 mg/kg-day group).

Two studies evaluated the fetotoxic potential of municipal drinking water in laboratory animals. Chernoff *et al*. (1979) reported a 28.1 percent incidence of supernumerary ribs on gestation day 18 in fetuses from CD-1 mice given municipal tapwater from Durham, North Carolina, comparied to a 21.1 percent incidence in fetuses from dams given distilled water, in an eight-month study with approximately 500 pregnant mice. F344 rats (N = 60 for each group) fed tapwater from Davis, California from gestation days 0 to 20 had a somewhat higher resorption frequency of 5.3 percent and frequency of affected fetuses of 6.5 percent than those fed bottled water, at 3.8 percent and 4.5 percent, respectively. The tap water group had results similar to those fed high pressure liquid chromatography grade (purified) water. There were no marked effects of dietary water source on maternal body weight, number of implantation sites, number of live fetuses, sex of offspring or number of resorptions per litter. Fetal length and weight, placenta weight, and soft tissue and skeletal malformations were also similar among the three groups (Keen *et al*., 1992; Rothman, 1992).

Immunotoxicity

Immunological effects of chloroform were studied by Munson *et al*. (1982). Male and female CD-1 mice were treated with 0, 50, 125, or 250 mg/kg-day of chloroform by daily gavage in aqueous Emulphor® solution for 14 days. Blood collected at sacrifice was used for studies of hematology, clinical chemistry, and humoral immunity. At necropsy, the major organs were weighed, and liver, kidney, and spleen were prepared for histopathological examination and assay of hepatic microsomal enzyme activities. Humoral immunity was studied in assays for primary IgM response to sheep red blood cells in the hemolytic plaque assay and by the hemagglutination technique. Cell-mediated immunity was evaluated by measuring delayed type hypersensitivity response,

popliteal lymph node response, and phagocytic activity of the fixed macrophage system, all in response to sheep red blood cells. Humoral immunity, assayed as the number of antibody-forming cells in response to sheep red blood cells, was significantly reduced in male and female mice of all dose groups in a dose-related manner. Hemagglutination was not affected by treatment, nor were measures of cell-mediated immunity. Hepatic effects were seen primarily at the higher doses in both males and females (increased relative and absolute liver weight ≥ 125 mg/kg-day and increased serum enzymes indicative of hepatotoxicity at 250 mg/kg-day).

The researchers also performed a 90-day study at the same doses (Munson *et al*., 1982). They found more mild changes in both the immune system and the liver than in the 14-day study. Humoral immunity (enumeration of antibody-forming cells) was still lower than controls in all male and female groups, but was not consistently related to dose and was only sporadically statistically significant. One assay of cell-mediated immunity, delayed-type hypersensitivity, was now significantly reduced in high dose females. In the liver, dose-related increases in absolute and relative liver weight were observed in females at all dose levels, but in males only at the highest dose, and serum chemistry indicators of hepatotoxicity were no longer increased in either sex. The researchers suggested that the reduced effect of chloroform in the 90-day study may indicate the development of tolerance to the compound.

Neurotoxicity

Although chloroform is a well-known central nervous system depressant, only one study was located that specifically examined the neurological effects of chloroform in experimental animals in detail. Balster and Borzelleca (1982) conducted neurobehavioral tests of adult male ICR mice exposed to chloroform by daily gavage in aqueous Emulphor® solution. Mice were tested for swimming endurance after 14 days of exposure to 3.1 or 31 mg/kg-day, motor performance and exploratory behavior after 90 days of exposure to 3.1 or 31 mg/kg-day, passive avoidance learning after 30 days of exposure to 100 mg/kg-day, and operant conditioning after 60 days of exposure to 100 or 400 mg/kg-day. Operant behavior was affected at both the 100 and 400 mg/kg-day doses of chloroform. Both response and reinforcement rates were significantly reduced in the treated groups. This effect was noticeable from the first day of treatment; partial tolerance developed over the 60-day test period. Chloroform had no effect on performance in any of the other tests.

Chronic Toxicity

Most chronic toxicity data on chloroform are from studies designed primarily as cancer bioassays, with doses too high to identify no effect levels and/or limited investigation of chronic toxicity endpoints. This section will focus on noncancer target organ effects observed in these studies as summarized in Table 15. In order to minimize repetition, methodological details and gross nonspecific effects (e.g., body weight, clinical signs, survival) are presented in more detail along with the cancer results in the following Carcinogenicity section.

Table 15. Summary of Significant Noncancer Effects in Chronic Toxicological Studies on Chloroform

Species, strain	Route	Sex	Duration	Doses	Effects	Reference
Rat F344	Inhalation	M, F	6 hours/day, 5 days/week for 104 weeks	0, 10, 30, 90 ppm	metaplasia of olfactory epithelium, goblet cell hyperplasia of respiratory epithelium, ossification of nasal turbinates	Nagano *et al*., 1998; U.S. EPA, 2001e; report on this study
Mouse BDF1	Inhalation	M, F	6 hours/day, 5 days/week for 104 weeks	0, 5, 30, 90 ppm	metaplasia of olfactory epithelium, respiratory goblet cell hyperplasia, ossification of nasal septum	Nagano *et al*., 1998; U.S. EPA, 2001e; report on this study
Rat Osborne-Mendel	Oral, gavage in corn oil	M, F	5 days/week for 78 wks, sacrificed at 111 week	0, 90, 180 mg/kg M, 0, 100, 200 mg/kg F TWAs	hepatic necrosis, urinary bladder hyperplasia, splenic hematopoiesis, decreased body weight gain and survival	NCI, 1976
Rat Osborne-Mendel	Oral, gavage in corn oil	M, F	5 days/week for 78 wks, sacrificed at 111 week	0, 90, 180 mg/kg M, 0, 100, 200 mg/kg F TWAs	above changes plus testicular atrophy in M heart thrombosis in high dose F	Reuber, 1979; reevaluation of NCI, 1976
Mouse $B6C3F_1$	Oral, gavage in corn oil	M, F	5 days/week for 78 wks, sacrificed at 92 or 93 week	0, 138, 277 mg/kg M, 0, 238, 477 mg/kg F TWAs	hepatic hyperplasia, some hepatic necrosis	NCI, 1976
Mouse $B6C3F_1$	Oral, gavage in corn oil	M, F	5 days/week for 78 wks, sacrificed at 92 or 93 week	0, 138, 277 mg/kg M, 0, 238, 477 mg/kg F TWAs	no increased noncancer findings noted	Reuber, 1979; reevaluation of NCI, 1976
Rat Sprague-Dawley	Oral, gavage in toothpaste	M, F	7 days/week for 52 weeks	0, 15, 75, 165 mg/kg-day	no treatment related effects	Palmer *et al*., 1979
Rat Sprague-Dawley	Oral, gavage in toothpaste	M, F	7 days/week for 95 weeks	0, 60 mg/kg-day	decreased body weight, serum butyryl ChE and relative liver weight in F	Palmer *et al*., 1979
Mouse ICI	Oral, gavage in toothpaste	M, F	unk. days/week for 80 weeks	0, 17, 60 mg/kg-day	no noncancer effects (kidney tumors)	Roe *et al*., 1979

Species, strain	Route	Sex	Duration	Doses	Effects	Reference
Mouse CFLP	Oral, gavage in toothpaste	M	unk. days/week for 80 weeks	0, 60 mg/kg-day	no noncancer effects (kidney tumors)	Roe *et al*., 1979
Mouse ICI	Oral, gavage in arachis oil	M	unk. days/week for 80 weeks	0, 60 mg/kg-day	moderate to severe kidney lesions (increased kidney tumors)	Roe *et al*., 1979
Mouse CFLP	Oral, gavage in toothpaste	M	unk. days/week for 80 weeks	0, 60 mg/kg-day	no noncancer effects (kidney tumors)	Roe *et al*., 1979
Mouse ICI	Oral, gavage in arachis oil	M	unk. days/week for 80 weeks	0, 60 mg/kg-day	moderate to severe kidney lesions (increased kidney tumors)	Roe *et al*., 1979
Mouse CBA and CF/1	Oral, gavage in toothpaste	unk.	unk. days/week for 80 weeks	0.60 mg/kg-day	moderate to severe kidney lesions (but no increased kidney tumors)	Roe *et al*., 1979
Dog Beagle	Oral, in toothpaste in a capsule	M, F	6 days/week for over 7 years	0, 15, or 30 mg/kg-day	increased fatty cysts in liver, plus increased markers of liver damage	Heywood *et al*., 1979
Rat Osborne-Mendel	Oral, in drinking water	M	7 days/week for 104 weeks	0, 200, 400, 900, 1800 ppm, or 0, 19, 38, 81, or 160 mg/kg-day	decreased body weight and food consumption and longer survival in 2 higher dose groups (and increased renal tumors)	Jorgenson *et al*., 1982, 1985
Mouse $B6C3F_1$	Oral, in drinking water	F	7 days/week for 104 weeks	0, 200, 400, 900, 1800 ppm, or 0, 34, 65, 130, or 263 mg/kg-day	early mortality in mice which wouldn't drink the water; dose-related increased liver fat (but no increased tumors)	Jorgenson *et al*., 1982, 1985
Rat Osborne-Mendel	Oral in drinking water	M	7 days/week for 104 weeks	0, 200, 400, 900, 1800 ppm, or 0, 19, 38, 81, or 160 mg/kg-day	dose-related increased renal lesions in two highest dose groups	Hard *et al*., 2000; reevaluation slides of Jorgenson *et al*., 1982, 1985
Rat F344	Oral, in drinking water	M	7 days/week for 100 weeks	0, 900, 1800 ppm, or 0, 45, or 90 mg/kg-day	proliferative liver lesions, no increase in kidney lesions	DeAngelo, 1995; as reported by U.S. EPA, 2001e

The only chronic inhalation toxicity data are from an inhalation cancer study by Nagano *et al.* (1998). In this study, groups of 50 male and female F344 rats and BDF1 mice had whole-body exposures to chloroform vapor six hours/day, five days/week for 104 weeks. Exposure concentrations were 0, 10, 30, or 90 ppm in rats and 0, 5, 30, or 90 ppm in mice. The paper by Nagano *et al*. (1998) discusses only cancer results. However, U.S. EPA (2001e) reported that this study found metaplasia of the olfactory epithelium and goblet cell hyperplasia of the respiratory epithelium in male and female rats and mice, and that ossification was observed in the nasal turbinates of rats and nasal septum of mice at the lowest exposure levels tested. Respiratory tumors were not increased (Nagano *et al*., 1998). U.S. EPA (2001b,d,e) and IARC (1999d) also reported that the 30 and 90 ppm exposure levels were acutely lethal to the mice, and that chronic exposure to these levels was achieved by first exposing the mice to lower concentrations and then progressively increasing the exposure concentration every two weeks (i.e., 5 ppm for two weeks, 10 ppm for two weeks, and in the high dose group 30 ppm for two weeks, before reaching the intended level).

Information on chronic toxicity by oral exposure comes from several studies. NCI (1976) conducted cancer bioassays of chloroform in rats and mice by gavage in oil. Groups of 50 Osborne-Mendel rats of each sex were gavaged with chloroform in corn oil five days/week for 78 weeks at time-weighted average doses of 90 or 180 mg/kg-day in males and 100 or 200 mg/kg-day in females, and sacrificed after 111 weeks. Both a matched control group of 20 rats of each sex and a colony control group of 99 male and 98 female rats were included in the study. Survival and body weight gain were reduced in a dose-related fashion in all chloroform-treated groups of male and female rats. A variety of clinical signs indicative of poor condition were observed in all groups, but more frequently in the treated groups. Several nonneoplastic lesions were considered potentially related to treatment by the researchers. Hepatic necrosis was observed in low and high dose male (3/50 and 4/40, respectively) and female (3/49 and 11/48) rats, but not in controls. Although inflammatory pulmonary lesions associated with pneumonia were seen in all groups (including controls), the incidence and severity of these lesions appeared to be increased in the treated groups (male and female, both doses). Apparent increases in urinary bladder hyperplasia, primarily in low dose males and females, and splenic hematopoiesis in low and high dose males, were also noted, although these lesions were observed to a lesser extent in controls as well. The only neoplastic effect was renal tumors in male rats.

The mouse study was conducted similarly to the rat study, except that colony controls comprised 77 males and 80 females and terminal sacrifice was performed at 92 to 93 weeks (NCI, 1976). Time weighted average doses were 138 or 277 mg/kg-day in males and 238 or 477 mg/kg in females. Survival, body weight, and clinical sign data did not indicate toxic effects in the treated mice prior to the onset of tumor formation. Hepatic necrosis was noted in one low dose male, four low dose females, and one high dose female (and no controls). Hyperplasia and tumors occurred with high incidence in the livers of treated mice.

Reuber (1979) separately evaluated the pathology data from the NCI (1976) study. He concluded that chloroform also produced testicular atrophy in male rats (33 percent of treated males versus two percent of controls) and thrombosis of the heart in high dose

female mice (23 percent, versus zero percent in all other groups). He noted that all nine individuals with heart thrombosis also had liver carcinomas. The author did not attempt to explain discrepancies between his interpretation of the data and the original report.

Target organ effects were not identified in rats treated with chloroform in toothpaste. (Palmer *et al*. (1979) treated male and female SD rats with 15, 75, or 165 mg/kg-day of chloroform by daily gavage in toothpaste for 52 weeks. There were no treatment-related effects on body weight, food consumption, hematology, serum chemistry, urinalysis, or histopathology (including the liver). A followup experiment included groups of 50 SD rats/sex treated with 0 or 60 mg/kg-day of chloroform by daily gavage in toothpaste for 95 weeks. Body weight was progressively reduced throughout the study in treated rats of both sexes, and there was a slight decrease in food consumption in treated females. Survival was not affected by treatment. Plasma cholinesterase activity was significantly decreased in treated females for much of the study, but there was no effect on red blood cell cholinesterase activity in females, or on plasma or red blood cell cholinesterase in males. The affected plasma enzyme in female rats was shown to be butyrylcholinesterase (pseudocholinesterase), which was not found in males in this study. The researchers did not consider this effect to be biologically significant. The only statistically significant change in organ weight was a slight decrease in relative liver weight in the female rats. Nonneoplastic histological findings in the liver, kidney, and lungs in treated groups were comparable to controls, and no tumors were associated with treatment.

Studies by Roe *et al*. (1979) found no adverse effects on survival, body weight, clinical signs hematology, organ weights, or nonneoplastic lesions in male and female ICI mice treated with 17 or 60 mg/kg-day of chloroform by gavage in toothpaste for 80 weeks, or in male CFLP (ICI redefined) mice treated with 60 mg/kg-day for 80 weeks. Male mice developed renal tumors in both experiments. When treated with 60 mg/kg-day of chloroform in arachis oil, the ICI males showed an increase in moderate to severe nonneoplastic kidney lesions in addition to increased renal tumors. The CBA and CF/1 strains of mice showed statistically significant increases in the incidences of moderate to severe nonneoplastic kidney lesions when treated with 60 mg/kg-day of chloroform in toothpaste, but did not develop renal tumors.

Heywood *et al*. (1979) performed a chronic study in dogs of chloroform in toothpaste. Groups of eight beagle dogs of each sex were treated with 15 or 30 mg/kg-day of chloroform in a toothpaste base by capsule six days/week for over seven years (376 weeks). A control group of 16 dogs/sex received toothpaste alone and an untreated control group of eight dogs/sex was also included. Clinical signs, food and water consumption, and body weight were monitored during the study. Ophthalmoscopy, clinical examinations, and comprehensive laboratory investigations were performed periodically. Treatment was followed by a 20 to 24 week recovery period before sacrifice at weeks 395 to 399. Organ weight data and tissues for gross and microscopic pathology were collected at necropsy. Survival, clinical signs, body weight, food and water intake, hematology, urinalysis, ophthalmoscopy, and organ weights were unaffected by chloroform treatment. Clinical chemistry measurements revealed statistically significant increases in serum alanine aminotransferase throughout the study at 30 mg/kg-day and after week 130 at 15 mg/kg-day. There were also apparent increases in other biochemical hepatotoxicity measures at the end of the exposure, including serum

aspartate aminotransferase, alkaline phosphatase (ALK), glutamate dehydrogenase (GDH), and gamma glutamyl transferase (GGT), although no statistical analysis was reported for these endpoints. Histopathological examination revealed a significant increase (Fisher exact test) compared with vehicle controls (M 0/12; F 1/15) in the incidence of moderate or marked fatty cysts in the liver of male and female dogs treated with 15 (M 6/7, $p < 0.001$; F 3/8, $p < 0.05$) or 30 (M 6/7, $p < 0.001$; F 7/8, $p < 0.001$) mg/kg-day of chloroform.

Chronic drinking water studies are available in rats and mice. Male Osborne-Mendel rats and female $B6C3F_1$ mice were exposed to 0, 200, 400, 900, or 1,800 ppm of chloroform in the drinking water for 104 weeks (Jorgenson *et al*., 1982, 1985). Additional control groups of 50 rats and 50 mice, matched for water intake to the high dose groups, were included. Time-weighted average doses, calculated by the researchers, were approximately 19, 38, 81, or 160 mg/kg-day in rats and 34, 65, 130, or 263 mg/kg-day in mice. Satellite groups of 10 male rats and 10 female mice per group (except matched control) were sacrificed for analysis of liver fat after three and six (mice only) months. Additional satellite groups of 20 male rats/group were sacrificed after six, 12, and 18 months, and at study termination, for hematology and clinical chemistry evaluation, as well as necropsy and histopathology (also liver fat analysis at six months). All animals were given complete necropsy at death, and the liver, kidney, urinary bladder, adrenals, spleen, esophagus, stomach, small intestine, colon, lung, lymph nodes, and any gross lesions or suspect tumors were examined for histopathology.

Rats in the two lower dose groups had water consumption, body weight, and survival roughly similar to controls (Jorgenson *et al*., 1982, 1985). Rats in the two higher dose groups had dose-related decreases in water consumption and body weight, and higher survival than controls. The researchers speculated that the increase in survival, which was also seen in the matched control group, was due to leaner body composition in these groups. Liver fat content in treated rats was similar to controls at three months, but was significantly increased in high dose rats at six months. Hematology and clinical chemistry findings were generally unremarkable. Hematology changes were observed only in the two higher dose groups and were consistent with hemoconcentration. Clinical chemistry changes occurred primarily in the two high dose groups, but were sometimes found in the lower dose groups as well. The matched control group displayed many of the same hematology and clinical chemistry changes as the treated groups, suggesting that the observed changes were secondary to reduced water intake and body weight, rather than a direct effect of chloroform. Nontumor pathology of the kidney was high in all groups, including controls (e.g., the incidence of nephropathy was over 90 percent in all groups). No other nonneoplastic observations were reported. Renal tumors were increased in the male rats.

Hard *et al*. (2000) reevaluated the pathology data from this study to investigate nonneoplastic effects in the kidney in more detail. The researchers were able to distinguish between spontaneous age-related chronic progressive nephropathy (foci of intense tubule basophilia associated with thickened basement membranes and sometimes accompanied by tubular atrophy) and lesions indicative of tubule injury caused by chloroform (nuclear crowding without thickening of basement membranes, cytoplasmic vacuolation, faint cytoplasmic basophilia in the mid to deep cortex). They found that

chloroform-related renal lesions did not occur in the control or low dose groups, had an incidence increasing from 25 percent after six months to 50 percent after 24 months in the second highest dose group, and had an incidence of 95 to 100 percent at all time points in the high dose group. The severity of the lesions was greater in the high dose group than the mid-dose group, and increased with duration of exposure in the high dose group. The researchers concluded that the chloroform-related lesions indicated sustained renal tubule cytotoxicity and compensatory regeneration in the rat kidneys.

In the mouse study, about 25 percent of the two higher dose groups and six percent of the 65 mg/kg-day group refused to drink the treated water and died early in the study (Jorgenson *et al*., 1982, 1985). However, after this early period, drinking water consumption, body weight, and survival in the treated mice were similar to controls. Liver fat content was significantly increased in the top three dose groups at three months and in the low dose group as well at six months. No results were reported regarding nonneoplastic lesions in the mice. Tumors were not increased.

U.S. EPA (2001e) summarized the results of an unpublished study by DeAngelo (1995) that found midzonal vacuolation in livers of male F344 rats treated with 900 or 1,800 ppm of chloroform in drinking water for 100 weeks (45 or 90 mg/kg-day, assuming water intake of 0.05 L/day-kg). Proliferative liver lesions were also increased. Kidney lesions were typical of aging rats and did not differ between treated and control groups.

Carcinogenicity

Chloroform produces kidney and liver tumors following ingestion in rats and mice (NCI, 1976; Jorgenson *et al*., 1985; Roe *et al*., 1979). These and other relevant studies are summarized below.

National Cancer Institute (NCI, 1976)

NCI (1976) sponsored the first comprehensive lifetime cancer bioassay of chloroform. Doses were selected based on preliminary toxicity tests in which B6C3F$_1$ mice and Osborne-Mendel rats were gavaged with chloroform five days/week for six weeks. These tests established an apparent maximum tolerated dose of chloroform in mice of 200 mg/kg-day (males), and 400 mg/kg-day (females). For rats, the maximum tolerated dose was 180 mg/kg-day for males and 250 mg/kg-day for females.

In the long-term study, Osborne-Mendel rats and B6C3F$_1$ mice were treated with USP grade chloroform (higher than 99.0 percent purity) by gavage in corn oil. For each species, treatment groups contained 50 animals of each sex. Control groups consisted of twenty animals/sex and species for matched controls and positive controls. Colony control groups (which included the chloroform matched controls as well as matched controls for other chemicals undergoing testing) consisted of 99 male and 98 female rats, and 77 male and 80 female mice. Mice in the colony control group were all housed in the same room; colony control rats were housed in two separate rooms. Positive controls were treated with carbon tetrachloride in corn oil by gavage.

Results in Mice

For the B6C3F$_1$ mice in the NCI (1976) study, dosing with chloroform began at five weeks of age, and continued for 78 weeks, five days/week. Mice were observed for nine to ten weeks, then sacrificed at age 92 or 93 weeks. Initially, male mice were given 200 or 100 mg/kg-day and female mice were given 400 or 200 mg/kg-day (the maximum tolerated dose and one half the maximum tolerated dose) of chloroform. During the 18th week of treatment, the chloroform doses were increased so that males received 150 or 300 mg/kg-day and females 250 or 500 mg/kg-day for the remaining 60 weeks. This corresponds to time-weighted average (TWA) doses of 138 or 277 mg/kg-day in males and 238 or 477 mg/kg-day in females.

Fifty-six percent of dosed male mice (both groups) and 50 percent of male vehicle controls survived until terminal sacrifice. Seventy five percent of the low dose and vehicle control females were alive at terminal sacrifice, compared to only 20 percent of the high dose females. The average terminal body weights of treated mice were 34 g for males and 31 g for females.

Mice of both sexes, in all treatment groups, had a high incidence of hepatocellular carcinoma (36 to 98 percent, Table 16), which was dose-related and statistically different from controls. In five animals (two low dose males, one high dose male, and two high dose females), the hepatocellular carcinomas metastasized to the lungs or kidneys. The total number of tumor-bearing animals was also significantly increased in chloroform-treated groups ($p < 0.05$, Fisher Exact Test). Only 1/18 vehicle control males had tumors, compared to 18/50 low dose males and 44/45 high dose males. Similarly, 0/20 vehicle control females had tumors; total tumor incidence in low and high dose females was 36/45 and 39/41, respectively. Chloroform treatment did not consistently decrease the time to appearance of the first tumor. In males, the first tumor was observed at 72 weeks in vehicle controls, compared to 66 and 54 weeks in low and high dose males, respectively. Among females, the first tumor in controls was seen at week 27, yet in the treated animals tumors were not observed until weeks 66 and 67, at the two doses.

Table 16. Carcinogenicity Studies of Chloroform Administered Orally to Mice

Sex	Applied dose mg/kg-day	Tumor site and type	Incidence	P value*
NCI (1976), B6C3F$_1$ mice				
Male	0 (colony), 0 (match), 138, 277	Liver, hepatocellular carcinoma	5/77, 1/18, 18/50, 44/45	0.011 0.0031
Female	0, 0, 238, 477	Liver, hepatocellular carcinoma	1/80, 0/20, 36/45, 39/41	4.0×10^{-10} 3.7×10^{-14}
Reuber (1979), B6C3F$_1$ mice (interpreting slides of NCI, 1976)				
Male	0, 0, 138, 277	Liver, hyperplastic nodule small hepatocellular carcinoma	 1/17, 2/17, 11/46, 0/44 1/17, 0/17, 3/46, 3/44	 0.25 0.38, 0.37

Sex	Applied dose mg/kg-day	Tumor site and type	Incidence	P value*
		large hepatocellular carcinoma hepatocellular carcinoma or hyperplastic nodule Hematopoietic system, malignant lymphoma	1/17, 0/17, 17/46, 41/44 3/17, 2/27, 31/46, 44/44 0/17, 0/17, 14/46, 10/44	0.00018 < 0.00001 < 0.00001 < 0.00001 0.0064 0.028
Female	0, 0, 238, 477	Liver, hyperplastic nodule small hepatocellular carcinoma large hepatocellular carcinoma Hematopoietic system, malignant lymphoma	0/20, 0/19, 1/45, 0/40 1/17, 0/17, 3/45, 1/40 0/20, 0/19, 9/45, 40/40 0/20, 0/20, 9/45, 4/40	0.34 0.68 < 0.00001 0.032 0.20
Jorgenson *et al.* (1985), B6C3F$_1$ mice				
Female	0, 0 (match), 25, 50, 112, 234	No statistically significant effects		
Roe *et al.* (1979), ICI mice				
Male	0, 17, 60[a]	Kidney, renal adenoma renal hypernephroma renal adenoma or hypernephroma	0/72, 0/37, 5/38 0/72, 0/37, 3/38 0/72, 0/37, 8/37	< 0.05[b] < 0.01[b] < 0.001[b]
Female	0, 17, 60	No statistically significant effects		
Male	0, 0 (match), 60[c] 0, 0 (match), 60[d] 0, 60[e]	Kidney renal adenoma renal hypernephroma renal adenoma or hypernephroma renal adenoma or hypernephroma renal adenoma or hypernephroma	1/45, 6/237, 9/4 0/45, 0/237, 2/49 1/45, 6/237, 9/49 0/83, 1/49, 5/47 1/50, 12/48	< 0.05[b] < 0.01[b] < 0.001[b] 0.093[b] < 0.001[b]
Roe *et al.* (1979), C57BL, CBA, and CF/1 mice				
Male	0, 60[c] each strain	No statistically significant effects		

*Significance by Fisher Exact Test
a, chloroform by gavage in toothpaste six d/wk for 80 weeks, terminal sacrifice at 96 weeks
b, values from U.S. EPA (1985a), statistical test not specified
c, dosing as in a, terminal sacrifice at 104 weeks
d, dosing as in a, terminal sacrifice at 97 or 99 weeks
e, chloroform by gavage in arachis oil six d/wk for 80 weeks, terminal sacrifice at 97 or 99 weeks

Results in Rats

Chloroform or vehicle treatments of Osborne-Mendel rats began when animals were seven weeks old, and continued for 78 weeks, five days/week. Rats were observed for an additional 26 weeks, and sacrificed at 111 weeks of age. Male rats received doses of 90 or 180 mg/kg-day of chloroform throughout the study period. Female doses were initially set at 125 and 250 mg/kg-day, but were reduced after 22 weeks to 90 and 180 mg/kg-day, for time-weighted average doses of 100 and 200 mg/kg-day. At the time of terminal sacrifice, 28 percent of all high dose rats remained alive compared to 44 or 48 percent in the low dose groups (females and males, respectively). Although survival was poor in both vehicle control groups (14 to 30 percent), only one vehicle control animal had died before week 90.

In male rats, treatment with chloroform was associated with a statistically significant increase ($p = 0.016$, Fisher exact test) in renal tubular cell adenomas and carcinomas (Table 17). These tumors were also noted in treated female rats, but the increase was not statistically significant. The combined incidence of Follicular cell and C-cell thyroid gland tumors (both adenomas and carcinomas) in males was 0/19, 4/50, and 12/50 (vehicle control, low dose, and high dose groups, respectively) and in females was 1/19, 8/49, and 10/49. Thyroid tumors were reported in 8/99 male and 1/98 female colony controls. The biological significance of the thyroid gland tumors is controversial because of the contrasting trends in incidence in males and females and because the two tumor types are derived from different cell types.

Conclusions

The NCI (1976) concluded that these data are not truly indicative of a carcinogenic response to chloroform. The U.S. EPA (1985a) concurred with the NCI's conclusions. However, Reuber (1979) concluded after examining the slides that chloroform had some carcinogenic activity in both Osborne-Mendel rats (renal tumors) and $B6C3F_1$ mice (hepatic tumors). Reuber also concluded that low and high dose male mice, and low dose female mice had a significantly elevated incidence of malignant lymphoma (Table 16). According to Reuber (1979), high dose male and female rats had a significantly higher incidence of hepatocellular carcinoma and hyperplastic nodules combined, as well as a significantly increased incidence of total liver tumors. He reported that male rats of both dosage groups had significantly greater numbers of total malignant tumors, and that high dose female rats had a significantly increased incidence of cholangiofibromas or cholangiocarcinomas, compared to controls (Table 17). This latter finding is of some interest, as Popp (1984) listed this as a rare type of carcinoma in both rats and mice.

The significance of Reuber's reevaluation of the NCI (1976) data is not immediately apparent. In some instances, his statistical analysis of tumor incidence was based on unconventional and possibly irrelevant combinations of lesions, i.e., equal weight was given to hepatocellular carcinomas and hyperplastic nodules. Even when one compares the treatment of equivalent data between Reuber (1979) and the NCI (1976), there are differences between the two. For example, Reuber's reported incidence of hepatocellular carcinoma (of both size classes) in male and female mice differs from the NCI values for three of the four groups (low dose males, low and high dose females). In all instances, the NCI interpretation resulted in lower tumor incidences than those determined by

Reuber (1979). A tendency of the NCI (1976) toward conservative data interpretation and statistical analysis may explain many of the differences, although Reuber's analyses have also been criticized as over-interpreting the data. In this regard, the U.S. EPA (1985a) attributed the different conclusions of Reuber (1979) and the NCI (1976) largely to a difference of opinion between pathologists.

Jorgenson *et al*. (1982, 1985)

Among the most controversial issues in interpretation of chloroform toxicity data has been the role of the corn oil vehicle in the induction of hepatic carcinomas in $B6C3F_1$ mice (NCI, 1976). Consequently, studies to examine the carcinogenicity of chloroform administered in drinking water at doses similar to those used by the NCI (1976) were conducted. Jorgenson *et al*. (1985) used the same strains of animals as the NCI (Osborne-Mendel rats and $B6C3F_1$ mice), but included a greater range of exposure levels to characterize better the low-dose chloroform response. Treatment was restricted to the one sex per species which developed a specific tumor type in the NCI study (i.e., male Osborne-Mendel rats and female $B6C3F_1$ mice).

Fresh drinking water solutions were prepared twice weekly from distilled pesticide-analysis quality chloroform. The 960 male Osborne-Mendel rats and 1,160 $B6C3F_1$ mice were divided into six groups given 0, 200, 400, 900, and 1,800 mg/L chloroform, plus a control group matched for water intake with the highest dose group. Treatment with chloroform began at seven weeks of age and continued for 104 weeks. Results for mice are given in Table 16 and for rats in Table 17. The chloroform doses listed in Tables 16 and 17 represent time-weighted average doses over the 104-week study period. Addition of chloroform to the drinking water resulted in a marked, concentration-dependent decrease in water intake in rats. Water consumption returned to near-normal levels in the two lowest dosage groups by week 80, but remained depressed in the other two groups.

Survival appeared to be directly related to the quantity of ingested chloroform. At the end of the study, 25, 29, 60, and 66 percent of the rats were alive in the 200, 400, 900, and 1,800 mg/L groups, respectively. Survival in matched controls was 54 percent, compared to only 12 percent in normal controls. The authors speculated that the differences in survival among the groups were due in part to decreased food and water consumption among chloroform-treated rats, which resulted in leaner animals.

The chloroform treatment had no effect on total tumor incidence in rats, and was associated with a decreased incidence of adrenal cortical adenomas, adrenal pheochromocytoma, thyroid C-cell adenomas, and combined incidence of thyroid C-cell adenoma and carcinoma. The incidences of renal tubular cell adenoma and combined renal tubular cell adenoma and carcinoma were significantly greater among high dose animals ($p < 0.01$, Peto Trend Test), compared to matched controls (Table 16). Total kidney tumors (adenoma, adenocarcinoma, and nephroblastoma) also were significantly greater ($p < 0.01$, Peto Trend Test) among this group. Although the incidence of renal tumors was not statistically different from controls among the other three dose groups, there was a consistent, dose-related increase in tumor incidence. Exposure to 1,800 mg/L of chloroform was also associated with a significantly increased combined incidence of lymphomas and leukemias ($p < 0.01$, Peto Trend Test), and all circulatory system tumors ($p < 0.05$, Peto Trend Test). Nontumor renal pathology was high in all groups, including

controls; the incidence of nephropathy was over 90 percent in all groups. No other nonneoplastic observations were reported.

Chiu *et al*. (1996) reevaluated the pathology data from this study and verified the tumor increase in the rat kidneys. They also suggested that marginal increases in circulatory tumors (hemangioma/hemangiosarcoma) in the higher dose groups may have been related to treatment, although discrepancies in the data for this tumor type prevent reaching any firm conclusions. Hard *et al*. (2000) also reevaluated the pathology data to investigate nonneoplastic effects in the kidney (described in the chronic noncancer section). Their conclusion that chloroform produce sustained renal tubule cytotoxicity and compensatory regeneration is relevant to carcinogenic mechanism. They noted the correspondence between these effects and renal tumors in the rats, i.e., significant increase in tumors at a dose where all rats had cytotoxicity and regenerative hyperplasia, marginal increase in tumors at the dose where half of the rats had lower level cytotoxicity/hyperplasia, and no increase at doses where none of the rats had cytotoxicity/hyperplasia.

In $B6C3F_1$ mice, large numbers of animals in the two highest dose groups refused to drink the water containing chloroform. Approximately 25 percent of mice in the 900 and 1,800 mg/L groups, and six percent of those in the 400 mg/L group died during the first week of the study as a result of water deprivation. After this time, the water consumption of treated animals varied from 78 to 90 percent of that in controls. As with rats, Jorgenson *et al*. (1985) established a group of matched controls for which water intake was restricted to that of the highest dose group. Following the deaths of the mice that refused to drink, survival among treated animals did not differ substantially from that of controls for the rest of the study.

Table 17. Carcinogenicity Studies of Chloroform Administered Orally to Rats

Sex	Applied dose mg/kg-day	Tumor site and type	Incidence	P value*
NCI (1976), Osborne-Mendel rats				
Male	0 (colony), 0 (match), 90, 180[a]	Kidney, renal carcinoma and adenoma	8/99, 0/19, 4/50 (38), 12/50(27)	0.27[b] 0.016
Female	0, 0, 100, 200	Thyroid, adenoma or carcinoma	1/98, 1/19, 8/49, 10/49	0.22 0.12
Reuber (1979), Osborne-Mendel rats (interpreting slides of NCI (1976)				
Male	0, 0, 90, 180	Liver, hyperplastic nodules hepatocellular carcinoma hyperplastic nodule or hepatocellular carcinoma all liver tumors Kidney, renal adenoma or carcinoma Total malignant tumors	 0/20, 1/19, 5/50, 8/49 0/20, 0/19, 0/50, 2/49 0/20, 1/19, 5/50, 10/49 0/20, 2/19, 5/50, 12/49 0/20, 0/19, 8/50, 14/49 6/20, 5/19, 25/50, 21/49	 0.045 0.049 0.0049 0.031
Female	0, 0, 100, 200	Liver,		

Sex	Applied dose mg/kg-day	Tumor site and type	Incidence	P value*
		hyperplastic nodule hepatocellular carcinoma hyperplastic nodule or hepatocellular carcinoma cholangiofibroma or cholangiocarcinoma all liver tumors Thyroid, adenoma or carcinoma	1/20, 2/20, 7/39, 12/39 0/20, 0/20, 2/39, 2/39 1/20, 2/20, 9/39, 14/39 0/20, 0/20, 3/39, 11/39 1/20, 2/20, 10/39, 20/39 3/20, 1/20, 11/39, 12/39	0.036 0.031 0.006 0.002 0.037, 0.021
Palmer *et al.* (1979), Sprague Dawley rats				
Male Female	0, 15, 75, 165[c] 0, 60[d]	No statistically significant effects		
Tumasonis *et al*. (1985), Wistar rats				
Male	0, 200[e]	Liver, hepatic adenofibrosis[f]	 0/22, 17/28	 < 0.001
Female	0, 240	Liver, hepatic adenofibrosis neoplastic nodules	 0/18, 34/40 0/18, 10/40	 < 0.001 < 0.03
Jorgenson *et al.* (1985), Osborne-Mendel rats				
Male	0, 0 (match), 18, 38, 79, 155[g]	Hematopoietic system; lymphomas and leukemia Circulatory system, all tumors Kidney, renal tubular cell adenoma renal tubular cell adenoma or adenocarcinoma all kidney tumors	5/303, 1/50, 19/316, 5/158, 2/48, 3/50 5/303, 0/50, 6/316, 3/148, 3/48, 3/50 4/301, 0/50, 2/313, 3/148, 2/48, 5/50 4/301, 1/50, 4/313, 4/148, 3/48, 7/50 5/301, 1/50, 6/313, 7/148, 3/48, 7/50	< 0.01 < 0.01 < 0.01 < 0.05 < 0.01 < 0.0001[h] < 0.01 < 0.0001[h] < 0.01 < 0.0001[h]

* Significance by Fisher Exact Test
a, chloroform in corn oil gavage five times/week for 78 weeks, sacrificed at 111 week
b, values from U.S. EPA, 1985a
c, six gavage doses per week of chloroform in toothpaste, study terminated at 52 week
d, dosing as in c. but treatment for 80 weeks, sacrificed at 95 week
e, chloroform in drinking water for 180 weeks, sacrifice time assumed 180 week, doses calculated as time weighted averages
f, hepatic adenofibrosis is a proliferative lesion of the bile duct (a.k.a. cholangiocellular carcinoma, Schauer and Kunze, 1976)
g, chloroform in drinking water for 104 weeks, sacrificed at 104 week; controls matched for water intake of highest dose; time weighted averages from calculation of Jorgenson *et al*. (1985)
h, Peto trend test

In notable contrast to the NCI (1976) bioassay, this study found no evidence of chloroform carcinogenicity to female B6C3F$_1$ mice. Data on "all tumors," hepatocellular

adenoma, and the combined incidence of hepatocellular adenoma and carcinoma in Table 16 show no effect of chloroform on the incidence of hepatic tumors in mice.

A reevaluation of the pathology data from this study by Chiu *et al*. (1996) confirmed the lack of effect on liver tumor incidence. The reevaluation found significant increases in circulatory and Harderian gland tumors in the lower dose groups, but concluded that the data were inadequate to assess the toxicological significance of these findings.

Roe *et al*. (1979)

Roe *et al*. (1979) evaluated carcinogenicity of chloroform administered to mice by gavage in a toothpaste base in three separate experiments,. Two of the experiments utilized ICI mice, and one compared the carcinogenicity of chloroform among ICI, CBA, C57BL, and CF/1 mice. Dose levels for these experiments were based on the results of a six-week study in which Schofield mice were gavaged with chloroform at 0, 60, 150, or 425 mg/kg-day. The two highest doses were lethal to 80 to 100 percent of the animals. The 60 mg/kg dose was selected as the maximum dose for the long-term studies based on "moderate retardation" of weight gain in both sexes.

The first experiment compared the tumor incidence in ICI mice treated with chloroform (17 or 60 mg/kg in toothpaste) with that in mice receiving toothpaste without the normal flavoring agents peppermint oil and eucalyptol. Each treatment group contained 52 males and 52 females, while the vehicle control group had 104 mice of each sex. Survival of animals was comparable among the three dose groups. At week 80, approximately 42 percent of the controls were still alive compared to 35 percent (17 mg/kg group) and 52 percent (60 mg/kg group). By the 95th week, only about 10 percent of the animals remained alive in any group. There were no statistically significant differences among treatment groups with respect to the number of mice with tumors or in the number of mice with malignant tumors. Eight high dose male mice of 38 examined had renal tumors. Of these, five were adenomas and three were hypernephromas (Table 16). Renal tumors were not found in any other group (0/72 in control males and 0/37 in low dose males). Renal adenoma was first observed in a mouse at week 88, and hypernephroma at week 92. Although Roe *et al*. (1979) did not statistically analyze these data, the combined incidence of these two tumors is significantly different from vehicle controls ($p < 0.001$, Fisher exact test).

Interest in effects of toothpaste flavoring agents on the possible carcinogenicity of chloroform prompted Roe *et al*. (1979) to conduct further studies. A second experiment utilized eight treatment groups to compare treatments with chloroform in toothpaste, toothpaste alone, and toothpaste with the flavorings, peppermint oil or eucalyptol. Untreated control groups were composed of 52 male and female ICI mice, while the vehicle control (toothpaste) group contained 260 animals (males only). Fifty-two male mice received 60 mg/kg-day of chloroform in toothpaste. The remaining four groups of mice received toothpaste containing 8 or 32 mg/kg of eucalyptol or 4 or 16 mg/kg of peppermint oil. Dosing was carried out over an 80-week period.

Survival patterns and body weight changes were similar among treatment groups; between 55 and 70 percent of all animals were alive at week 80. Necropsies revealed a number of nonneoplastic lesions in mice from all groups, but no apparent correlation with

treatments. Lung and liver tumors and malignant lymphomas occurred with approximately the same frequency in all eight groups. As in the first experiment, an elevated incidence of renal tumors was documented in chloroform-treated mice. Of the nine mice with renal tumors, seven had adenomas and two had hypernephromas (Table 16). The U.S. EPA (1985a) calculated that the incidence of these tumors was significantly different from controls when evaluated by individual tumor type ($p < 0.05$, adenomas; $p < 0.01$, hypernephromas), or in combination ($p < 0.001$) (test not specified).

When Roe *et al*. (1979) administered chloroform (in toothpaste) to four different strains of mice (experiment three), they found a marked, strain-dependent variation in survival. For each of the strains, C57BL, CBA, CF/1, and ICI, 52 males received 60 mg/kg-day of chloroform, and 52 mice served as vehicle controls. Additional groups of ICI mice were either untreated (100 animals), received chloroform in arachis oil (52 animals), or received arachis oil alone (52 animals). At terminal sacrifice, only 12 percent of the CF/1 mice and 31 percent of the ICI mice remained alive versus 21 and 27 percent in the respective control groups. This is in contrast to 69 and 79 percent survival among treated C57BL and CBA mice, and 52 and 69 percent survival in the corresponding controls. None of the animals that were dosed with chloroform in toothpaste had a statistically significant increase in the incidence of tumors of any type. However, administration of chloroform in arachis oil was associated with an increase in the incidence of benign and malignant renal tumors. The U.S. EPA (1985a) found that the increase in malignant renal tumors in animals given chloroform and arachis oil was significantly different from controls ($p < 0.01$, test not specified), as was the combined incidence of benign and malignant renal tumors ($p < 0.001$, test not specified) (Table 16).

Tumasonis *et al*. (1985, 1987)

Tumasonis *et al*. (1985, 1987) administered chloroform to Wistar rats in drinking water. Treatment began when the animals were weanlings, and continued for 180 weeks. Based on three week-long preliminary toxicity studies, Tumasonis *et al*. (1985, 1987) determined that the maximum acceptable concentration of chloroform in water for rats was 2.9 g/L, and this was the amount initially provided. Over the course of treatment, it was noted that the rats gradually increased their daily intake of water. Consequently, after 72 weeks, the concentration of chloroform was decreased to 1.45 g/L. The calculated time-weighted average doses of chloroform received by animals in this study were 200 mg/kg-day (males) and 240 mg/kg-day (females).

Rats treated with chloroform weighed less than untreated controls throughout the study. At terminal sacrifice, the average body weight of treated males was 230 g and treated females was 180 g. Control males weighed approximately 500 g and females weighed 300 g. Treated females had a statistically significant ($p < 0.03$, Fisher Exact Test) increased incidence of neoplastic nodules (site not specified) and a significantly increased incidence of hepatic adenofibrosis (Table 17). Of the 28 treated male rats necropsied, 17 had this type of tumor ($p < 0.001$, Fisher Exact Test). In treated females, hepatic adenofibrosis occurred in 34/40 animals ($p < 0.001$, Fisher Exact Test). This tumor type was not observed in male or female controls. As Tumasonis *et al*. (1985, 1987) noted, their classification of these hepatic lesions as "adenofibrosis" is conservative, and follows the classification scheme of the Institute of Laboratory Animal Resources of the National

Academy of Science (NRC, 1980). The authors of the NRC (1980) report did not consider it likely that rat bile duct cells could develop into adenomatous tumors. Others, however, have classified rat liver lesions "similar" to those observed by Tumasonis *et al*. (1985, 1987) as cholangiocellular carcinomas, i.e., carcinomas of the bile duct (Terao and Nakano, 1974; Schauer and Kunze, 1976, as cited in Tumasonis *et al*., 1985, 1987). In contrast to the findings of the NCI (1976), only one renal adenoma and one renal carcinoma were documented in treated males. Three renal tumors were observed in treated females. Because the study protocol limited histopathology to the liver and gross lesions, tumors may have been missed in tissues other than the liver.

Palmer *et al*. (1979)

Palmer *et al*. (1979) reported the results of a long-term study of chloroform in toothpaste in SD rats, conducted as one of a series of chloroform bioassays sponsored by Beecham Products (see also Roe *et al*., 1968, 1979; Heywood *et al*., 1979). Chloroform was administered daily by gavage in a toothpaste base at 15, 75, and 165 mg/kg.. Each treatment group contained 25 males and 25 females, while the concurrent vehicle control group had 75 rats of each sex. Rats in all groups developed "severe" respiratory and renal disease, and the experiment was terminated after one year. Tissue histological examination revealed one malignant tumor of the mediastinum in a high dose male, and a mammary fibroadenoma in a vehicle control female.

Subsequently, a second experiment was initiated which utilized Caesarian-derived, specific pathogen free SD rats. In this study, 50 male and 50 female rats were treated by gavage with 60 mg/kg of chloroform in a toothpaste base, six days/week for 80 weeks. An additional 50 animals of each sex received toothpaste alone. Rats were observed for 15 weeks after the end of treatment, then sacrificed. The age of animals at the onset of treatment was not specified. The percentage of animals surviving declined rapidly after the 40th week of treatment, and by the 95th week, only 26 percent of the treated females and 32 percent of the treated males were alive. Survival among controls was also poor; 14 percent of females and 22 percent of males survived until the end of the experiment.

Thirty eight chloroform-treated rats and 34 vehicle controls had tumors of various sites. Nine tumors in chloroform-treated rats were malignant, compared to eight in controls. Mammary tumors were the most frequently observed tumor type (found in 21 treated and 16 control females). Of these, 6/21 and 1/16 were malignant, respectively (no significant difference by Fisher Exact Test). No renal or hepatic tumors were found in any group, and there were no statistically significant differences between treated and untreated animals in the total number of rats with tumors (Table 17). Palmer *et al*. (1979) concluded that chloroform had "no adverse effect on survival" and did not "significantly influence the age of onset, malignancy, or location of tumors." However, the poor survival provided low sensitivity for detecting any effects.

Other Studies

Heywood *et al*. (1979) conducted a bioassay with purebred beagle dogs exposed to chloroform for a major part of their lifetime. Chloroform was given in a toothpaste base in gelatin capsules at 15 or 30 mg/kg-day, seven days/week to 48 male and 48 female beagles 18 to 24 weeks old, with eight animals/sex/dose plus vehicle and untreated

controls. Dosing continued for 376 weeks (7.2 years), followed by an observation period of "several months." Serum analysis during treatment documented a dose-related increase in serum glutamic pyruvic transaminase and serum glutamic oxaloacetic transaminase activity. These values returned to normal in all but one dog during the posttreatment period.

At the time of terminal sacrifice, 84 of the original 96 dogs were still alive. Of the 11 deaths, nine occurred in control animals. Heywood *et al*. (1979) found evidence of chloroform induced hepatotoxicity among treated animals, including bile duct hyperplasia, "minimal" hepatic fibrosis, and vacuolated histiocytes. Both treated and control animals also exhibited a high incidence of chronic interstitial nephritis. No neoplastic lesions were found in animals that died prior to completion of the study, and animals that survived the entire 376 weeks of treatment had no significant differences in tumor incidence compared to vehicle or untreated controls. No liver or kidney tumors were observed in any of the dogs, although two to four animals from each group developed hyperplastic liver nodules. One of the criticisms of this study is that the seven year dosing period is far less than the average 13 to 14 year lifespan of a beagle (U.S. EPA, 1985a). Consequently, the treatment regimen may have been inadequate to evaluate the lifetime carcinogenic risk of chloroform in dogs.

In his review of chloroform carcinogenicity, Reuber (1979) included a brief overview of Heywood *et al*. (1979). He concluded that chloroform treatment caused an increase in tumors in male and female dogs, based on an analysis of the combined tumor incidence from both sexes and/or both dosage groups. However, the only statistically significant difference was an increase by the Cochran-Armitage Trend Test ($p = 0.0378$) in the total (combined) incidence of neoplasms in females from both treatment groups.

Only one study was located regarding the carcinogenicity of inhaled chloroform. Nagano *et al*. (1998) exposed groups of 50 male and 50 female F344 rats and BDF1 mice to chloroform vapor six hours/day, five days/week for 104 weeks. Exposure concentrations were 0, 10, 30, or 90 ppm in rats and 0, 5, 30, or 90 ppm in mice. Animals were observed daily for clinical signs and mortality, and weighed periodically throughout the study. Surviving animals were sacrificed at week 105. All animals that died during the study or were sacrificed at study termination were necropsied, and all organs and gross lesions were prepared for histopathological examination. No results were presented regarding mortality, body weight, clinical signs, or nonneoplastic lesions. No increase in tumor incidence was observed in male or female rats at any concentration. In mice, there was a dose-related increased incidence of renal cell tumors in males (carcinoma: 0/50, 1/50, 4/50, and 11/48; adenoma: 0/50, 0/50, 3/50, and 1/48), and a slight apparent increased incidence of hepatocellular carcinomas in females (1/50, 1/50, 0/50, and 3/50). The researchers did not report the results of statistical tests, but the increase in renal cell carcinomas in high dose males was statistically significant ($p < 0.001$) in a Fisher exact test conducted for this assessment. Although it was not mentioned in the Nagano *et al*. (1998) paper, U.S. EPA (2001e) and IARC (1999d) reported that the high exposure levels of 30 and 90 ppm were acutely lethal to the mice, and that chronic exposure to these levels was achieved by first exposing the mice to lower concentrations and then progressively increasing the exposure concentration every two weeks until the desired

concentrations were reached (i.e., 5 ppm for two weeks, 10 ppm for two weeks, and in the high dose group 30 ppm for two weeks, before reaching the intended level).

U.S. EPA (2001e) reported the results of an unpublished drinking water study by DeAngelo (1995). Groups of 50 male F344 rats were treated with chloroform in drinking water at concentrations of 0, 900 or 1,800 ppm for 100 weeks (approximately 0, 45, or 90 mg/kg-day, assuming water intake of 0.05 L/kg-day). Satellite groups of six rats per group were sacrificed at 26, 52, and 78 weeks. Liver and kidney were examined for gross and microscopic pathology. There was a marginally significant ($p < 0.1$) increased prevalence of hepatocellular proliferative lesions (apparently comprising hyperplastic nodules as well as neoplasms) in the liver in the high dose group at 100 weeks (20.5 percent) versus controls (5.6 percent). No kidney tumors were found.

Rudali (1967) administered chloroform to 24 NLC mice by gavage in oil in two doses of 2,800 mg/kg each and observed the animals for 10 months. Five of the mice survived, and three of the five had hepatomas. The report did not mention the use of controls. U.S. EPA (2001e) reported results of a study by Voronin *et al*. (1987a) that is available only as an abstract. Mice treated with chloroform in oil had an increased incidence of unspecified tumors at 250 mg/kg-day, but not at 15 mg/kg-day. Mice treated with chloroform in the drinking water (0.0042 to 42 mg/kg-day) had no increase in tumors.

Dow Chemical Company (1999) observed no tumors in 13 and 26 week studies in P53± transgenic mice at doses up to 140 mg/kg-day in males or 240 mg/kg-day in females (the maximum tolerated dose in each sex, based on hepatotoxicity). Findings in the transgenic mice were similar to findings in wild type mice. P53± transgenic mice are predisposed to a high cancer risk because only a single mutational event is required. This model has proven useful for detection of genotoxic carcinogens (U.S. EPA, 1999c).

Tumor Promotion Studies

Deml and Oesterle (1985, 1987) examined the promoting activity of chloroform in juvenile female SD rats. Following a single initiating dose of 8 mg/kg diethylnitrosamine (DEN), chloroform was administered by gavage (25, 100, 200, or 400 mg/kg) twice a week for 11 weeks. Compared to diethylnitrosamine controls, chloroform significantly ($p = 0.01$, test not specified) increased the number and area of ATPase-deficient foci in the liver in a dose-dependent manner at doses equal to and greater than 100 mg/kg. Treatment with chloroform after initiation with diethylnitrosamine also caused a 3 - 4.5-fold increase in the number and area of gamma glutamyl transpeptidase-positive foci and glycogen-positive foci.

Pereira *et al*. (1982) also used a liver foci bioassay to evaluate promoting activity of chloroform. When a single dose (1.5 mmol/kg) of chloroform was given by gavage twice weekly for eight weeks to diethylnitrosamine-initiated rats, the results were equivocal. The incidence of gamma glutamyl transpeptidase-positive foci was significantly ($p < 0.05$, Fisher exact test) higher in animals treated with both DEN and chloroform (compared to controls), but was not significantly different when compared to rats that received diethylnitrosamine or chloroform alone.

Both Pereira *et al*. (1985) and Klaunig *et al*. (1986) have reported that chloroform administered in drinking water inhibits tumorigenesis in mice. Pereira *et al*. (1985) gave

15 day old mice initiating doses of ethylnitrosourea (ENU). At five weeks of age, treatment with chloroform (1,800 ppm) or sodium phenobarbital (500 ppm) in drinking water began, and continued until animals were 51 weeks old; animals were sacrificed one week later. Ethylnitrosourea alone (20 mg/kg) induced an increase in liver tumors in male mice, and in lung tumors in mice of both sexes (neither increase was statistically significant). Male mice treated with chloroform after initiation with ethylnitrosourea had significantly ($p \leq 0.01$, Student's t test) fewer liver tumors than ethylnitrosourea controls. Treatment with chloroform had no significant effect on lung tumor incidence in ethylnitrosourea-initiated animals, and chloroform alone did not significantly affect lung tumor incidence in mice of either sex. Klaunig *et al*. (1986) gave groups of 30 day old $B6C3F_1$ mice drinking water containing 0 or 10 mg/L diethylnitrosamine for four weeks; animals then received water containing 600 or 1,800 mg/L of chloroform for 51 weeks. In diethylnitrosamine-initiated animals, chloroform significantly decreased the average size and number of liver tumors, compared to animals given diethylnitrosamine alone (significance level not given).

Capel *et al*. (1979) found that chloroform administered in drinking water at a dose of 15 mg/kg for 14 days enhanced the growth of introduced tumor cells (Ehrlich ascites tumor cells, B16 melanoma cells, and Lewis lung tumor cells) in mice. A study in which chloroform (25 to 400 mg/kg-day) was given by gavage in oil twice/week for 11 weeks also produced positive results; there was an increased incidence of enzyme altered foci in livers of female SD rats previously initiated with N-nitrosodiethylamine, although not in uninitiated rats (Deml and Oesterle, 1985, 1987).

Herren-Freund and Pereira (1986, 1987) found that exposure to 1,800 ppm of chloroform in drinking water for 10 weeks had no effect on the incidence of hepatic gamma glutamyl transpeptidase positive foci in hepatectomized rats previously initiated with N-nitrosodiethylamine, that concurrent treatment with chloroform and N-nitrosodiethylamine did not significantly increase altered foci or tumors, and that initiation with single doses of 130 or 260 mg/kg of chloroform and promotion with phenobarbital also had no effect on gamma glutamyl transpeptidase positive foci. In fact, Reddy *et al*. (1992) observed a dose-related decrease in enzyme altered liver foci in partially hepatectomized male F344 rats initiated with N-nitrosodiethylamine and promoted with chloroform concentrations of 200, 400, 900, or 1,800 ppm in the drinking water for 12 weeks. Similarly, Daniel *et al*. (1989) observed a decrease in the incidence of gastrointestinal tumors in male F344 rats initiated with 1,2-dimethylhydrazine when promoted with chloroform (900 or 1,800 ppm in the drinking water for 39 weeks).

Toxicological Effects in Humans

The database for toxicological effects of chloroform in humans is limited. The primary source of exposure to chloroform is the ingestion of tap water, where it occurs as a disinfection byproduct. Consequently, the most relevant human data are from epidemiological studies on reproductive outcomes associated with intake of disinfected water. Studies that examined potential associations between exposure to total THMs or chloroform and adverse reproductive outcome are summarized below. Because the industrial production and use of chloroform are limited, there are limited toxicity data

available from occupational studies. Limited clinical or case report data were identified in the materials reviewed in preparation of this document. No published experimental or epidemiological data were located on genotoxicity or immunotoxicity through oral or inhalation exposure to chloroform by humans.

Acute Toxicity

Acute inhalation exposure to chloroform is most prominently associated with central nervous system depression and induction of anesthesia in humans (U.S. EPA, 1985a). Most human experience with chloroform derives from its use as an inhalation anesthetic. Concentrations used to induce anesthesia were in the range of one to three percent (10,000 to 30,000 ppm). Higher concentration (40,000 ppm) could be lethal. Once induced, anesthesia could be maintained using lower concentrations (5,000 ppm) that produced only light anesthesia if not preceded by exposure to higher levels. Concentrations lower than 1,500 ppm do not produce anesthesia, although more subtle signs of central nervous system depression have been reported at these levels. Deaths occurring during chloroform anesthesia have been attributed to respiratory and cardiac failure (Adriani, 1970). Respiratory rate increases under light chloroform anesthesia and initially during induction, but becomes depressed under deep, prolonged anesthesia, leading in some cases to respiratory failure (Adriani, 1970). The cardiovascular system is affected by chloroform in two different ways. Chloroform sensitizes the heart muscle to epinephrine, resulting in arrhythmias and in some cases, ventricular tachycardia and fibrillation. In addition, under deep anesthesia, chloroform directly depresses the myocardium, potentially resulting in asystole and cardiac failure (Adriani, 1970). Decreases in blood pressure occur secondary to depression of the myocardium and central nervous system. Smith *et al*. (1973) observed cardiac arrhythmias in 29 of 58 (50 percent) patients undergoing elective surgery and anesthetized with chloroform concentrations of 20,000 ppm or less. Hypotension (defined as a 20 percent or more decrease in blood pressure compared with preexposure levels) was observed in seven of the 58 patients (12 percent). Whitaker and Jones (1965) reported bradycardia in eight percent and cardiac arrhythmia in 1.3 percent of 1,500 patients anesthetized with chloroform. Hypotension was observed in 27 percent of the patients, and was found to be related to the duration of the exposure, as well as pretreatment with thiopentone.

Chloroform anesthesia has also been known to produce late deaths, subsequent to the anesthesia. Symptoms of delayed chloroform poisoning include progressive weakness, prolonged vomiting, jaundice, enlarged liver, hemorrhage, delirium, coma, and death secondary to hepatic dysfunction. The symptoms are first noticed one to three days after anesthesia, with death occurring on the fourth or fifth day (Wood-Smith and Stewart, 1964). In such cases, degeneration and necrosis of the liver are observed at autopsy. The effect on the liver begins immediately; 75 percent of glycogen stores are depleted after 30 minutes of chloroform anesthesia (Adriani, 1970). Subsequent changes include impaired performance in liver function tests, decreased bile formation, increased bile pigments in blood and urine, reduced blood prothrombin, and prolonged prothrombin time. The degree of liver impairment is related to the concentration used to induce anesthesia.

Other effects associated with chloroform anesthesia include inhibition of secretory activity, motility, and muscle tone in the gastrointestinal tract; nausea and vomiting

during recovery; increased blood glucose; metabolic acidosis; oliguria during anesthesia, followed by polyuria, and sometimes albuminuria and glycosuria, during recovery; and in severe cases, renal tubular necrosis (Adriani, 1970; Wood-Smith and Stewart, 1964).

Oral chloroform poisoning is rare and has usually been associated with intentional (attempted suicide, substance abuse) or accidental ingestion (Boyer *et al*., 1998; Schroeder, 1965). The generally accepted minimal lethal dose for chloroform in humans is 28 mL (400 mg/kg for a 70 kg person), although doses as low as 10 mL (143 mg/kg) have proven fatal (Schroeder, 1965). Effects seen in oral poisoning cases are generally similar to those observed with inhalation anesthesia, although there may also be severe irritation of the gastrointestinal tract (Boyer *et al*., 1998; Schroeder, 1965). Ingestion of high doses is followed within a few minutes by unconsciousness and in some cases death due to respiratory or cardiac failure. Delayed effects in patients that survive the initial exposure can include persistent vomiting, anorexia, metabolic acidosis, hepatotoxicity (jaundice, enlarged liver, elevated serum bilirubin and aspartate aminotransferase, necrosis), renal toxicity (increased blood urea nitrogen, oliguria, albuminuria, glucosuria, tubular lesions), and death four or five days after ingestion due to liver failure.

Chloroform is irritating to the skin and eyes upon direct contact. Dermal application resulted in burning pain associated with erythema, hyperemia, and vesication of the skin (U.S. EPA, 1985a). Repeated brief daily applications resulted in complete destruction of the stratum corneum after six days in two young volunteers, but milder changes in two older individuals (Malten *et al*., 1968; ATSDR, 1997). Chloroform splashed into the eye produced burning pain, conjunctival redness, and in some cases mild damage to the cornea (Winslow and Gerstner, 1978).

Subchronic Toxicity

Phoon *et al*. (1983) reported two separate outbreaks of toxic jaundice associated with subchronic chloroform exposure in workers. In the first outbreak, 13 of 102 workers of an electronics factory in Singapore developed symptoms of hepatitis, including jaundice, nausea, vomiting, and anorexia, over a nine month period. Chloroform was used as a degreaser in their work area, and airborne concentrations of the chemical exceeded the maximum range of the detector used at 400 ppm. The jaundiced workers had been exposed to chloroform for one to five months. In the second outbreak, 18 workers from another Singapore electronics factory were diagnosed with hepatitis. All of the affected workers were employed in a department where chloroform was used as a plastic adhesive and air measurements of chloroform ranged from 14 to 50 ppm. Affected workers had been exposed to chloroform for one to four months. Every one of these patients was originally diagnosed with viral hepatitis, but due to the absence of fever (which is usually associated with viral hepatitis) in most patients and the epidemiological evidence (all had high exposure to chloroform), the diagnosis was made retrospectively of toxic hepatitis resulting from chloroform exposure.

Developmental and Reproductive Toxicity

Many epidemiological studies have investigated potential links between exposure to THMs as disinfections byproducts in drinking water and adverse reproductive or

developmental outcomes in humans. These are discussed in a separate section below. Studes of human effects of exposures to chloroform itself are few.

Wennborg *et al*. (2000) studied adverse pregnancy outcomes in biomedical laboratory workers exposed to solvents in Sweden from 1990 to 1994 in a questionnaire-based study (N = 1,052). There was a marginally significant increase in risk of spontaneous abortion of pregnancies in women who had worked with chloroform with an odds ratio adjusted for mother's age and previous miscarriages of 2.3 and 95 percent CI of 0.9 to 5.9, based on 13 cases in 856 pregnancies with exposure to chloroform and 60 cases in 770 pregnancies without chloroform exposure. Exposure to chloroform presumably occurred primarily by inhalation in the laboratory workers, but was not quantified. In addition to chloroform, laboratory workers were exposed to other solvents, bacteria and viruses, and radioactive isotopes, none of which, however, showed any relationship to spontaneous abortion in this cohort. The odds ratio for large for gestational age infants in association with the mother's laboratory work was 1.9 with a 95 percent CI of 0.7 to 5.2. This study found no evidence for a relationship between maternal chloroform exposure and offspring birth weight or malformations.

Neurotoxicity

Chloroform is a well known central nervous system depressant, but no studies were located that specifically examined the neurological effects of chloroform in humans in detail. The neurological effects observed in human studies (e.g., self-reported neurological symptoms, such as dizziness or impaired memory) are described in the sections on acute and chronic toxicity of chloroform.

Chronic Toxicity

Several studies have investigated health effects in workers with chronic occupational exposure to chloroform (Challen *et al*., 1958; Bomski *et al*., 1967; Li *et al*., 1993). Exposure of these workers presumably occurred mainly by inhalation exposure, although dermal and oral exposure may have occurred in some cases as well. Challen *et al*. (1958) observed symptoms indicative of neurological effects in workers exposed to chloroform in a British factory where throat lozenges containing the chemical were produced. Complaints reported by nine out of 10 "long service operators" with exposure durations ranging from three to 10 years and exposure concentrations estimated to have been 77 to 237 ppm included nausea, loss of appetite, thirst, lassitude, sleepiness, inability to concentrate, mental "slowness," depression, and irritability, both during and after exposure. Some of the employees in question had been noticed by managers to be staggering about at times while at work. Similar, but less severe, complaints were received from eight out of 10 "short service operators" with 10 to 24 month exposure to estimated chloroform levels of 23 to 71 ppm. No similar complaints were reported by five matched control workers from other departments. Liver function tests (serum bilirubin, thymol turbidity, urinary urobilinogen) found no evidence of hepatotoxicity in any of these workers.

Similar neurological symptoms were reported in a group of 61 workers exposed to chloroform at various factories in China (Li *et al*., 1993). Estimated chloroform levels in

air ranged from 0.9 to 29 ppm (based on three representative work sites), and exposure durations ranged from one to 15 years (mean = 7.8 years). Compared with unexposed controls, significant increases were found for reports of anorexia, palpitations, fatigue, somnolence, dizziness, impaired memory, anger, and depression. Neurological testing conducted in some workers revealed significant deficiencies in tests for simple visual reaction time, digital symbol substitution, and digit span, among others. Slight liver damage in the exposed workers was indicated by increased levels of serum prealbumin and transferrin. The workers in this study were most likely exposed to an assortment of other chemicals in addition to chloroform, so it is not clear to what extent the observed effects can be attributed to chloroform exposure.

Overt hepatotoxic effects were seen in some workers with long-term occupational exposure to chloroform. Hepatomegaly was observed in 17 of 68 workers at a Polish chemical factory where chloroform was the main solvent used (Bomski *et al*., 1967). Exposure concentrations were estimated to be in the range of 2 to 205 ppm. Exposure durations ranged from one to four years. Although some other solvents (methanol, methylene chloride) were used in the plant, they were found only in trace amounts in air samples. Nausea, headache, and lack of appetite were common complaints in chloroform-exposed workers. Three of the 17 workers with enlarged livers were considered to have toxic hepatitis, based on increases in serum alanine aminotransferase, aspartate aminotransferase, and gamma globulin. The other 14 were diagnosed with fatty liver, although biopsies were not performed. Splenomegaly was found in 10 workers. The incidence of viral hepatitis was several times higher among the exposed workers than the incidence in the surrounding city, and had been for the previous few years. The researchers hypothesized that toxic liver damage from chloroform may have increased the susceptibility of workers to the viral infection.

No effects on clinical chemistry parameters indicative of effects on the liver or kidney were found in state hospital inmates exposed to chloroform in toothpaste or mouthwash for one or more years (De Salva *et al*., 1975). In the first experiment, 118 patients brushed their teeth twice daily with 1 g of toothpaste containing either 3.4 percent or zero percent chloroform for five years. In the second experiment, a group of 57 patients brushed with the 3.4 percent chloroform toothpaste and rinsed with a mouthwash containing 0.425 percent chloroform twice daily for one year, while a control group of 54 patients used toothpaste and mouthwash without chloroform. Actual doses of chloroform ingested in these studies are unknown. No differences from controls were found in either study in serum enzyme indicators of hepatic or renal toxicity (alkaline phosphatase, alanine aminotransferase, aspartate aminotransferase, blood urea nitrogen).

Carcinogenicity

Linde and Mesnick (1979) performed an historical cohort mortality study of anesthesiologists presumed to have been occupationally exposed to chloroform vapor during the years 1880 to 1890 who died during the years 1930 to 1946. There was no clear evidence of increased cancer mortality in the anesthesiologists studied. Several epidemiological studies on the association between disinfection byproducts in drinking water and cancer are summarized in a separate section below.

The U.S. EPA (1994c, 1997b, 1998h) reviewed these epidemiological studies, examining the relationship between exposure to THMs in chlorinated drinking water and cancer mortality. The most notable findings of these studies have been weak, but fairly consistent associations between exposure to chlorination byproducts in drinking water and cancers of the bladder, colon, rectum. However, none of these studies have provided a definitive conclusion about the relationship between chloroform exposure and cancer, due to the presence of numerous other potentially carcinogenic chemicals in the chlorinated drinking water. These data have been found to be inconclusive with regard to the potential carcinogenicity of chloroform in humans by IARC (1999d), ATSDR (1997), and U.S. EPA (2001e).

Bromoform

Toxicological Effects in Animals

The toxicological effects of bromoform in experimental animals have been comprehensively reviewed by U.S. EPA (1980a, 1987b, 1994b) and ATSDR (1990). Additional information is available on U.S. EPA (2008a) IRIS and in monographs prepared by the IARC (1991b, 1999b). Significant studies on the acute, short-term, subchronic, and chronic toxicity of bromoform are summarized in Table 18 below.

Table 18. Summary of Significant Noncancer Toxicological Studies on Bromoform

Reference	Species, strain	Route	Sex	N	Duration	NOAEL (mg/kg-day)	LOAEL (mg/kg-day)
Acute Studies							
Bowman *et al*. (1978)	Mouse ICR Swiss	Gavage (aqueous)	M, F	10	Single dose	500-	1,000 central nervous system effects
Chu *et al*. (1980)	Rat Sprague-Dawley	Gavage (corn oil)	M, F	10	Single dose	-	546 central nervous system effects, gross pathology
NTP (1989a)	Rat F344/N	Gavage (corn oil)	M, F	5	Single dose	500	1,000 mortality
	Mouse $B6C3F_1$	Gavage (corn oil)	M, F	5	Single dose	250	500 mortality in males
Short-term studies							
Munson *et al*. (1982)	Mouse CD-1	Gavage (aqueous)	M, F	6-12	14 days	125	250 increased serum enzyme activity, decreased immune response in males
Chu *et al*. (1982a)	Rat Sprague-Dawley	Drinking water	M	10	28 days	80	--
Condie *et al*. (1983)	Mouse CD-1	Gavage (corn oil)	M	8-16	14 days	145	289 kidney, liver histopathology

Reference	Species, strain	Route	Sex	N	Duration	NOAEL (mg/kg-day)	LOAEL (mg/kg-day)
NTP (1989a)	Rat F344/N	Gavage (corn oil)	M, F	5	14 days	200	400 decreased body weight gain in males
	Mouse $B6C3F_1$	Gavage (corn oil)	M, F	5	14 days	200	400 stomach nodules in males
Aida *et al*. (1992a)	Rat Wistar	Diet	M	7	1 month	62	187 hepatic vacuolization
			F	7	1 month	56	208 hepatic vacuolization
Potter *et al*. (1996)	Rat F344	Gavage (aqueous)	M	4	1, 3, or 7 days	379	--
Melnick *et al*. (1998)	Mouse $B6C3F_1$	Gavage (corn oil)	F	10	3 weeks (5 d/wk)	-	200 liver histopathology, increased labeling index
Coffin *et al*. (2000)	Mouse $B6C3F_1$	Gavage (corn oil)	F	10	11 days	-	200 increased labeling index
		Drinking water	F	10	11 days	-	301 increased labeling index
Subchronic Studies							
Chu *et al*. (1982b)	Rat Sprague-Dawley	Drinking water	M	20	90 days	57	218 hepatic vacuolization and other lesions
			F	20	90 days	55	283 hepatic vacuolization and other lesions
NTP (1989a)	Rat F344/N	Gavage (corn oil)	M, F	10	13 weeks (5 d/wk)	25	50 hepatic vacuolization in males
	Mouse $B6C3F_1$	Gavage (corn oil)	M, F	10	13 weeks (5 d/wk)	100	200 hepatic vacuolization in males
Chronic Studies							
Tobe *et al*. (1982)	Rat Wistar SPF	Diet	M	40	2 years	22	90 serum enzyme change, altered liver appearance
			F	40	2 years	38	152 serum enzyme change, altered liver appearance
NTP (1989a)	Rat F344/N	Gavage (corn oil)	M, F	50	103 weeks (5 d/wk)	-	100 decreased terminal body weight, liver histopathology

Reference	Species, strain	Route	Sex	N	Duration	NOAEL (mg/kg-day)	LOAEL (mg/kg-day)
	Mouse $B6C3F_1$	Gavage (corn oil)	M	50	103 weeks (5 d/wk)	100	--
			F	50	103 weeks (5 d/wk)	-	100 decreased body weight, fatty change in the liver

Acute Toxicity

Bowman *et al*. (1978) assessed the acute toxicity of bromoform in ICR Swiss mice. Male and female mice (10/sex/group) were treated with single bromoform doses ranging from 500 to 4,000 mg/kg. At least seven dose levels were utilized in each experiment. Bromoform was solubilized in a solution of Emulphor®:alcohol:saline (1:1:8) and administered by gavage to fasted animals. The post-treatment observation period was 14 days. LD_{50}s were 1,400 (95 percent CI 1,205 to 1,595) and 1,550 mg/kg (95 percent CI 1,165 to 2,062) for males and females, respectively. Ataxia, sedation, and anesthesia occurred within 60 minutes of treatment at doses of 1,000 mg/kg and above. The sedation lasted approximately four hours. Published results on the acute oral toxicity (LD_{50}) of bromoform in rats and mice are summarized in Table 19.

Chu *et al*. (1980, 1982a) evaluated the acute toxicity of bromoform in male and female SD rats (10/sex/dose). Fasted adults received doses of 546, 765, 1,071, 1,500, or 2,100 mg/kg by gavage in corn oil. The post-treatment observation period was 14 days. The LD_{50}s in male and female rats were 1,388 (95 percent CI 1,167 to 1,693) and 1,147 mg/kg (95 percent CI 890 to 1,524), respectively. Clinical signs observed in treated rats included sedation, flaccid muscle tone, ataxia, piloerection, and hypothermia. Gross pathological examination revealed liver and kidney congestion in treated animals.

Table 19. Oral LD_{50} Values for Bromoform

Species	Strain	Route (vehicle)	Sex	Number per dose group	LD_{50} (95% Confidence Interval) mg/kg	Reference
Mouse	ICR Swiss	Gavage (aqueous)	M F	10 10	1,400 (1,205-1,595) 1,550 (1,165-2,062)	Bowman *et al*. (1978)
Rat	Sprague-Dawley	Gavage (corn oil)	M F	10 10	1,388 (1,167-1,693) 1,147 (890-1,524)	Chu *et al*. (1980)
Rat	F344/N	Gavage (corn oil)	M F	5 5	933 (669-1,301) 933 (669-1,301)	NTP (1989a)
Mouse	$B6C3F_1$	Gavage (corn oil)	M F	5 5	707 (404-1,239) 1,072 (768-1,495)	NTP (1989a)

Chu *et al*. (1982a) reported results for growth, food intake, organ weight, histopathology, hematological indices, liver microsome aniline hydroxylase activity, and serum chemistry in surviving rats from the study conducted by Chu *et al*. (1980). Lymphocyte counts were decreased in male rats at 765 and 1,071 mg/kg and in female rats at 765 mg/kg, but the effect was not dose-dependent. Female rats administered a 765 mg/kg dose had elevated aniline hydroxylase levels.

The NTP (1989a) investigated the acute oral toxicity of bromoform in male and female F344/N rats. Five rats/sex/group received a single oral dose of 125, 250, 500, 1,000, or 2,000 mg/kg by gavage in corn oil. Fasting status was not reported. The posttreatment observation period was 14 days. At sacrifice, necropsy was performed on at least one animal from each sex and dose group. An LD_{50} value of 933 mg/kg (95 percent CI 669 to 1,301) was obtained for both male and female rats. Shallow breathing was observed in rats that received the 1,000 or 2,000 mg/kg doses. No other clinical signs were reported.

NTP (1989a) investigated the acute oral toxicity of bromoform in male and female $B6C3F_1$ mice. Five mice/sex/dose received a single 125, 250, 500, 1,000, or 2,000 mg/kg oral dose by gavage in corn oil. LD_{50} values of 707 mg/kg (95 percent CI 404 to 1,239) and 1,072 mg/kg (95 percent CI 768 to 1,495) were obtained for male and female mice, respectively. The final mean body weight of mice that survived to the end of the study period was unaffected by bromoform exposure. Male mice that received doses of 500, 1,000, or 2,000 mg/kg and females that received 1,000 or 2,000 mg/kg were lethargic. Shallow breathing was noted in male mice at the 1,000 or 2,000 mg/kg dose.

Short-Term Toxicity

Chu *et al*. (1982a) administered bromoform (5, 50, and 500 ppm) to male SD rats (10/group) in drinking water containing 0.25 percent Emulphor® for 28 days. Water intake was measured twice/week; body weight and food consumption were measured weekly. Serum biochemical and hematological parameters, hepatic microsomal enzyme activities, selected nonmicrosomal enzyme activities, gross pathology, and histopathology were assessed at study termination. Estimated daily doses were 0, 0.7, 8.5, or 80 mg/kg-day, as calculated by the study authors. There were no treatment-related deaths in the study. The study authors reported that no dose-related biochemical, hematological, or histologic changes occurred in treated animals, identifying a NOAEL of 80 mg/kg-day.

Munson *et al*. (1982) administered bromoform (0, 50, 125, and 250 mg/kg-day) in water by gavage to male and female CD-1 mice (6-12/sex/group) for 14 days. Changes in body and organ weights, clinical chemistry and hematological parameters, and humoral- and cell-mediated immune system functions were evaluated. Effects on immune system response are discussed in this document under Immunotoxicity. Body weights were significantly decreased in high dose females, while body weights in males were significantly increased at the mid and high doses. Absolute and relative liver weights were significantly increased in males at the mid and high dose and in females at the high dose. Absolute spleen weight was decreased in mid and high dose females. Fibrinogen was significantly decreased in males at the high dose. Statistically significant changes in clinical chemistry results included decreased glucose levels (high dose males), increased

aspartate aminotransferase activity (high dose males and females), and decreased blood urea nitrogen levels (high dose males). A NOAEL of 125 mg/kg-day and a LOAEL of 250 mg/kg-day were identified, based on changes in clinical chemistry parameters.

Condie *et al*. (1983) dosed 8-16 male CD-1 mice per dose group) with 0, 72, 145, or 289 mg/kg-day of bromoform by gavage in corn oil for 14 days. Body weight was measured on days one and 14. Blood was collected for clinical chemistry at study termination. Kidney cortical slices were collected for measurement of *para*-aminohippurate uptake and liver and kidney tissue were examined for histopathology. No treatment-related clinical signs were reported. Body weight was unaffected by bromoform treatment. Biochemical evidence of liver damage (elevated alanine aminotransferase) and kidney damage (decreased *para*-aminohippurate uptake by kidney slices) was observed at the high dose. Minimal to moderate liver and kidney injury were observed at the high dose. Lesions that appeared to be dose-related included epithelial hyperplasia and mesangial nephrosis in the kidney, and centrilobular pallor, mitotic figures, and focal inflammation in the liver. A NOAEL of 145 mg/kg-day and a LOAEL of 289 mg/kg-day were identified on the basis of histopathological changes in the kidney and liver.

NTP (1989a) investigated short-term oral toxicity of bromoform in F344/N rats and $B6C3F_1$ mice. Groups of male and female rats (five/sex/group) were administered 0, 100, 200, 400, 600, or 800 mg/kg-day of bromoform in corn oil by gavage for 14 days. All rats that received 600 or 800 mg/kg-day died before the end of the study. One death was observed at 400 mg/kg-day. These rats exhibited lethargy, labored breathing, and ataxia. Increased lacrimation was observed in the 800 mg/kg group. Mean final body weights at 400 mg/kg-day were decreased by 14 percent in male rats and by four percent in female rats relative to controls. A NOAEL of 200 mg/kg-day and a LOAEL of 400 mg/kg-day were identified in this study, based on mortality and decreased body weight.

The female mice (five/group) were administered doses of 0, 100, 200, 400, 600, or 800 mg/kg-day of bromoform in corn oil by gavage for 14 days. Male mice received doses of 0, 50, 100, 200, 400, or 600 mg/kg-day. The mice were observed for effects on clinical signs, body weight and mortality. One male and one female in the high dose groups died before study termination. Ataxia and lethargy were noted at doses of 600 mg/kg-day and above. Mean final body weights were comparable to those of the controls. Raised stomach nodules were observed in males at 400 and 600 mg/kg-day and in females at 600 and 800 mg/kg-day. A NOAEL of 200 mg/kg-day and a LOAEL of 400 mg/kg-day were identified in this study, based on an increased incidence of stomach nodules in male mice.

Aida *et al*. (1992a) administered bromoform to Slc:Wistar rats (seven/sex/group) for one month at dietary levels of 0, 0.068, 0.204, or 0.612 percent for males and 0, 0.072, 0.217, or 0.651 percent for females. Bromoform was micro-encapsulated and mixed with powdered feed; the control groups received placebo capsules. Clinical effects, body weight, food consumption, hematology parameters, serum chemistry, and histopathology of all major organs were evaluated. Based on the mean food intakes, the study authors calculated mean compound intakes of 0, 61.9, 187.2, or 617.9 mg/kg-day for males and 0, 56.4, 207.5, or 728.3 mg/kg-day for females. No deaths were observed in any dose groups. High dose animals of both sexes exhibited slight piloerection and emaciation. Mean final body weight was significantly reduced (24 percent decrease) in high dose males relative to the controls. There were no significant changes in food consumption for

any group. Relative liver weight was significantly increased in mid and high dose males and females. No changes in hematological parameters were observed as a result of treatment. Observed dose-related changes in serum chemistry included significant decreases in serum glucose in low and high dose males and in mid and high dose females; triglycerides in high dose males and in mid and high dose females; cholinesterase activity in high dose males and in all female treatment groups; lactate dehydrogenase in mid and high dose females, and blood urea nitrogen in mid and high dose females. Gross and histopathological findings were limited to the liver. Discoloration was observed in all males and females in the high dose group. The incidence and severity of liver cell vacuolization and swelling were dose-related. Severe hepatic cell vacuolization was observed in 5/7 high dose males and in 6/7 females at the mid and high doses. Slight to moderate liver cell swelling was observed in three high dose males, while all high dose females displayed slight signs of liver cell swelling. NOAELs of 61.9 mg/kg-day for males and 56.4 mg/kg-day for females, and LOAELs of 187.2 mg/kg-day for males and 207.5 mg/kg-day for females were identified based on liver histopathological changes.

Potter *et al*. (1996) evaluated the effect of bromoform on hyaline droplet formation and cell proliferation in the kidney of male F344 rats. Four rats per dose received 0.75 or 1.5 mmol/kg of bromoform in four percent Emulphor® by gavage for one, three, or seven days. These doses correspond to 190 or 379 mg/kg-day, respectively. Cell proliferation in the kidney following bromoform exposure was measured *in vivo* by [^{3}H]-thymidine incorporation. No significant effects were noted following exposures of up to seven days duration, except a reduced hyaline droplet formation on day seven which might be explained by a decrease in serum testosterone concentrations.

Coffin *et al*. (2000) examined the effect of bromoform administered in corn oil by gavage or in drinking water on liver toxicity, cell proliferation and DNA methylation in female B6C3F$_1$ mice. Bromoform doses of 0, 200, or 500 mg/kg were administered by gavage to ten animals/group five days/week for two weeks. The high dose was selected because it had been demonstrated to cause cancer in female mice. Bromoform was administered in drinking water at approximately 75 percent of the saturation level, resulting in an average daily dose of 1.19 mmol/kg (301 mg/kg). The mice were sacrificed 24 hours after the last gavage dose and the livers were removed, weighed, and processed for histopathological examination, proliferating cell nuclear antigen-labeling index analysis, and determination of *c-myc* methylation status. A significant, dose-dependent increase in relative liver weight was observed in animals dosed by gavage when compared to the control. Relative liver weight was unaffected in animals administered the compound in drinking water. A slight but statistically significant increase in the severity of liver histopathology was reported in animals treated by both routes of administration. The histopathology findings for animals receiving bromoform in the drinking water were similar to those observed in the low dose gavage group. Bromoform administered by gavage caused a significant, dose-dependent increase in the proliferating cell nuclear antigen-labeling index. Bromoform also significantly enhanced cell proliferation when the compound was administered in drinking water.

Subchronic Toxicity

Three published studies have addressed the subchronic oral toxicity of bromoform. Chu *et al*. (1982b) evaluated toxicity in male and female SD rats exposed to bromoform in drinking water. NTP (1989a) evaluated bromoform toxicity in male and female F344/N rats and $B6C3F_1$ mice administered bromoform in corn oil. Summaries of these studies are provided below. No subchronic inhalation studies are available for bromoform.

Chu *et al*. (1982b) administered bromoform to male and female SD rats (20/sex/group) in drinking water at levels of 0, 5, 50, 500, or 2,500 ppm for 90 days beginning right after weaning. Doses were approximately 0, 0.65, 6.1, 57, and 218 mg/kg-day for males and 0, 0.64, 6.9, 55, and 283 mg/kg-day for females, as calculated by the study authors. Half of each group (10/sex/dose) was sacrificed at the end of the exposure period, and the remaining animals were given tap water for a 90 day recovery period. The study authors did not report the frequency of water renewal, measures taken to minimize the loss of bromoform by volatilization, or analytical verification of bromoform concentrations in the drinking water. At 2,500 ppm, food consumption was depressed in both males and females, with the decrease reaching statistical significance in males. Body weight gain was also decreased at the high dose, but the difference was not statistically significant. Lymphocyte counts were significantly decreased in high dose males and females when evaluated 90 days after cessation of treatment. The only change observed in serum biochemistry parameters was a significant decrease in lactate dehydrogenase in both males and females at the high dose. This effect was also noted 90 days after cessation of treatment. Mild histologic changes occurred in the liver and thyroid of both sexes. Although neither incidence nor severity was clearly dose-related, these parameters tended to increase with dose. The severity of hepatic lesions was significantly increased in high dose males and in females at 500 and 2,500 ppm. Hepatic lesions included increased cytoplasmic volume and vacuolation due to fatty infiltration. Thyroid lesions included decreased follicular size and colloid density and occasional focal collapse of follicles. The severity of thyroid lesions in the treated animals was not significantly different from that observed in the controls. Although the authors noted that histologic changes were mild and similar to controls when evaluated after the 90 day recovery period, males in the high dose group continued to exhibit an increased incidence of hepatic lesions with greater severity relative to control. A NOAEL of 57 mg/kg-day and a LOAEL of 218 mg/kg-day for hepatic effects in males, and a NOAEL of 55 mg/kg-day and a LOAEL of 283 mg/kg-day for hepatic effects in females were identified.

NTP (1989a) exposed male and female F344/N rats to bromoform in corn oil by gavage for five days/week for 13 weeks. Ten animals/sex/dose received doses of 0, 12, 25, 50, 100, or 200 mg/kg-day. The rats were observed twice per day and weighed weekly. At sacrifice, all animals were necropsied and tissues from the vehicle control and high dose groups were examined histologically. Although not stated in the methods section, liver tissue of males and females in other dose groups was also examined for histopathology, as indicated by results reported in the text. None of the rats died before the end of the study. Final mean body weights were similar in dosed and control groups. All high dose animals and males dosed with 100 mg/kg-day were lethargic. A dose-dependent increase in the frequency of hepatocellular vacuolation was observed in male rats (vehicle control, 3/10; 12 mg/kg-day, 6/10; 25 mg/kg-day, 5/10; 50 mg/kg-day, 8/10; 100 mg/kg-day,

8/10; 200 mg/kg-day, 10/10). This response reached statistical significance at 50 mg/kg-day when independently analyzed using the Fisher Exact test. Severity data were not reported for this lesion, but the study authors noted that vacuoles were more numerous in the 200 mg/kg-day group. Corresponding hepatic effects were not observed in females. A NOAEL of 25 mg/kg-day and a LOAEL of 50 mg/kg-day were identified based of hepatic vacuolization observed in male rats.

In a parallel study, NTP (1989a) exposed male and female $B6C3F_1$ mice to bromoform in corn oil by gavage five days/week for 13 weeks. Ten animals/sex/dose received doses of 0, 25, 50, 100, 200, or 400 mg/kg-day. The mice were observed twice per day and weighed weekly. At sacrifice, all animals were necropsied and tissues from the vehicle control and high dose groups were examined for histopathology. In addition, the liver and spleen of 200 mg/kg-day male mice and liver of 100 mg/kg-day male mice were examined for histopathology. One female who received 100 mg/kg-day died. Body weights in surviving animals were not significantly affected; although males receiving 400 mg/kg-day had final mean body weights that were approximately eight percent less than those observed in control. A dose-related increase in the incidence of hepatocellular vacuoles was seen in male mice (incidences of 5/10 at 200 mg/kg and 8/10 at 400 mg/kg reported in text; incidence in controls not explicitly stated). The severity of this effect was reported to be minimal to moderate and the response involved only a few cells or was diffuse. A corresponding hepatic response was not observed in females. A NOAEL of 100 mg/kg-day and a LOAEL of 200 mg/kg-day were identified based on hepatocellular vacuolization in male mice.

Genetic Toxicity

Although the overall data are mixed, positive results have been obtained for mutagenicity in several test strains of *S. typhimurium*; mutagenicity in cultured mouse lymphoma cells; induction of sister chromatid exchange and chromosome aberrations *in vivo* and *in vitro*; sex linked recessive lethal mutations in *Drosophila*; DNA damage in bacteria and mammalian cells; and aneuploidy in *Aspergillus nidulans*. Thus, the weight of the available evidence indicates that bromoform is mutagenic and genotoxic.

In Vitro Assays

Genotoxicity of bromoform has been evaluated in numerous *in vitro* assays with bacteria and eukaryotic cells (Table 20). Relevant studies are briefly described by endpoint below. A potential limitation of the database on bromoform *in vitro* genotoxicity is the failure of many authors to indicate whether the tests were conducted in a closed system to prevent volatilization loss of bromoform from the incubation mixture. Use of a sealed test system is noted in cases where the study authors provided this information.

Table 20. Summary of *In Vitro* Genotoxicity Studies on Bromoform

Endpoint	Assay system	Results (with/without metabolic activation)	References
Gene mutation – Bacteria *(Salmonella typhimurium)*	TA100[a], TA1535	NT/+	Simmon *et al*. (1977), Simmon and Tardiff (1978)
	TA100	NT/+	Rapson *et al*. (1980)
	TA100 TA98, TA1535, TA1537	-/(+) -/-	Haworth *et al*. (1983)
	TA100 TA98, TA1535, TA1537	-/(+) -/-	Varma *et al*. (1988)
	TA100, TA102 TA98, TA97	-/- -/+	Mersch-Sunderman (1989)
	TA1535, TA1537[b] TA100 TA97, TA98	-/- -/±[b] ±[b]/-	NTP (1989a)
	TA100	-/+	Ishidate *et al*. (1982)
	TA98 TA100, TA1538[a]	+/+ -/-	Zeiger (1990)
	TA100 (fluctuation test)	-/+	LeCurieux *et al*. (1995)
	Arabinose resistance assay (*Ara*) BA13/BAL13	-/+[b]	Roldan-Arjona and Pueyo (1993)
	RSJ 100	NT/+	DeMarini *et al*. (1997)
Gene mutation – Mammalian cells	Mouse lymphoma cells	+/+	NTP (1989a)
Chromosome aberration	Chinese hamster fibroblasts	+/-	Ishidate *et al*. (1982)
	Chinese hamster ovary cells	-/±	Galloway *et al*. (1985), NTP (1989a)
DNA damage	*Escherichia coli* PQ37, SOS chromotest	+/+	LeCurieux *et al*. (1995)
	Human lymphocytes in whole blood culture	NT/+	Landi *et al*. (1999b)
Sister chromatid exchange	Toadfish leukocytes [a]	NT/-	Maddock and Kelly (1980)
	Human lymphocytes	NT/+	Morimoto and Koizumi (1983)
	Chinese hamster ovary cells	-/±	Galloway *et al*. (1985), NTP (1989a)
	Human lymphocytes	NT/-	Landi *et al*. (1999a)

NT = Not Tested
[a] Assay was conducted in a closed system.
[b] Equivocal results were obtained.

Gene Mutation in Bacteria

Multiple studies have reported positive results for mutagenicity in *S. typhimurium* strain TA 100 in the absence of metabolic activation. Simmon *et al*. (1977) and Simmon and Tardiff (1978) reported that bromoform was mutagenic in this test strain when assayed as

a vapor in a desiccator at a minimum amount of 570 µmol. Rapson *et al*. (1980) obtained positive results in TA 100 in the absence of metabolic activation when tested at 500 µg/mL. Ishidate *et al*. (1982) also obtained positive results, but did not report an effective concentration. LeCurieux *et al*. (1995) obtained positive results using the fluctuation test procedure when bromoform was tested at 300 µg/L.

Varma *et al*. (1988) evaluated the mutagenic potential of bromoform in *S. typhimurium* strains TA 98, TA 100, TA 1535, and TA 1537. A slight increase in revertants (230 versus 130 spontaneous revertant colonies) was observed for TA 100 in the absence of metabolic activation. The results of the other assays were negative.

Mersch-Sunderman (1989) obtained negative results for mutagenicity in S. *typhimurium* strain TA 100 in the absence of metabolic activation, in contrast to the positive results reported in the studies above. However, positive results were obtained in this study for test strains TA 97 and TA 98 in the absence of metabolic activation.

NTP (1989a) evaluated the mutagenic potential of bromoform in *S. typhimurium* strains TA 100, TA 1535, and TA 1537. Concentrations of 0.04 to 13 µmol/plate (10 to 3,333 µg/plate) produced no evidence of mutagenicity in strains TA 1535 or TA 1537 with or without exogenous metabolic activation by rat or hamster liver S9 fraction. Equivocal evidence of mutagenicity was noted in strain TA 100 without activation, and in strains TA 97 and TA 98 in the presence of liver microsomes prepared from Aroclor-induced Syrian hamsters. Results reported by NTP (1989a) were reevaluated and published in Haworth *et al*. (1983). Haworth and coworkers concluded that bromoform is mutagenic in the absence of metabolic activation, based on results obtained in test strain TA 100.

Zeiger (1990) reported positive results for mutagenicity in *S. typhimurium* strain TA 98 when bromoform was tested as a vapor in a closed system using a preincubation protocol. In contrast, results were negative when tested in an open system. Positive results were observed at levels of at least 114 µmol/desiccator, in the presence and absence of S9 prepared from rat or hamster liver. Bromoform was negative in the closed system in strains TA 100 and TA 1538 with or without rat or hamster liver S9 fraction.

Roldan-Arjona and Pueyo (1993) evaluated bromoform in the *S. typhimurium* arabinose resistance test *(Ara)* forward mutation assay at concentrations up to 25 µmol/plate (6.3 mg/plate), utilizing a preincubation protocol. Although a clear dose-related response was observed in the absence of activation, the results were classified as questionable because a doubling of background levels was not achieved. There was no evidence of mutagenicity in the presence of exogenous metabolic activation. Although no attempt was made to minimize volatilization of the test compound, cytotoxicity observed at the high exposure level indicated that the test material reached the cells.

Gene Mutation in Mammalian Cells

NTP (1989a) evaluated the genotoxic potential of bromoform in mouse L5178Y cells. Exposure to bromoform concentrations greater than or equal to 2,300 µM in the absence of S9 activation or concentrations of at least 300 µM with S9 activation resulted in forward mutations at the thymidine kinase (tk) locus.

Sister Chromatid Exchange

Maddock and Kelly (1980) reported that bromoform did not induce sister chromatid exchange when toadfish leukocytes were exposed to bromoform concentrations of 0.4 to 400 µM. In contrast, Morimoto and Koizumi (1983) obtained positive results for induction of sister chromatid exchange in human lymphocytes in the absence of S9 activation. NTP (1989a) reported equivocal results for induction of sister chromatid exchange in Chinese hamster ovary (CHO) cells exposed to bromoform in the absence of metabolic activation. One of two laboratories conducting the NTP assays reported increased frequency of sister chromatid exchange in Chinese hamster ovary cells exposed to 3,800 µM of bromoform. Both laboratories observed negative results for induction of sister chromatid exchange in the presence of metabolic activation. Landi *et al*. (1999a) examined induction of sister chromatid exchange in whole blood cultures of human peripheral blood lymphocytes exposed to bromoform *in vitro*. No differences in sister chromatid exchanges per cell were noted in lymphocytes from glutathione *S*-transferase theta gene *GSTT1-1* positive or negative donors.

Chromosome Aberration

Induction of chromosomal aberrations by bromoform has been evaluated in two *in vitro* studies. Ishidate *et al*. (1982) observed chromosomal aberrations in Chinese hamster fibroblasts in the presence, but not in the absence, of exogenous metabolic activation by S9 fraction. This pattern contrasts with the results for mutagenicity in bacteria, where positive results were noted in the absence (rather than the presence) of metabolic activation. Test concentrations were not reported. NTP (1989a) obtained equivocal results for chromosome aberrations in Chinese hamster ovary cells in the absence of exogenous metabolic activation and negative results in the presence of S9 fraction.

Other *In Vitro* Genotoxicity

Positive results were reported in two *in vitro* tests of DNA damage induction. LeCurieux *et al*. (1995) obtained positive results for the SOS chromotest in *Escherichia coli* strain PQ37 with and without metabolic activation. Landi *et al*. (1999b) obtained weakly positive results for DNA damage in human lymphocytes cultured *in vitro* using the Comet assay. CCRF-CEM human lymphoblastic leukemia cell lines exposed to brominated THMs at 5 or 10 mM for two hours produced DNA strand breaks *in vitro* with bromoform the highest potency, followed by DBCM, then BDCM (Geter *et al*., 2004). However, Geter *et al*. (2004) reported negative results of *in vitro* DNA strand breaks in primary rat hepatocytes exposed to bromoform at 5 or 10 mM for four hours.

The brominated THMs are genotoxic in some *in vitro* test systems (LeCurieux *et al*., 1995). These authors used the SOS chromotest, the *S. typhimurium* fluctuation test, and the newt micronucleus test. With the SOS chromotest all the THMs except chloroform were found to induce primary DNA damage in *Escherichia coli* PQ37. In the fluctuation test with *S. typhimurium* TA100 only, bromoform showed mutagenic activity.

The newt micronucleus assay detected a clastogenic effect on the peripheral blood erythrocytes of *Pleurodeles waltl* larvae from BDCM and bromoform. Benigni *et al*. (1993) obtained positive results for induction of aneuploidy in the mold *Aspergillus nidulans*. The lowest effect concentration was 3.43 mmol.

In Vivo Assays

In vivo data for bromoform genotoxicity are available for chromosome aberrations, induction of micronuclei, sister chromatid exchange, DNA damage, and other endpoints. The results of *in vivo* genotoxicity tests on bromoform are summarized in Table 21.

Chromosome Aberration

Chromosome aberration data on bromoform are available from two *in vivo* studies. NTP (1989a) obtained negative results for chromosomal aberrations in bone marrow cells following intraperitoneal injection of mice with doses up to 800 mg/kg bromoform. Fujie *et al*. (1990) analyzed chromosome aberrations in bone marrow from Long-Evans rats following oral (males only) or intraperitoneal (males and females) exposure to bromoform. Oral doses were administered by gavage in saline for five consecutive days. Bromoform induced a dose-related increase in the incidence of aberrant cells, with a statistically significant increase at 253 mg/kg-day. A more pronounced increase in clastogenic activity was observed following a single intraperitoneal dose, with a statistically significant effect at 25.3 mg/kg. The predominant types of induced aberrations were chromatid and chromosome breaks for both administration routes.

Table 21. Summary of *In Vivo* Genotoxicity Studies on Bromoform

Endpoint	Assay system	Result	References
Micronucleus induction	Mouse, bone marrow cells	-	Hayashi *et al*. (1988)
	Mouse, bone marrow cells	-	Stocker *et al*. (1997)
	Mouse, bone marrow cells	+	NTP (1989a)
	Newt, peripheral erythrocytes	+	LeCurieux *et al*. (1995)
Chromosome aberrations	Mouse, bone marrow cells	-	NTP (1989a)
	Rat, bone marrow cells (oral) Rat, bone marrow cells (i.p.)	+ +	Fujie *et al*. (1990)
Sister chromatid exchange	Mouse, bone marrow cells	+	Morimoto and Koizumi (1983)
	Mouse, bone marrow cells	+	NTP (1989a)
DNA damage	Rat, renal cells	-	Potter *et al*. (1996)
Unscheduled DNA synthesis	Rat, hepatocytes	-	Stocker *et al*. (1997)
Sex linked recessive lethal mutations	*Drosophila melanogaster*	+	Woodruff *et al*. (1985), NTP (1989a)
Heritable translocations	*Drosophila melanogaster*	-	Woodruff *et al*. (1985), NTP (1989a)
Aneuploidy	*Aspergillus nidulans*	+	Benigni *et al*. (1993)
Initiation	Rat, liver	-	Herren-Freund and Pereira (1986)

Sister Chromatid Exchange

Data for sister chromatid exchange are available from two studies conducted in mice. Morimoto and Koizumi (1983) evaluated sister chromatid exchange in bone marrow cells of ICR/SJ mice administered bromoform in olive oil by gavage. Positive results were obtained in mice administered four 25 mg/kg doses. NTP (1989a) evaluated sister chromatid exchange in bone marrow cells of $B6C3F_1$ mice administered 200 to 800 mg/kg doses of bromoform ip. Positive results were obtained at the 800 mg/kg dose.

Micronucleus Induction

Micronucleus induction has been measured in at least four *in vivo* studies. NTP (1989a) tested bromoform in $B6C3F_1$ mice, obtaining positive results following two 800 mg/kg doses ip. Hayashi *et al*. (1988) obtained negative results following ip administration of a 1,000 mg/kg dose of bromoform in ddY mice. Stocker *et al*. (1997) administered gavage doses of 250 to 1,000 mg/kg bromoform in aqueous one percent methylcellulose to Swiss CD-1 mice. No significant difference was noted in the frequency of micronucleus induction in bromoform-treated animals at 24 and 48 hours after dosing. LeCurieux *et al*. (1995) obtained positive results in newt larvae (*Pleurodeles waltl*) peripheral erythrocytes following a six day exposure to 2.5 µg/mL of bromoform.

Other *In Vivo* Genotoxicity

Herren-Freund and Pereira (1986) assessed the initiating potency of bromoform using the rat liver gamma glutamyl transpeptidase negative foci assay. A 250 mg/kg oral dose of bromoform in an unspecified vehicle did not initiate gamma glutamyl transpeptidase positive foci under the conditions used in this test.

NTP (1989a) studied the genotoxic potential of bromoform in *Drosophila melanogaster*. Adult male fruit flies fed a 1,000 ppm solution of bromoform exhibited an increased frequency of sex-linked recessive lethal mutations. No significant effect on the frequency of reciprocal translocations was observed. These data are also reported in Woodruff *et al*. (1985).

Potter *et al*. (1996) evaluated the effect of bromoform on the incidence of DNA strand breaks in the kidney. Male F344 rats received 190 or 379 mg/kg of bromoform in four percent Emulphor® by gavage for one, three, or seven days. No effect was observed on DNA strand breaks evaluated with the alkaline unwinding assay one day after the final dose. No DNA strand breaks in liver, kidney, or duodenum epithelial cells were observed *in vivo* in male F344/N rats administered 0.3 or 0.6 mM/kg as a single gavage dose of bromoform in 0.5 percent Emulphor® for four hours (Geter *et al*., 2004).

Stocker *et al*. (1997) assessed *in vivo* genotoxicity of bromoform by measuring unscheduled DNA synthesis in the livers of bromoform-treated rats. Male SD rats received single doses of 324 or 1,080 mg/kg bromoform by gavage in aqueous one percent methylcellulose. The authors selected these doses to correspond to 30 and 100 percent of the calculated maximum tolerated dose. Hepatocytes were examined for unscheduled DNA synthesis two and 14 hours after treatment. Bromoform was also tested in the mouse bone marrow micronucleus test at 0, 250, 500, and 1,000 mg/kg; the maximum tolerated dose over a four day observation period was 1,000 mg/kg.

Bromoform did not cause unscheduled DNA synthesis in rat liver nor induce micronuclei in mouse bone marrow.

Mode of Action Studies

DeMarini *et al*. (1997) investigated the role of glutathione *S*-transferase activity in the mutagenicity of bromoform in *Salmonella typhimurium*. Strains of *Salmonella* utilized for this investigation included RSJ 100, which has been engineered to express the rat glutathione *S*-transferase theta 1-1 (*GSTT1-1*) gene, and TPT 100, which has the rat *GSTT1-1* gene inserted in a nonfunctioning orientation. Exposure to 1,600 ppm bromoform induced a 95-fold increase in revertant colonies in the RSJ 100 compared to background revertant formation. Bromoform-induced mutations at the *his*G46 allele in strain RSJ 100 were analyzed using the colony probe hybridization method. DeMarini *et al*. (1997) also studied the glutathione *S*-transferase mediated induction of GC → AT transitions by dichloromethane, BDCM, and DBCM in RSJ 100. All four halomethanes were mutagenic in RSJ 100. Molecular analysis showed that 96 to 100 percent of the base pair substitutions induced by the halomethanes at the *hisG46* allele in RSJ 100 were GC → AT transitions, and 87 to 100 percent of these were at the second position of the CCC/GGG target. Bromoform also induced GC → AT transversions (2.8 percent), at the second position of the CCC target. Dichloromethane was tested in *S. typhimurium* strain TA 100 (which does not express *GSST1-1*) for comparison. In contrast to the bromoform-induced mutations in RSJ 100, only 15 percent of the dichloromethane-induced mutations in TA 100 were GC → AT. This suggests that overexpression of *GSTT1-1* in RSJ 100 mediated bromoform mutagenicity and induced a specific type of mutational lesion in *Salmonella*. The proposed pathway for bromoform bioactivation is illustrated in Figure 2 in the metabolism section.

Developmental and Reproductive Toxicity

There are just two studies on the reproductive or developmental toxicity of bromoform. Ruddick *et al*. (1983) investigated the reproductive and developmental toxicity of bromoform in SD rats. NTP (1989b) investigated the effect of bromoform on fertility and reproduction in Swiss CD-1 mice using a two generation continuous breeding protocol. The methodology used in each study was generally consistent with standard guidelines for reproductive or developmental studies (U.S. EPA, 1998a,b). The significant results are summarized in Table 22.

In the Ruddick *et al*. (1983) study, pregnant SD rats (14-15/dose group) were administered 0, 50, 100, or 200 mg/kg-day of bromoform in corn oil by gavage on gestation days six to 15. Body weights were measured on gestation day one, days six through 15, and before and after pups were delivered by caesarean section on gestation day 22. Maternal blood samples were evaluated for standard hematology and clinical chemistry parameters. The dams were sacrificed on gestation day 22 and tissues were removed for pathological examination. Weights of liver, heart, brain, spleen, and one kidney were determined. Standard histopathology was conducted on five control and high dose females per group.

Table 22. Significant Reproductive and Developmental Studies on Bromoform (ATSDR, 1990; IARC, 1991b, 1999b; U.S. EPA, 1980a, 1987b, 1994b, 2005a)

Reference	Species, strain	Route	Sex	N	Duration	NOAEL (mg/kg-day)	LOAEL (mg/kg-day)
Ruddick *et al*. (1983)	Rat Sprague-Dawley	Gavage (oil)	F	14-15	Gestation day 6 - 15 (prenatal toxicity protocol)	100	200 sternebral variations
NTP (1989a)	Mouse ICR Swiss	Gavage (oil)	M, F	20	105 days (continuous breeding protocol)	100	200 decreased postnatal survival, organ weight changes, and liver histopathology in F_1 mice

Ruddick *et al*. (1983) evaluated each dam for number of resorption sites and number of fetuses. Fetuses in all groups were individually weighed, and evaluated for viability and external malformations. Histopathologic examination was performed on two fetuses per litter. Of the remaining live fetuses, approximately two-thirds were examined for skeletal alterations and one third for visceral abnormalities. No information was provided on food and water consumption and on the sex and sex ratio of fetuses. Maternal weight gain, organ weights, hematology, and clinical chemistry were unaffected by bromoform treatment. No significant treatment-related differences were observed for resorption sites, fetuses per litter, and fetal weights, gross malformations, and visceral abnormalities. No treatment-related histopathological effects were noted in dams or fetuses. However, an increased incidence of several skeletal variations was reported, including 14th rib, wavy ribs, interparietal deviations, and sternebra aberrations (Table 23). The incidence of affected fetuses per number of affected litters in the 0, 50, 100, and 200 mg/kg-day groups, respectively, was 3/3, 4/3, 4/3 and 7/5 for a 14th rib; 1/1, 5/3, 6/5, and 13/8 for sternebra aberrations; 1/1, 1/1, 6/3, and 6/4 for interparietal variations; and 1/1, 0/0, 0/0, and 6/4 for wavy ribs.

Table 23. Summary Data for Skeletal Variations in Ruddick *et al*. (1983)

Dose	Fetuses examined	Litters examined	Incidence of skeletal variations (fetuses affected/litters affected)			
			14th Rib	Sternebra variations	Interparietal variations	Wavy ribs
0	112	15	3/3	1/1	1/1	1/1
50	115	15	4/3	5/3	1/1	0/0
100	107	14	4/3	6/5	6/3	0/0
200	115	15	7/5	13/8*, **	6/4	6/4

* Statistically significant ($p < 0.05$) by the Fisher Exact test
** Statistically significant trend with dose ($p < 0.02$) by Cochran-Armitage trend test.

Statistical significance for skeletal variations was not reported by the study authors. OEHHA analyzed the data using the Fisher Exact test and the Cochran-Armitage trend test. The incidence of sternebra aberrations was significantly different from controls at the highest dose of bromoform tested (200 mg/kg-day) and there was a statistically significant dose-related trend ($p < 0.002$) for this endpoint. These findings suggest that the LOAEL and NOAEL for fetotoxicity are 200 and 100 mg/kg-day, respectively. The NOAEL for maternal toxicity was 200 mg/kg-day, the highest dose tested.

NTP (1989b) investigated the effect of bromoform on fertility and reproduction in Swiss CD-1 mice using the Reproductive Assessment by Continuous Breeding (RACB) protocol. Twenty male-female pairs received daily doses of 50, 100, or 200 mg/kg-day by gavage in corn oil. An additional forty male-female pairs were dosed with the corn oil vehicle only. Dose selection was based on a 14 day range finding study. Both males and females were dosed for seven days precohabitation and 98 day during cohabitation. The parameters evaluated were fertility, litters/pair, live pups/litter, proportion of pups born alive, sex of live pups, and pup body weights. The last litters born (generally the fifth litter) in control and 200 mg/kg-day groups were reared by the dams, weaned and raised to sexual maturity (ca. 74 days). Gavage treatment was initiated on postnatal day 22, with offspring receiving the same treatment (vehicle control or 200 mg/kg-day bromoform) as their parents. At sexual maturity, males and females from different litters within the same treatment group were cohabited for seven days and then housed individually until delivery. The endpoints for this mating trial were the same as for the parental generation. At study termination, the F_1 mice were weighed, necropsied and evaluated for selected organ weights, epididymal sperm motility, and sperm morphology. Selected organs were fixed for histopathological examination. This version of the RACB protocol is considered compatible with U.S. EPA's two-generation reproductive and developmental toxicity protocol (U.S. EPA, 1996a).

The fertility index for the parental generation was 100 percent for the control and treated groups (a breeding pair was designated as fertile if they produced at least one live or dead pup). In the 200 mg/kg-day group, the dam body weights at delivery were consistently less than control. The reduction in body weight was statistically significant after delivery of the first, second, fourth, and fifth litters. There was no detectable effect of treatment on the number of litters per pair, live pups per litter, proportion of pups born alive, sex of live pups, or pup body weights. The gestational period was similar across groups. Postnatal survival of F_1 pups at 200 mg/kg-day was significantly lower than in controls. The study authors reported that this difference was primarily attributable to three dams that lost all of their pups by postnatal day four. One dam in the control group also lost her litter by postnatal day four. The study authors noted that this result is consistent with a treatment effect on early maternal behavior, early lactational failure, and/or postnatal development. When F_1 mice were cohabited for one week, no effect of treatment on mating index or fertility was observed. There were no significant differences relative to control for live pups per litter (male, female, or combined), proportion of live pups, proportion of male pups, or pup weight at birth. At sacrifice, male and female F_1 mice administered 200 mg/kg-day exhibited increased relative liver weights and decreased relative kidney weights compared to controls. The mean body weight of F_1 males was significantly less than that of the controls. Minimal to moderate hepatocellular degeneration was noted in the livers of high dose F_1 males and females. Bromoform

treatment had no effect on epididymal sperm density, motility, or morphology in F_1 males. No treatment-related histologic lesions were observed in the seminal vesicles, coagulating glands, or prostate glands of males, or in the lung, kidney, or thyroid gland of either sex. Based on liver histopathology, decreased postnatal survival, and other signs of toxicity (e.g., changes in organ weights) in F_1 mice of both sexes at the highest dose tested, a LOAEL of 200 mg/kg-day and a NOAEL of 100 mg/kg-day were identified. Based on consistently decreased body weights of pregnant dams at delivery, the LOAEL for maternal toxicity is 200 mg/kg-day and the NOAEL is 100 mg/kg-day.

Immunotoxicity

Few experimental data are available on the immunotoxicity of bromoform. Munson *et al*. (1982) investigated potential immunological effects of bromoform in male and female CD-1 mice. The mice (6-12/sex/dose) received 0, 50, 125, or 250 mg/kg-day by gavage in 10 percent Emulphor® for 14 days. Humoral immune system function was assessed by the primary IgM response to sheep erythrocytes as estimated by hemolytic plaque assay and by plasma antibody titer determined by hemagglutination. Cell-mediated immunity was assessed by the delayed-type hypersensitivity response to sheep erythrocytes. Humoral immune function in males was significantly depressed at the high dose, as indicated by decreased numbers of antibody-forming cells, expressed either as AFC/spleen or AFC/10^6 cells. Cell-mediated immune response was also decreased at the high dose, as indicated by a significantly decreased stimulation index. A clear dose response pattern was not observed for either humoral or cell-mediated responses. The authors reported no treatment-related effects on immune system function in the females. Based on the results of this study, a NOAEL and a LOAEL for immunotoxicity of 125 mg/kg-day and 250 mg/kg-day, respectively, were identified.

Neurotoxicity

Bromoform is a central nervous system toxicant at higher doses. In an acute study, ataxia, sedation, and anesthesia occurred in mice within 60 minutes after a single gavage dose of 1,000 mg/kg (Bowman *et al*., 1978). Sedation persisted for four hours after treatment. Clinical signs observed in rats treated with up to 2,100 mg/kg included sedation, flaccid muscle tone, ataxia, piloerection, and hypothermia (Chu *et al*., 1980, 1982a). Signs observed in rats dosed with 100 or 200 mg/kg-day for two years included lethargy in males and females and aggressiveness in males (NTP, 1989a). Central nervous system effects are believed to result from a nonspecific anesthetic effect, similar to that produced by various other volatile halocarbons (ATSDR, 1990).

Balster and Borzelleca (1982) administered a screening battery of behavioral tests to adult male ICR mice exposed to bromoform under a variety of dosage regimens. The experiments examined acute dose effects; 14 and 90 day treatments at 300 or 3,000 times the estimated average human daily intake of bromoform in disinfected tap water (0.9 and 9.7 mg/kg-day, respectively); 30 days of treatment at 100 mg/kg-day; and 60 days of treatment at 100 or 400 mg/kg-day. In each case, bromoform was administered by gavage to 6-8 mice per group in a 1:8 mixture of Emulphor® and distilled water. A nongavaged control group was included in the 14 and 90 day experiments in addition to a vehicle control group.

The acute effects of bromoform were evaluated using the screen test, a test of motor performance. Five or six differenct dose levels were administered (individual dose levels were not reported). Performance was evaluated at 30, 60, and 90 minutes after treatment. Effects on performance were noted at 30 minutes, with little evidence of change at the longer durations. An ED_{50} of 431 mg/kg (95 percent CI 238 to 788) was calculated for this response. Clinical signs of ataxia and incoordination were observed at higher doses. Anesthesia was observed at the highest dose.

The 14-day experiment evaluated the effect of repeated daily doses of 0, 0.9, or 9.2 mg/kg-day on swimming endurance, evaluated 24 hours after the final treatment. No adverse effect on performance was observed at either dose of bromoform.

The 90-day experiment evaluated the effect of bromoform on the cling test, screen test, and holeboard test. Doses of 0, 0.9, or 9.2 mg/kg-day were administered for 90 consecutive days. Performance on the three behavioral measures was evaluated 24 hours after the last dose. No significant effect of bromoform treatment on performance was observed for any of the tests.

The 30-day experiment examined the effect of bromoform administration on latency to enter the dark compartment in a passive avoidance learning test. Doses of 0 or 100 mg/kg-day were administered for 30 consecutive days. The learning test was conducted 24 hours after the final treatment. Bromoform had no effect on passive avoidance learning or the initial step-through latency on this test.

In the final experiment, Balster and Borzelleca (1982) evaluated operant conditioning in mice treated daily with bromoform at doses of 0, 100, or 400 mg/kg-day for 60 days. Performance was measured daily during a three-day pretreatment period. The mice were treated only with the vehicle during this period. Test animals were subsequently given bromoform 30 minutes prior to daily behavioral testing for 60 consecutive days. The behavioral measurements were continued for three additional days after termination of bromoform treatment. Treatment with 100 or 400 mg/kg-day significantly decreased response rates for the first day of treatment compared with those for the last day of pretreatment. The study authors noted no evidence for progressive deterioration and reported that partial tolerance occurred over the course of the experiment (no data were presented in support of these statements).

The results of these studies indicate that bromoform has low potential for eliciting adverse effects in several standard tests of behavioral toxicity. A LOAEL of 100 mg/kg-day was identified based on a nonprogressive decrease (with development of partial tolerance) in response rates in a test of operant conditioning.

Chronic Toxicity

The chronic oral toxicity of bromoform has been assessed in three studies: a dietary study conducted in rats and gavage studies in rats and mice. No data are available on the chronic inhalation toxicity of bromoform.

Tobe *et al*. (1982) evaluated the chronic effects of microencapsulated bromoform administered in the diet to male and female Slc:Wistar SPF rats (40/sex/group) for 24 months at dietary levels of 0.0, 0.04, 0.16, or 0.65 percent. The control groups (70/sex)

received microcapsules without bromoform. Body weights and food consumption were monitored weekly for the first 6 months, every two weeks from six to 12 months, and every four weeks during the second year of the study. Interim findings were reported from the sacrifice of nine animals/sex in the control group and five animals/sex/dose in the exposure groups at 18 months; all surviving animals were sacrificed at 24 months. Hematology and serum biochemistry measurements were conducted prior to the interim and terminal sacrifices. All animals were necropsied and examined for gross pathological effects at sacrifice. Although the authors indicate that tissues were collected for microscopic examination, no histopathology findings were reported.

Tobe *et al*. (1982) did not estimate bromoform intake on a mg/kg-day basis. Based on reported body weights (150 to 475 g for males and 100 to 215 g for females) and food consumption (15 to 20 g/day for males and 10 to 20 g/day for females), the dietary levels administered in this experiment correspond to doses of about 0, 20, 90, and 360 mg/kg-day for males and 0, 40, 150, and 620 mg/kg-day for females. Marked suppression of body weight gain (35 to 41 percent) was seen in males and females at the high dose, and moderate suppression of body weight gain (about 15 percent) was seen in males and females at the mid dose. Dose-related decreases in nonesterified fatty acids were observed in all treated males and in females at the mid and high dose. Females also exhibited a dose-related increase in levels of γ-glutamyl transpeptidase (γ-GTP) with the increases significant at the mid and high dose. Other changes in serum biochemistry at the high dose included decreased serum triglyceride and increased alanine aminotransferase and aspartate aminotransferase activity. Triglyceride levels were significantly decreased by 86 and 80 percent in the male and female high dose groups, respectively, at study termination. Alanine aminotransferase and aspartate aminotransferase activities at study termination were increased by 1.6 to 2.6-fold at the high dose compared to controls. The enzyme activity changes were statistically significant, with the exception of aspartate aminotransferase activity in males. Yellowing, small white spots, and roughening of the surface were observed in the livers of the mid and high dose animals. Based on the necropsy findings and the serum biochemistry, NOAEL and LOAEL of 20 and 90 mg/kg-day, respectively, for males and 40 and 150 mg/kg-day, respectively, for females were identified.

NTP (1989a) administered bromoform to male and female F344/N rats (50/sex/group) in corn oil by gavage for 103 weeks (five days/week) at doses of 0, 100, or 200 mg/kg-day. Animals were observed for clinical signs throughout the study. Body weights were measured weekly for 12 weeks and monthly thereafter. At termination, all study animals were necropsied. Full histopathological examination was performed on all control and high dose animals and on low dose males. Gross lesions and selected tissues including esophagus, kidney, liver, lymph nodes, mammary gland, pancreas, pituitary gland, salivary glands, thyroid gland, trachea, and uterus were examined in low dose females.

Mean final body weight was decreased by eight percent and 21 percent in low and high dose males, respectively, and by four percent and 25 percent in low and high dose females, respectively, relative to control. Survival of the high dose males was significantly decreased relative to control. Treatment-related clinical signs included lethargy in males and females and aggressiveness in males. Treatment-related lesions including fatty change, active chronic inflammation, and necrosis were observed in the

liver (Table 24). The incidence of fatty change was increased at the low and high dose in both sexes. The incidence of active chronic inflammation was increased in low and high dose females and high dose males. The incidence of hepatic necrosis was increased in high dose males, but was decreased in dosed females. Other lesions included increased incidences of forestomach ulcers in males (vehicle control, 1/49; 100 mg/kg, 5/50; 200 mg/kg, 10/50); lung inflammatory changes in males (1/50, 7/50, 15/50); squamous metaplasia of the salivary gland ducts (males: 0/50, 15/50 31/48; females: 0/49, 10/49, 16/50) and inflammatory changes (male: 0/50, 16/50, 25/48; female: 0/49, 9/49, 18/50) in the salivary glands of both sexes; and squamous metaplasia in the prostate gland of males (2/49, 6/46, 12/50). Lesions in the lungs and salivary glands were reported to be consistent with infection by sialodacryloadenitis (SDA) virus. However, since the occurrence of these lesions was clearly dose-related, the investigators concluded that they were likely to represent a combination of viral- and chemical-related effects. Lesions in the prostate gland were characterized as a possible chemical effect associated with inflammatory lesions in that organ. A LOAEL of 100 mg/kg-day in both male and female rats was identified, based on hepatic lesions.

Table 24. Selected Hepatic Lesions Observed in Fischer 344/N Rats Administered Bromoform by Gavage in Corn Oil for Two Years

Lesion	Vehicle control	100 mg/kg-day	200 mg/kg-day
Males			
Fatty Change	23/50	49/50	50/50
Active Chronic Inflammation	0/50	29/50	23/50
Necrosis	7/50	3/50	20/50
Females			
Fatty Change	19/50	39/49	46/50
Active Chronic Inflammation	9/50	8/49	27/50
Necrosis	11/50	3/49	2/50

Source: NTP (1989a)

NTP (1989a) administered bromoform to groups of male or female B6C3F$_1$ mice (50/sex/dose) by gavage in corn oil five days/week for 103 weeks. Males received doses of 0, 50, or 100 mg/kg-day and females received doses of 0, 100, or 200 mg/kg-day. Mice were observed for clinical signs twice daily throughout the study. Body weight was measured weekly for 12 weeks and monthly thereafter. At study termination, all animals were necropsied and examined for gross pathology. A complete histological examination of tissues was performed on all vehicle control and high dose animals. Selected tissues were examined in low dose males (bone, gross lesions, liver, lungs, stomach, and trachea) and females (gross lesions, liver, stomach, thyroid gland, and trachea).

Significantly decreased survival was observed in low and high dose females, but not in males. Decreased survival of females was attributed in part to an uteroovarian infection.

No treatment-related clinical signs were noted. Mean final body weights of low and high dose females were reduced by 10 and 16 percent, respectively, compared to controls. Neither mean final body weight nor body weight gain was affected in males. Nonneoplastic lesions considered by the study authors to be treatment-related included hyperplasia of the glandular stomach in males (vehicle control, 1/50; 50 mg/kg, 5/50; 100 mg/kg, 6/49) and follicular cell hyperplasia of the thyroid gland in females (vehicle control, 5/49; 100 mg/kg, 4/49; 200 mg/kg, 19/47) and minimal to mild cytoplasmic vacuolization of hepatocytes in females (vehicle control, 1/49; 100 mg/kg, 9/50; 200 mg/kg, 24/50). A LOAEL of 100 mg/kg-day (the lowest dose tested) for female mice was identified, based on decreased body weight and fatty changes of the liver. A NOAEL of 100 mg/kg-day (the highest dose tested) was identified for male mice.

Carcinogenicity

Bromoform has been demonstrated to be a colon carcinogen in male and female F344/N rats following administration by corn oil gavage. The carcinogenicity database for bromoform consists of three bioassays. No studies were identified that examined carcinogenicity in animals exposed to bromoform by inhalation.

Theiss *et al*. (1977) gave bromoform by ip injection up to three times weekly for eight weeks.to male Strain A mice (20/group) at doses of 0, 4, 48, or 100 mg/kg. A positive control group (20 animals) was also included in the study design. Mice were sacrificed 24 weeks after the first injection and the frequency of lung tumors in each test group was compared with vehicle-treated controls. Bromoform produced a significant increase ($p = 0.041$) in tumor incidence only at the intermediate dose. These results may be suggestive of carcinogenic potential, but do not form an adequate basis for the assessment of cancer risk, based on the use of an injection route for bromoform administration, lack of a clear dose-response relationship for tumorigenicity, and a less than lifetime exposure.

The NTP (1989a) evaluated bromoform carcinogenicity in male and female $B6C3F_1$ mice. Males (50/group) received doses of 0, 50, or 100 mg/kg-day of bromoform in corn oil by gavage for 103 weeks, five days/week; females (50/group) received doses of 0, 100, or 200 mg/kg-day bromoform by the same protocol. Animals were housed five/cage. Body weights were measured weekly for the first 12 weeks and monthly thereafter. At study termination, all animals were necropsied and tissues and organs examined for visible lesions. Complete histopathologic examinations were performed on all mice in the control and high dose groups. For the low dose group, histopathologic examinations were performed on animals dying through month 21 of the study, organs with visible lesions, and potential target organs identified in short-term studies or by literature review. Bone, liver, lungs, stomach, and trachea were examined in low dose males, while liver, stomach, thyroid gland, and trachea were examined in low dose females. Histopathology findings were verified by an independent quality assurance assessment.

Mean body weights for vehicle control and dosed male mice were similar throughout the study. No significant differences in survival of males were noted between treatment groups and controls. Histopathological examination did not identify treatment-related

increases in incidence of nonneoplastic or neoplastic lesions. On the basis of these data, it does not appear that a maximum tolerated dose was achieved for males.

Mean final body weights for low and high dose female mice were 10 and 16 percent less, respectively, than the mean body weight of the vehicle controls. Survival was significantly reduced after week 77 for low dose females and between weeks 77 and 100 for high dose females. The study authors attributed decreased survival of females in part to an uteroovarian bacterial infection, as indicated by presence of cysts and positive microbial culture. High dose females had a statistically significant (by independent analysis) increase in incidence of thyroid follicular cell hyperplasia. However, there were no statistically significant increases in incidence of neoplastic lesions at any dose.

Based on the results of this study, NTP (1989a) concluded there was no evidence of carcinogenic activity of bromoform in male or female mice. However, based on the absence of effects on weight gain or other endpoints of toxicity, it is questionable whether a sufficiently high dose was tested in males.

In a parallel experiment, the NTP (1989a) evaluated the carcinogenicity of bromoform in male and female F344/N rats (50/sex/dose). Bromoform was administered in corn oil by gavage at doses of 0, 100, or 200 mg/kg-day five days/week for 103 weeks. Complete histopathologic examinations were performed on all animals in the control and high dose groups. For the low dose groups, histopathologic examinations were performed on animals dying through month 21 of the study, on organs with visible lesions, and on potential target organs identified in short-term studies or by literature review. A complete histopathological examination was to be conducted on the low dose group if mortality in the highest dose group exceeded that in the vehicle control group by more than 15 percent, which was the case for male rats. Esophagus, kidney, liver, lymph nodes, mammary gland, pancreas, pituitary gland, salivary glands, thyroid gland, trachea, and uterus were examined in low dose female rats.

Mean final body weights of low and high dose males were eight and 21 percent less, respectively, than the control group. Mean final body weights of low and high dose females were four and 25 percent less, respectively, than controls. Clinical signs observed included lethargy in males and females and aggressiveness in males. Survival of high dose males was significantly lower than the vehicle control group after week 91 (control, 34/50; low dose, 30/50; high dose, 11/50). No significant differences in survival were noted between any groups of female rats.

Adenomatous polyps in females and adenomatous polyps or adenocarcinomas (combined) of the large intestine in both males and females showed statistically significant positive trends (Table 25). An adenocarcinoma of the large intestine was diagnosed in one high dose male and two high dose females. All three adenocarcinomas arose from adenomatous polyps. Although the number of tumors was small, their occurrence was considered to be biologically significant because intestinal tumors are rare in F344/N rats administered corn oil by gavage. The historical control incidences (as of 1987) for intestinal tumors in male F344/N rats were 0/285 for the contract laboratory conducting the study and 3/1,873 (0.2 percent) overall. The corresponding incidences for female F344/N rats were 0/282 for the contract laboratory and 0/1,888 overall.

Table 25. Tumor Incidence in the Large Intestine of Fischer 344/N Rats Exposed to Bromoform in Corn Oil for Two Years (NTP, 1989a)

Tumor	Tumor frequency		
Male rat	**Control**	**100 mg/kg**	**200 mg/kg**
Adenocarcinoma	0/50	0/50	1/50
Polyp (adenomatous)	0/50	0/50	2/50
Adenomatous polyp or adenocarcinoma	0/50	0/50	3/50
Female rat	**Control**	**100 mg/kg**	**200 mg/kg**
Adenocarcinoma	0/50	0/50	2/50
Polyp (adenomatous)	0/50	1/50	6/50
Adenomatous polyp or adenocarcinoma	0/50	1/50	8/50

Source: Table 13 in NTP (1989a)

Neoplastic nodules of the liver were observed in four low dose and two high dose female rats. The incidence of neoplastic nodules was not significantly increased in high dose females or in dosed males. The majority of the lesions observed in females did not meet the current NTP morphological criteria for classification as hepatocellular adenomas. For these reasons, the study authors did not consider the marginally increased incidence of neoplastic nodules in females to be compound-related. NTP (1989a) concluded that there was some evidence for carcinogenic activity of bromoform in male rats and clear evidence for carcinogenic activity in female rats, based on increased incidences of uncommon neoplasms of the large intestine. The low survival rate in high dose males decreased the power of the study to detect a carcinogenic response.

Classification of Carcinogenic Potential

Bromoform is currently listed by the State of California (OEHHA, 2009) as a chemical known to cause cancer (listed April 1, 1991). The Carcinogenic Risk Assessment Verification Endeavor (CRAVE) group of the U.S. EPA (2008a) reviewed the available evidence on carcinogenicity of the brominated THMs and assigned bromoform to Group B2, probable human carcinogen, in 1993. The B2 was assigned "Based on inadequate human data and sufficient evidence of carcinogenicity in animals, namely an increased incidence of tumors after oral administration of bromoform in rats and intraperitoneal administration in mice. Bromoform is genotoxic in several assay systems. Also, bromoform is structurally related to other THMs (e.g., chloroform, BDCM, DBCM) which have been verified as either probable or possible carcinogens."

The IARC (1999b) has concluded that there is limited evidence of carcinogenicity in experimental animals and inadequate evidence in humans for bromoform. Bromoform was therefore categorized as Group 3, not classifiable as to carcinogenicity in humans.

Toxicological Effects in Humans

The database for toxicological effects of bromoform in humans is limited. The primary source of exposure to bromoform is the ingestion of tap water, where it occurs as a byproduct of disinfection. Consequently, the most relevant human data are from epidemiological studies on reproductive outcomes associated with intake of disinfected water. These studies are summarized in a THM epidemiology section below. Because the industrial production and use of bromoform are limited, no toxicity data are available from occupational studies. No clinical or case report data were identified in the materials reviewed in preparation of this document. No published experimental or epidemiological data were located for subchronic or chronic toxicity, immunotoxicity, or carcinogenicity through oral or inhalation exposure to bromoform in humans.

Acute Toxicity

Bromoform was used historically as a sedative for children with whooping cough. Typical doses were approximately one drop, given three to six times per day. Accidental overdoses and a few deaths have been reported. The estimated lethal dose for a 10 to 20-kg child is approximately 300 mg/kg, and the LOAEL for mild sedation is approximately 54 mg/kg-day (U.S. EPA, 1994a).

Genetic Toxicity

Two studies have investigated the potential role of human polymorphisms in the glutathione *S*-transferase theta gene (*GSTT1-1*) using *in vitro* techniques. These studies were motivated by the findings of DeMarini *et al*. (1997), which suggest that *GSTT1-1* may bioactivate THMs to mutagenic compounds. The working hypothesis in each study was that individuals with at least one copy of *GSTT1-1* would be more sensitive to the genotoxic effects of brominated THMs than individuals lacking the gene. Bromoform was used as the model THM to test this hypothesis.

Landi *et al*. (1999a) measured the ability of bromoform to induce sister chromatid exchange in whole blood cultures of human peripheral lymphocytes obtained from glutathione *S*-transferase theta positive ($GSTT\text{-}1^{+}$) and negative ($GSST1\text{-}1^{-}$) donors. Lymphocytes from each donor were exposed to 5 mM bromoform at zero, 16, or 24 hours after the initiation of cultures. No significant differences were noted in the frequency of sister chromatid exchange in lymphocytes from $GSTT\text{-}1^{+}$ or $GSTT1\text{-}1^{-}$ individuals. The study authors noted that glutathione *S*-transferase theta 1-1 is expressed in the red blood cells that were cocultured with the lymphocytes (the target cell), but not in the lymphocytes themselves. Thus, lack of glutathione *S*-transferase theta 1-1 expression in lymphocytes may account for the results of this study.

In a subsequent study, Landi *et al*. (1999b) tested the ability of bromoform to induce DNA damage *in vitro* in human lymphocytes from $GSST1\text{-}1^{+}$ and $GSST1\text{-}1^{-}$ individuals. Whole blood cultures were exposed to 10^{-2} to 10^{-4} M bromoform and assayed for DNA breaks using the Comet assay. The DNA damaging potency of bromoform did not differ significantly between lymphocytes (the target cell for the Comet assay) collected from $GSST1\text{-}1^{+}$ and $GSST1\text{-}1^{-}$ donors. However, as noted for the previous study, lymphocytes

do not express GSST1-1, even in *GSST1-1*$^{+}$ individuals, and this may account for the lack of effect. When data were combined from both genotypic groups, there was a weak but statistically significant induction of Comets following treatment with bromoform.

Developmental and Reproductive Toxicity

Bromoform is considered as one of the THM disinfection byproducts in epidemiology studies summarized in a separate section below.

Neurotoxicity

No experimental or epidemiological data on the neurotoxicity of bromoform in humans were available. Clinical observations are consistent with central nervous system depression (summarized in U.S. EPA, 1994a).

Bromodichloromethane

Toxicological Effects in Animals

The toxicological effects of BDCM in experimental animals have been comprehensively reviewed in documents prepared by U.S. EPA (1980b, 1985b, 1987b, 1994b) and ATSDR (1989). Additional information is available on IRIS (U.S. EPA, 2008b) and in IARC monographs (1991a, 1999a). Because the data for BDCM are extensive, individual summary tables are provided by duration or toxicity category, e.g., reproductive and developmental toxicity.

Acute Toxicity

Summaries of the acute oral toxicity studies on BDCM in rats and mice are provided in Table 26 and Table 27 below. Bowman *et al*. (1978) assessed the acute toxicity of BDCM in ICR Swiss mice. Groups of male and female mice (10/sex/group) were treated with single doses ranging from 500 to 4,000 mg/kg. At least seven dose levels were utilized in each experiment. BDCM was solubilized in a 1:1:8 solution of Emulphor®, alcohol, and saline, administered by gavage to fasted animals. The posttreatment observation period was 14 days. The LD_{50}s were 450 (95 percent CI 326 to 621) and 900 mg/kg (95 percent CI 811 to 999) for males and females, respectively. Sedation and anesthesia occurred within 30 minutes of treatment at doses of 500 mg/kg and above. The observed sedation lasted approximately four hours.

Chu *et al*. (1980, 1982a) evaluated the acute toxicity of BDCM in male and female SD rats (10/sex/dose). Fasted adults received doses of 390, 546, 765, 1,071, or 1,500 mg/kg in corn oil by gavage. The posttreatment observation period was 14 days. The LD_{50} values for male and female rats were 916 (95 percent CI 779 to 1,083) and 969 mg/kg (95 percent CI 764 to 1,198), respectively. Clinical signs observed in treated rats included sedation, flaccid muscle tone, ataxia, piloerection, and hypothermia. Gross pathological examination revealed liver and kidney congestion in treated animals.

Table 26. Summary of Acute Studies on BDCM

Reference	Species	Route	Sex	N	Dose (mg/kg)	NOAEL	LOAEL
Bowman *et al*. (1978)	Mouse ICR Swiss	Gavage (aqueous)	M, F	10	500 – 4,000 (individual doses not given)	-	500 anesthesia, sedation
NTP (1987)	Rat F344/N	Gavage (corn oil)	M, F	5	150, 300, 600, 1,250, 2,500	300	600 mortality
NTP (1987)	Mouse $B6C3F_1$	Gavage (oil)	M, F	5	150, 300, 600, 1,250, 2,500	300	600 lethargy, mortality
Lilly *et al*. (1994)	Rat F344	Gavage (corn oil) (aqueous)	M	6	0, 200, 400	-	200 renal tubule degeneration and necrosis; altered renal function markers
Lilly *et al*. (1996)	Rat F344	Gavage (corn oil or water)	M	6	0, 200, 400	-	200 renal tubule necrosis; altered renal function markers
Lilly *et al*. (1997b)	Rat F344	Gavage (aqueous)	M	5	0, 123, 164, 246, 328, 492	164	246 decreased body weight; altered liver and renal markers
Keegan *et al*. (1998)	Rat F344	Gavage (aqueous)	M	6	0, 21, 31, 41, 82, 123, 164, 246	41	82 altered renal markers; decreased liver weight

Chu *et al*. (1982a) reported results for growth, food intake, organ weight, histopathology, hematological indices, liver microsome aniline hydroxylase activity, and serum chemistry in surviving rats from the Chu *et al*. (1980) study. Food intake and body weight gain were significantly decreased in surviving males at the 765 and 1,071 mg/kg doses (only one animal survived at the highest dose). Relative liver and kidney weights were significantly reduced at 765 mg/kg in males. Lactate dehydrogenase activity was decreased in males administered 1,071 mg/kg, but a dose-related trend was not evident. BDCM had no significant effect on any measured biochemical parameter in females. Reduced hemoglobin concentration, hematocrit, and/or red blood cell counts were observed in male rats at doses ranging from 390 to 1,071 mg/kg. Hemoglobin concentration was significantly decreased in females treated with 546 or 765 mg/kg. No clearly dose-related histological effects were noted in the liver or kidney of either sex.

Table 27. Oral LD_{50} Values for BDCM

Species	Strain	Route (vehicle)	Sex	Number per dose group	LD_{50} (95 percent confidence interval) mg/kg	Reference
Mouse	ICR Swiss	Gavage (aqueous)	M F	10 10	450 (326 - 621) 900 (811 - 999)	Bowman *et al*. (1978)
Rat	Sprague-Dawley	Gavage (corn oil)	M F	10 10	916 (779 - 1,083) 969 (764 - 1,198)	Chu *et al*. (1980)
Rat	F344/N	Gavage (corn oil)	M F	5 5	651 (462 - 917) 751 (568 - 993)	NTP (1987)
Mouse	$B6C3F_1$	Gavage (corn oil)	M F	5 5	-[a] 651 (462 - 917)	NTP (1987)

[a]The pattern of mortality precluded calculation of an LD_{50} value.

NTP (1987) administered single doses of BDCM in corn oil to male and female F344/N rats (five/sex/dose) at doses of 150, 300, 600, 1,250, or 2,500 mg/kg by gavage. The animals were observed for 14 days post-treatment and at least one male and one female in each dose group were necropsied at study termination. All animals dosed with 1,250 or 2,500 mg/kg died before the end of the study. At 600 mg/kg, deaths occurred in two of five males and one of five females. The LD_{50}s determined for male and female rats were 651 (95 percent CI 462 to 917) and 751 mg/kg (95 percent CI 568 to 993), respectively. Clinical signs observed at 1,250 or 2,500 mg/kg included lethargy and labored breathing. At necropsy, the liver from animals dosed with 1,250 or 2,500 mg/kg appeared pale. No dose-related effects were seen on body weight gain in surviving animals.

NTP (1987) also administered single gavage doses of BDCM in corn oil to male and female $B6C3F_1$ mice (five/sex/dose) at doses of 150, 300, 600, 1,250, or 2,500 mg/kg. Animals were observed for 14 days, and a necropsy was performed on at least one male and one female in each dose group. All animals dosed with 1,250 or 2,500 mg/kg died before the end of the study. At 600 mg/kg, death occurred in all males and two of five females. The male survival pattern prevented the calculation of an LD_{50}. The LD_{50} for females was 651 (95 percent CI 462 to 917). Lethargy was observed in males at 600 and 1,250 mg/kg and in females at 600 mg/kg and above. At necropsy, the liver appeared pale and the cranial cavity contained blood in animals dosed with 1,250 or 2,500 mg/kg. No dose-related effects were seen on body weight gain in surviving animals during the post-treatment observation period.

Other acute studies on BDCM have examined effects of different vehicles (Lilly *et al*., 1994), pretreatment with a corn oil vehicle (Lilly *et al*., 1996), and/or induction of biochemical and histological effects following a single oral dose by gavage (Lilly *et al*., 1994, 1996, 1997b; Keegan *et al*., 1998). Lilly *et al*. (1994) administered a single dose of 0, 200, or 400 mg/kg BDCM to male F344 rats (six/group) by gavage in corn oil or an

aqueous 10 percent Emulphor® solution. Urine samples were collected prior to dosing and at 6 to 12 hour intervals after dosing, up to 48 hours. The rats were sacrificed at 48 hours post-dosing for measurement of serum chemistry and histopathological examination of the liver and kidney.

Body weights were significantly decreased in rats treated with 400 mg/kg BDCM in the aqueous vehicle. Absolute and relative kidney weights were significantly increased at 400 mg/kg in both vehicles, but the effect was greater with corn oil. Significant increases were observed in urinary markers of renal toxicity at 24 and/or 48 hours after dosing with 200 or 400 mg/kg BDCM in either vehicle. Renal toxicity at 200 mg/kg did not show a marked vehicle effect. At 400 mg/kg, renal toxicity was greater in animals administered BDCM in corn oil. Renal tubule degeneration and necrosis were observed at both doses. The incidence of renal tubule degeneration was greater in animals receiving the low dose in an aqueous vehicle, but the severity of renal degeneration and necrosis was greater in the animals receiving the high dose in corn oil. Serum markers of hepatotoxicity were significantly increased at 400 mg/kg for both vehicles. Hepatocellular degeneration and necrosis were observed at 400 mg/kg in both vehicles, but were more severe and the incidence of centrilobular necrosis was higher in animals receiving the corn oil vehicle. The results indicate that the kidney may be a more sensitive target for a single dose of BDCM than the liver. The authors attributed the vehicle-related differences in toxicity at the high dose to slower gastrointestinal uptake of BDCM from corn oil, compared to the aqueous vehicle. It was postulated that at the high dose, more BDCM would be converted to a reactive metabolite with corn oil dosing, while saturation of metabolizing enzymes would occur with the aqueous gavage. The difference in uptake would be less pronounced at the low dose, resulting in comparable levels of toxicity. A LOAEL of 200 mg/kg, the lowest dose tested, was identified for each vehicle based on minimal renal tubule degeneration and changes in biochemical markers of renal function.

Lilly *et al*. (1996) investigated the effect of corn oil pretreatment on BDCM toxicity. Male F344 rats (six/group) received corn oil or water by gavage five days/week for six weeks prior to BDCM treatment . At the end of the pretreatment period, the animals were given a single dose of 0, 200, or 400 mg BDCM/kg in 10 percent Emulphor® by gavage. Urine was collected at 24, 36, and 48 hours after dosing for measurement of indicators of renal toxicity. The rats were sacrificed at 48 hours and serum was analyzed for indicators of hepatic toxicity. All animals were necropsied and organ weights were collected. Other parameters analyzed included serum levels of bile acids, triglycerides, cholesterol, and albumin, and urinary levels of N-acetylglucosaminidase and gamma glutamyl transpeptidase activity. Activities of CYP2E1 and CYP2B1/B2 were measured in the microsomal fraction of the liver to determine whether these cytochrome P450 isoforms were induced by corn oil.

Liver weight was significantly reduced in the water pretreatment group at the high dose. Kidney weight was reduced in both pretreatment groups at the high dose. Activities of serum aspartate aminotransferase and lactate dehydrogenase were significantly elevated in both pretreatment groups at 400 mg/kg. Alanine aminotransferase activity increased in a dose-dependent manner in the water pretreatment group, but significant elevations were noted only at the 400 mg/kg dose in animals pretreated with corn oil. Activities of urinary aspartate aminotransferase and lactate dehydrogenase in both pretreatment groups

were greater than the control at all time points. Alkaline phosphatase levels were significantly increased in both pretreatment groups at 24 hours. At 36 and 48 hours, alkaline phosphatase levels were elevated only in water-pretreated animals. High incidences of renal tubular necrosis occurred at 200 and 400 mg/kg in both pretreatment groups and the severity of lesions was similar. No significant differences were noted in the hepatic activity of CYP2E1 or CYP2B1/B2 in the corn oil pretreated animals compared to the water controls. The weight of evidence indicates that six weeks of pretreatment with corn oil did not significantly enhance the acute hepatic or renal toxicity of BDCM. The study authors concluded that vehicle-related differences in toxicity observed in other BDCM studies most likely result from pharmacokinetic differences in absorption, as suggested by Lilly *et al*. (1994), rather than induction of metabolizing enzymes by corn oil. An acute LOAEL of 200 mg/kg-day was identified in this study on the basis of renal tubular necrosis.

Lilly *et al*. (1997b) administered single doses of 0, 123, 164, 246, 328, or 492 mg/kg of BDCM to male F344 rats (five/dose) by gavage in aqueous 10 percent Emulphor®. Urine samples were collected for 12 hours prior to dosing and over four successive 12 hour intervals after dosing. The rats were sacrificed at 24 and 48 hours post-dosing for serum chemistry measurements. No histopathology data were collected. Body weights were significantly decreased only after 48 hours at doses at or above 246 mg/kg. At 24 hours after treatment, absolute kidney weights were increased at doses of 328 and 492 mg/kg and relative kidney weights were significantly increased at doses at or above 246 mg/kg. At 48 hours, only relative kidney weight at the high dose was significantly increased. Serum enzyme activities were decreased from the 24 hour levels, but statistically significant elevations relative to controls were noted at or above 246 mg/kg for aspartate aminotransferase and alanine aminotransferase and at 123 mg/kg and above for sorbitol dehydrogenase. No changes in urinary markers of renal toxicity were found at either 123 or 164 mg/kg. N-acetylglucosaminidase, lactate dehydrogenase, and aspartate aminotransferase activity in urine were significantly elevated (with some exceptions) at 24, 36 and 48 hours post-treatment at 246 mg/kg and above. Based on these results, the kidney did not appear to be more sensitive than the liver to acute toxic effects of BDCM as previously reported by Lilly *et al*. (1994). A LOAEL of 123 mg/kg was identified based on a biochemical indicator, increased serum sorbitol dehydrogenase activity, without supporting hepatic histopathology. A NOAEL was not identified.

Keegan *et al*. (1998) investigated acute toxicity of BDCM in male F344 rats. Six animals/group were gavaged with a single dose of 0, 20.5, 30.7, 41.0, 81.9, 123, 164, and 246 mg/kg dissolved in a 10 percent aqueous solution of Alkamuls EL-620. The rats were sacrificed 24 hours later and liver, kidneys, and serum were collected. Histopathological examination was not indicated in the methods. Significant decreases in body weight were observed at doses of 123 mg/kg and above. Decreases in absolute liver weight were observed at doses of 81.9 mg/kg and above. Relative kidney weights were significantly increased at doses of 164 and 246 mg/kg. Dose-dependent increases in alanine aminotransferase, aspartate aminotransferase, and sorbitol dehydrogenase were observed at doses of 81.9 mg/kg and above. The study authors identified a NOAEL and LOAEL of 41.0 mg/kg and 81.9 mg/kg, respectively, based on changes in absolute liver weight and biochemical indications of hepatic toxicity.

Short-Term Toxicity

The short-term toxicity of BDCM has been investigated in at least twelve studies ranging from three to 35 days in duration (Table 28). Munson *et al*. (1982) administered BDCM by aqueous gavage to male and female CD-1 mice (8-12/sex/group) for 14 days at 0, 50, 125, or 250 mg/kg-day. Endpoints evaluated included body and organ weights, hematology, serum enzyme levels, and immune system functions (discussed in the Immunotoxicity section of this document). No compound-related deaths occurred. Body weights were significantly decreased in males and females at the high dose. Significant organ weight changes included increased relative liver weight (mid and high dose groups), decreased absolute spleen weight (high dose males and mid and high dose females), and decreased relative spleen weight (mid and high dose females). Fibrinogen levels were significantly decreased in high dose males and in mid and high dose females. Significant alterations in clinical chemistry occurred at the high dose and included decreased glucose levels (males), increased alanine aminotransferase and aspartate aminotransferase activities (males and females), and increased blood urea nitrogen levels (males and females). A NOAEL of 50 mg/kg-day and a LOAEL of 125 mg/kg-day were identified based on changes in organ weight.

Table 28. Summary of Short-Term Toxicity Studies on BDCM

Reference	Species	Route	Sex	N	Duration	Doses (mg/kg-day)	NOAEL (mg/kg-day)	LOAEL (mg/kg-day)
Chu *et al*. (1982a)	Rat Sprague-Dawley	Drinking water	M	10	28 days	0, 0.8, 8, 68	68	-
Munson *et al*. (1982)	Mouse CD-1	Gavage (aqueous)	M, F	8-12	14 days	0, 50, 125, 250	50	125 decreased immune function; organ weights
Condie *et al*. (1983)	Mouse CD-1	Gavage (corn oil)	M	8-16	14 days	0, 37, 74, 148	74	148 liver, kidney histopathology
NTP (1987)	Rat F344/N	Gavage (corn oil)	M, F	5	14 days	0, 38, 75, 150, 300, 600	150	300 decreased final body weight
	Mouse $B6C3F_1$	Gavage (corn oil)	M, F	5	14 days	0, 19, 38, 75, 150, 300	75	150 mortality, gross renal pathology
Aida *et al*. (1992a)	Rat Wistar	Diet	M	7	1 month	0, 21, 62, 189)	62	189 liver histopathology
			F	7	1 month	0, 21, 66, 204	66	204 liver histopathology

Reference	Species	Route	Sex	N	Duration	Doses (mg/kg-day)	NOAEL (mg/kg-day)	LOAEL (mg/kg-day)
Thornton-Manning *et al*. (1994)	Rat F344	Gavage (aqueous)	F	6	5 days	0, 75, 150, 300	75	150 liver, renal histopathology; serum hepatotoxicity markers
	Mouse C57BL/6J	Gavage (aqueous)	F	6	5 days	0, 75, 150	75	150 serum hepatotoxicity markers
Potter *et al*. (1996)	Rat F344	Gavage (aqueous)	M		1, 3, or 7 days	0, 123, 246	246 hyaline droplet formation; cell proliferation	-
Melnick *et al*. (1998)	Mouse $B6C3F_1$	Gavage (corn oil)	F	10	3 weeks (5 d/wk)	0, 75, 150, 326	75	150 hepatocyte degeneration; increased labeling index; serum hepatotoxicity markers
NTP (1998a)	Rat Sprague-Dawley	Drinking water	M, F	6	2 weeks	0, 11, 45, 91, 124	45	91 transient reduction in weight gain
				5-13	35 days	Group A males 0, 9, 38, 67	9	38 single cell hepatic necrosis in Group A males
Coffin *et al*. (2000)	Mouse $B6C3F_1$	Gavage (Corn oil)	F	10	11 days	0, 150, 300	-	150 hydropic degeneration in liver; increased proliferating cell nuclear antigen - labeling index
		Drinking water	F	10	11 days	0, 138	-	138 hydropic degeneration in liver

Chu *et al*. (1982a) administered BDCM to male SD rats (10/group) in drinking water for 28 days at concentrations of 0, 5, 50, or 500 ppm, resulting in average daily doses of 0, 0.8, 8.0, or 68 mg/kg-day, as calculated by the authors based on recorded fluid intake. Effects on body weight, water and feed consumption, clinical chemistry, and histopathology were evaluated. No compound-related effects on growth rate or food consumption, clinical signs of toxicity, biochemical alterations, or histological changes were reported. A NOAEL of 68 mg/kg-day was identified.

Condie *et al*. (1983) administered BDCM at 0, 37, 74, or 148 mg/kg-day to male CD-1 mice (8-16/dose) in corn oil by gavage for 14 days. Effects on body weight, clinical signs, clinical biochemistry, and histopathology were investigated. Alanine aminotransferase activity was significantly elevated at the high dose. Significantly decreased *para*-aminohippurate uptake by kidney slices (an indication of kidney damage) was observed at the mid and high dose. Significantly decreased blood urea nitrogen was observed at the low and mid dose, but not at the high dose. Minimal to moderate liver and kidney injury was observed in the majority of animals at the high dose. Dose-related hepatic lesions included centrilobular pallor and focal inflammation. Dose-related renal lesions included intratubular mineralization, epithelial hyperplasia, and cytomegaly. A NOAEL of 74 mg/kg-day and a LOAEL of 148 mg/kg-day were identified, based on dose-related histopathological changes in the liver and kidney.

NTP (1987) administered doses of 0, 38, 75, 150, 300, or 600 mg/kg-day of BDCM in corn oil by gavage to male and female F344/N rats (five/sex/dose) in a 14 day range-finding study. One low dose and one high dose female died during the exposure period. All high dose animals were hyperactive after dosing and either lost weight or failed to gain weight during the study. Final mean body weights of males and females administered 300 mg/kg were decreased by 21 percent and seven percent, respectively, relative to the vehicle controls. At 600 mg/kg-day, body weights of males and females were decreased by 44 percent and 22 percent, respectively. At necropsy, renal medullae were reddened in all high dose males and in one female in each of the control, low dose, and high dose groups. A NOAEL of 150 mg/kg-day and a LOAEL of 300 mg/kg-day in rats were identified, based on decreased body weight.

In a parallel experiment, NTP (1987) administered doses of 0, 19, 38, 75, 150, or 300 mg/kg-day in corn oil by gavage to male and female $B6C3F_1$ mice (five/sex/dose) for 14 days. All male mice at the two highest doses died during the exposure period. Compound-related clinical signs included lethargy, dehydration, and hunched posture. The final mean body weights of surviving mice did not differ significantly from the controls. At necropsy, the renal medullae were reddened in four males in the 150 mg/kg-day group, all males in the 300 mg/kg-day group, and one female in the 150 mg/kg-day group. A NOAEL and LOAEL of 75 mg/kg-day and 150 mg/kg-day, respectively, were identified, based on mortality, clinical signs, and necropsy results. A comparison of the results obtained in this study to those obtained by Condie *et al*. (1983) in male CD-1 mice suggest possible strain-specific differences in sensitivity to BDCM.

Aida *et al*. (1992a) exposed Slc:Wistar rats (seven/sex/group) to BDCM in the diet at levels of 0, 0.024, 0.072, or 0.215 percent for males and 0, 0.024, 0.076, or 0.227 percent for females for one month. BDCM was microencapsulated and mixed with powdered feed. Placebo granules were mixed with the diet of the control groups. Effects on body weight, food consumption, hematology parameters, serum chemistry, and histopathology and other parameters were determined. The average daily doses were estimated to be 0, 20.6, 61.7, or 189 mg/kg-day for males and 0, 21.1, 65.8, or 204 mg/kg-day for females, based on the authors' calculations using mean food intakes. High dose animals exhibited clinical signs of slight piloerection and emaciation. Body weights were significantly decreased in high dose males and females by 18 and 12 percent, respectively, relative to the controls. Relative liver weight was significantly increased in high dose females.

Biochemical parameters that showed statistically significant, dose-related changes included serum lactate dehydrogenase, serum triglycerides, and serum glucose in males (decreasing trend) and cholinesterase and serum triglycerides (decreasing trend) in females. Compound-related histopathological lesions were limited to the liver. The severity of all observed lesions was categorized as very slight or slight. The highest incidences were observed for hepatic vacuolization (mid and high dose males and females) and hepatic swelling (mid and high dose males, high dose females). Other observed lesions included swelling of hepatocytes (high dose males, mid and high dose females), single cell necrosis (high dose males), hepatic cord irregularity (high dose males), and bile duct proliferation (mid and high dose males, high dose females). LOAELs of 189 mg/kg-day for males and 204 mg/kg-day for females were identified based on histopathological changes in the liver. The corresponding NOAELs are 62 mg/kg-day for males and 66 mg/kg-day for females.

Thornton-Manning *et al*. (1994) gave doses of 0, 75, 150, or 300 mg/kg-day of BDCM to groups of 6 female F344 rats by gavage for five consecutive days, in an aqueous vehicle containing 10 percent Emulphor®. Effects on body weight, clinical chemistry, organ weight, histopathology, and cytochrome P450 activity in hepatic microsomes were investigated. Two animals in the high dose group died during the exposure period. Final body weights of the high dose group were significantly decreased compared to controls. Absolute and relative kidney and liver weights were significantly increased at 150 and 300 mg/kg-day except for a nonsignificant increase in absolute liver weight at 150 mg/kg-day. Increased activities or concentrations of lactate dehydrogenase, aspartate aminotransferase, sorbitol dehydrogenase, creatinine, and blood urea nitrogen at 300 mg/kg-day were suggestive of renal and hepatic toxicity. Centrilobular vacuolar degeneration was observed in the liver at both 150 and 300 mg/kg-day and the severity of the lesion was dose-related. Centrilobular hepatocellular necrosis was observed in one high dose animal. In the kidney, renal tubular vacuolar degeneration and renal tubule regeneration were observed at 150 and 300 mg/kg-day; incidence and severity increased with dose. Mild to moderate renal tubule necrosis was observed in all animals at the high dose. Significant decreases in the hepatic microsomal activity of the CYP1A and CYP2B markers ethoxyresorufin-*O*-dealkylase and pentoxyresorufin-*O*-dealkylase were observed in all dosed groups, but did not show a dose-related trend. Total P450 levels were reduced at the mid and high doses. No effect was observed on the CYP2E1 marker pNP-hydroxylase. A NOAEL of 75 mg/kg-day and a LOAEL of 150 mg/kg-day were identified, based on the histopathological lesions in the kidney and liver.

Thornton-Manning *et al*. (1994) conducted an analogous experiment with female C57BL/6J mice. Six mice/group were given doses of 0, 75 and 150 mg/kg-day of BDCM suspended in aqueous 10 percent Emulphor® by gavage for five consecutive days. Effects on body weight, clinical chemistry, organ weight, histopathology, and hepatic cytochrome P450 activity in hepatic microsomes were investigated. All mice survived to terminal sacrifice. No effects on body, kidney, or liver weight were observed with the exception of a significant increase in absolute liver weight at 150 mg/kg-day. No significant change in cytochrome P450 levels was evident, although a nonsignificant dose-related decrease in total P450 content was observed. Alanine aminotransferase was significantly increased at 150 mg/kg-day, and there was a significant dose-related increase in sorbitol dehydrogenase activity. Creatinine and blood urea nitrogen were not

significantly increased. No kidney or liver lesions were observed at either dose. A NOAEL of 75 mg/kg-day and a LOAEL of 150 mg/kg-day were identified based on increases in serum enzyme activity without evident changes in histopathology.

Potter *et al.* (1996) investigated hyaline droplet formation and cell proliferation in the kidney of male F344 rats treated with BDCM. Four rats/dose were gavaged with 0, 123 or 246 mg/kg-day BDCM in four percent Emulphor® for one, three, or seven days. Cell proliferation in the kidney was assessed by [^{3}H]-thymidine incorporation. No exposure-related increase in hyaline droplet formation was observed at either dose. A reduced hyaline droplet formation on day seven was observed which might be explained by a decrease in serum testosterone concentrations. No statistically significant effects on tubular cell proliferation were observed following exposures of up to seven days. A NOAEL of 246 mg/kg-day was identified, the highest dose tested.

Melnick *et al*. (1998) exposed female B6C3F$_1$ mice (10/group) to doses of 0, 75, 150, or 326 mg/kg-day BDCM in corn oil by gavage for three weeks, five days/week. Effects on body and organ weights, water intake, hydropic degeneration, labeling index and other parameters were investigated. No compound-related signs of toxicity were observed during the study. Body weight and water intake did not differ significantly from control at any tested dose. Significant dose-related increases in absolute liver weight and liver weight to body weight ratio occurred in the 150 and 326 mg/kg-day dose groups. Serum alanine aminotransferase activity was significantly increased in the mid and high dose groups. Serum sorbitol dehydrogenase activity was increased at all tested doses. Hydropic degeneration was evident in the livers of animals treated with the mid and high doses. The labeling index was significantly increased at the mid and high doses, indicating hepatocyte proliferation. A NOAEL and LOAEL of 75 and 150 mg/kg-day, respectively, were identified, based on histological findings.

NTP (1998a) examined the effect of BDCM on food and water consumption by SD rats in a range finding experiment for a study of developmental and reproductive effects. BDCM was administered to six rats/sex/dose at nominal concentrations of 0, 100, 500, 1,000, and 1,500 ppm in drinking water for two weeks. Feed and water consumption were measured twice weekly. The average BDCM doses were 11, 45, 91 and 124 mg/kg-day for the respective dose groups, estimated by the study authors on the basis of water consumption. All animals were observed twice daily for clinical signs of toxicity. Body weights were recorded twice weekly and at the termination of the experiment. No necropsies were conducted at the end of the experiment. No mortality or treatment-related clinical signs were observed in any dose group. Body weights and weight gains were comparable among all groups, except for reduced body weight gains on the first day of treatment (study day five) in the 1,000 and 1,500 ppm groups. The reductions in weight gain for these groups were 27.5 and 18.5 percent, respectively. Male rats in the 1,000 and 1,500 ppm groups showed decreases in feed consumption of 31 percent and 41 percent, respectively, on study days one to five. Water consumption was reduced in the 500, 1,000, and 1,500 ppm groups, suggesting that BDCM is unpalatable at these concentrations. The greatest reduction in water intake was noted on study days one to five. Intake was reduced by 61 and 62 percent for males in the 1,000 ppm and 1,500 ppm groups, respectively, and by 38, 40, and 52 percent for females in the 500, 1,000 and 1,500 ppm groups, respectively.

NTP (1998a) conducted a short-term reproductive and developmental toxicity screen in SD rats to evaluate the potential toxicity of BDCM in drinking water for 35 days. Male and female rats (5-13/sex/dose) were exposed to drinking water concentrations of 0, 100, 700 and 1,300 ppm BDCM. The main study used two groups of male rats designated as Group A (non-bromodeoxyuridine (BrdU)-treated, 10 rats/dose group) and Group B (bromodeoxyuridine[3]-treated, five or eight rats/dose), and three groups of female rats designated as Group A (periconception exposure, 10 animals/dose), B (gestational exposure, 13/dose), and C (periconception exposure, BrdU-treated, five or eight/dose group). Test animals were dosed for 25 to 30 days, except for Group B females, which were dosed from gestation day six to evidence of littering or birth (about 15 to 16 days). Effects on feed and water consumption, body weight, hematological parameters, clinical chemistry, cell proliferation, and pathology were evaluated in addition to developmental and reproductive endpoints.

Based on water consumption and BDCM analyses in the drinking water, average daily doses were 0, 9, 38, and 67 mg/kg-day for Group A males; 0, 7, 43, and 69 mg/kg-day for Group B males; and 0, 14, 69, or 126 mg/kg-day for Group C females. Reproductive and developmental effects are reported in the reproductive toxicity section of this document. No significant differences were noted in organ weights or hematological parameters. A 14 percent decrease in creatinine concentration in the 100 ppm Group A males and a 43 percent increase in 5'-nucleotidase in the 1,300 ppm Group A males versus controls did not appear to be toxicologically significant. Cytoplasmic vacuolization of hepatocytes and mild liver necrosis were observed in Group A males treated with 700 and 1,300 ppm BDCM and in Group B males treated with 1,300 ppm. Hepatic necrosis was dose-dependent, with incidences of 0/10, 0/10, 4/9, and 10/10 observed at 0, 100, 700, and 1,300 ppm, respectively. Hematopoietic cell proliferation in the spleen was observed in Group A males at all BDCM doses and may have been a response to general stress. Evidence of mild kidney necrosis was observed in Group A males in the 1,300 ppm group, but may have resulted from decreased water intake. BrdU labeling index, a measurement of cell proliferation, was unchanged in the livers and kidneys of Group B males at all doses. A small but statistically significant increase in the labeling index was noted in the livers and kidneys of Group C females in the 1,300 ppm group. Based on average daily doses for Group A males at the 100 and 700 ppm concentrations (Table 6A in NTP, 1998a), a NOAEL and LOAEL of 9 mg/kg-day and 38 mg/kg-day, respectively, were identified based on the hepatic cell necrosis in Group A males.

Coffin *et al*. (2000) examined the effect of BDCM administered in corn oil by gavage or in drinking water on cell proliferation and DNA methylation in the liver of female $B6C3F_1$ mice. Doses of 0, 150, or 300 mg/kg of BDCM were administered to 10 mice/group by gavage for five days. Treatment was discontinued for two days and then resumed for four more days. The high dose was selected on the basis that it had previously been shown to be carcinogenic in female mice. In a separate experiment, BDCM was administered in drinking water for 11 days at approximately 75 percent of the saturation level, resulting in an average daily dose of 138 mg/kg. The mice were

[3] Bromodeoxyuridine, BrdU, test animals are pretreated with this compound prior to determination of labeling index.

sacrificed 24 hours after the last gavage dose and the livers were removed, weighed, and processed for histopathological examination, proliferating cell nuclear antigen-labeling index analysis, and determination of *c-myc* methylation status.

A significant, dose-dependent increase in relative liver weight was observed in animals dosed by gavage; however, relative liver weight was unaffected in animals given BDCM in drinking water, compared to controls. Histopathological findings in gavage-dosed animals included hydropic degeneration at the low dose and necrosis, fibrosis, and giant cell reaction at the high dose. No severity or incidence data were provided. The histopathology findings for animals receiving BDCM in the drinking water were similar to those observed in the low dose gavage group. BDCM administered by gavage caused a dose-dependent increase in the proliferating cell nuclear antigen-labeling index, which was significant at each dose, compared to the control. There was no significant effect when the compound was administered in drinking water. Administration of BDCM by gavage or in drinking water decreased methylation of the *c-myc* gene. A LOAEL of 150 mg/kg, the lowest dose tested, was identified based on hydropic degeneration in liver in animals given BDCM by gavage in corn oil. A NOAEL was not identified.

Subchronic Toxicity

Two studies addressed subchronic oral toxicity of BDCM (Table 29). Chu *et al*. (1982b) evaluated toxicity in male and female SD rats exposed to BDCM in drinking water. NTP (1987) evaluated BDCM toxicity in male and female F344/N rats and $B6C3F_1$ mice administered the compound in corn oil. No subchronic inhalation studies are available.

Chu *et al*. (1982b) administered BDCM to male and female SD rats (20/sex/dose) in drinking water containing one percent Emulphor® for 90 days at concentrations of 0, 5, 50, 500, or 2,500 ppm. Half of each group (10/sex/dose) were sacrificed at the end of the exposure period, and the remaining animals were given tap water for a 90 day recovery period (only results in animals sacrificed immediately after the treatment period are summarized below). Effects on food and water consumption, body weight, clinical chemistry, hematology, and histopathology were investigated.

The estimated average daily doses were 0, 0.61, 6.9, 56, and 212 mg/kg-day for males and 0, 0.89, 8.2, 67, and 220 mg/kg-day for females, based on daily water intake, initial body weight, and body weight gain at the high dose. At 2,500 ppm, food consumption and body weight gain were significantly depressed in males and females. No effect was noted on organ weights, biochemical parameters, or hematological parameters (during the treatment period) at any dose. Mild histological changes were observed in the liver and thyroid of the males. Incidence of hepatic lesions increased in males at concentrations ≥ 50 ppm (incidence: vehicle control, 2/9; 5 ppm, 1/10; 50 ppm, 8/10; 500 ppm, 8/10; 2,500 ppm, 9/10). Significant increases in the severity of hepatic lesions were noted at ≥ 50 ppm compared to control. The incidence of hepatic lesions in exposed females (vehicle control, 0/10; 3/10; 5/10; 3/10; 4/10) increased relative to that of the control group, but did not show a dose-related trend. Severity of the lesions was significantly increased in the 50 and 2,500 ppm groups, but not in the 5 or 500 ppm groups.

Table 29. Summary of Subchronic Toxicity Studies on BDCM

Reference	Species	Route	Sex	N	Duration	Doses (mg/kg-day)	NOAEL (mg/kg-day)	LOAEL (mg/kg-day)
Chu *et al*. (1982b)	Rat Sprague-Dawley	Drinking water	M	20	90 days	0, 0.6, 6.5, 53, 212	0.6	6.5 hepatic lesions
			F	20	90 days	0, 0.8, 6.9, 57, 219	219 non dose-dependent hepatic and thyroid lesions	-
NTP (1987)	Rat F344/N	Gavage (corn oil)	M, F	10	13 weeks	0, 19, 38, 75, 150, 300	75	150 reduced body weight gain
	Mouse $B6C3F_1$	Gavage (corn oil)	M	10	13 weeks	0, 6.3, 13, 25, 50, 100	50	100 focal necrosis of proximal renal tubular epithelium
		Gavage (corn oil)	F	10	13 weeks	0, 25, 50, 100, 200, 400	100	200 hepatic micro-granulomas

Both sexes exhibited slightly increased incidence of thyroid lesions at ≥ 50 ppm (males: vehicle control, 3/9; 2/10; 5/10; 5/10; 4/10; females: 0/10; 0/10; 1/10; 3/10; 2/10). The severity of thyroid lesions in exposed animals was comparable to that observed in vehicle controls. A NOAEL and LOAEL of 0.61 mg/kg-day and 6.9 mg/kg-day, respectively, were identified, based on increased incidence and severity of combined hepatic lesions in male rats. Limitations of this study include 1) lack of documentation on the preparation and renewal of the test medium and chemical analysis to verify concentrations, and 2) lack of information on the specific type of lesions observed in the liver and thyroid.

NTP (1987) studied subchronic toxicity of BDCM in male and female F344/N rats (10/sex/dose) given doses of 0, 19, 38, 75, 150, or 300 mg/kg-day in corn oil by gavage five days/week for 13 weeks. The 19 mg/kg-day group received 1.9 mg/kg-day for the first three weeks of the study. Body weight was recorded weekly. Necropsy was performed on all animals at study termination and histopathological examinations on the vehicle control, 150 mg/kg-day, and 300 mg/kg-day groups. Five of ten male rats and 2/10 female rats in the high dose group died prior to study termination. Final mean body weight of male rats was reduced by 30 and 55 percent, respectively, in the 150 and 300

mg/kg-day dose groups. Final mean body weight of female rats was reduced by 12 and 21 percent, respectively, in the 150 and 300 mg/kg-day dose groups. Compound-related lesions were observed only at the high dose. Centrilobular degeneration of the liver with occasional necrotic cells and mild bile duct hyperplasia was observed in 4/9 high dose males. Kidney lesions in high dose males consisted of degeneration of renal proximal tubular epithelial cells (incidence: 4/9) and definite foci of coagulative necrosis of the tubular epithelium (2/9). Other lesions reported in high dose males included lymphoid degeneration of the thymus, spleen, and lymph nodes (4/9) and mild to moderate atrophy of the seminal vesicles and/or prostate (4/9). Enlarged hepatocytes were observed in high dose females (2/9). Although degeneration of the spleen, thymus, and lymph nodes was noted in high dose females, the extent of the atrophy was much less than that observed in males. A NOAEL of 75 mg/kg-day and a LOAEL of 150 mg/kg-day were identified based on reduced body weight gain in male and female animals.

NTP (1987) also assessed the toxicity of BDCM in male and female B6C3F$_1$ mice (10/sex/dose) with BDCM doses of 0, 6.25, 12.5, 25, 50, or 100 mg/kg-day for males and 0, 25, 50, 100, 200, or 400 mg/kg-day for females in corn oil by gavage, five days/week for 13 weeks. Body weights were measured weekly. Necropsy was performed on all animals, and histopathology on the vehicle control and 50, 100, 200, and 400 mg/kg-day groups. All animals survived to the end of the study. No compound-related clinical signs were noted. The final mean body weight of males in the 100 mg/kg-day group was decreased by 8.5 percent relative to control. The final mean body weights of females in the 200 and 400 mg/kg-day groups were reduced by five percent and six percent, respectively. Compound related lesions were observed at 100 mg/kg-day in male mice and at 200 and 400 mg/kg-day in female mice. Increased incidences of focal necrosis of the proximal renal tubular epithelium (6/10) and nephrosis of minimal severity (2/10) were observed in male mice. Hepatic microgranulomas were observed in 7/10 females at 200 mg/kg-day and 8/10 females at 400 mg/kg-day. A NOAEL of 50 mg/kg-day and LOAEL of 100 mg/kg-day were identified based on renal histopathology in male mice.

DeAngelo *et al*. (2002) demonstrated that brominated THMs, especially BDCM, administered in the drinking water significantly induced preneoplastic aberrant crypt foci, a type of early putative preneoplastic lesions, in the colon of male F344/N rats. Rats were exposed to isomolar concentrations of the THMs at 0.5 g/L chloroform, 0.7 g/L BDCM, 0.9 g/L DBCM, or 1.1 g/L bromoform, for 13 weeks. Deionized water and 0.25 percent Alkamuls EL-620 were the negative and vehicle controls. Aberrant crypt foci incidence (percent) and number (aberrant crypt foci/colon) for the rat were: combined controls, 0; 100 percent Alkamuls EL-620, 27.17 ± 6.28 ($p < 0.01$); chloroform, 16.7 percent, 0.17 ± 0.17; BDCM, 83.3 percent, 1.50 ± 0.56 ($p < 0.01$); DBCM, 50 percent, 1.17 ± 0.65 ($p < 0.01$); bromoform, 66.7 percent, 1.17 ± 0.40 ($p < 0.01$). THM-induced aberrant crypt foci occurred primarily (92 percent) in the rectal segment of the colon.

Genetic Toxicity

Although the overall data are mixed, positive results were obtained for mutagenicity in several strains of *S. typhimurium*, induction of sister chromatid exchange in at least three studies, and chromosome aberrations *in vivo* and *in vitro*. Positive or equivocal results

were obtained for mutagenicity in cultured mouse lymphoma cells. Thus, the weight of the available evidence suggests that BDCM has mutagenic and genotoxic potential.

In Vitro Assays

The genotoxicity of BDCM has been evaluated in numerous *in vitro* assays conducted in bacteria and eukaryotic cells (Table 30). A potential limitation of the BDCM *in vitro* genotoxicity database is the failure of many authors to indicate whether the tests were conducted in a closed system to prevent volatilization loss of BDCM from the incubation mixture. Use of a sealed test system is noted in cases where the study authors provided this information.

Table 30. Summary of *In Vitro* Genotoxicity Studies on BDCM

Endpoint	Assay System	Results (with/without metabolic activation)	References
Gene mutation – Bacteria *(Salmonella typhimurium)*	TA100[a]	NT/+	Simmon *et al*. (1977), Simmon and Tardiff (1978)
	TA100	-/+	Ishidate *et al*. (1982)
	TA98, TA100, TA1535, TA1537	-/-	NTP (1987), Mortelmans *et al*. (1986)
	TA97, TA100 TA98 TA104	+/- (+)/- (+)/(+)	Strobel and Grummt (1987)
	TA1537 TA98, TA100, TA1535, TA1537	-/+ -/-	Varma *et al*. (1988)
	TA100, TA97 TA98 TA102	+/- +/+ -/-	Mersch-Sundermann (1989)
	TA100 (fluctuation test)	-/+	LeCurieux *et al*. (1995)
	TA1535[a]	NT/+	Pegram *et al*. (1997)
Gene mutation – Mammalian cells	Mouse lymphoma cells	+/-	NTP (1987), McGregor *et al*. (1988)
	Mouse lymphoma cells	?/-	Sofuni *et al*. (1996)
Chromosome aberrations	Chinese hamster fibroblast cells	+/-	Ishidate *et al*. (1982); Ishidate (1987)
	Chinese hamster FAF cells	NT/+	Strobel and Grummt (1987)
	Chinese hamster ovary cells	-/-	NTP (1987), Anderson *et al*. (1990)
	Chinese hamster lung fibroblasts[a]	(+)/(+)	Matsuoka *et al*. (1996)
DNA damage	*Escherichia coli* PQ37, SOS chromotest	-/-	Mersch-Sundermann *et al*. (1989)
	Escherichia coli PQ37, SOS chromotest	+/+	LeCurieux *et al*. (1995)
Sister	Human lymphocytes	NT/+	Morimoto and Koizumi (1983)

Endpoint	Assay System	Results (with/without metabolic activation)	References
chromatid exchange	Human Lymphocytes Rat liver cells	+/NT (+)/NT	Sobti (1984)
	Chinese hamster ovary cells	-[b]/-	NTP (1987), Anderson *et al*. (1990)
	Chinese hamster FAF cells	NT/-	Strobel and Grummt (1987)
	Rat erythroblastic leukemia K_3D cells	?/+	Fujie *et al*. (1993)
Other	*Saccharomyces cerevisiae* strains D7 (conversion) and XV185-14C (reversion)	-/(+)	Nestmann and Lee (1985)
	Aneuploidy	-/-	Matsuoka *et al*. (1996)

NT = Not Tested
(+) weakly positive response
[a] Assay was conducted in a closed system.
[b] Equivocal results were obtained.

Gene Mutation in Bacteria

The mutagenic potential of BDCM has been investigated in *S. typhimurium* reverse mutation assays in multiple test strains. Simmon *et al*. (1977) and Simmon and Tardiff (1978) determined that BDCM vapor was mutagenic in *S. typhimurium* strain TA100 when assayed in a desiccator without metabolic activation. The minimum amount required to elicit a mutagenic response was 600 µmol. Ishidate *et al*. (1982) assayed mutagenicity of BDCM in TA100 in the presence and absence of rat liver S9 fraction. Increased mutation frequencies were observed only in the absence of S9 activation. The concentrations tested in these assays were not reported. NTP (1987, also reported in Mortelmans *et al*., 1986) reported that BDCM was nonmutagenic in strains TA1535, TA1537, TA98, or TA100 when tested in the presence or absence of metabolic activation using a preincubation protocol at concentrations up to cytotoxic levels. The study authors concluded that their negative results might have been due to volatilization of BDCM from the test system. Strobel and Grummt (1987) obtained positive results for mutagenicity in TA97 and TA100 with exogenous metabolic activation. Weakly positive results were obtained for TA104 with and without exogenous metabolic activation and TA98 with metabolic activation. Varma *et al*. (1988) tested for mutagenicity in strains TA1535, TA1537, TA98, and TA100. BDCM at concentrations of 2.4 to 3.2 µmol/plate induced mutations in TA1537 in the absence of metabolic activation. No effect was observed in the other tested strains. Mersch-Sundermann (1989) obtained positive results in the absence of metabolic activation for TA100 and TA97; positive results with and without metabolic activation in TA98; and negative results with and without metabolic activation in TA102. LeCurieux *et al.* (1995) obtained negative results in TA100 in the fluctuation test modification of the reverse mutation assay.

Gene Mutation in Mammalian Cells

Two studies have examined mutagenicity in cultured mammalian cells. NTP (1987, also reported in McGregor *et al*., 1988) found that BDCM induced dose-related increases in forward mutations in the mouse lymphoma L5178Y/TK$^{+/-}$ assay when tested with metabolic activation at concentrations ≥ to 300 µg/mL. Negative results were obtained for mutagenicity without metabolic activation. Sofuni *et al*. (1996) tested BDCM in the mouse lymphoma L5178Y/TK$^{+/-}$ assay as part of an international collaborative program under the auspices of the Japanese Ministry of Health and Welfare. The results of this assay were equivocal. BDCM evoked a marginal dose-dependent response in the presence of exogenous metabolic activation in one of two test laboratories.

Sister Chromatid Exchange

The effect of BDCM on sister chromatid exchange has been examined in several studies. Morimoto and Koizumi (1983) observed a dose-dependent increase in frequency of sister chromatid exchange in cultured human lymphocytes in the absence of metabolic activation. The response was statistically significant at concentrations ≥ 400 µM. Sobti (1984) reported that 100 µM BDCM significantly increased the frequency of sister chromatid exchange in CCRF-CEM human lymphoid cells and RL_4 rat liver cells. The response in rat liver cells was considered marginal. Strobel and Grummt (1987) reported no induction of sister chromatid exchange in Chinese hamster FAF cells tested in the absence of metabolic activation. NTP (1987, also reported in Anderson *et al*., 1990) found no evidence for induction of sister chromatid exchange in CHO cells exposed to BDCM without metabolic activation. With S9 metabolic activation, one of three assays resulted in a positive response at ≥ 4,000 µg/mL, a concentration of BDCM that caused cytotoxicity in the other trials. Fujie *et al*. (1993) observed a statistically significant, dose-related increase in sister chromatid exchange in rat erythroblastic leukemia K_3D cells exposed to BDCM without exogenous activation. A comparison of sister chromatid exchange with and without metabolic activation at only one concentration showed increased sister chromatid exchange in the presence of metabolic activation.

Chromosome Aberration

Ishidate *et al*. (1982) and Ishidate (1987) reported induction of chromosomal aberrations in Chinese hamster fibroblasts by BDCM with but not without metabolic activation. The BDCM concentrations were not reported. Strobel and Grummt (1987) obtained positive results in Chinese hamster FAF cells in the absence of metabolic activation. NTP (1987, also reported by Anderson *et al*., 1990) found no evidence of induction of chromosomal aberrations in CHO cells with up to 5,000 µg/mL BDCM with or without exogenous metabolic activation. Matsuoka *et al*. (1996) found weak induction of chromosome aberrations in Chinese hamster lung fibroblast (CHL/IU) cells exposed to BDCM in tightly capped flasks in the presence and absence of exogenous metabolic activation.

Other *In Vitro* Genotoxicity

Nestmann and Lee (1985) investigated mutagenicity of BDCM in *Saccharomyces cerevisiae* strains D7 (gene conversion) and XV185-14C (reverse mutation) at 0.001 to 0.1 µg/mL with and without S9 activation. A weakly positive increase in convertants and in revertants was observed for BDCM in the absence of metabolic activation.

LeCurieux *et al*. (1995) reported induction of DNA damage by BDCM in the presence and absence of exogenous activation, as determined in the *E. coli* PQ37 SOS chromotest. CCRF-CEM human lymphoblastic leukemia cell lines exposed to BDCM at 5 or 10 mM for two hours produced DNA strand breaks (Geter *et al*., 2004). In contrast, Mersch-Sundermann *et al*. (1989) found no induction of DNA damage by BDCM using the same assay. Geter *et al*. (2004) reported negative results for DNA strand breaks in primary rat hepatocytes exposed to BDCM at 5 or 10 mM for four hours.

In Vivo Assays

In vivo data for BDCM genotoxicity are available for chromosome aberrations, sister chromatid exchange, induction of micronuclei, and DNA damage and repair. The results of *in vivo* genotoxicity tests on BDCM are summarized in Table 31.

Table 31. Summary of *In Vivo* Genotoxicity Studies on BDCM

Endpoint	Assay System	Result	References
Micronucleus induction	Mouse, rat, bone marrow cells	-	Ishidate *et al*. (1982)
	Mouse, bone marrow cells	-	Hayashi *et al*. (1988)
	Mouse, peripheral erythrocytes	-	Hayashi *et al*. (1992)
	Newt, peripheral erythrocytes	(+)	LeCurieux *et al*. (1995)
Chromosome aberrations	Rat, bone marrow cells (oral) Rat, bone marrow cells (i.p.)	+ +	Fujie *et al*. (1990)
Sister chromatid exchange	Mouse, bone marrow cells	+	Morimoto and Koizumi (1983)
DNA damage	Rat, renal cells	-	Potter *et al*. (1996)
Unscheduled DNA synthesis	Rat, hepatocytes	-	Stocker *et al*. (1997)

(+) = weakly positive

Chromosome Aberration

Fujie *et al*. (1990) assessed chromosome aberrations in the bone marrow of Long-Evans rats following oral (males only) or intraperitoneal (males and females) exposure to BDCM. Oral administration of BDCM induced dose-related increases in chromatid and chromosome breaks. A more pronounced response was observed following a single intraperitoneal dose with statistically significant effects occurring at 16.4 mg/kg.

Sister Chromatid Exchange

Morimoto and Koizumi (1983) examined the frequency of sister chromatid exchange in bone marrow cells from male ICR/SJ mice following exposure to BDCM. The animals received gavage doses of 0, 25, 50, 100, or 200 mg/kg-day in olive oil for four days.

BDCM exposure resulted in a dose-dependent increase in sister chromatid exchange frequency, statistically significant at 50 mg/kg-day and above.

Micronucleus Induction

Ishidate *et al*. (1982) examined induction of micronuclei in ddY mice, MS mice, and Wistar rats following BDCM doses of 125 to 500 mg/kg-day in olive oil by ip injection, with animals sacrificed 18, 24, 30, 48, or 72 hours later. No significant induction of micronucleus formation in bone marrow was observed in either mice or rats at any tested dose. Hayashi *et al*. (1988) found no induction of micronuclei in bone marrow of ddY mice following BDCM at single ip doses up to 500 mg/kg in corn oil. Hayashi *et al*. (1992) studied induction of micronuclei in peripheral blood erythrocytes by BDCM using manual and flow cytometric methods. No evidence of induction was observed at ip doses up to 100 mg/kg BDCM given once/week for five weeks. LeCurieux *et al*. (1995) obtained weakly positive results for induction of micronuclei in *Pleurodeles waltl* larvae.

Other *In Vivo* Genotoxicity

Potter *et al*. (1996) examined the effect of BDCM on DNA strand breakage in kidney cells of male F344 rats given 123 or 246 mg/kg-day of BDCM by gavage in four percent Emulphor® for one, three, or seven days. No compound-related DNA strand breaks were observed (Potter *et al*., 1996). No DNA strand breaks in liver, kidney, or duodenum epithelial cells were observed four hours after male F344/N rats were gavaged with 0.3 or 0.6 mM/kg BDCM in 0.5 percent Emulphor® (Geter *et al*., 2004). Rats exposed to BDCM in drinking water at 0.6, 1.2, or 2.4 g/L for five weeks showed no induction of DNA strand breaks in liver, kidney, or duodenum epithelial cells (Geter *et al*., 2004).

Stocker *et al*. (1997) evaluated unscheduled DNA synthesis in hepatocytes harvested from BDCM-treated rats. Male SD rats were gavaged with a single dose of 0, 135, or 450 mg/kg BDCM in aqueous one percent methylcellulose. The doses were selected to deliver 0, 30 and 100 percent, respectively, of the maximum tolerated dose of BDCM. Unscheduled DNA synthesis was evaluated two and 14 hours after treatment. No evidence of increased unscheduled DNA synthesis was obtained at either dose.

Foureman *et al*. (1994) found DBCM to be nonmutagenic in the sex-linked recessive lethal test in *Drosophila melanogaster*. The flies were both fed and injected with DBCM however the doses (units) administered are unclear in the tabular summary of results.

Micronucleus tests with BDCM and DBCM (Hayashi *et al*., 1988) and a chromosome aberration test with BDCM (NTP, 1987) did not show clastogenicity in the mouse bone marrow. Although NTP (1987) reported weak activity for bromoform in the mouse micronucleus test, Hayashi *et al*. (1988) found no activity in the same system.

Benigni *et al*. (1993) evaluated induction of aneuploidy and toxicity in *Aspergillus nidulans* treated with the THMs and several other halomethanes. The most active THM in inducing whole chromosome segregants (nondisjunctional diploids and haploids) was DBCM (5.62 percent segregants less background at 0.02 percent by volume) followed by BDCM (8.56 percent at 0.06 percent), bromoform (2.32 percent at 0.03 percent), and chloroform (6.36 percent at 0.15 percent). All the other halomethanes except bromotrichloromethane (3.11 percent at 0.015 percent) exhibited less aneuploid induction

than the THMs. All these values were statistically significant with the X^2 test ($p < 0.001$). The authors also described quantitative structure activity relationships (QSAR) using physical chemical and molecular orbital parameters: the dose able to block mitotic growth (ARR); the dose with 37 percent survival (D_{37}); and the lowest efficient concentration in aneuploidy induction (LEC). For the THMs the observed LECs were: DBCM, 1.47 mM; bromoform, 3.43 mM; BDCM, 6.00 mM versus predicted LECs of 2.24 mM; 0.86 mM; and 4.00 mM, respectively. The QSAR model correctly predicted the aneugenic activity of five out of six halomethanes. According to the QSAR model, induction of aneuploidy in *A. nidulans* depends on both the electrophilic and steric properties of the chemicals, whereas toxicity mainly depends on steric factors.

Mode of Action Studies

Two studies have examined the mutagenicity of BDCM in strains of *S. typhimurium* engineered to express the rat *GSTT1-1* gene. These studiesare discussed in the section on metabolism earlier in this document. The pathways proposed for bioactivation of brominated THMs (based in part on these data) are presented in Figure 2.

Developmental and Reproductive Toxicity

At least ten oral exposure studies on the reproductive and developmental toxicity of BDCM have been conducted in rats, mice, or rabbits (Table 32). These studies include prenatal and two-generation studies and studies on the mode of action for induction of full litter resorption in F344 rats. Studies by NTP (1998a) and Christian *et al*. (2001a,b; 2002) followed established protocols and were Good Laboratory Practice compliant.

Ruddick *et al*. (1983) assessed developmental toxicity of BDCM in SD rats. Females, 15/dose, received BDCM in corn oil at 0, 50, 100, or 200 mg/kg-day by gavage on gestation days six to 15. Body weights were recorded on gestation days one through 15, and before and after C-section on day 22, when the dams were sacrificed and tissues were removed for pathological examination. Hematology and clinical chemistry parameters were evaluated in maternal blood. Dam liver, heart, brain, spleen, and one kidney were weighed. Each dam was examined for resorption sites and viable fetuses. All fetuses were weighed, and examined for viability and external malformations. Histopathologic examination was performed on control and high dose females (five/group) and on two pups per litter. The remaining live fetuses were examined for skeletal alterations and visceral abnormalities.

One animal died in the control group. The number of litters ranged from nine to 14 per dose group. Maternal body weight gain was reduced 13, 15, and 38 percent at the three doses, respectively, statistically significant only at the highest dose. Relative maternal liver weight was significantly increased in all dosed groups and relative maternal kidney and brain weights were significantly reduced at the high dose. No significant compound-related changes in hematology, clinical chemistry, maternal or fetal histopathology, resorptions, or fetuses per litter were noted.

Table 32. Summary of Developmental and Reproductive Toxicity Studies on BDCM

Reference	Species, strain, sex	N	Dose (mg/kg-day)	Route	Duration	NOAEL (mg/kg-day)	LOAEL (mg/kg-day)
Ruddick *et al*. (1983)	Rat Sprague-Dawley F	9-14	0, 50, 100, 200	Gavage (oil)	Gestation days 6-15	200	-
Narotsky *et al*. (1997)	Rat F344 F	13-14	0, 75, 100	Gavage (oil) (water)	Gestation days 6-15	25	50 (full litter resorption)
NTP (1998a)	Rat Sprague-Dawley M, F	10-13	Males - 0, 8, 41, 68 Females - 0, 14, 72, 116	Drinking water	25-30 days	68 (males) 116 (females)	-
Klinefelter *et al*. (1995)	Rat F344 M	7	0, 22, 39	Drinking water	52 weeks	22	39 (decreased sperm velocities)
Bielmeier *et al*. (2001)	Rat F344 F		8-11	Gavage (aqueous)	Gestation day 9	-	75 (full litter resorption)
Christian *et al*. (2001b)	Rabbit NZ White F	5	0, 4.9, 13.9, 32.3, 76.3	Drinking Water	Gestation days 6-29	76	-
Christian *et al*. (2001a)	Rabbit F	25	0, 1.4, 13, 36, 55	Drinking water	Gestation days 6-29	55 developmental	-
Christian *et al*. (2001b)	Rat M, F	10	Variable by physiological stage	Drinking water	Males 64 days Females 74 days	50 ppm 16.3 - 41.4[a]	150 ppm 23.5 - 40.3[a] (decreased pup body weight)
Christian *et al*. (2001a)	Rat Sprague-Dawley F	25	0.0, 2.2, 18.4, 45.0, 82.0	Drinking water	Gestation days 6-21	45	82 (reduced ossification assoc. with maternal toxicity)
Christian *et al*. (2002)	Rat Sprague-Dawley M, F	30	Variable by physiological stage	Drinking water	Two generation	50 ppm 4.1-12.6[a]	150 ppm 11.6-40.2[a] (delayed sexual maturation assoc. with reduced body weight)

[a] The estimated average daily dose is presented as a range because it varied widely across physiological stage; therefore, a biologically meaningful average dose could not be calculated for the duration of treatment.

No differences were reported for fetal weights, gross malformations, or visceral anomalies; increased sternebral aberrations were observed in all treated groups. The numbers of affected litters out of total litters were 2/9, 4/14, 7/13, and 6/10 for control, low, mid, and high dose groups; our analysis using the Fisher exact test indicates that none of these increases differs significantly from control. The developmental toxicity NOAEL was 200 mg/kg-day. The maternal NOAEL and LOAEL are 100 and 200 mg/kg-day, respectively, based on decreased maternal body weight gain.

Klinefelter *et al*. (1995) evaluated the effects of BDCM on male reproduction during a chronic cancer bioassay study. Seven F344 rats/dose were provided with drinking water containing 0, 330, or 620 mg/L of BDCM. An interim sacrifice was conducted at 52 weeks and included an evaluation of epididymal sperm motion parameters and histopathological examination of the testes and epididymides. The estimated daily doses were 0, 22, and 39 mg/kg-day based on measured body weight and water consumption. Sperm velocities (mean straight-line, average path, and curvilinear) were significantly decreased at 39 mg/kg-day, but not at 22 mg/kg-day. No histopathological alterations were observed in any reproductive tissues. A NOAEL of 22 mg/kg-day and a LOAEL of 39 mg/kg-day were identified, based on decreased sperm velocities in the absence of histopathological change. However, this finding is different from that of a shorter duration rat study reported later where sperm measures including motility endpoints were not altered by BDCM exposure in drinking water for up to 35 days (NTP, 1998a).

Narotsky *et al*. (1997) evaluated developmental toxicity of BDCM in F344 rats gavaged with an aqueous 10 percent Emulphor® solution, versus a corn oil vehicle. Test animals (12-14/group) received doses of 0, 25, 50, or 75 mg/kg-day on gestation days six to 15. Effects on maternal body weight and other parameters were monitored. Dams were allowed to deliver naturally and pups were examined and weighed on postnatal days one and six. Skeletal and visceral anomalies in the pups were not evaluated. The dams were sacrificed after examination of pups on postnatal day six and the number of uterine implantation sites per female was recorded. The uteri of females that did not deliver litters were evaluated histologically to detect any cases of full litter resorption.

One animal that received the high dose in corn oil died before study termination. Clinical signs of toxicity that were observed only for the corn oil vehicle included kyphosis (humpback) in high dose animals and chromodacryorrhea/lacrimation in mid dose animals. Piloerection was observed in high-dose rats given either vehicle and in mid-dose rats given the aqueous vehicle. Maternal weight gain on gestation days six to eight was significantly reduced in all aqueous vehicle-dosed groups and in the mid- and high-dose groups administered the corn oil vehicle. Statistical analysis of maternal weight gain indicated no interaction between vehicle and dose. Full litter resorption was observed at the mid and high doses in rats administered the aqueous (8 and 83 percent, respectively) and corn oil (17 and 21 percent, respectively) vehicles. Statistical analysis of the full litter resorption incidence showed a significant vehicle-dose interaction. One high dose corn oil vehicle litter was carried to term but was delivered late and all pups died by postnatal day six. No significant effects were observed on duration of gestation, pre- or postnatal survival, or pup morphology in surviving litters. A NOAEL and a LOAEL for developmental toxicity of 25 mg/kg-day and 50 mg/kg-day, respectively, were identified for both vehicles based on full litter resorption. A LOAEL for maternal

toxicity of 25 mg/kg-day was identified, based on significantly reduced body weight gain on gestation days six to eight in dams receiving the aqueous vehicle.

Narotsky *et al*. (1997) calculated both an ED_{05} (the effective dose producing a five percent increase in response rate above background) and a benchmark dose (BMDL, the lower confidence limit of the ED_{05}) for each vehicle based on the incidence of full litter resorption. For the corn oil vehicle, the ED_{05} and BMDL were 48.4 and 39.3 mg/kg-day, respectively. For the aqueous vehicle, the ED_{05} and BMDL were 33.3 and 11.3 mg/kg-day, respectively. Thus the corn oil vehicle yielded a less conservative (i.e., higher) BMDL than the aqueous vehicle, reflecting the different confidence intervals around the estimated five percent response levels.

NTP (1998a) conducted a short-term reproductive and developmental toxicity screen in SD rats on BDCM in drinking water at concentrations of 0, 100, 700, and 1,300 ppm, based on decreased water consumption in a 14 day range finding study. The formulation and assay of the test solutions were described in detail. The study assessed development, female and male reproduction, hematology, clinical chemistry, and pathology. The reproductive parameters evaluated included: males, testis and epididymis weight, sperm morphology, density and motility; females, mating index, pregnancy index, fertility index, gestation index, live births, resorptions, implants/litter, corpora lutea and pre- and post-implantation. Nonreproductive endpoints are discussed in the Short-Term Toxicity section in this document. The main study used two groups of male rats designated as Group A (non-BrdU-treated, 10 rats/dose group) and Group B (BrdU-treated, five or eight rats/dose) and three groups of female rats designated as Groups A (periconception exposure, 10 rats/dose group), B (gestational exposure, 13 rats/group), and C (periconception exposure, BrdU-treated, five or eight rats/group). Rats were dosed for 25 to 30 days, except for Group B females, which were dosed from gestation day six to birth (approximately 15 to 16 days).

All animals survived the treatment period except one 700 ppm Group A male. Body weight and food and water consumption were decreased at many of the time points for 700 or 1,300 ppm BDCM groups. Body weights in dosed groups were decreased by 5 to 13 percent, food consumption was decreased by 14 to 53 percent, and water consumption was decreased by 7 to 86 percent compared to control. Based on measured water consumption, the 0, 100, 700, and 1,300 ppm concentrations were equivalent to 0, 8, 41, and 68 mg/kg-day for male rats, 0, 14, 72, and 116 mg/kg-day for female rats in groups A and C, and 0, 13, 54, and 90 mg/kg-day for Group B females.

BDCM exposure did not alter any reproductive parameter investigated, except for a non-dose-related increase in live fetuses per birth at 100 ppm in Group C females, and a slight decrease in corpora lutea in Group A females at 700 ppm. NTP (1998a) concluded that BDCM was not a short-term developmental or reproductive toxicant at any of the doses tested. NOAELs for reproductive and developmental toxicity of 68 and 116 mg/kg-day for male and female rats, respectively, were identified.

Bielmeier *et al*. (2001) conducted a series of experiments to examine the mode of action of BDCM-induced full litter resorption, including a comparison of F344 and SD rats, a critical period study, and two hormone profile studies. In the strain comparison experiment, 13-14 female SD rats/group were gavaged with 0, 75, or 100 mg/kg-day

BDCM in 10 percent Emulphor® on gestation days six to 10. F344 rats (12-14/dose group) were concurrently dosed with 0 or 75 mg/kg-day in the same vehicle. The incidence of full litter resorption in SD rats treated with 75 or 100 mg/kg-day of BDCM was zero percent, compared to 62 percent in the F344 rats. The two strains showed similar signs of maternal toxicity, and the percent body weight loss after the first day of dosing was comparable for SD rats (no resorption observed) and the F344 rats that resorbed their litters. F344 rats that maintained their pregnancies generally did not lose weight after the first dose, although they gained significantly less weight than controls. The two rat strains had similar incidences of piloerection. The strains showed different ocular responses; one half (7/14) of the treated F344 rats showed lacrimation and/or excessive blinking shortly after dosing during the first two days of BDCM administration compared to only 1/28 of the SD rats. The study authors reported that lacrimation was not predictive of full litter resorption in F344 rats. The rats were allowed to deliver and pups were examined on postnatal days one and six. Surviving litters appeared normal and no effects on postnatal survival, litter size, or pup weight were observed.

The second experiment by Bielmeier *et al*. (2001) examined the critical period during pregnancy for induction of full litter resorption by BDCM. F344 rats (12-13/dose group) were dosed over two different five day periods during organogenesis and the incidences of litter resorption were compared. The rats were gavaged with 75 mg/kg-day BDCM in 10 percent Emulphor® on gestation days six to 10 (which includes the luteinizing hormone-dependent period of pregnancy) or on gestation days 11 to 15 (a luteinizing hormone-independent period). Additional groups of 8-10 rats dosed with 0 or 75 mg/kg-day on gestation days six to 15 served as negative and positive controls, respectively. Full litter absorption occurred only in rats treated during the luteinizing hormone-dependent period (gestation days six to 10 or six to 15). In contrast, all rats treated with BDCM on gestation days 11 to 15 maintained their litters. Surviving litters appeared normal with no effects on postnatal survival, litter size, or pup weight. The study authors interpreted the results as evidence for an effect of BDCM on luteinizing hormone secretion or signal transduction.

To further investigate endocrine responses that might be associated with BDCM-induced full litter resorption, Bielmeier *et al*. (2001) examined the serum profiles of luteinizing hormone and progesterone in two experiments. In the first, 8 to 10 F344 rats/dose were gavaged with 100 mg/kg BDCM in 10 percent Emulphor® on gestation days eight or nine. Progesterone and luteinizing hormone concentrations were measured in tail blood samples collected once daily on gestation days nine through 12. Full litter resorption occurred in 0, 60, and 100 percent of the control, gestation day eight and gestation day nine groups, respectively. When progesterone was measured 24 hours after dosing, all rats that resorbed their litters had markedly reduced levels of progesterone compared to the control rats or to rats that retained their litters.

In the second hormone profile study, Bielmeier *et al*. (2001) gave 0, 75, or 100 mg/kg-day of BDCM to F344 rats (8-11/dose) on gestation day nine by gavage in 10 percent Emulphor®. Blood samples were collected at 0, 6, 12, and 24 hours after dosing for determination of progesterone and luteinizing hormone concentrations. Full litter resorption in the three dose groups was 0, 64, and 90 percent, respectively. Peak progesterone levels occurred at six hours post-administration in all groups, including the

controls. The peak progesterone concentration was significantly reduced in the dams given 75 mg/kg (who resorbed their litters) compared to the peak concentration in the control group. The peak progesterone concentration in animals receiving 100 mg/kg was slightly reduced, but the effect did not reach statistical significance. At 12 and 24 hours after dosing, the progesterone levels of dams that resorbed their litters were significantly reduced in both BDCM dose groups compared to controls. Mean progesterone levels in dams that retained their litters were comparable to controls. Luteinizing hormone levels were comparable in control and treatment groups at all time points. A significant decline in luteinizing hormone levels was observed within all groups over the 24 hour period with no significant differences noted between groups.

Bielmeier *et al*. (2001) demonstrated that BDCM caused pregnancy loss, i.e., full-litter resorption, in F344 rats when given on gestational days six to 10, encompassing the luteinizing hormone-dependent period of gestational days seven to 10, considering the plug day as gestational day zero. Pregnancy loss was associated with reduced serum progesterone levels, but an effect on serum luteinizing hormone was not identified. The high full-litter resorption rate during the luteinizing hormone-dependent period, the lack of response thereafter, and the reduced progesterone without an associated reduction in luteinizing hormone suggests that BDCM disrupts luteal responsiveness to luteinizing hormone. A LOAEL of 75 mg/kg-day, the lowest dose tested, was identified for full litter resorption in F344 rats. A NOAEL could not be identified.

Using a more sensitive fluorometric technique (DELFIA®), with more frequent sampling to better define the temporal pattern of endocrine disruption during pregnancy caused by BDCM, Bielmeier *et al*. (2004) demonstrated that the chemical-induced pregnancy loss was associated with marked reductions in serum luteinizing hormone and corresponding decreases in progesterone on gestational day 10. F344 rats were gavaged with 75 mg/kg-day BDCM on gestational days six to 10. The decrease in serum luteinizing hormone consistently preceded the decrease in progesterone. Both progesterone and human chorionic gonadotropin prevented BDCM-induced pregnancy loss. Coadministration of progesterone or human chorionic gonadotropin, a luteinizing hormone agonist, prevented pregnancy loss. These results support the hypothesis that BDCM-induced pregnancy loss in the rat occurs via a luteinizing hormone-mediated mode of action.

Christian *et al*. (2001a) conducted developmental toxicity tests in SD rats and New Zealand White rabbits. Both studies were sponsored by the Chlorine Chemistry Council and were conducted in accordance with U.S. EPA Health Effects Test Guidelines OPPTS 870.3700: Prenatal Developmental Toxicity Study (U.S. EPA, 1998a) and U.S. EPA Good Laboratory Practice Standards. For the rat study, female SD rats (25/group) were exposed to BDCM in drinking water at 0, 50, 150, 450, or 900 ppm on days six to 21 of gestation. These concentrations were based on the NTP (1998a) results and a 14-day range-finding study conducted by Christian *et al*. (2001b), which found 1,350 ppm to cause excessive maternal toxicity. Procedures for preparation, storage and assay of the test solutions were described in the publication. Effects on viability, clinical signs, body weight, water consumption, and feed consumption were investigated. All study animals were sacrificed on gestation day 21 and caesarean sectioned. Each animal was necropsied and gross lesions were identified. Effects on gravid uterus weight (with cervix), number of corpora lutea per ovary, number and distribution of implantation sites,

live and dead fetuses, early and late resorption, and placental abnormalities were examined. Individual fetuses were weighed, sexed, and examined for external, soft tissue, and skeletal abnormalities. Approximately one half of the fetuses in each litter were examined for soft tissue alterations and the other half for skeletal alterations.

Dosages over the 16 day exposure period were calculated by the study authors from measured water consumption and body weights. The average doses were 0, 2.2, 18.4, 45.0, and 82.0 mg/kg-day in the 0, 50, 150, 450, and 900 ppm exposure groups, respectively. No adverse clinical signs, aborted fetuses, or premature deliveries were observed. All rats survived until scheduled sacrifice. No compound-related gross lesions were identified at necropsy. Absolute (g/day) and relative (g/kg-day) water consumption were significantly reduced in each exposure group for the entire exposure period. The effect was greatest on the first two exposure days and gradually decreased in severity with continued exposure. Reductions in feed consumption and body weight gain were observed and were considered secondary to reduced water consumption. Exposure-related decreases in absolute and relative feed consumption occured in the 150, 450, and 900 ppm groups. In the 150 ppm group, the effects were statistically significant only on gestation days 12 to 15 and were considered to be of little toxicological importance by the study authors. Absolute and relative feed consumption in the 450 ppm and 900 ppm groups were significantly reduced for the entire exposure period. Body weight gain and weight gain corrected for gravid uterus weight were significantly reduced for the entire exposure period in the 450 ppm and 900 ppm groups. Average maternal body weights on gestation day 21 were significantly reduced in the same exposure groups after correction for gravid uterine weights, although the gravid uterine weights alone were not affected.

No effects on litters were observed in dams exposed to BDCM concentrations of up to 900 ppm. Pregnancy occurred in all but two rats in the control group. Litter averages for corpora lutea, implantations, litter sizes, proportion of live fetuses, early or late resorptions, fetal body weights, reabsorbed conceptuses, and live fetuses were comparable among all groups. No full litter resorption were observed and there were no dead fetuses. Late resorption occurred in one control group litter. All placentas appeared normal. All measures of the examined litters were within the historical range of the test facility (Argus Research Laboratories). No compound-related gross external, soft tissue or skeletal malformations were identified. Statistically significant changes were observed in skeletal variations consisting of reversible delays in ossification. An increased incidence of wavy ribs (one control group fetus and four fetuses in the 900 ppm group) was considered a marginal or noneffect because the litter incidence (the more relevant measure of effect) was not significantly different from control and the value was within the historical range of the test facility. The control group fetus and one fetus in the 900 ppm also had hypoplastic ribs. Significant decreases in the average number of ossification sites per fetus for the forepaw phalanges and hindpaw phalanges and metatarsals in the 900 ppm exposure group indicated an effect on fetal growth.

A maternal NOAEL and LOAEL of 18 and 54 mg/kg-day, respectively, were identified, based on significant reductions in maternal body weight and body weight gains. A developmental NOAEL and LOAEL of 45 mg/kg-day (450 ppm) and 82 mg/kg-day (900 ppm), respectively, were identified, based on significantly decreased number of ossification sites/fetus for forepaw phalanges and hindpaw metatarsals and phalanges.

In the Christian *et al*. (2001a) prenatal developmental toxicity study in New Zealand White rabbits, BDCM was provided to presumed pregnant rabbits (25/exposure group) at 0, 15, 150, 450, or 900 ppm in drinking water on gestation days six to 29. Viability, clinical signs, feed consumption, water intake, and body weight were monitored daily during the exposures. Doses calculated from water intake and body weights averaged 0, 1.4, 13.4, 35.6, and 55.3 mg/kg-day, respectively, over the 14-day treatment. Changes in gross lesions, uterine weight, abnormal placentas, number of corpora lutea, number of implantation sites, live and dead fetuses, and early and late resorption were investigated at Caesarean sectioning on gestation day 29. Each fetus was examined for weight, sex, fetal ossification sites, and external, soft tissue and skeletal abnormalities. One rabbit in the 900 ppm group was sacrificed because of a back injury, and another rabbit in the 900 ppm group had a dead litter as a result of a uterine abnormality. These occurrences were not considered compound-related. No compound-related clinical signs or necropsy results were observed. The 450 and 900 ppm groups had significantly reduced feed and water consumption rates throughout the exposure period. Body weight gains and body weight gains corrected for the weight of gravid uterus in the 450 ppm and 900 ppm exposure groups were reduced for the entire exposure period. The 900 ppm group lost weight on gestation days 21 to 27. Average corrected maternal body weights were affected, but gravid uterine weights were not significantly affected.

BDCM had no observable effect on litter averages for implantations, corpora lutea, live litter size, early or late resorptions, percentage of male fetuses, percentage of resorptions, or fetal body weight. No instances of full litter resorption were observed. Statistically significant increases in number of fused sterna centra were observed in the 150 and 450 ppm groups, but this effect was not exposure-dependent and the incidences were within the historical range for the test facility. The NOAEL and LOAEL for maternal toxicity in this study were 13.4 mg/kg-day (150 ppm) and 35.6 mg/kg-day (450 ppm), respectively, based on decreased body weight gain. The developmental NOAEL was 55.3 mg/kg-day (900 ppm) based on absence of statistically significant, dose-related effects.

Christian *et al*. (2001b) reported the results of range-finding developmental toxicity studies in SD rats and New Zealand White rabbits. Male and female SD rats (10/sex/group) were randomly assigned to five exposure groups. Additional rats (six males/group and 15 females/group) were assigned to satellite groups for analysis of BDCM in selected tissues and fluids. BDCM exposure in drinking water at 0, 50, 150, 450, or 1,350 ppm began 14 days before cohabitation and continued through gestation and lactation to give total exposure durations of 63 to 70 days. Viability and clinical signs were monitored daily. Water consumption was monitored daily, except during cohabitation and after lactation day 15 (when intakes were confounded by multiple animals consuming water). Body weights were recorded weekly and, for females, on lactation days 1, 5, 8, 11, 15, 22, and 29. All rats were evaluated for gross lesions at necropsy. Reproductive parameters evaluated included estrous cycling, mating performance, duration of gestation, fertility indices, gestation indices, average numbers of live and dead pups, and viability and body weights of the pups on selected lactation days. Viability and lactation indices, percent survival, and sex ratios were evaluated in the litters. Two male and female weanlings/litter were selected for additional observations, including daily feed and water consumption, weekly body weights and necropsy. The

remaining pups were sacrificed and examined for gross lesions on lactation day 29. No histopathology was performed on the F_0 or F_1 generations.

In the F_0 generation, all male rats and all but one female rat survived to scheduled sacrifice. Exposure-dependent reductions in relative water consumption were attributed to apparent taste aversion. The reductions were most evident in the 450 and 1,350 ppm groups, and was reported to be accompanied by reduced feed consumption and reduction of body weight (data not provided). The average doses calculated from body weight and water consumption measurements varied by physiological stage. No gross lesions attributable to BDCM were reported in male or female F_0 rats at necropsy. No effects of BDCM were observed on any of the reproductive parameters in the F_0 male or female rats. However, exposure to BDCM was associated with a concentration-dependent reduction in F_1 pup body weights in the 150, 450, and 1,350 ppm groups. Statistical analysis was not reported in this dose range-finding study. A LOAEL and a NOAEL for developmental toxicity of 150 ppm and 50 ppm, respectively, were identified, based on decreased pup weight. A LOAEL and a NOAEL for the F_0 generation of 450 ppm and 150 ppm, respectively, was identified, based on reduced body weight gain. These concentrations correspond to doses of 16.3 to 41.7 mg/kg-day and 23.5 to 90.3 mg/kg-day, respectively, for the females, expressed as a range because of marked changes in drinking water consumption by F_0 female rats during the different stages (premating, mating, gestation, and lactation). For F_0 males, exposure to 150 ppm and 450 ppm correspond to administered doses of about 12 and 28 mg/kg-day, respectively.

In the second Christian *et al*. (2001b) range-finding study, New Zealand White presumed pregnant rabbits (five/group) were exposed to BDCM in drinking water at 0, 50, 150, 450, and 1,350 ppm on gestation days six to 29. Four additional rabbits/dose were treated for assay of BDCM in tissues. Viability, clinical signs, body weight, and feed and water consumption were monitored daily during exposure. The rabbits were euthanized on gestation day 29. Effects on fetal body weight, viability, sex ratio, and external alterations were investigated. The BDCM doses were 0.0, 4.9, 13.9, 32.3, and 76.3 mg/kg-day, estimated from body weights and water consumption. Effects on water and feed consumption and body weight gain were reported to be similar to those observed in rats (no data provided). No adverse effects on the fetus were observed at exposures up to 1,350 ppm. Insufficient data were provided for identification of a maternal LOAEL and NOAEL. The NOAEL for developmental effects of 76 mg/kg-day (1,350 ppm), the highest dose tested, was identified, based on the absence of adverse effects on fetus.

Christian *et al*. (2002) evaluated reproductive toxicity of BDCM in a two-generation study conducted in SD rats. BDCM was provided to animals (30/sex/concentration in each generation) in the drinking water at 0, 50, 150, or 450 ppm. F_0 generation males were exposed for about 106 days, while parental generation female rats were exposed for about 116 days. F_0 and F_1 generation rats were evaluated for viability, clinical signs, water and feed consumption body weights, gross pathology, organ weights, histopathology (control and high dose groups, 10 animals/sex; 50 and 150 ppm rats suspected of reduced fertility), and reproductive endpoints (mating, fertility, abortions, premature deliveries, duration of gestation, litter sizes, sex ratios, viability, reproductive organ weights, sperm parameters, and implantations). F_1 rats were evaluated for age at vaginal latency or preputial separation. Three F_1 and F_2 weanling rats/sex/litter were

evaluated for organ weights. Offspring were evaluated for implantation and pup numbers, sex ratio, viability, body weight, external gross alterations and reproductive parameters in F_1 adults.

The BDCM dose for each drinking water concentration varied by sex and reproductive status. The 50, 150 and 450 ppm concentrations corresponded to 4.1 to 12.6, 11.6 to 40.2, and 29.5 to 109 mg/kg-day, respectively, as calculated by the study authors. One death in the 150 ppm group and three deaths (including one humane sacrifice) in the 450 ppm group may have been compound-related. Adverse clinical signs occurred in F_0 females and F_1 males and females in the 150 and 450 ppm exposure groups. Compound-related signs included chromorhinorrhea, pale extremities, urine-stained abdominal fur and coldness to touch. These signs were considered to be associated with reduced water consumption. Body weight and body weight gain were significantly reduced in the 450 ppm F_0 males and 150 and 450 ppm F_1 males and females. Significantly reduced final body weight in F_0 females exposed to 450 ppm was associated with decreased absolute organ weights and increased relative organ weights. Absolute and relative water consumption rates were significantly reduced in F_0 and F_1 males and females at all BDCM concentrations. Absolute and relative feed consumption rates were reduced in males and females of both generations at 150 and 450 ppm. There were no gross pathological or histopathological indications of compound-related toxicity.

Most indicators of reproductive or developmental toxicity examined by Christian *et al*. (2002) were unaffected by BDCM treatment. However, at 150 and 450 ppm, the F_1 pups had statistically significant reductions in body weight at weaning on lactation day 22, while the F_2 pup body weights were also reduced, but non-significantly. Sexual maturation was slightly delayed in the 150 and 450 ppm groups as determined by vaginal latency and preputial separation, and estrus was delayed in F_1 females at 450 ppm. The study authors considered these effects to be a secondary response associated with reduced pup weights. A NOAEL and LOAEL for reproductive effects of 50 ppm (4.1 to 12.6 mg/kg-day) and 150 ppm (11.6 to 40.2 mg/kg-day), respectively, were identified based on delayed sexual maturation associated with reduced body weight. However, the study authors questioned whether delayed sexual maturation should be treated as reproductive toxicity or general toxicity, since the root cause appears to be dehydration brought about by taste aversion to BDCM. A NOAEL and LOAEL of 50 and 150 ppm respectively were identified in the F_0 generation based on reduced body weight and body weight gain.

Immunotoxicity

Limited animal studies on immunotoxicity of BDCM were identified. Munson *et al.* (1982) gave doses of 0, 50, 125, or 250 mg/kg-day BDCM to CD-1 male and female mice (8-12/sex/dose) by gavage for 14 days. BDCM treatment resulted in decreased antibody-forming cells in serum and decreased hemagglutination titers. These changes were statistically significant at the high dose in both sexes. Decreased hemagglutination was noted in mid-dose females. A NOAEL of 50 mg/kg-day and a LOAEL of 125 mg/kg-day were identified, based on decreased immune function in female mice.

French *et al*. (1999) evaluated immunotoxicity of BDCM in four experiments conducted in mice and rats. In the first experiment, six female C57BL/6 mice/group were exposed

to drinking water containing 0, 0.05, 0.25, or 0.5 g/L BDCM with 0.25 percent Emulphor® for 14 or 28 days. The immunological endpoints examined were antibody response to injected sheep red blood cells and T and B lymphocyte proliferation. Mitogens used in the proliferation assay were concanavalin A or phytohemagglutinin-p for T cells and lipopolysaccharide for B cells. The average BDCM doses were estimated by the authors to be 0, 10, 37, or 62 mg/kg-day. There were no significant differences in the number of antibody forming cells, antibody production, spleen weights, or splenic and mesenteric lymph node cell proliferative responses to T and B cell mitogens. A NOAEL of 62 mg/kg-day was identified based on lack of effect at the highest dose.

In the second experiment of French *et al*. (1999), six female C57BL/6 mice/dose group received 0, 50, 125, or 250 mg/kg-day of BDCM in 10 percent aqueous Emulphor® by gavage once a day for 16 days. No compound-related differences were observed in antibody-forming cells, antibody titers, or mitogen-induced proliferation in any treatment group. Decreased spleen weight and spleen to body weight ratio were observed at 125 mg/kg-day compared to the Emulphor® control, but a dose-dependent trend was not observed. A NOAEL of 250 mg/kg-day was based on lack of effects at the highest dose.

French *et al*. (1999) investigated immunotoxicity of BDCM in male F344 rats in two experiments. In each case, the immunological parameters examined were antibody response to injected sheep red blood cells and T and B lymphocyte proliferation. The mitogens used in the proliferation assay were Concanavalin A or phytohemagglutinin-p for T cells and *S. typhimurium* mitogen for B cells. In the first rat study, six animals/group were exposed to drinking water containing 0, 0.07 or 0.7 g/L BDCM and 0.25 percent Emulphor® for 26 weeks, which resulted in average doses of 0, 5, or 49 mg/kg-day, respectively, as estimated by the study authors. A significant suppression of Concanavalin A-stimulated proliferation of spleen cells occurred in the 49 mg/kg-day dose group. No other compound-related effects on measured parameters were reported. These data suggest NOAEL and LOAEL values of 5 and 49 mg/kg-day, respectively.

In the final experiment, French *et al*. (1999) administered doses of deionized water, 10 percent Emulphor®, or 75, 150, or 300 mg/kg BDCM in 10 percent Emulphor® by gavage to six female F344 rats/group for five days. Two rats in the 300 mg/kg-day group died during the exposure period. Decreased body, spleen, and thymus weights were observed in surviving high dose animals relative to either control group. Concanavalin A and phytohemagglutinin-p responses were depressed in spleen cells isolated from high dose animals. Thymus weight was decreased in the 150 mg/kg-day group. Concanavalin A responses were significantly depressed in both spleen and mesenteric lymph node cells in the 300 mg/kg-day group. All three dose groups exhibited suppression of phytohemagglutinin-p stimulated mesenteric lymph node cells compared to the vehicle (but not the water) control. The Emulphor® vehicle alone significantly elevated proliferative response to phytohemagglutinin-p in mesenteric lymph node cells relative to the deionized water group. In contrast to the T cell responses, there was a significant increase in antibody production and proliferative responses to *S. typhimurium* mitogen (B cells) from spleen cells at 300 mg/kg-day. A NOAEL of 150 mg/kg-day and a LOAEL of 300 mg/kg-day were identified, based on depression of immune response.

Neurotoxicity

BDCM is a central nervous system toxicant at higher doses. In an acute study, sedation occurred in mice within 30 minutes after administration of 500 mg/kg by gavage (Bowman *et al*., 1978) and persisted for four hours after treatment. Clinical signs observed in rats dosed with up to 1,500 mg/kg included sedation, flaccid muscle tone, ataxia, and piloerection (Chu *et al*., 1980, 1982a). Central nervous system effects are believed to result from a nonspecific anesthetic effect, similar to that produced by various other volatile halocarbons (ATSDR, 1989).

Balster and Borzelleca (1982) administered a screening battery of behavioral tests to adult male ICR mice exposed to BDCM under a variety of dosage regimens. The experiments examined acute dose effects; 14 and 90 day treatments at 300 or 3,000 times the estimated average human daily intake of BDCM in disinfected tap water (1.2 and 11.6 mg/kg-day, respectively); 30 days of treatment at 100 mg/kg-day; and 60 days of treatment at 100 or 400 mg/kg-day. In each case, BDCM was given by gavage in a 1:8 mixture of Emulphor® and distilled water. A nongavaged control group was included in the 14 and 90 day experiments in addition to a vehicle control group. Six to eleven animals were used/treatment.

In the acute study, five or six doses were administered but the dose levels were not reported. Motor performance on a screen test was evaluated at 30, 60, and 90 minutes after treatment. Effects were noted at 30 minutes, with little evidence of change at 60 or 90 minutes. An ED_{50} of 524 mg/kg (95 percent CI 273 to 1,007) was calculated for this response. Clinical signs of ataxia and incoordination were observed at higher doses, and anesthesia at the highest dose. The 14-day experiment evaluated the effect of BDCM doses of 0, 1.2, or 11.6 mg/kg-day for 14 consecutive days on swimming endurance, evaluated 24 hours after the final treatment. No effect on performance was observed. The 90-day experiment evaluated the effect of 0, 1.2, or 11.6 mg/kg-day BDCM, administered for 90 consecutive days, on the cling test, screen test, and holeboard, evaluated 24 hours after the last dose. No significant effect on performance was observed for any of the tests. The 30-day experiment examined the effect of 0 or 100 mg/kg-day BDCM on latency to enter the dark compartment in a passive avoidance learning test, 24 hours after the final treatment. BDCM had no effect on passive avoidance learning or initial step-through latency (Balster and Borzelleca, 1982).

The final experiment in the series evaluated operant conditioning in mice exposed to BDCM doses of 0, 100, or 400 mg/kg-day for 60 days. Performance was measured daily during a three-day vehicle pretreatment period. The mice were then treated with BDCM 30 minutes prior to daily behavioral testing for 60 consecutive days. Behavioral measurements were continued for three days after termination of BDCM treatment. Treatment with 400 mg/kg-day significantly decreased response rates at the last day of pretreatment compared with the first day of treatment. The study authors noted no evidence for progressive deterioration and reported that partial tolerance occurred over the course of the experiment (no data were presented in support of these statements).

This study indicates that BDCM has low potential for eliciting adverse effects in several standard tests of behavioral toxicity. A LOAEL of 400 mg/kg-day was identified, based

on a nonprogressive decrease (with development of partial tolerance) in response rates in the operant conditioning test.

Chronic Toxicity

The chronic oral toxicity of BDCM has been assessed in four studies: a dietary study conducted in male and female rats, two gavage studies (one per species) in male and female rats and mice, and a drinking water study in male rats (Table 33). No data are available on the chronic inhalation toxicity of BDCM.

Table 33. Summary of Chronic Toxicity Studies on BDCM

Reference	Species, strain, sex	N	Dose, (mg/kg-day)	Route	Duration	NOAEL (mg/kg-day)	LOAEL (mg/kg-day)
NTP (1987)	Rat F344/N M, F	50	0, 50, 100	Gavage (oil)	102 weeks	-	50 kidney and liver lesions
NTP (1987)	Mouse $B6C3F_1$ M, F	50	0, 25, 50	Gavage (oil)	102 weeks	-	25 liver, kidney, and thyroid lesions
Aida *et al*. (1992b)	Rat Wistar M, F	40	Male 0, 6, 26, 138 Female 0, 8, 32, 168	Diet	24 months	-	6 liver fatty degeneration and granuloma
Klinefelter *et al*. (1995)	Rat F344 M	7	0, 330, 620	Drinking water	52 weeks	-[a]	-

[a]The NOAEL and LOAEL for this study are based on reproductive parameters

Tobe *et al*. (1982) administered BDCM in the diet to male and female Slc:Wistar SPF rats (40/sex/group) for 24 months. BDCM was microencapsulated and mixed with powdered feed at concentrations of 0.0, 0.014, 0.055, or 0.22 percent. Control groups (70/sex) received microcapsules without the test compound. Body weight and food consumption were monitored weekly for the first six months, every two weeks from six to 12 months, and every four weeks thereafter. Interim sacrifices were conducted at six, 12, and 18 months. All surviving animals were sacrificed at two years. Hematology, serum biochemistry measurements and necropsy were performed at each time of sacrifice. Histopathology, body weight, organ weight, hematology, and clinical chemistry results from this study are reported in Aida *et al*. (1992b).

The estimated average doses were 0, 6, 26, or 138 mg/kg-day for males and 0, 8, 32, or 168 mg/kg-day for females based on measured food intake and body weight. Marked reduction of body weight gain was seen in males and females at the high dose. Animals

in the high dose groups exhibited persistent mild piloerection and emaciation from month one until study termination. Relative liver weight was significantly increased ar the mid and high doses at each time point. Absolute kidney weight decreased and relative kidney weight increased at the high dose. No compound-related changes in hematological parameters were noted in either sex. Dose-dependent reductions in serum triglycerides and cholinesterase activity and increased γ-glutamyl transpeptidase were noted in one or more dose groups at all time points. Decreased serum glucose was noted at all time points for females. Gross necropsy revealed dose-related yellowing and roughening of the liver surface in high dose and some mid dose animals. Compound-related microscopic lesions were limited to the liver. Selected lesion incidences at 24 months are shown in Table 34. Dose-related fatty degeneration and granuloma were observed in males and females at 24 months. Cholangiofibrosis was observed at the high dose. A LOAEL was identified of 6 mg/kg-day for histopathological changes in both sexes.

Table 34. Incidence of Hepatic Lesions in Wistar Rats Exposed for 24 Months to Microencapsulated BDCM in the Diet (Aida *et al*., 1992b)

Lesion	Dietary concentration of BDCM (percent)			
	0	**0.014**	**0.055**	**0.22**
Males				
Fatty degeneration	0/24	5/14	12/13	19/19
Granuloma	0/24	4/14	9/13	19/19
Cholangiofibrosis	0/24	0/14	0/13	4/19
Females				
Fatty degeneration	2/32	8/19	18/18	18/18
Granuloma	0/32	8/19	18/18	18/18
Cholangiofibrosis	0/32	0/19	0/18	12/18

NTP (1987) assessed chronic effects of BDCM in male and female F344/N rats (50/sex/dose). BDCM was administered in corn oil by gavage at 0, 50, or 100 mg/kg-day five days/week for 102 weeks. Clinical signs were recorded weekly. Body weights were recorded weekly for the first 12 weeks of the study and monthly thereafter. Necropsy was performed on all animals, including those found dead. Complete histopathology was performed on all females and on high dose and vehicle control males. Histopathological examinations were conducted on low dose males in instances of premature death, on all gross lesions, and on tissues and organs where chemically-related lesions were identified in the high dose male rats. Tissues examined in low dose males were adrenal glands, colon, heart, kidney, liver, lung, pancreas, preputial gland, and rectum. Survival of dosed rats was comparable to that of vehicle control. Mean body weights of high dose male and female rats were decreased compared to the corresponding vehicle control starting at 15 and 32 weeks, respectively. Final mean body weights in high dose males and females were 88 and 79 percent of control, respectively. No compound-related clinical signs were reported. In males, compound-related nonneoplastic effects included cytomegaly of

renal tubular epithelial cells (vehicle control, 0/50; low dose, 18/50; high dose, 44/50); renal tubular cell hyperplasia (0/50; 1/50; 3/50); hepatic necrosis (1/50; 4/50; 6/50); and fatty metamorphosis (36/50; 48/50; 47/50). In females, compound-related changes included eosinophilic cytoplasmic change (0/50; 1/50; 11/50); clear cell change (4/50; 6/50; 39/50); focal cellular change (4/50; 4/50; 11/50); fatty metamorphosis of the liver (7/50; 22/50; 13/50); and renal tubular cell hyperplasia (0/50; 1/50; 6/50). A LOAEL of 50 mg/kg-day was identified based on histopathological changes in the liver and kidney.

NTP (1987) also assessed chronic effects of BDCM in male and female $B6C3F_1$ mice (50/sex/dose) gavaged five days/week for 102 weeks with 0, 25, or 50 mg/kg-day for males and 0, 75, or 150 mg/kg-day for females. Clinical signs were recorded weekly. Body weights were recorded weekly for the first 12 weeks and monthly thereafter. Necropsy was performed on all animals, including those found dead. Complete histopathologic examinations were performed on all animals. Final survival of treated male mice was comparable to that of vehicle control. The survival of low (after week 79) and high dose female mice (after week 61) was significantly lower than that of the vehicle controls. Final survival rates were 26/50, 13/50, and 15/50 for the 0, 5, and 50 mg/kg-day groups, respectively. Decreased survival was associated in part with ovarian abscesses (8/50, 19/47, 18/49). Mean body weights of high dose male mice were within 10 percent of the control throughout the study. Mean body weights of low dose male mice were comparable to those of vehicle control. Mean body weight of high dose female mice decreased progressively during the study, with a low value of 64 percent relative to vehicle control at weeks 92 and 96. Final mean body weights for low and high dose females were 91 and 75 percent of the control, respectively. No compound-related clinical signs were reported. Compound-related nonneoplastic lesions in males included fatty metamorphosis of the liver (incidence: vehicle control, 4/49; low dose, 8/50; high dose, 19/50); renal cytomegaly (0/49; 41/50; 47/50), and thyroid follicular cell hyperplasia (0/48; 3/44; 5/49). Compound-related thyroid follicular cell hyperplasia was observed in females (6/50; 18/45; 21/48). A LOAEL of 25 mg/kg-day was identified, based on histopathological alterations in male and female mice.

Klinefelter *et al*. (1995) reported 52-week interim necropsy and histopathology findings from a cancer bioassay in male F344 rats. Seven rats/dose were given drinking water containing average BDCM concentrations of 0, 330, or 620 ppm. At sacrifice, the testis, epididymis, liver, spleen, kidney, thyroid, stomach, intestine, and bladder were examined for histopathology. Average doses of 0, 22, and 39 mg/kg-day were estimated by the authors from measurements of water consumption and body weight. BDCM had no effect on body weight or on kidney, liver, spleen, or thyroid weight. There was no histopathological evidence of compound-related effects on any of the organs. High levels of nephropathy and interstitial cell hyperplasia were observed, but were not considered compound-related. A NOAEL and LOAEL were identified based on reproductive endpoints (summarized in the section on developmental and reproductive toxicity).

Carcinogenicity

The database on the carcinogenic potential of BDCM consists of eight oral and one ip bioassays (Table 35). No studies were identified that examined carcinogenicity in animals exposed to BDCM by inhalation.

George *et al*. (2002) investigated carcinogenicity of BDCM in male F344/N rats and male $B6C3F_1$ mice. Groups of 50 animals/dose were administered BDCM at 0.05, 0.25 or 0.5 g/L for mice and 0.07, 0.35 or 0.70 g/L for rats in drinking water containing 0.25 percent Emulphor®. Average doses calculated by the investigators were 8.1, 27.2 or 43.4 mg/kg-day for mice and 3.9, 20.6 or 36.3 for rats, based on measured BDCM levels and water consumption. In several interim sacrifices, animals were examined for gross lesions and given a complete necropsy. All gross lesions and samples of thyroid, liver, stomach, duodenum, jejunum, ileum, colon, rectum, spleen, kidneys, urinary bladder, and testis were examined microscopically for histopathology. Five days prior to sacrifice, BrdU was implanted subcutaneously to measure hepatocyte and renal tubular cell proliferation using a labeling index.

Table 35. Summary of Carcinogenicity Studies on BDCM

Reference	Species, strain sex	N	Dose (mg/kg-day)	Route	Exposure duration	Response
George *et al*. (2002)	Rat	50	0, 3.9, 20.6, 36.3	Drinking water	104 weeks	significant increase in hepatocellular adenomas and carcinomas in low and mid doses
George *et al*. (2002)	Mouse	50	0, 8.1 27.2, 43.4	Drinking water	100 weeks	no statistically significant increase in any tumor type
NTP (1987)	Rat F344/N M, F	50	0, 50, 100	Gavage (corn oil)	102 weeks	significantly increased tumors in large intestine and kidney in males and females
NTP (1987)	Mouse $B6C3F_1$ M, F	50	0, 25, 50	Gavage (corn oil)	102 weeks	significantly increased tumors in male kidneys and female livers
Voronin *et al*. (1987b)	Mouse CBA × C57B1/6 M, F	50-75	0, 0.0076, 0.76, 76	Drinking water	104 weeks	no statistically significant increase in incidence observed for any tumor type
Tumasonis *et al*. (1987)	Rat Wistar M, F	58	Males 100 Females 175	Drinking water	185 weeks	significant increases in hepatic neoplastic nodules, hepatic adenofibrosis, and lymphosarcoma in females
Aida *et al*. (1992b)	Rat Wistar M, F	40	Male 0, 6, 26, 138 Female 0, 8, 32, 168	Diet	24 months	low incidence of tumors in liver and kidney
Theiss *et al*. (1977)	Mouse Strain A Male	20	0, 20, 40, 100	IP Injection (up to 3 times/wk)	8 weeks	increased incidence of lung tumors at high dose, not statistically significant

In the male mice, water consumption decreased in the high dose groups, but food consumption did not differ among the groups. No significant effects on survival, body weight or weights of liver, spleen or testis were observed. Significant decreases in kidney weights that appeared to be dose-related were observed. There were no significant alterations in clinical chemistry. Mild nonneoplastic changes were reported in the liver but no changes in hepatocyte or renal tubular cell proliferation or increases in hepatocellular or renal tubular cell neoplasms were observed.

In the male rats, water and food consumption were comparable among the dose groups. No compound-related changes in survival and body weight were apparent. Liver, spleen, testes and thyroid weights were not significantly different from control, but kidney weights were reduced in the high dose group. No alterations in clinical chemistry were observed at 26, 52 or 78 weeks and no evidence of hepatocellular or renal toxicity was observed. Hepatocellular cell proliferation was unaffected by histopathological examination and renal cell proliferation appeared to be decreased as measured by BrdU incorporation at the interim sacrifice. Hepatocellular adenomas were significantly increased at the low dose (7/45 in the 3.9 mg/kg-day group) and combined hepatocellular adenomas and carcinomas were increased in the low and mid dose groups but not at the high dose (8/45 at 3.9 mg/kg-day and 7/48 at 20.6 mg/kg-day). No increases in large bowel cancers were observed. Renal tubular cell hyperplasia was significantly increased in the mid and high dose groups compared to control.

The increase in combined liver tumors in male rats observed by George *et al*. (2002) was statistically significant at the low dose, and marginal at the mid dose (along with no effect at the high dose). The authors speculate that these observations may have resulted from a decrease in metabolism (to an active metabolite) at higher doses. Other investigators have observed a decrease in microsomal enzyme activity as measured by ethoxyresorufin-*O*-dealkylase and pentoxyresorufin-*O*-dealkylase activity (CYP1A and CYP1B) with repeated BDCM dosing (75 mg/kg-day or 150 mg/kg-day) in the rat but not in the mouse (Thornton-Manning *et al*., 1994). A reduction of P450 levels was observed at doses greater than 150 mg/kg-day. Allis *et al*. (2001) noted an increase in ethoxyresorufin-*O*-dealkylase and pentoxyresorufin-*O*-dealkylase activity and CYP2B1 levels measured using Western Blot techniques with inhalation exposures at or below 100 ppm in F344 rats. Decreased enzyme activity and a decrease in CYP2B1 levels were observed following inhalation of greater than 100 ppm of BDCM. No effects were noted on p-nitrophenol hydroxylase activity or CYP2E1 levels with a similar exposure in the male F344 rat. Cytochrome P450 levels mirrored effects on CYP2B1 levels.

NTP (1987, also reported in Dunnick *et al*., 1987) evaluated carcinogenic potential of BDCM in male and female F344/N rats. Animals (50/sex/group) received 0, 50, or 100 mg/kg-day BDCM in corn oil by gavage five days/week for 102 weeks. Clinical signs were recorded weekly. Body weights were recorded weekly for the first 12 weeks and monthly thereafter. Necropsy was performed on all animals, including those found dead. Complete histopathology was performed on all female rats and on high dose and vehicle control males. Histopathological examinations were conducted on low dose males in instances of premature death, on all gross lesions, and on tissues and organs where chemically-related lesions were identified in the high dose male rats. Tissues examined in low dose males were adrenal glands, colon, heart, kidney, liver, lung, pancreas,

preputial gland, and rectum. Non-cancer effects are discussed above under chronic toxicity studies. Statistically significant increases in the incidences of adenomatous polyps and adenocarcinoma of the large intestine and tubular cell adenoma and carcinoma in the kidney were observed in male and female rats (Table 36). The neoplasms of the large intestine and kidney are considered biologically significant because they are uncommon tumors in F344/N rats. Historical incidence of tumors of the large intestine in NTP studies is less than 0.2 percent in male rats and zero percent in female rats. Historical incidence of kidney tumors in NTP studies is 0.6 percent in male rats and 0.1 percent in female rats. The study authors considered these uncommon tumors to be clear evidence of carcinogenic activity under the conditions of this study.

Table 36. Tumor Frequencies in Fischer 344/N Rats Exposed to BDCM in Corn Oil for Two Years (NTP, 1987)

Sex	Site	Tumor Type	Tumor Incidence		
			Control	50 mg/kg	100 mg/kg
Male	Large intestine [a]	Adenomatous polyp	0/50	3/49	33/50[b]
		Adenocarcinoma	0/50	11/49[b]	38/50[b]
		Combined adenoma and carcinoma[d]	0/50	13/49[b]	45/50[b]
	Kidney [a]	Tubular cell adenoma	0/50	1/49	3/50
		Tubular cell adenocarcinoma	0/50	0/49	10/50[b]
		Combined adenoma and carcinoma[e]	0/50	1/49	13/50[b]
Female	Large intestine [c]	Adenomatous polyp	0/46	0/50	7/47[b]
		Adenocarcinoma	0/46	0/50	6/47[b]
		Combined adenoma and carcinoma[f]	0/46	0/50	12/47[b]
	Kidney	Tubular cell adenoma	0/50	1/50	6/50[b]
		Tubular cell adenocarcinoma	0/50	0/50	9/50[b]
		Combined adenoma and carcinoma[g]	0/50	1/50	15/50[b]

[a] One low dose rat died accidentally at week 33 and was dropped from the incidence calculation.
[b] Statistically significant at $p < 0.05$, compared to controls.
[c] The intestine was not examined in four vehicle controls and three high dose rats.
[d] Historical incidence at study laboratory (mean): 0/250; historical incidence in NTP studies: 3/1,390 (0.2 percent)
[e] Historical incidence at study laboratory (mean): 1/250 (0.4 percent); historical incidence in NTP studies: 8/1,448 (0.6 percent)
[f] Historical incidence at study laboratory: 0/236; historical incidence in NTP studies: 0/1,400
[g] Historical incidence at study laboratory (mean): 0/250; historical incidence in NTP studies: 2/1,447 (0.1 percent)

NTP (1987, also reported in Dunnick *et al*., 1987) evaluated carcinogenic potential of BDCM in male and female B6C3F$_1$ mice. The animals (50/sex/dose) received doses of 0, 25, or 50 mg/kg-day (males) or 0, 75, or 150 mg/kg-day (females) in corn oil by gavage for five days/week for 102 weeks. Clinical signs were recorded weekly. Body weights

were recorded weekly for the first 12 weeks and monthly thereafter. Necropsy was performed on all animals, including those found dead. Complete histopathological examinations were performed on all animals. Non-cancer effects are discussed above under chronic toxicity studies. Adenoma and adenocarcinoma of the kidney in male mice and adenoma and carcinoma of the liver in female mice showed significant positive trends (Table 37). NTP (1987) considered these tumors to be clear evidence of carcinogenic activity under these experimental conditions.

Table 37. Tumor Frequencies in B6C3F$_1$ Mice Exposed to BDCM in Corn Oil for two Years (NTP, 1987)

Sex	Site	Tumor type	Tumor incidence		
			Control	**25 mg/kg**	**50 mg/kg**
Male	Kidney [b]	Tubular cell adenoma	1/46	2/49	6/50
		Tubular cell adenocarcinoma	0/46	0/49	4/50
		Combined adenoma and adenocarcinoma[c]	1/46	2/49	9/50[a]
			Control	**75 mg/kg**	**150 mg/kg**
Female	Liver	Hepatocellular adenoma	1/50	13/48[a]	23/50[a]
		Hepatocellular carcinoma	2/50	5/48	10/50[a]
		Combined adenoma and carcinoma[d]	3/50	18/48[a]	29/50[a]

[a] Statistically significant at $p < 0.05$, compared to controls.
[b] Mice not included in the tumor incidence were two control that died in week one, one that died during week nine, and one that escaped in week 79; one low dose mouse died in the first week.
[c] Historical incidence at study laboratory (mean): 2/299 (0.7 percent); historical incidence in NTP studies: 5/1,490 (0.3 percent).
[d]Historical incidence at study laboratory (mean ± standard deviation): 30/298 (10 ± 5 percent); historical incidence in NTP studies: 116/1,489 (8 ± 6 percent).

Tumasonis *et al*. (1987) exposed male and female Wistar rats (58/dose) to BDCM in drinking water from weaning until all study animals had died (approximately 185 weeks). The BDCM concentration in drinking water was 2,400 mg/L for the first 72 weeks, then was reduced to 1,200 mg/L for the remaining 113 weeks. The average doses to the rats were not reported, but appeared to be about 175 mg/kg-day for females and 100 mg/kg-day for males, as estimated from a graph provided in the publication. Exposed animals of both sexes weighed less than controls at all ages. Incidence of neoplastic hepatic nodules was significantly increased in exposed females relative to controls (control, 0/18; exposed, 17/53), but not in males (control, 5/22; exposed, 6/47). Significant increases were also noted in incidence of hepatic adenofibrosis and lymphosarcoma in exposed females. One renal adenocarcinoma each was found in the male and female exposure groups. Pituitary tumors were nonsignificantly increased in exposed males. Statistically significant decreases in incidence of mammary and pituitary tumors in females and lymphosarcomas in males may have been related to decreased body weight.

Voronin *et al*. (1987b, as cited in U.S. EPA, 2005b) examined carcinogenicity of BDCM in CBA × C57Bl/6 mice. Animals (50-55 mice/sex) were exposed to BDCM in drinking water at 0.04, 4.0, or 400 mg/L for 104 weeks, along with untreated control groups of 75 male and 50 female mice. The concentrations used resulted in average daily BDCM doses of about 0.0076, 0.76, or 76 mg/kg-day. Total tumor incidences, based on the number of mice surviving until detection of the first tumor, were 4/63, 3/35, 1/16, and 1/18 for males, and 3/34, 1/45, 1/18, and 1/13 for females in the control, low, mid, and high dose groups, respectively. According to U.S. EPA (2005b), the study authors concluded that the results were not statistically significant by chi square analysis, and that BDCM was not carcinogenic under the conditions used in the bioassay.

BDCM was administered to male and female Slc:Wistar rats (40/sex/treatment and 70/sex for controls) at dietary levels of 0, 0.014, 0.055, or 0.22 percent for up to 24 months (Tobe *et al.*, 1982; Aida *et al*., 1992b). The test material was microencapsulated and mixed with powdered feed. Placebo capsules were added to the control diet. The estimated mean daily doses were 0, 6.1, 25.5, or 138.0 mg/kg-day for males and 0, 8.0, 31.7, or 168.4 mg/kg-day for females, as calculated using mean food intake rates. Body weight was decreased in high dose males and females at six, 12, and 18 months. A low tumor incidence was observed in the liver and kidney. Hepatocellular adenoma was observed in one low and one high dose male and in one control and two high dose females. Renal cell carcinoma and nephroblastoma were each observed in one high dose male. Cholangiocarcinoma was observed in one high dose male and three high dose females. Additional tumors were observed at other sites at low incidence or did not appear dose-related. The study authors concluded that there was no clear evidence for carcinogenic activity under the conditions of this study.

Theiss *et al*. (1977) examined the carcinogenic potential of BDCM in male Strain A mice. Mice (20/group) were administered BDCM iv up to three times/week with doses of 20, 40, or 100 mg/kg for eight weeks; concurrent positive and negative controls (20/group) were included. Mice were sacrificed 24 weeks after the first injection, and the frequency of lung tumors in each group was compared with vehicle-treated controls. The frequency of lung tumors/mouse was increased at the high dose (0.85 ± 0.27) compared to the controls (0.20 ± 0.11), but did not reach statistical significance ($p = 0.067$).

Mode of Action Studies

Melnick *et al*. (1998) investigated the relationship between liver toxicity and tumorigenicity of BDCM. Female $B6C3F_1$ mice (10/group) were gavaged with BDCM in corn oil five days/week for three weeks at doses of 0 (vehicle only), 75, 150, or 326 mg/kg-day. A significant dose-related increase in absolute liver weight and liver weight/body weight ratio was noted for the 150 and 326 mg/kg-day dose groups. Serum alanine aminotransferase activity was significantly increased at the two highest doses and serum sorbitol dehydrogenase activity was elevated at all doses tested. At necropsy, there was clear evidence of hepatocyte hydropic degeneration in animals treated with 150 and 326 mg/kg-day. BrdU was administered to the animals during the last six days of the study, and hepatocyte labeling index was analyzed. The 150 and 326 mg/kg-day doses resulted in significantly elevated hepatocyte proliferation as measured by the labeling index. These authors compared the BDCM dose response for liver toxicity (enzyme and

labeling index data) and tumorigenicity (data from previous NTP bioassays) using the Hill equation model, finding that the shape of the dose response as well as the Hill exponents were different for liver toxicity and tumorigenicity. The study authors concluded that their results do not support a causal relationship between liver toxicity/reparative hyperplasia and tumor development for BDCM.

Classification of Carcinogenic Potential

BDCM has been listed by the State of California as a chemical known to cause cancer since January 1, 1990. IARC (1991a, 1999a) has classified BDCM as a Group 2B carcinogen, possibly carcinogenic to humans (sufficient evidence of the carcinogenicity of BDCM in experimental animals, but inadequate evidence in humans).

The Carcinogenic Risk Assessment Verification Endeavor group of the U.S. EPA assigned BDCM to Group B2, a probable human carcinogen (U.S. EPA, 2008b). Assignment to this category is appropriate for chemicals where there are no, or inadequate, human data, but which have sufficient animal data to indicate carcinogenic potential. NTP (1998b) classified BDCM as reasonably anticipated to be a human carcinogen based on sufficient evidence of carcinogenicity in experimental animals.

Toxicological Effects in Humans

The database for toxicological effects of BDCM in humans is limited. The primary source of BDCM exposure is ingestion of tap water, where it occurs as a byproduct of disinfection. Epidemiological studies on reproductive outcomes associated with intake of disinfected water are summarized in a separate section below. Because the industrial production and use of BDCM are limited, there are no toxicity data available from occupational studies and no clinical or case report data were identified. No published experimental or epidemiological data were located for acute, subchronic, or chronic toxicity, genotoxicity, immunotoxicity, neurotoxicity, or carcinogenicity through oral or inhalation exposure to BDCM in humans.

As described in the section on bromoform, there have been two studies (DeMarini *et al*., 1997; Landi *et al*., 1999a,b, 2000) on the potential role of human polymorphisms in the *GSTT1-1* gene using *in vitro* techniques. The working hypothesis was that individuals with at least one copy of *GSTT1-1* would be more sensitive to the genotoxic effects of brominated THMs than individuals lacking the gene. The bromoform data are relevant to BDCM because these compounds are assumed to share common metabolism pathways.

Developmental and Reproductive Toxicity

Chen *et al*. (2003, 2004), in studies spurred by the epidemiological associations of BDCM and other THMs in drinking water with adverse reproductive outcomes, suggested that placenta is a likely target of human BDCM toxicity. Chen *et al*. (2003) found that secretion of immunoreactive chorionic gonadotropin and bioactive gonadotropin was reduced in primary cultures of human placental term syncytiotrophoblasts exposed to 20 to 2,000 nM BDCM. The lowest effective concentration of 20 nM is about 35 times higher than the maximum concentration reported in human blood at 0.57 nM. Chen *et al*. (2004) showed that BDCM

concentrations of 20 nM and 2,000 nM to mononucleated cytotrophoblast cells undergoing morphological differentiation to multinucleated syncytiotrophoblast-like colonies inhibited the formation of multinucleated colonies in a dose-dependent manner, as determined by immunocytochemical staining for desmosomes and nuclei. Secretion of bioactive and immunoreactive chorionic gonadotropin was significantly inhibited in a dose-dependent manner, and cellular levels of gonadotropin were also reduced. These results indicated that BDCM disrupts syncytiotrophoblast formation and inhibits gonadotropin secretion *in vitro*.

Dibromochloromethane

Toxicological Effects in Animals

The toxicological effects of DBCM in experimental animals have been comprehensively reviewed in documents prepared by U.S. EPA (1980b, 1987b, 1994b) and ATSDR (1990). Additional information is available in U.S. EPA (2008d) and in monographs prepared by the IARC (1991c, 1999c).

Acute Toxicity

The acute oral toxicity of DBCM has been studied in rats and mice. Acute lethality results are summarized in Table 38. Bowman *et al*. (1978) assessed acute toxicity of DBCM in groups of 10 male and female ICR Swiss mice, with at least seven dose levels from 500 to 4,000 mg/kg in each experiment. DBCM was solubilized in a 1:1:8 solution of Emulphor®:alcohol:saline and administered by gavage to fasted animals. The posttreatment observation period was 14 days. LD_{50} values were 800 mg/kg (95 percent CI 667 to 960 mg/kg) and 1,200 mg/kg (95 percent CI 945 to 1,524 mg/kg) for males and females, respectively. Ataxia, sedation, and anesthesia occurred within 30 minutes of treatment at 500 mg/kg and above. Sedation lasted approximately four hours. Necropsy on animals that died revealed apparent fatty infiltration of the liver, pale kidneys, and hemorrhage in the adrenals, lungs, and brain.

Chu *et al*. (1980, 1982a) evaluated acute toxicity of DBCM in groups of 10 SD ratsof both sexes. Fasted adults were gavaged with 546, 765, 1,071, 1,500, or 2,100 mg/kg in corn oil. The post-treatment observation period was 14 days. The LD_{50}s for males and females were 1,186 (95 percent CI 997 to 1,421) and 848 mg/kg (95 percent CI 576 to 1,090), respectively. Clinical signs in treated rats included sedation, flaccid muscle tone, and prostration. Gross pathological examination revealed liver and kidney congestion in treated animals. The results for growth, food intake, organ weight, histopathology, hematological indices, liver microsome aniline hydroxylase activity, and serum chemistry in surviving rats were reported in Chu *et al*. (1982a). DBCM significantly reduced feed intake and body weight gain of males at 1,500 mg/kg (the highest dose with surviving animals). Serum cholesterol was significantly increased in males at 1,500 mg/kg.

Table 38. Oral LD_{50} Values for DBCM

Species	Strain	Route (vehicle)	Sex	Number per dose group	LD_{50} (95 percent CI) mg/kg	Reference
Mouse	ICR Swiss	Gavage (aqueous)	M F	10 10	800 (667-960) 1,200 (945-1,524)	Bowman *et al*. (1978)
Rat	Sprague-Dawley	Gavage (corn oil)	M F	10 10	1,186 (997-1,421) 848 (576-1,090)	Chu *et al*. (1980)
Rat	F344/N	Gavage (corn oil)	M F	5 5	Not reported	NTP (1985)
Mouse	$B6C3F_1$	Gavage (corn oil)	M F	5 5	Not reported	NTP (1985)
Golden Hamster	--	Gavage (olive oil)	M	Not reported	145 (118-187)	Korz and Gatterman (1997)

The National Toxicology Program (NTP, 1985) assessed the acute oral toxicity of DBCM in male and female F344/N rats and $B6C3F_1$ mice, but did not compute LD_{50}s. Five rats and mice/sex/group received a single dose of 0, 160, 310, 630, 1,250, or 2,500 mg/kg by gavage in corn oil. One or two animals from each sex and dose group were necropsied. All rats at the highest dose died by day three. Four male rats and one female rat died at 1,250 mg/kg. One female rat died in the 630 mg/kg group. Doses ≥ 310 mg/kg produced lethargy for three hours after dosing. Gross necropsy of rats found no compound-related effects. For the mice, all males in the 1,250 and 2,500 mg/kg groups died. Three males given 630 mg/kg and one male given 310 mg/kg died. All female mice given 2,500 mg/kg and four females given 1,250 mg/kg died. No clinical signs were reported. At necropsy, livers with discolored foci and kidneys with dark red or pale medullae were seen more frequently in dosed mice than in the vehicle controls.

Korz and Gatterman (1997) reported results of acute toxicity tests in male golden hamsters, but did not provide experimental details. They estimated an LD_{50} of 145 mg/kg (95 percent CI 118 to 187 mg/kg). These data, if verifiable, indicate that golden hamsters are considerably more sensitive to the acute effects of DBCM than are rats and mice.

Müller *et al*. (1997) assessed cardiotoxic effects of acute DBCM exposure and time course for recovery. Male Wistar rats received a DBCM dose of 0, 83, 167, 333, or 667 mg/kg by gavage in olive oil. Heart rate, blood pressure, body temperature, and physical activity were monitored in conscious rats (six/group) by telemetry. Heart rate and blood pressure were also measured 25 minutes following administration in rats anesthetized with urethane (10/group). Conscious rats exhibited premature ventricular contractions one minute following administration of 667 mg/kg DBCM. Heart rate and body temperature initially decreased in all treatment groups following DBCM, recovering to control values within 24 hours in rats administered 83 to 333 mg/kg. Heart rate remained depressed in high dose rats for up to 48 hours. Body temperature in high dose animals

decreased to 4.5 °C below controls by 72 hours after exposure. Blood pressure increased initially in all treatment groups following dosing. Recovery toward control values was noted within 48 hours at doses up to 333 mg/kg. Blood pressure in the high dose group remained below control values 72 hours after exposure. Physical activity was decreased for the entire observation period in conscious rats dosed with 333 and 667 mg/kg. Negative effects on muscle contractility were observed in anesthetized rats at 333 and 667 mg/kg; negative effects on the rate of contraction were observed at 333 mg/kg, and negative dromotropic effects were observed at 167 to 667 mg/kg. Results for heart rate, blood pressure, and several contractility parameters did not exhibit dose-related trends.

Short-Term Toxicity

The short-term toxicity of DBCM has been evaluated in nine studies ranging from three days to one month in duration (Table 39).

Table 39. Summary of Short-Term Toxicity Studies on DBCM

Reference	Species	Route	Sex	N	Duration	Dose (mg/kg-day)	NOAEL (mg/kg-day)	LOAEL (mg/kg-day)
Munson *et al*. (1982)	Mouse CD-1	Gavage (aqueous)	M, F	8-12	14 days	0, 50, 125, 250	50	125 decreased body weight and immune function
Chu *et al*. (1982a)	Rat Sprague-Dawley	Drinking water	M	10	28 days	0, 0.7, 8.5, 68	68	--
Condie *et al*. (1983)	Mouse CD-1	Gavage (corn oil)	M	8-16	14 days	0, 37, 74, 147	74	147 liver, kidney, and stomach histopathology
NTP (1985)	Rat F344/N	Gavage (corn oil)	M, F	5	14 days	0, 60, 125, 250, 500, 1,000	125	250 reduced male body weight
	Mouse $B6C3F_1$	Gavage (corn oil)	M, F	5	14 days	0, 30, 60, 125, 250, 500	60	125 liver and kidney gross pathology
Aida *et al*. (1992a)	Rat Wistar	Diet	M	7	1 month	0, 18, 56, 173	18	56 liver histopathology
	Rat Wistar	Diet	F	7	1 month	0, 34, 101, 333	34	101 liver histopathology

Reference	Species	Route	Sex	N	Duration	Dose (mg/kg-day)	NOAEL (mg/kg-day)	LOAEL (mg/kg-day)
Potter *et al*. (1996)	Rat F344	Gavage (aqueous)	M	4	1, 3, or 7 days	0, 156, 312	156	312 decreased body weight after 7 d
Melnick *et al*. (1998)	Mouse $B6C3F_1$	Gavage (corn oil)	F	10	3 weeks (5 d/wk)	0, 50, 100, 192, 417	100	192 liver histopathology
Coffin *et al*. (2000)	Mouse $B6C3F_1$	Gavage (corn oil)	F	10	11 days	0, 100, 300	--	100 increased labeling index, liver histopathology
		Drinking water	F	10	11 days	0, 171	--	171 liver histopathology

Chu *et al*. (1982a) administered DBCM to male SD rats (10/group) in drinking water containing 0.25 percent Emulphor® for 28 days at DBCM concentrations of 0 (drinking water and Emulphor® controls), 5, 50, and 500 ppm. Water intake was measured twice per week and body weight and food consumption was measured weekly. Serum biochemical and hematological parameters, hepatic microsomal enzyme activities, selected nonmicrosomal enzyme activities, gross pathology, and histopathology were assessed at study termination. The estimated daily doses of DBCM were 0, 0.7, 8.5, or 68 mg/kg-day, calculated by the authors using water consumption and body weight data. No compound-related effects were reported; data were not provided for any endpoint. The NOAEL in this study was apparently 68 mg/kg-day.

Munson *et al*. (1982) administered DBCM by aqueous gavage to male and female CD-1 mice (8-12/sex/group) for 14 days at 0, 50, 125, or 250 mg/kg-day. Data were collected for body and organ weights, clinical chemistry and hematological parameters, humoral- and cell-mediated immune system functions, and hepatic microsomal enzyme activities. Effects on immune responses are discussed in the Immunotoxicity section. All dosed animals survived to study termination. Mean final body weight was significantly decreased in high dose males. Relative liver weight was significantly increased in males and females at the mid and high doses. Absolute and relative spleen and thymus weights were significantly decreased in males only at the high dose. Statistically significant changes in high dose males and females were decreased fibrinogen, decreased serum glucose, and increased serum aspartate aminotransferase and alanine aminotransferase activities. Hexobarbital sleeping time was increased in high dose females. The NOAEL and LOAEL are 125 mg/kg-day and 250 mg/kg-day, respectively, based on changes in body weight in males and clinical chemistry parameters in both sexes.

Condie *et al*. (1983) dosed male CD-1 mice (10/dose) with 0, 37, 74, or 147 mg/kg-day of DBCM by gavage in corn oil for 14 days. Body weight was measured on days one and 14. Blood was collected for clinical chemistry at study termination. Renal cortical slices were collected for measurement of *para*-aminohippurate uptake and samples of liver and kidney tissue were collected for histopathological examination. No treatment-related clinical signs were reported. Body weight was unaffected by DBCM treatment. Biochemical evidence of liver damage (elevated alanine aminotransferase) and kidney damage (decreased PAH uptake by kidney slices) was observed at the high dose. Compound-related increases of tubular epithelial hyperplasia and mesangial hypertrophy were observed in the kidney. Dose related changes were observed in the incidence of mitotic figures and cytoplasmic vacuolization. The severity of lesions in both organs was generally minimal to slight. The NOAEL and LOAEL were 74 mg/kg-day and 147 mg/kg-day, respectively, based on histopathological changes in the kidney and liver.

NTP (1985) evaluated short-term oral toxicity of DBCM in F344/N rats. Male and female rats (five/sex/group) were gavaged with 0, 60, 125, 250, 500, or 1,000 mg/kg-day in corn oil for 14 days. Clinical signs, body weight and mortality were evaluated. Rats receiving 500 and 1,000 mg/kg-day exhibited lethargy, labored breathing, and ataxia, and all rats dosed at 1,000 mg/kg-day and females dosed at 500 mg/kg-day died before the end of the study. Mean terminal body weight was reduced by 11 percent in males dosed with 250 mg/kg-day and by 20 percent in the two surviving animals at 500 mg/kg-day. At necropsy, mottled livers and reddened, darkened medullae were observed in males and females receiving ≥ 500 mg/kg-day. A NOAEL of 125 mg/kg-day and a LOAEL of 250 mg/kg-day were identified, based on decreased body weight in male rats.

NTP (1985) also evaluated the short-term oral toxicity of DBCM in $B6C3F_1$ mice. Five mice/sex received gavage doses of 0, 30, 60, 125, 250, or 500 mg/kg-day in corn oil for 14 days. Clinical signs, body weight, gross pathology, and mortality data were collected. Animals given the high dose exhibited ataxia, lethargy, and labored breathing, and four males and three females at the high dose died before study termination. Mean final body weights were comparable to those of the controls. Mottled livers and reddened renal medullae were observed in both sexes at 500 mg/kg-day. White papillomatous nodules were observed in the stomachs of males at ≥ 125 mg/kg-day and in females at ≥ 250 mg/kg-day. The NOAEL and LOAEL are 60 mg/kg-day and 125 mg/kg-day, respectively, based on gross pathological changes in the stomach, liver, and kidney.

Aida *et al*. (1992a) administered DBCM to Slc:Wistar rats (seven/sex/group) for one month at dietary levels of 0, 0.020, 0.062, or 0.185 percent for males and 0, 0.038, 0.113, or 0.338 percent for females. DBCM was microencapsulated and mixed with powdered feed; the control groups received feed containing placebo capsules. Clinical effects, body weight, food consumption, hematology parameters, serum chemistry, and histopathology of all major organs were evaluated. Based on the mean food intakes, the study authors calculated average daily doses of 0, 18.3, 56.2, and 173.3 mg/kg-day for males and 0, 34.0, 101.1, and 332.5 mg/kg-day for females. All animals survived to study termination. Slight piloerection and emaciation were observed in high dose females. Mean final body weight was significantly reduced (eight percent decrease) in high dose females relative to the controls. There were no significant changes in food consumption for any group. Absolute and relative liver weights were significantly increased in high dose males.

Absolute liver weight was significantly increased in mid and high dose females and relative liver weight was increased in all dosed females versus the controls. Relative kidney weights were increased in high dose females.

The treatments did not affect hematological parameters. Statistically significant and dose-related changes in serum chemistry observed in males included decreased nonesterified fatty acids (high dose), triglycerides (high dose), alkaline phosphatase activity (mid and high dose) and cholinesterase (high dose) and significantly increased total cholesterol (mid and high dose). Significant, dose-related changes in females included total triglycerides (high dose), total cholesterol (all doses), alkaline phosphatase (mid and high dose), cholinesterase (all doses) and lactate dehydrogenase (all doses). Gross and histopathological findings were limited to liver, which showed discoloration in males and females at the high dose. The incidence and severity of liver cell vacuolization, swelling, and single cell necrosis were dose-related in males. In females, the incidence and severity of swelling and single cell necrosis and the severity of liver cell vacuolization were dose-related. Liver cell vacuolization appeared to be the most sensitive response. The response at the low dose in males and females was not considered adverse, because the incidence of this lesion was similar to the controls and the severity was very slight. The NOAELs in this study are therefore 18.3 mg/kg-day for males and 34.0 mg/kg-day for females, on the basis of histopathological changes (vacuolization) in the liver. The corresponding LOAELs are 56.2 mg/kg-day for males and 101.1 mg/kg-day for females.

Potter *et al*. (1996) evaluated the effect of DBCM on hyaline droplet formation, testosterone levels, and cell proliferation in the kidney of male F344 rats. Four rats/dose were gavaged with 156 or 312 mg/kg-day of DBCM in four percent Emulphor® for one, three, or seven days. Body weights were recorded at the beginning and end of the study. Organ weights were collected at necropsy. Effects on hyaline droplet formation were determined histologically. Renal cell proliferation following DBCM exposure was measured *in vivo* by [^{3}H]-thymidine incorporation. Mean final body weight was significantly reduced (14 percent) at the high dose. DBCM reduced or eliminated hyaline droplet formation in renal tubules. Testosterone levels decreased relative to controls on day seven, which may have accounted for the changes in hyaline droplet formation. No significant differences in labeling index were noted following DBCM exposures for up to seven days. The NOAEL and LOAEL in this study are 156 and 312 mg/kg-day, respectively, based on reduced body weight after seven days of treatment.

Coffin *et al*. (2000) examined the effect of DBCM given by gavage in corn oil or in drinking water on liver toxicity, cell proliferation and DNA methylation in female B6C3F$_1$ mice. Doses of 0, 100, or 300 mg/kg were administered to 10 animals/group five days/week for two weeks. The high dose in the gavage study was selected because it had been demonstrated to induce tumors in female mice (NTP, 1985). DBCM was administered in drinking water at approximately 75 percent of the saturation level, which resulted in an average daily dose of about 171 mg/kg-day. The mice were sacrificed 24 hours after the last gavage dose and the livers were removed, weighed, and processed for histopathological examination, proliferating cell nuclear antigen-labeling index analysis, and determination of *c-myc* methylation status. A statistically significant, dose-dependent increase in relative liver weight was observed in animals dosed by gavage

compared to the control. Relative liver weight was unaffected in animals given the compound in drinking water. A slight but significant increase in the severity of liver histopathology was reported in animals exposed via drinking water and gavage at the high dose. No incidence data were reported for any dose group and severity data were not provided for the control group. DBCM administered by gavage caused a significant, dose-dependent increase in the labeling index, but cell proliferation did not increase significantly when the compound was administered in drinking water.

Melnick *et al*. (1998) exposed 10 female B6C3F$_1$ mice/dose to DBCM at 0 (vehicle only), 50, 100, 192, or 417 mg/kg-day by gavage in corn oil five days/week for three weeks. Data were collected on clinical signs, body weight, water intake, serum indicators of hepatotoxicity, organ weights, gross pathology, and histopathology. BrdU was given to the animals during the last six days of the study for determination of hepatocyte labeling index. No compound-related clinical signs of toxicity were observed. Body weight and water intake were not significantly altered at any dose tested. Significant, dose-related increases in relative liver weight were seen at 100, 192 and 417 mg/kg-day. Serum alanine aminotransferase activity was significantly increased at the two highest doses. Serum sorbitol dehydrogenase was significantly elevated at ≥ 100 mg/kg-day. However, the increase in activity (shown graphically) was very small relative to the control at 100 and 192 mg/kg-day. At necropsy, there was clear evidence of hepatocyte hydropic degeneration in the 192 and 417 mg/kg-day groups. A significant increase in hepatocyte proliferation, as measured by the labeling index, was evident only at 417 mg/kg-day. The LOAEL was 192 mg/kg-day, based on hepatocyte hydropic degeneration and elevated serum enzyme activities. The NOAEL was 100 mg/kg-day.

Subchronic Toxicity

Four published studies have addressed the subchronic toxicity of DBCM through oral exposure in rats or mice (Table 40). Chu *et al*. (1982b) evaluated toxicity in male and female SD rats exposed via drinking water. NTP (1985) evaluated toxicity in male and female F344/N rats and B6C3F$_1$ mice given DBCM by gavage in corn oil. Daniel *et al*. (1990) assessed toxicity in male and female SD rats administered the compound by gavage in corn oil. No subchronic inhalation studies are available for DBCM.

Chu *et al*. (1982b) assessed subchronic toxicity of DBCM in male and female SD rats. Twenty rats/sex/group were provided drinking water containing one percent Emulphor® and DBCM at 0, 5, 50, 500, or 2,500 ppm for 90 days. Corresponding doses were about 0, 0.57, 6.1, 49, and 224 mg/kg-day for males and 0, 0.69, 7.5, 59.2, and 237 mg/kg-day for females, as estimated from mean water intake and body weight. Half of the the rats were sacrificed at the end of the exposure period and the remaining animals were given tap water for a 90-day recovery period. Data were collected on clinical signs, body weight, feed and water consumption, organ weights, clinical chemistry, hematological parameters, microsomal enzyme activities, and gross and histopathology. The authors did not report analytical verification of the DBCM concentrations in water, the frequency of water renewal, or other measures taken to minimize loss of DBCM by volatilization.

Table 40. Summary of Subchronic Toxicity Studies on DBCM

Reference	Species	Route	Sex	N	Duration	Dose (mg/kg-day)	NOAEL (mg/kg-day)	LOAEL (mg/kg-day)
Chu *et al*. (1982b)	Rat Sprague-Dawley	Drinking water	Male	20	90 days	0, 0.57, 6.1, 49, 224	49	224 hepatic lesions
	Rat Sprague-Dawley	Drinking water	Female	20	90 days	0, 0.69, 7.5, 59, 237	59	237 hepatic lesions
NTP (1985)	Rat F344/N	Gavage (corn oil)	Male, Female	10	13 weeks	0, 15, 30, 60, 125, 250	30	60 hepatic lesions in males
	Mouse $B6C3F_1$	Gavage (corn oil)	Male, Female	10	13 weeks	0, 15, 30, 60, 125, 250	125	250 hepatic and renal lesions in males
Daniel *et al*. (1990)	Rat Sprague-Dawley	Gavage (corn oil)	Male, Female	10	90 days	0, 50, 100, 200	-	50 hepatic lesions in males and renal lesions in females

One male in the 5 ppm group died during the exposure period. At 2,500 ppm, food consumption was depressed in both males and females, with the decrease reaching statistical significance in males. Body weight gain was decreased by 12 percent at the high dose, but the difference was not statistically significant compared to the control. Lymphocyte counts were significantly decreased in high dose males at the end of the 90 day recovery period. No changes were observed in serum biochemistry parameters. The incidence and severity of hepatic lesions (increased cytoplasmic volume and vacuolation due to fatty infiltration) were increased in exposed animals compared to the vehicle control. The response was weakly dose-related in males (incidence: vehicle control, 5/9; 5 ppm, 3/10; 50 ppm, 4/10; 500 ppm, 5/10; 2500 ppm, 6/9), but not in females (0/10, 3/10, 5/10, 3/10, 4/10). The severity of hepatic lesions was significantly increased in 2500 ppm males and in 50 and 2,500 ppm females. Although the authors noted that histologic changes were mild and similar to controls when evaluated after the 90 day recovery period, the high dose males continued to exhibit an increased incidence of hepatic lesions with greater severity relative to the control. These data identify a NOAEL for hepatic effects of 49 mg/kg-day and a LOAEL of 224 mg/kg-day in males and a NOAEL of 59 mg/kg-day and a LOAEL of 237 mg/kg-day in females.

NTP (1985) exposed male and female F344/N rats to DBCM by gavage in corn oil for 5 days/week for 13 weeks. Ten rats/sex/dose received 0, 15, 30, 60, 125, or 250 mg/kg-day, were observed for clinical signs twice/day and were weighed weekly. At sacrifice, all animals were necropsied and tissues from the vehicle control and high dose groups

were examined histologically. Other selected tissues were also examined histologically, including livers of all male rats and 125 mg/kg female rats and kidney and salivary glands of 125 mg/kg male and female rats.

At the highest dose, 9/10 males and females died before study termination. Final mean body weight for 125 mg/kg-day males was seven percent less than the vehicle controls. Final mean body weights for other dose groups were similar to the vehicle controls. No compound-specific clinical signs were reported. Compound-related lesions were identified in the liver, kidney, and salivary gland. A dose-dependent increased frequency of hepatocellular vacuolation, interpreted as severe fatty metamorphosis, was observed in male rats (vehicle control, 4/10; 15 mg/kg-day, 7/10; 30 mg/kg-day, 8/10; 60 mg/kg-day, 10/10; 125 mg/kg-day, 10/10; 250 mg/kg-day, 10/10). This response reached statistical significance at ≥ 60 mg/kg-day using the Fisher exact test. Hepatocellular vacuolation was also noted in all females treated with the high dose. Hepatocellular centrilobular necrosis was observed in high dose males (8/10) and females (7/9). Toxic nephropathy in the renal cortex, characterized by tubular cell degeneration, regeneration, and cast formation, was observed in 8/10 males and 9/9 females at the high dose. Other compound-related lesions found in high dose males and females included acute inflammation (5/10, 5/8) and squamous metaplasia (9/10, 6/8) of the salivary glands. The NOAEL was 30 mg/kg-day and the LOAEL was 60 mg/kg-day, based on hepatic vacuolation in male rats.

NTP (1985) also exposed male and female $B6C3F_1$ mice to DBCM by gavage in corn oil five days/week for 13 weeks. Ten animals/sex/dose received doses of 0, 15, 30, 60, 125, or 250 mg/kg-day. The mice were observed twice/day for clinical signs of toxicity and weighed weekly. At sacrifice, all animals were necropsied and tissues from the vehicle control and high dose groups were examined for histopathology, as well as livers of male mice in the 125 mg/kg group.

Sporadic deaths in males were considered unrelated to DBCM exposure. No clinical signs of toxicity were reported. Mean final body weights in surviving animals were not significantly affected at any dose. Necrosis and vacuolar change (interpreted by the study authors as fatty metamorphosis) were observed in males (5/10) receiving the high dose. In addition, toxic nephropathy characterized by tubular degeneration or mineralization was observed in the high dose males (5/10). Corresponding hepatic or renal lesions were not observed in the females. These data indicate a NOAEL of 125 mg/kg-day and a LOAEL of 250 mg/kg-day, on the basis of hepatocellular vacuolation in male mice.

Daniel *et al*. (1990) assessed the subchronic toxicity of DBCM in male and female SD rats. Ten animals/sex/dose received gavage doses of 0, 50, 100, or 200 mg/kg-day in corn oil for 90 consecutive days. Dosages were adjusted weekly based on individual body weights. Data were collected on clinical signs of toxicity, body weight, feed consumption, clinical chemistry, and hematological parameters. Urine was collected for analysis during the final week of the study following an overnight fast. Ophthalmoscopic examinations were performed prior to treatment and during the last week of the study. A complete histopathologic examination was conducted on all animals at the high dose and five controls/sex. This initial evaluation identified the liver, kidney and testes as target organs. These organs were subsequently evaluated in all study animals.

No deaths, clinical signs of toxicity, or compound-related changes in ophthalmology or hematology were observed. Mean final body weights were significantly reduced in the high dose groups by 32 percent in males and by 13 percent in females. Decreases in mean final body weight in the other treatment groups were less than 10 percent of the vehicle control values. Feed intake by males was significantly reduced during weeks one to five and 10 to 13. The absolute and relative weights of several organs were decreased in males and females and these changes were considered related to decreased body weights. Decreases in brain and thymus weight and increases in liver and kidney weight were considered compound-related. Serum hepatotoxicity indicators included elevated alanine aminotransferase (mid and high dose males) and alkaline phosphatase (high dose males and females). Serum indicators of renal toxicity included elevated creatinine (mid and high dose males and high dose females) and decreased potassium (high dose males). Compound-related lesions were observed in the liver and kidneys. The most prominent finding in the liver was a high incidence of centrilobular lipidosis (fatty change) in males (vehicle control, 0/10; 50 mg/kg-day, 9/10; 100 mg/kg-day, 9/10; 200 mg/kg-day, 10/10) and females (0/10, 0/10, 1/10, 9/10). The severity of this lesion was dose-related. Other compound-related hepatic lesions included centrilobular necrosis (mid and high dose males and high dose females) and centrilobular chronic necrosis (high dose males and females). Slight to moderate degeneration within the kidney proximal tubular cells occurred in all high dose males and females and to a lesser extent in mid dose males and low and mid dose females. The LOAEL in this study is 50 mg/kg-day, the lowest dose tested, based on liver histopathology in males and kidney histopathology in females.

Genetic Toxicity

Although the overall data are mixed, positive results have been obtained for mutagenicity in several test strains of *S. typhimurium*; mutagenicity in cultured mouse lymphoma cells; induction of sister chromatid exchange and chromosome aberrations *in vivo* and *in vitro*; DNA damage in bacteria; and aneuploidy in mammalian cells and *Aspergillus nidulans*. Negative results have also been obtained *in vitro* and *in vivo*. For some *in vitro* tests, negative findings may have occurred because measures were not taken to prevent volatilization of the test compound. The overall weight of evidence indicates that DBCM has mutagenic and genotoxic potential.

In Vitro Assays

The genotoxicity of DBCM has been evaluated in numerous *in vitro* assays in bacteria and eukaryotic cells (Table 41). A potential limitation of the database is the failure of many authors to indicate whether the tests were conducted in a closed system to prevent volatilization of DBCM from the incubation mixture. Use of a sealed test system is noted in cases where the study authors provided this information.

Table 41. Summary of *In Vitro* Genotoxicity Studies on DBCM

Endpoint	Assay system	Results (with/without metabolic activation)	References
Gene mutation – Bacteria *(Salmonella typhimurium)*	TA100[a]	NT/+	Simmon *et al*. (1977), Simmon and Tardiff (1978)
	TA100	-/+	Ishidate *et al*. (1982)
	TA98, TA100, TA1535, TA1537	-/-	NTP (1985)
	TA1535, TA1537 TA98, TA100	+/+ +/-	Varma *et al*. (1988)
	TA97, TA98, TA100, T 102	-/-	Mersch-Sundermann (1989)
	TA100 (fluctuation test)	-/-	LeCurieux *et al*. (1995)
Gene mutation – Mammalian cells	Mouse lymphoma cells[a]	NT/+	McGregor *et al*. (1991)
	Mouse lymphoma cells	+/(+)	Sofuni *et al*. (1996)
Chromosome aberrations	Chinese hamster fibroblast cells	+/-	Ishidate *et al*. (1982)
	Chinese hamster ovary cells	+/+	Loveday *et al*. (1990)
	Chinese hamster lung fibroblasts[a]	(+)/-	Matsuoka *et al*. (1996)
DNA damage	*E. coli* PQ37, SOS chromotest	-/-	Mersch-Sundermann *et al*. (1989)
	E. coli PQ37, SOS chromotest	+/+	LeCurieux *et al*. (1995)
Sister chromatid exchange	Human lymphocytes	NT/+	Morimoto and Koizumi (1983)
	Human Lymphocytes Rat liver cells	+/NT +/NT	Sobti (1984)
	Chinese hamster ovary cells	+/-	Loveday *et al*. (1990)
	Rat erythroblastic leukemia K_3D cells	+/+	Fujie *et al*. (1993)
Other	*S. cerevisiae* strains D7 (conversion)	-/(+)	Nestmann and Lee (1985)
	S. cerevisiae strain XV185-14C (reversion)	-/-	
	Drosophila melanogaster sex-linked recessive lethal mutation	NA/-	Foureman *et al*. (1994)
	Chinese hamster lung fibroblast Aneuploidy	-/+	Matsuoka *et al*. (1996)
	Aspergillus nidulans Aneuploidy	NT/+	Benigni *et al*. (1993)

NT = Not Tested; NA = Not Applicable
(+) weakly positive response
[a] Assay was conducted in a closed system.

Gene Mutation in Bacteria

Simmon *et al*. (1977) and Simmon and Tardiff (1978) showed that DBCM vapor was mutagenic in *S. typhimurium* TA100 assayed in a desiccator without metabolic activation.

The minimum amount required to elicit a mutagenic response was 57 µmol. Ishidate *et al*. (1982) assayed DBCM mutagenicity in strain TA100 in the presence and absence of rat liver S9 fraction and observed increased mutation frequencies only in the absence of S9 activation. NTP (1985) reported that DBCM was nonmutagenic in TA1535, TA1537, TA98, or TA100 tested with or without metabolic activation using a preincubation protocol at concentrations up to cytotoxic levels. The study authors suggested that the negative results may have been due to volatilization of DBCM from the test system. Varma *et al*. (1988) tested for mutagenicity of DBCM in TA1535, TA1537, TA98, and TA100, and found a significantly increased mutation frequency at the lowest metabolically activated concentration (0.12 µmol/plate) in all four strains. DBCM at the same concentration also resulted in increased mutation frequencies in TA1535 and TA1537 in the absence of metabolic activation. Higher concentrations had no clear effect on mutation frequency. Mersch-Sundermann (1989) obtained negative results in the presence and absence of metabolic activation for TA98, TA100, TA102, and TA97. LeCurieux *et al*. (1995) obtained negative results with and without metabolic activation in TA100 in the fluctuation test modification of the reverse mutation assay.

Gene Mutation in Mammalian Cells

Two studies have examined the mutagenic potential of DBCM in cultured mammalian cells. McGregor *et al*. (1991) reported that DBCM induced dose-related increases in forward mutations in the mouse lymphoma L5178Y/TK$^{+/-}$ assay in the absence of metabolic activation in sealed tubes at concentrations greater than or equal to 480 µM. Sofuni *et al*. (1996) tested DBCM in the mouse lymphoma L5178Y/TK$^{+/-}$ assay as part of an international collaborative program. Positive results were obtained for mutagenicity with and without metabolic activation in two test laboratories.

Sister Chromatid Exchange

Morimoto and Koizumi (1983) assessed induction of sister chromatid exchange in cultured human lymphocytes in the absence of metabolic activation and observed a dose-dependent increase in frequency, which was statistically significant at concentrations greater than or equal to 400 µM. Sobti (1984) reported induction of sister chromatid exchange by DBCM in CCRF-CEM human lymphoid cells and RL_4 rat liver cells at 100 µM. Loveday *et al*. (1990) found no evidence for DBCM induction of sister chromatid exchange in CHO cells in the absence of metabolic activation but with S9 metabolic activation, a positive response was obtained at 3,600 µM. Fujie *et al*. (1993) observed a statistically significant, dose-related increase in sister chromatid exchange in rat erythroblastic leukemia K_3D cells exposed to DBCM in the absence of metabolic activation. Their comparison of sister chromatid exchange in the presence and absence of metabolic activation at a single concentration suggested that DBCM may also induce sister chromatid exchange in the presence of metabolic activation.

Chromosome Aberrations

Ishidate *et al*. (1982) reported induction of chromosomal aberrations in Chinese hamster fibroblasts with but not without metabolic activation. The concentrations tested were not reported. Loveday *et al*. (1990) found no evidence of induction of chromosomal aberrations with or without exogenous metabolic activation. Matsuoka *et al*. (1996) observed a marginally positive increase in chromosome aberrations in Chinese hamster

lung fibroblast (CHL/IU) cells with but not without metabolic activation when exposed to DBCM in tightly capped flasks.

Other *In Vitro* Genotoxicity

Nestmann and Lee (1985) investigated mutagenicity of DBCM in *S. cerevisiae* strains D7 and XV185-14C at 11 to 5,700 µM in the presence and absence of exogenous metabolic activation. The results for conversion in strain D7 were weakly positive in the absence of metabolic activation. Results for strain XV185-14C were negative with and without exogenous metabolic activation.

LeCurieux *et al*. (1995) found that DBCM induced DNA damage in the presence and absence of exogenous activation in the *E. coli* PQ37 SOS chromotest. CCRF-CEM human lymphoblastic leukemia cell lines exposed to DBCM at 5 or 10 mM for two hours produced DNA strand breaks (Geter *et al*., 2004). In contrast, Mersch-Sundermann *et al*. (1989) found no induction of DNA damage by BDCM using the same assay. Geter *et al*. (2004) reported negative results for DNA strand breaks in primary rat hepatocytes exposed to 5 or 10 mM DBCM for four hours.

Positive results for aneuploidy were obtained in Chinese hamster lung fibroblasts in the absence, but not the presence of exogenous metabolic activation (Matsuoka *et al*., 1996). Benigni *et al*. (1993) obtained positive results for aneuploidy in *Aspergillus nidulans*.

In Vivo Assays

In vivo data for DBCM genotoxicity are available for chromosome aberrations, sister chromatid exchange, induction of micronuclei, and DNA damage and repair. The results of *in vivo* genotoxicity tests on DBCM are summarized in Table 42.

Table 42. Summary of *In Vivo* Genotoxicity Studies on DBCM

Endpoint	Assay system	Result	References
Micronucleus induction	Mouse, rat, bone marrow cells	-	Ishidate *et al*. (1982)
	Mouse, bone marrow cells	-	Hayashi *et al*. (1988)
	Newt, peripheral erythrocytes	-	LeCurieux *et al*. (1995)
Chromosome aberrations	Rat, bone marrow cells (oral) Rat, bone marrow cells (i.p.)	+ +	Fujie *et al*. (1990)
Sister chromatid exchange	Mouse, bone marrow cells	+	Morimoto and Koizumi (1983)
DNA damage	Rat, renal cells	-	Potter *et al*. (1996)
Unscheduled DNA synthesis	Rat, hepatocytes	-	Stocker *et al*. (1997)

Chromosome Aberration

Fujie *et al*. (1990) assessed chromosome aberrations in bone marrow of Long-Evans rats following oral (males only) or ip (males and females) DBCM exposure. Oral

administration induced dose-related increases in chromatid and chromosome breaks. A more pronounced response was observed following a single ip dose, with statistically significant effects occurring at 20.8 mg/kg.

Sister Chromatid Exchange

Morimoto and Koizumi (1983) examined frequency of sister chromatid exchange in male ICR/SJ mice following DBCM exposure. Animals received 0, 25, 50, 100, or 200 mg/kg-day for four days by gavage in olive oil. DBCM exposure resulted in a dose-dependent increase in sister chromatid exchange frequency, which achieved statistical significance at 25 mg/kg-day and above.

Micronucleus Induction

Ishidate *et al*. (1982) examined induction of micronuclei in ddY mice, MS mice, and Wistar rats following exposure to DBCM. No significant induction of micronucleus formation in bone marrow was observed in either mice or rats. Hayashi *et al*. (1988) found no induction of micronuclei in bone marrow of ddY mice with DBCM at single ip doses up to 500 mg/kg in corn oil. LeCurieux *et al.* (1995) obtained negative results for induction of micronuclei in *Pleurodeles waltl* larvae.

Other *In Vivo* Genotoxicity

Potter *et al*. (1996) examined the effect of DBCM on DNA strand breakage in kidney cells of male F344 rats. Animals received 156 or 312 mg/kg-day of DBCM by gavage in four percent Emulphor® for one, three, or seven days. No compound-related DNA strand breaks were observed at either dose. No DNA strand breaks in liver, kidney, or duodenum epithelial cells were observed *in vivo* in male F344/N rats administered 0.3 or 0.6 mM/kg of DBCM as a single oral gavage dose in 0.5 percent Emulphor® for four hours (Geter *et al*., 2004).

Stocker *et al*. (1997) evaluated unscheduled DNA synthesis in hepatocytes from male SD rats given a single gavage dose of DBCM at 0, 135 or 450 mg/kg in aqueous one percent methylcellulose. The doses were selected to deliver 30 or 100 percent of the maximum tolerated dose. Unscheduled DNA synthesis was analyzed two and 14 hours after treatment. No evidence of increased unscheduled DNA synthesis was obtained.

DBCM was positive in the comet assay in mouse liver and brain, and in rat stomach, colon, liver, kidney, bladder, and lung (Sekihashi *et al*., 2002), even though it has been shown to be carcinogenic to mice but not rats. Groups of four male ddY mice or male Wistar rats were treated once orally with DBCM in olive oil at half of the LD_{50}, that is, 400 mg/kg in mice or 200 mg/kg in rats, and the organs were sampled three, eight, and 24 hours after treatment.

Mode of Action Studies

DeMarini *et al*. (1997) investigated the mutational spectrum of DBCM in *S. typhimurium* at the *hisG46* allele by performing colony probe hybridizations of about 100 revertants induced in strain RSJ 100 by DBCM. Approximately 100 percent of the mutations were GC→AT transitions, and about 91 percent of the mutations occurred at the second position of the CCC/GGG DNA target sequence. Dichloromethane was tested in *S. typhimurium* strain TA100 (a homologue of the TA1535 parent strain which contains the

plasmid pKM101 and which does not express GSST1-1) for comparison. In contrast to the DBCM induced mutations in RSJ 100, only 15 percent of the mutations induced by dichloromethane in TA100 were GC→AT mutations. This result suggests that overexpression of GSTT1-1 in strain RSJ 100 mediated the mutagenicity of DBCM and induced a specific type of mutation in *Salmonella*.

Developmental and Reproductive Toxicity

The database for DBCM reproductive and/or developmental toxicity consists of three oral studies, summarized in Table 43.

Table 43. Summary of Developmental and Reproductive Toxicity Studies on DBCM

Reference	Species	Route	Sex	N	Duration	Dose (mg/kg-day)	NOAEL (mg/kg-day)	LOAEL (mg/kg-day)
Borzelleca and Carchman (1982)	Mouse ICR Swiss	Drinking water	M F	10 30	25 to 27 weeks	0, 17, 171, 685	--	17 marginal - reduced F/2b body weight on postnatal days 14 & 21
Ruddick *et al*. (1983)	Rat Sprague-Dawley	Gavage (corn oil)	F	10 - 12	Gestation days 6-15	0, 50, 100, 200	200	--
NTP (1996)	Rat Sprague-Dawley	Drinking water	M	10	29 days	4.2, 12.4, 28.2	28.2	--
			F	10	35 days	6.3, 17.4, 46.0	46.0	--
			F	13	16 days Gestation days 6 - parturition	7.1, 20.0, 47.8	47.8	--

Borzelleca and Carchman (1982) conducted a modified multigeneration study of DBCM in ICR Swiss mice. Groups of 10 males and 30 females (F_0 generation) were provided with drinking water containing 0.1 percent Emulphor® and DBCM at 0, 0.1, 1.0, or 4.0 mg/mL for seven weeks, then mated to produce the F_{1a} litters. Each male mouse was cohoused for seven days with three randomly-selected females. Two weeks after weaning of F_{1a} litters, F_0 mice were randomly remated to produce F_{1b} litters. A similar protocol was followed to produce F_{1c} litters. The F_{1a} and F_{1c} litters were sacrificed and necropsied after a 21-day postnatal observation period and the F_{1b} generation was culled. Surviving F_{1b} animals (10 males and 30 females) were provided with drinking water with 0, 0.1, 1.0 or 4.0 mg/mL DBCM for 11 weeks and subsequently mated to produce the F_{2a} and F_{2b} generations. A two week interval occurred between weaning of the F_{2a} generation and remating of the F_{1b} generation to produce the F_{2b} generation. Under this protocol, the total continuous exposures of the F_0 and F_{1b} parental generations to DBCM

in drinking water during premating, mating, gestation, and lactation were 27 and 25 weeks, respectively. Following weaning of the final litters, both parental generations were sacrificed and necropsied. No histopathological examinations were conducted of the F_0 and F_{1b} generations. The F_{2a} and F_{2b} generations were sacrificed and necropsied 21-day postpartum. Approximately one third of the pups from the F_{1c} and F_{2b} generations were screened for dominant lethal mutations or teratological abnormalities.

Body weight gain and drinking water consumption were recorded weekly and semiweekly for the F_0 and F_{1b} generations, respectively. Mating, gestation, gestation survival, and lactation survival indices were determined for each mating. A 21-day survival study was performed on pups of all generations and viability and lactation indices were calculated. After sacrifice of all litters except F_{1b} on day 21, one male and one female pup/litter were randomly selected for necropsy. Treated dams from the F_0 and F_{1b} generations were sacrificed on gestation day 18 for teratology screening. The implantations, resorptions, and live and dead fetuses were counted. Fetuses were individually weighed and examined for gross malformations and randomly selected subsamples of fetuses were examined for either skeletal or visceral anomalies.

Although data were collected for body weight and water consumption, the study authors did not estimate the average daily DBCM dose for most treatment groups. The authors indicated that the highest drinking water concentration (4.0 mg/mL) corresponded to a nominal dose of 685 mg/kg-day. Because the study authors indicated that water consumption was not affected by treatment (data not provided), this dose was used to estimate intake at other concentrations. Thus, 0.1 and 1.0 mg/mL correspond to average daily doses of 17 and 171 mg/kg-day, respectively. Final body weights were significantly reduced in the high dose males and the mid and high dose females of the F_0 and F_{1b} generations. Water consumption data suggest that taste aversion to DBCM was not a causative factor for decreased body weights. Animals in both the F_0 and F_{1b} generations had enlarged livers with dose-related gross morphological changes suggestive of hepatotoxicity. Mating index was significantly decreased only for the F_{2a} high dose group. The gestational index was significantly decreased at the high dose for all three F_1 generations. Parental ingestion of 4.0 mg/mL DBCM resulted in statistically significant decreased litter size in all generations; decreased viability index in the F_{1a}, F_{1b}, F_{1c} and F_{2a} generations; decreased lactation index in the F_{2b} generation; and decreased postnatal body weight in the F_{2b} generation. Parental ingestion of 1.0 mg/mL DBCM resulted in a statistically significant decreased litter size in the F_{1c} generation; decreased viability index in the F_{1b} generation; decreased lactation index in the F_{1b} and F_{2b} generations; and decreased postnatal body weight in the F_{2b} generation. Parental ingestion of 0.1 mg/L resulted in a statistically significant reduction in postnatal body weight in the F_{2b} generation on postnatal day 14 and 21, but not on postnatal day seven. No statistically significant increases in dominant lethal or teratogenic effects were reported in either the F_{1c} or F_{2b} generations.

The LOAEL for developmental and reproductive toxicity in this study is approximately 17 mg/kg-day (the lowest dose tested), based on reduced mean body weights in the F_{2b} generation on postnatal day 14 and 21. For parental toxicity, liver alterations indicative of hepatotoxicity were clearly evident at the two higher doses in both parental generations. At the lowest dose, hepatic changes were mainly limited to discoloration,

possibly due to the accumulation of fat deposits as suggested by the study authors. Gross morphology of the liver was normal; therefore, the adversity of this effect is uncertain. Based on these considerations, 17 mg/kg-day (the lowest dose tested) is considered to be a marginal LOAEL for parental toxicity.

Ruddick *et al*. (1983) investigated the prenatal toxicity of DBCM in SD rats. Presumed pregnant dams (10-12/dose group) were gavaged with 0, 50, 100, or 200 mg/kg-day in corn oil on gestation days six to 15. Body weights were measured on gestation days one through 15, and before and after caesarean section on gestation day 22. The fetuses were removed, weighed individually, and examined for viability and external malformations. Two pups/dam were given histopathological examination. Approximately two thirds of the remaining live fetuses were examined for skeletal abnormalities and one third for visceral alterations. The dams were sacrificed on gestation day 22 and organs were examined for pathological changes. Maternal blood was analyzed for hematological and clinical chemistry parameters. Liver, heart, brain, spleen, and one kidney were wieghed. Tissues from five control and high dose dams/group were examined for histopathological changes. When compound-related effects were observed in the high dose group, the affected tissues were also examined in the mid dose group.

Maternal weight gain was depressed by 25 percent at the high dose relative to the vehicle control. No significant effects on maternal organ weights, hematology or clinical chemistry, number of resorption sites, number of fetuses/litter, or mean fetal body weight gain were observed in any of the dose groups. No compound-related histopathology was noted in either dams or fetuses and there were no skeletal or visceral fetal anomalies attributed to DBCM. No dose-related trends are apparent in the fetal endpoints. The NOAEL for developmental toxicity in this study was 200 mg/kg-day, the highest dose tested. The maternal LOAEL and NOAEL for maternal toxicity were 200 and 100 mg/kg-day, respectively, based on significantly decreased maternal body weight gain.

Potter *et al*. (1996) evaluated the effect of DBCM on hyaline droplet formation and cell proliferation in the kidney of male F344 rats. Four rats/dose received 0.75 or 1.5 mmol/kg of DBCM in four percent Emulphor® by gavage for one, three, or seven days. No significant effects were noted following exposures up to seven days, except a reduced hyaline droplet formation on day seven which might be explained by a decrease in serum testosterone concentrations.

NTP (1996) conducted a short-term reproductive and developmental toxicity screen in SD rats, administering drinking water containing DBCM at 0, 50, 150, or 450 ppm during a 35-day study period. Males (10/group) were exposed on study days six through 34. Group A females (periconception, 10/group) were treated on study days one through 34. Group A females were mated to treated males on study days 13 through 18 and necropsied on study day 34. Group B females (gestational exposure, 13/group) were mated on study day one to untreated males, treated from gestation day six through parturition, and necropsied on postnatal day five. Data were collected on clinical signs of toxicity, body weight, and water and feed consumption for all groups. At study termination, males were examined for hematology, clinical chemistry, gross pathology, histopathology, and a complete sperm evaluation (count, density, motility, and morphology). Hematology parameters, clinical chemistry, and histopathology were not evaluated in females. Endpoints measured in Group A females included pregnancy

status, number and position of live and dead fetuses, number and position of early and late resorptions, and number of corpora lutea in the ovaries. Group B females were allowed to deliver. Postpartum endpoints included numbers of males, females and live and dead pups, total body weight of males and females, and number of implantation sites.

All animals survived to study termination. No clinical signs of toxicity were observed in any group. Mean absolute body weights, feed consumption, and gross findings were comparable across all study groups. Water consumption was reduced by 13 to 44 percent at most measurement intervals in all treated groups. Based on measured water consumption, the study authors estimated doses to be 4.2, 12.4, and 28.2 mg/kg-day for males, 6.3, 17.4, and 46.0 mg/kg-day for Group A females, and 7.1, 20.0, and 47.8 mg/kg-day for Group B females. Absolute and relative organ weights were comparable in all male dose groups. Alkaline phosphatase and 5'-nucleotidase were increased at all doses in males, but reached statistical significance only at the low dose for alkaline phosphatase and at the mid and high dose for 5'-nucleotidase. Total serum proteins were also decreased at the high dose in males. No compound-related microscopic lesions were observed. No statistically significant effects were observed on sperm parameters.

None of the reproductive or fertility measures in Group A or B females was significantly affected at any dose. The proportion of male pups was significantly decreased in Group B females at the high dose compared to the control value. This result was not considered to be compound-related by the study authors, because the control value (0.61) was unusually high compared to historical values, and the result at the high dose (0.44) fell within the historical range for this endpoint. Thus, DBCM did not produce reproductive effects at doses up to 28.2 mg/kg-day in males or up to 47.8 mg/kg-day in females. The study authors stated that DBCM may have resulted in general toxicity at all doses in the males, based on clinical chemistry changes. However, the adversity of the clinical chemistry changes is questionable based on absence of a clear dose-related response, the small magnitude of changes, and absence of histopathological lesions. Therefore, the NOAELs are 28.2 mg/kg-day and 47.8 mg/kg-day for males and females, respectively, for reproductive and systemic effects.

Immunotoxicity

Few experimental data are available on the immunotoxicity of DBCM. Munson *et al*. (1982) investigated potential immunological effects of DBCM in male and female CD-1 mice. The mice (6-12/sex/dose) were gavaged with 0, 50, 125, or 250 mg/kg-day DBCM in 10 percent Emulphor® for 14 days. Humoral immune system function was assessed by the primary IgM response to sheep erythrocytes as estimated by hemolytic plaque assay and by plasma antibody hemagglutination titer. Cell-mediated immunity was assessed by measuring delayed-type hypersensitivity response to sheep erythrocytes. Humoral immune function in males was significantly depressed at the mid and high doses, as indicated by decreased numbers of antibody-forming cells, expressed either as AFC/spleen or AFC/10^6 cells. Cell-mediated immune response was also decreased at the high dose, as indicated by a significantly decreased stimulation index. A clear dose response pattern was noted for either humoral, but not cell-mediated responses. The authors stated that no treatment-related effects on immune system function were observed

in female mice (data not reported). The NOAEL and LOAEL values for immunotoxicity in males were 50 mg/kg-day and 125 mg/kg-day, respectively.

Neurotoxicity

DBCM is a central nervous system toxicant at higher doses. In an acute study, ataxia, sedation, and anesthesia occurred in mice within 30 minutes after gavage with 500 mg/kg (Bowman *et al*., 1978), and sedation persisted for four hours. Clinical signs in rats dosed with up to 2,100 mg/kg included sedation, flaccid muscle tone, ataxia, piloerection, and hypothermia (Chu *et al*., 1980, 1982a). Signs observed in rats dosed with ≥ 300 mg/kg-day for longer periods include lethargy, labored breathing, and ataxia (NTP, 1985). Central nervous system effects are believed to result from a nonspecific anesthetic effect, similar to that produced by various other volatile halocarbons (ATSDR, 1990).

Balster and Borzelleca (1982) administered a screening battery of behavioral tests to adult male ICR mice exposed to DBCM in a variety of dosage regimens, including acute dosing; 14 and 90 day treatments at 300 or 3,000 times the estimated average human daily intake of DBCM in disinfected tap water (1.0 and 10.0 mg/kg-day, respectively); 30 days of treatment at 100 mg/kg-day; and 60 days of treatment at 100 or 400 mg/kg-day. In each case, DBCM was administered by gavage in 1:8 Emulphor®:distilled water. A nongavaged control group was included in the 14 and 90 day experiments in addition to a vehicle control group. Six to eight mice were used per treatment.

Acute effects of DBCM on motor performance were evaluated using the screen test. Five or six doses were administered (dose levels not reported). Performance was evaluated at 30, 60, and 90 minutes after treatment. Effects were noted at 30 minutes, with little evidence of change at the longer durations. An ED_{50} of 524 mg/kg (95 percent CI 273 to 1,007 mg/kg) was calculated for this response. Clinical signs of incoordination and ataxia were observed at higher doses, with anesthesia at the highest dose.

In the 14-day experiment, Balster and Borzelleca (1982) evaluated the effect of repeated doses on swimming endurance. DBCM doses of 0, 1.0, or 10.0 mg/kg-day were administered for 14 consecutive days. Swimming endurance was evaluated 24 hours after the final treatment. No adverse effect on performance was observed at either dose. The 90-day experiment evaluated the effect of DBCM on the cling test, screen test, and holeboard. Doses of 0, 1.0, or 10.0 mg/kg-day were administered for 90 consecutive days, and performance was evaluated 24 hours after the last dose. No significant effect of treatment on performance was observed for any of the tests. The 30-day experiment examined the effect of DBCM on latency to enter the dark compartment in a passive avoidance learning test. Doses of 0 or 100 mg/kg-day were administered for 30 consecutive days and the learning test was conducted 24 hours after the final treatment. DBCM had no effect on passive avoidance learning or the initial step-through latency.

The final Balster and Borzelleca (1982) experiment evaluated operant conditioning in mice exposed to 0, 100, or 400 mg/kg-day DBCM for 60 days. Performance was measured daily during a three day pretreatment period, after treatment with the vehicle only. Mice were subsequently treated with DBCM 30 minutes prior to daily behavioral testing for 60 consecutive days. The behavioral measurements were continued for three days after the end of the DBCM treatment. Treatment with 100 or 400 mg/kg-day

significantly decreased response rates for the first day of treatment compared to the last day of pretreatment. The study authors noted no evidence for progressive deterioration and reported that partial tolerance occurred over the course of the experiment (no specific data for DBCM were presented in support of these statements).

The results of these studies suggest that DBCM has low potential for eliciting adverse effects in several standard tests of behavioral toxicity. These data identify a LOAEL of 100 mg/kg-day, based on a nonprogressive decrease (with development of partial tolerance) in response rates in a test of operant conditioning.

Korz and Gatterman (1997) assessed behavioral alterations in male golden hamsters exposed to acute or repeated doses of DBCM. The test animals were initially observed during a 10 day pretreatment period (days −9 to zero). For the repeated dose experiment, nine hamsters/group received gavage doses of 0 or 5 mg/kg in olive oil for 14 consecutive days. For the acute exposure experiment, 6-7 animals/group received a single gavage dose of 0 or 50 mg/kg. Nonbehavioral parameters measured included selected organ weights, hematocrit and hemoglobin values, and food and water consumption. Home cage activities, wheel running, locomotion, and contact with the water bottle teats were monitored continuously during the study. Open field tests were conducted on study days −6, −3, one, four, seven, 10, and 13. The elements recorded in the open field were defecation, locomotion, rearing, urination, and flank marking. An intruder test was conducted on day 14. The elements recorded were chase, follow, retreat, sniff, and upright stance. None of the nonbehavioral parameters differed significantly from the control. The repeated-dose animals significantly increased water bottle contacts on study days four through seven compared to vehicle controls. Acutely dosed animals showed significantly increased locomotor activity on days three to six and decreased wheel running activity on days six through nine. In the open field test, the acutely exposed animals showed significantly more flankmark movements at study days four and seven than did the vehicle control animals. In the intruder test, repeated-dose males approached and bit the intruder significantly less often than did the control animals. The LOAEL for behavioral effects in this study is 5 mg/kg-day, based on results from the repeated-dose experiment.

Chronic Toxicity

The chronic oral toxicity of DBCM has been assessed in three studies: a dietary study conducted in rats and gavage studies (one per species) conducted in rats and mice (Table 44). No data are available on the chronic inhalation toxicity of DBCM.

Tobe *et al*. (1982) administered DBCM to male and female Slc:Wistar SPF rats (40/sex/group) in the diet for 24 months. DBCM was microencapsulated and mixed with feed at dietary levels of 0.0, 0.022, 0.088, or 0.35 percent. Control groups (70/sex) received placebo capsules. Body weight and food consumption were monitored weekly for the first six months, every two weeks from 6-12 months, and every four weeks thereafter. Interim data were reported from the sacrifice of nine rats/sex/control group and five rats/sex/dose group at 18 months. Surviving rats were sacrificed at 24 months and necropsies, hematology, and serum biochemistry tests were conducted. No histopathology data were published.

Table 44. Summary of Chronic Studies on DBCM

Reference	Species	Route	Sex	N	Duration	Dose (mg/kg-day)	NOAEL (mg/kg-day)	LOAEL (mg/kg-day)
Tobe *et al*. (1982)	Rat Wistar SPF	Diet	M	40	2 years	0, 12, 49, 196	12	49 decreased body weight gain
			F	40	2 years	0, 17, 70, 278	17	70 decreased body weight gain
NTP (1985)	Rat F344	Gavage (corn oil)	M, F	50	2 years	0, 40, 80	--	40 hepatic lesions
	Mouse $B6C3F_1$	Gavage (corn oil)	M, F	50	105 weeks	0, 50, 100	--	50 hepatic and thyroid lesions

Based on body weights and food consumption, the doses were approximately 0, 12, 49, and 196 mg/kg-day for males and 0, 17, 70, and 278 mg/kg-day for females. A dose response relationship was not evident for deaths during the study. Mild piloerection and emaciation were observed in high dose males and females from the first month through study termination. Marked suppression of body weight gain was seen in males and females at the high dose, and mild suppression (about 10 percent) was seen in mid-dose males and females. No effect was observed on hematological parameters. Significant serum biochemistry changes, compared to controls, were seen sporadically in both sexes at 18 and 24 months. Apparent treatment-related changes included decreased total triglycerides and serum cholinesterase activity at the high dose, yellowing of the liver surface at the mid and high doses, and roughening of the liver surface in high dose males. The results suggest NOAELs of 12 and 17 mg/kg-day and LOAELs of 49 and 70 mg/kg-day for males and females, respectively, based on decreased body weight.

NTP (1985) evaluated the chronic oral toxicity of DBCM in male and female F344/N rats. Fifty animals/sex/dose were gavaged at 0, 40, or 80 mg/kg-day in corn oil five days/week for 104 weeks. Clinical signs were recorded weekly. Body weights were recorded weekly for the first twelve weeks and monthly thereafter. Necropsy and histopathological examination was conducted on all dose groups. Survival was comparable in all groups. Body weight gain was decreased in high dose males after week 20; final weight gain was 88 percent of the control value. Final mean body weight of males was eight percent less than the vehicle control, but not different from controls in the females. Compound-related hepatic lesions were observed in the livers of males and females at both doses (Table 45), including fatty change and "ground glass" appearance of the cytoplasm. A dose-related increased nephrosis was observed in female kidneys

(vehicle control, 7/50; low dose, 11/50; high dose; 14/50), but not in males. The LOAEL in this study is 40 mg/kg-day, the lowest dose tested, based on liver histopathology.

Table 45. Selected Hepatic Lesions Observed in Fischer 344/N Rats Administered DBCM by Gavage in Corn Oil for Two Years (NTP, 1985)

Lesion	Vehicle control	40 mg/kg-day	80 mg/kg-day
Males			
Fatty change	27/50	47/50	49/50
Ground glass cytoplasmic change	12/50	23/50	50/50
Females			
Fatty change	19/50	39/49	46/50
Ground glass cytoplasmic change	0/50	1/50	12/50

NTP (1985) also conducted a chronic oral exposure study of DBCM in male and female B6C3F$_1$ mice. Fifty animals/sex/dose were gavaged at 0, 50, or 100 mg/kg-day in corn oil five days/week for 105 weeks. Clinical signs were recorded weekly. Body weights were recorded weekly for the first twelve weeks and monthly thereafter. Necropsy and histopathological examination was conducted on all dose groups. Survival in high dose males was significantly decreased compared to controls. Survival in females did not differ from controls. A dosing error at week 58 killed 35 of 50 low dose males. The data for this group are inadequate for our risk assessment and are not reported here. No compound-related clinical signs were observed. Mean final body weight was decreased in high dose males and females, but not in low dose females. Compound-related hepatocytomegaly and hepatic focal necrosis were observed in high dose males. Females showed hepatic calcification at the high dose and fatty metamorphosis and increased incidence of thyroid follicular cell hyperplasia at both doses (Table 46). Thyroid lesions were not observed in treated males. The LOAEL is 50 mg/kg-day, the lowest dose, based on histopathological changes in female liver and thyroid.

Table 46. Selected Nonneoplastic Lesions Observed in Female B6C3F$_1$ Mice Administered DBCM by Gavage in Corn Oil for Two Years (NTP, 1985)

Lesion	Vehicle control	50 mg/kg-day	100 mg/kg-day
Liver			
Fatty change	23/50	49/50	50/50
Calcification	0/50	29/50	23/50
Thyroid			
Follicular cell hyperplasia	1/49	13/46	31/50

Carcinogenicity

The database on the carcinogenic potential of DBCM consists of two year oral bioassays in F344/N rats and B6C3F$_1$ mice (NTP, 1985). These data are also summarized in a publication by Dunnick *et al*. (1985). No studies were identified on carcinogenicity in animals exposed to DBCM by inhalation.

In the NTP (1985) two year bioassay of DBCM in male and female F344/N rats, 50 rats/sex/dose were gavaged with 0, 40, or 80 mg/kg-day in corn oil five days/week for 104 to 105 weeks. Clinical signs were recorded weekly. Body weights were recorded weekly for the first twelve weeks and monthly thereafter. All animals were necropsied and histopathological examination was conducted on tissues from all dose groups. Survival was comparable in all study groups. Body weight gain was decreased in high dose males after week 20; final weight gain was 88 percent of the control value. Final mean body weight of males was eight percent less than the vehicle control value. Final mean body weights of dosed females were similar to the control value. Compound-related nonneoplastic lesions (fatty metamorphosis and groundglass cytoplasmic changes) are discussed in the Chronic Toxicity section of this document. These data indicate that an adequately high dose was tested. There were no statistically significant increases in incidence of any neoplastic lesion. The study authors concluded that this study provided no evidence for carcinogenicity of DBCM in rats.

In the NTP (1985) chronic oral study of DBCM in male and female B6C3F$_1$ mice, 50 mice/sex/dose were gavaged with 0, 50, or 100 mg/kg-day in corn oil five days/week for 104 to 105 weeks. Clinical signs were recorded weekly. Body weights were recorded weekly for the first twelve weeks and monthly thereafter. All animals were necropsied and histopathological examination was conducted on tissues from all dose groups. Survival in high dose males was significantly decreased compared to the control group. Survival in females did not different from controls. A dosing error at week 58 killed 35 of 50 male mice in the low dose group, but did not have apparent effects on females. Too few animals were left in this male group for analysis of tumorigenicity. At week 82, nine high dose male mice died of an unknown cause. No compound-related clinical signs were observed in dosed animals. Mean final body weight was decreased in high dose males and females. Compound-related nonneoplastic lesions in the livers and thyroids are discussed above in the Chronic Toxicity section. At the highest dose, a statistically significant increase in incidence of hepatocellular adenomas and adenomas and carcinomas combined was observed in females, and a statistically significant increased incidence of hepatocellular carcinomas and adenomas and carcinomas combined in the males (Table 47).. A negative trend in incidence of malignant lymphomas was evident in high dose males. The study authors concluded that there was equivocal evidence for carcinogenicity in males and some evidence of carcinogenicity in females.

Table 47. Tumor Incidence in the Liver of B6C3F$_1$ Mice Exposed to DBCM by Gavage in Corn Oil for Two Years (NTP, 1985)

Tumor	Tumor frequency		
Males	**Control**	**50 mg/kg**	**100 mg/kg**
Adenoma	14/50	--	10/50
Carcinoma	10/50	--	19/50
Adenoma or carcinoma	23/50	--	27/50
Females	**Control**	**50 mg/kg**	**100 mg/kg**
Adenoma	2/50	4/49	11/50
Carcinoma	4/50	6/49	8/50
Adenoma adenocarcinoma	6/50	10/49	19/50

Mode of Action Studies

It has been suggested that liver tumors following treatment with THMs occur as a secondary response to cytotoxicity and regenerative hyperplasia induced by oxidative metabolism to reactive dihalocarbonyl metabolites. To address this issue, Melnick *et al*. (1998) compared the dose response relationships for hepatotoxicity, replicative DNA synthesis, and hepatocarcinogenicity in female B6C3F$_1$ mice. Ten female B6C3F$_1$ mice/group were given DBCM doses of 0 (vehicle), 50, 100, 192, or 417 mg/kg-day in corn oil via gavage five days/week for three weeks. No compound-related signs of toxicity were observed and body weight and water intake were not significantly altered at any dose. A statistically significant, dose-related increase in relative liver weight was seen at 100, 192 and 417 mg/kg-day. Serum alanine aminotransferase activity was significantly increased in the two highest dose groups. The serum sorbitol dehydrogenase activity was significantly elevated at all doses above 50 mg/kg-day, although the increases were small at 100 and 192 mg/kg-day. Clear evidence of hepatocyte hydropic degeneration was observed at 192 and 417 mg/kg-day. BrdU was administered to the animals during the last six days of the study for analysis of hepatocyte labeling index. Only the 417 mg/kg-day dose significantly elevated hepatocyte proliferation as measured by the labeling index. The study authors compared the DBCM dose response for liver toxicity (enzyme and labeling index data) and tumorigenicity (data from the NTP bioassays) using the Hill equation model. Melnick *et al*. (1998) concluded that the shape of the dose response and the Hill exponents were different for liver toxicity and tumorigenicity, and did not support a causal relationship between liver toxicity and reparative hyperplasia and tumor development.

Classification of Carcinogenic Potential

DBCM was listed on January 1, 1990 by the State of California as a chemical known to cause cancer on the Proposition 65 list, and was delisted on October 29, 1999. The Carcinogenic Risk Assessment Verification Endeavor group of the U.S. EPA (2008d) reviewed the available evidence on the carcinogenicity of the brominated THMs and

assigned DBCM to Group C, possible human carcinogen, in 1992. Assignment to this category is based on inadequate human data and limited evidence for carcinogenicity in animals, plus consideration of positive data for mutagenicity and structural similarity to other THMs which are known animal carcinogens. NTP (1985) found equivocal evidence for carcinogenicity in male $B6C3F_1$ mice, based on increased incidences of hepatocellular carcinomas and some evidence for carcinogenicity in female $B6C3F_1$ mice based on a compound-related increase in incidence of hepatocellular adenomas and in the combined incidence of hepatocellular adenomas and carcinomas. The use of a corn oil vehicle in this study is a source of uncertainty, since corn oil is suspected of a role in the induction of liver tumors in mouse bioassays. NTP (1985) found no evidence for carcinogenicity in F344/N rats. The tumor data in mice are supplemented by positive genotoxicity data for DBCM both *in vitro* and *in vivo*.

The IARC (1991c, 1999c) concluded that there is limited evidence of carcinogenicity in experimental animals and inadequate evidence in humans for DBCM, which corresponds to Group 3, not classifiable as to human carcinogenicity.

Toxicological Effects in Humans

The database for toxicological effects of DBCM in humans is limited. The primary DBCM exposure is through ingestion of tap water, where it occurs as a byproduct of disinfection. Consequently, the most relevant human data are from epidemiological studies on reproductive outcomes associated with intake of disinfected water. These studies are described in a separate section below. Because industrial production and use of DBCM are limited, there are no toxicity data from occupational studies. No clinical or case report data were identified. No experimental or epidemiological data were located for acute, subchronic, or chronic toxicity, genotoxicity, immunotoxicity, neurotoxicity, or carcinogenicity of DBCM through oral or inhalation exposure in humans.

As described in the bromoform section, two studies (DeMarini *et al*., 1997; Landi *et al*., 1999a,b) have investigated the potential role of human polymorphisms in the *GSTT1-1* gene using *in vitro* techniques with bromoform. The working hypothesis in each study was that individuals with at least one copy of *GSTT1-1* would be more sensitive to the genotoxic effects of brominated THMs than individuals lacking the gene. The data on bromoform are relevant to DBCM because these compounds are assumed to share common pathways for metabolism.

EPIDEMIOLOGICAL INVESTIGATIONS OF THMS IN DRINKING WATER

Cancer Studies

A large number of studies have examined the association between consumption of tap water containing THMs and incidence of cancer in the intestine, rectum, or bladder. The exposure metric in these studies was usually total THMs; some of the studies examined potential associations between chloroform and cancer incidence, although this is invariably complicated by the coexistence of the other disinfection byproducts.

The Safe Drinking Water Act of 1974 mandated that the U.S. EPA conduct a national survey to measure the concentration of various volatile and halogenated compounds in raw and finished drinking water supplies of major U.S. cities. Results of the National Organics Reconnaissance Survey (NORS) showed that THMs, especially chloroform, were ubiquitous in finished drinking water. Quantitative data from the NORS also demonstrated that chloroform was present in finished water at concentrations far greater than those of other THMs, including those of industrial origin (Symons *et al*., 1975). Because chloroform had been identified as an animal carcinogen as shown in the preceding discussion, the NORS data precipitated a controversy over the contribution of chloroform and other THMs to the incidence of human cancer.

In the 1970's, several municipalities and the Environmental Defense Fund independently sponsored a series of epidemiology studies on the association between cancer incidence, cancer mortality, and the presence of THMs in drinking water. The majority of these reports were never published. However, Wilkins *et al*. (1979) and the NRC (1980) published in-depth, critical reviews of the studies. The U.S. EPA (1985a) reviewed these and several additional epidemiological studies on cancer mortality in relation to THMs in drinking water. The results of these studies are noted in the following paragraphs, but are not discussed in depth here due to the thoroughness of the earlier reviews.

Harris (1974) reported a statistically significant, positive correlation between ingestion of chlorinated municipal drinking water taken from the Mississippi River and genitourinary tract and gastrointestinal cancer in Louisiana residents, without specifying a significance level. However, when De Rouen and Diem (1975) analyzed the Harris (1974) cancer mortality and water quality data, but restricted their analysis to southern Louisiana, they reported an equal number of significant ($p < 0.05$) positive and negative associations between ingestion of Mississippi River water and site-specific cancer incidence.

A more detailed study by Page *et al*. (1976) analyzed the association between cancer mortality over a 20 year period in Louisiana and the proportion of residents in each parish that received treated municipal drinking water from the Mississippi River. Other independent variables included proportion of the population that was rural or urban, and median family income. An attempt was made to control for the effects of employment in the petroleum, mining, and chemical industries. Total cancer mortality in white and nonwhite males and in nonwhite females was significantly correlated with ingestion of river water ($p < 0.005$). Urinary organ cancer in white males and nonwhite females, and cancer of the gastrointestinal organs in males and females of all racial groups were also significantly associated with exposure to river water ($p < 0.05$).

A similar study by Kuzma *et al*. (1977) focused on cancer mortality among individuals who drank treated municipal water from the Ohio River, and found a statistically significant ($p < 0.05$) increased mortality from stomach cancer (males and females) and bladder cancer (males only). A re-analysis of these results by Harris *et al*. (1977) concurred with the conclusions of the original study. Salg (1977) also looked at cancer mortality in a seven-county area served by the Ohio River. Of the 19 anatomic cancer sites studied, she reported a positive association ($p < 0.2$) for 13 sites in at least one of the four gender and race population groups, and found that white males had a significantly elevated incidence of colon and bladder cancer.

Alavanja *et al*. (1977, as cited in NRC, 1980), compared the incidence of gastrointestinal and genitourinary cancer in women from upstate New York who obtained their municipal drinking water either chlorinated or nonchlorinated from both surface and groundwater sources. They found that the risk of developing gastrointestinal or genitourinary cancer increased two-fold in urban women who drank chlorinated water, but an increase was not apparent in rural women who ingested chlorinated water. A companion study (Alavanja *et al*., 1978) reported that men and women in three New York counties with chlorinated drinking water had a greater risk of mortality due to gastrointestinal and genitourinary tract cancers than their cohorts who lived in areas without chlorinated water.

Mah *et al*. (1977), Kruse (1977), and Carlson and Andelman (1977), all as cited in NRC (1980), evaluated cancer mortality in relation to water chlorination in California, Maryland, and Pennsylvania, but with equivocal results.

Cantor *et al*. (1978) examined the association between cancer mortality and THM concentration in drinking water in Caucasian residents of 923 counties that were more than 50 percent urban, based on 1970 census data. Following adjustment of cancer mortality rates for several socioeconomic and demographic factors, Cantor *et al*. (1978) reported a positive but nonsignificant correlation between THM concentration and brain and bladder cancer (males and females), and nonHodgkin's lymphoma and kidney cancer in males. A negative association was found between stomach cancer and THM concentration in females. The authors noted that bladder cancer mortality showed the strongest and most consistent association with THM levels.

Hogan *et al*. (1979), using a weighted regression analysis to cancer mortality data for every county in the U.S., found a positive, statistically significant correlation between chloroform concentration in finished drinking water and mortality due to cancer of the bladder, rectum, and large intestine in white females; and stomach cancer in white males. An unweighted regression analysis of the same data showed a significant correlation between chloroform levels and bladder, rectal, thyroid, and breast cancer in white females and significant correlation with pancreatic and rectal cancers among males. The statistical significance level was not stated. Although both the weighted and unweighted analyses demonstrated associations between chloroform levels and cancer mortality for rectum and bladder, the results were dependent on the weighting scheme of the analysis, the appropriateness of the data, and the specific statistical model. Therefore, the author concluded that causal interpretation of these results would be questionable.

Young *et al*. (1981) evaluated cancer mortality in white females from a 28 county area of Wisconsin in relation to chlorine concentration in drinking water. For the five year period 1972 to 1977 the 8,029 cancer deaths among white Caucasian women were matched with an equal number of noncancer deaths with respect to age, county, and year of death. Data on water chlorination practices and chlorine content provided by the various municipal suppliers was used to calculate the average daily chlorine concentration in water ingested by each member of the study population. These values were separated into four exposure categories, none (less than 0.01 ppm), low (0.01 to 0.99 ppm), medium (1.0 to 1.70 ppm), and high (1.71 to 7.0 ppm). Young *et al*. (1981) found a significant ($p < 0.05$) association between colon cancer and all three chlorine-exposure levels. The odds ratio for colon cancer was essentially the same among the three chlorine categories, between 1.51 and 1.53. Among individuals who lived in areas

where drinking water was contaminated by runoff, the odds ratio for colon cancer increased to 2.94, 3.68, and 3.43 (low, medium, and high chlorine content, respectively).

Brenniman *et al*. (1978) conducted a case control study of mortality from gastrointestinal and genitourinary tract cancer in white residents of Illinois communities supplied by groundwater. The chloroform concentration in chlorinated water supplies ranged from 1 to 50 mg/L. Females who drank chlorinated groundwater had a significantly increased risk of developing cancer of the large intestine and rectum ($p < 0.05$) and a significantly greater ($p < 0.05$) risk of "total digestive tract cancer." Females who lived in "standard metropolitan" or urban areas with chlorinated water had a significantly increased risk of total gastrointestinal and urinary tract cancer ($p < 0.025$), compared to females with nonchlorinated water supplies. Because the authors did not control for smoking, diet, or occupation, they discounted these findings as inconclusive.

Wilkins and Comstock (1981) examined the relationship between drinking water chlorination and the risk of human cancer in a predominantly rural county in Maryland. Survey information was available on the source of drinking water for 98 percent of the population, as well as information on a number of personal and socioeconomic variables of the residents which was used to provide the reference population for a concurrent prospective study of cancer incidence. The total study population consisted of 14,553 males and 16,227 females. Potential cancer cases were identified from the regional cancer registry and death certificates for residents whose first diagnosis of cancer was made from 1963 through 1975. The authors combined census data and cancer case identification information to develop sex and site specific disease rates for white residents over 25 years of age. The study population was separated into three groups depending on the source of drinking water - a large municipal water system, private wells, or one of several small municipal suppliers. The city regularly chlorinated its water, which was obtained primarily from surface sources, and was considered to have the highest THM levels. A house to house sampling program found that the average concentration of chloroform in city tap water was 107 mg/L (Wilkins, 1979, as cited in Wilkins *et al*., 1979). Residents who obtained water from wells were considered to have the lowest exposure to THMs among the three groups, because water from these relatively deep private wells was not chlorinated. Study participants who used water from small municipal water delivery systems received a mixture of chlorinated water from surface sources and untreated groundwater. The authors noted that it was not possible to define the THM exposure of this group with any certainty.

Cancer incidence rates in the three exposure cohorts were examined with respect to age, marital status, education, smoking history, and year of residence in the 1963 household survey (Wilkins and Comstock, 1981). In the 12 year study period, 45 county residents developed liver cancer, 31 developed kidney cancer, and 80 developed bladder cancer. Both crude and adjusted rates for cancer of the kidney (males and females) and the liver (males) were similar, regardless of water source. Females from the city group had an approximately two-fold greater relative risk of developing liver or bladder cancer than females from the two other groups, which, the authors noted, could occur by chance. Similar results were reported for males from the city group for relative risk of bladder cancer. Considering duration of exposure to a specific water source, men who had resided in their 1963 homes approximately 12 years, and who ingested chlorinated

surface water (the city cohort), had a significantly increased risk ($p < 0.05$) of bladder cancer (relative risk 6.46). Women in this exposure group had elevated but not statistically significant risks for cancer of liver (relative risk 3.35) and bladder (relative risk 2.13). As with all studies of this type, the inability to account for many confounding variables (such as actual THM exposures) make it impossible to draw firm conclusions, especially with regard to a single THM such as chloroform. However, the results are consistent with those of other studies that reported a positive association between THMs in drinking water and an increased risk of bladder, colon, kidney, or liver cancer.

Lawrence *et al*. (1984) conducted a case control study on the association between THMs in drinking water and mortality due to colorectal cancer. Subjects were white, female members of the New York State Teachers Retirement System who lived in the "central geographic corridor" of New York. Screening of fatal cancer cases between 1968 and 1978 revealed 395 total cases including 319 cases of colon cancer and 76 cases of rectal cancer. The same number of controls was selected, matched for age, sex, and year of death. Exposure to THMs was estimated from residence and employment data for the 20-year period prior to the death of each member of the study population. A survey of THM concentration in water systems supplied solely by surface water was used to develop a statistical model to estimate chloroform concentration in relation to total THM concentration. Operational records for all water treatment facilities that served the study population were used to calculate the average daily values of THMs, and the statistical model provided an estimate of each individual's cumulative 20-year exposure to chloroform. Cases and controls were compared for cumulative chloroform exposure for the year prior to death, and for the year of diagnosis. The two groups were also analyzed to determine the relation between cancer outcome, cumulative chloroform exposure and dose, and source of water (lake, reservoir, river, and stream), and a number of demographic variables. None of the variables, including exposure to chloroform, was significantly associated with the development of colorectal cancer.

Data from a population-based case control interview study (Cantor *et al*., 1987) of bladder cancer in 10 areas of the U.S. were used to estimate relative risks among white men (2,116 cases, 3,892 controls) and women (689 cases, 1,366 controls) according to beverage intake level and type of water source. Individual year-by-year profiles of water source and treatment were developed by linking lifetime residential information with historical data from an ancillary water utility survey. Risk of bladder cancer increased with intake level of beverages made with tap water. The odds ratio for the highest versus lowest quintile of tap water consumption was 1.43 and 95 percent CI 1.23 to 1.67. The risk gradient with intake was restricted to persons with at least a 40-year exposure to chlorinated surface water and was not found among long-term users of nonchlorinated ground water. The odds ratios for the highest versus lowest quintiles of tap water intake were 1.7 and 2.0, respectively, among subjects with 40 to 59 and ≥ 60 years' exposure. Duration of exposure to chlorinated surface water was associated with bladder cancer risk among women and nonsmokers of both sexes. Among nonsmoking respondents with tap water consumption above the population median, the odds ratio increased with exposure duration to 3.1 and 95 percent CI 1.3 to 7.3 for ≥ 60 years of residence at places served by chlorinated surface water, versus nonchlorinated ground water users.

Lynch *et al*. (1989) concluded that misclassification of chlorination exposure signified the difference between observing and not observing an association with bladder cancer. This conclusion was derived using data from the Iowa portion of the National Bladder Cancer Case Control Study with four methods of quantifying chlorination exposure with sequentially decreasing degrees of misclassification for the 268 bladder cancer cases and 658 population-based controls. This demonstrated the effect of misclassification on depressing odds ratio estimates for years of exposure to chlorinated drinking water and bladder cancer.

Morris *et al*. (1992) presented a meta-analysis of pooled data from 10 cancer epidemiology studies in which there was a presumed exposure to chlorinated water and its byproducts. The authors reported a combined relative risk estimate of 1.21 (95 percent CI 1.09 to 1.34) for bladder cancer and 1.38 (95 percent CI 1.01 to 1.87) for rectal cancer. Odds ratios of ten other specific sites including colon and combined colon and rectum were not statistically significantly elevated. A calculated upper-bound estimate of approximately 10,000 cases of rectal and bladder cancer cases/year was the chief conclusion of the study. It should be noted that the studies included in the meta-analysis had methodological issues and significant design differences (Murphy, 1993; Poole, 1997; U.S. EPA, 1994c). Notwithstanding the methodological shortcomings that make quantitative use of this study for risk assessment difficult, the study cannot be dismissed. U.S. EPA has used the study for estimating the potential benefits from regulations controlling disinfection byproducts in finished drinking water. A range of cancer cases that could be attributed to exposure to chlorinated waters was estimated by U.S. EPA (1997b, 1998c,h,i,k) to be from less than one cancer case/year based on animal bioassays of THMs to 10,000 cases/year based on human epidemiological studies.

Vena *et al*. (1993) investigated fluid intake and the consumption of specific beverages in 351 cases of transitional cell carcinoma of the bladder in white males in western New York state with a matching group of 855 white male controls selected from Erie, Niagara, and Monroe counties. Total fluid consumption was found to be a strong factor for bladder cancer when a number of potential confounding factors were controlled. Risks were higher among those less than 65 year of age (odds ratio 6.3, 95 percent CI 2.8 to 14.0). The odds ratio was 3.4 (95 percent CI 1.8 to 6.2) for the highest quartile of fluid consumption among those 65 years of age and older. The tap water component was associated with increased risk in both age categories exhibiting a dose response relationship. Risks were higher among those who had never smoked cigarettes. The majority of the study population spent 90 percent of their life using public water supplies. The public water supplies in the three county study area are obtained from Lakes Erie and Ontario, and the Niagara River, all of which have historically been treated with chlorine for disinfection and oxidation. The authors speculate that the observed association of bladder cancer and fluid intake is related to the intake of disinfection byproducts.

Ijsselmuiden *et al*. (1992) carried out a case control study of the association of drinking water and pancreatic cancer in Washington County, Maryland. There were 101 cases and 206 controls. Chlorinated municipal water was used by 79 percent of cases and 63 percent of controls. The crude odds ratio was 2.23 (95 percent CI 1.24 to 4.10) and the adjusted odds ratio was 2.18 (95 percent CI 1.20 to 3.95). Adjustment for age and

smoking had little effect on the risk. The study provided no information on the individual amount of water consumed or dosage of disinfection byproducts.

King and Marrett (1996) conducted a case control study of bladder cancer and chlorination byproducts in treated water in Ontario, Canada. Residence and water source histories and data from municipal water supplies were used to estimate individual exposure according to source, chlorination status, and THM concentration. Exposures were estimated for the 40 year prior period using 696 cases of diagnosed bladder cancer between 1992 and 1994 and 1,545 controls with at least 30 year exposure information. Odds ratios adjusted for potential confounders were used to estimate relative risk. Study subjects exposed to chlorinated surface water for ≥ 35 year had an odds ratio of 1.41 (95 percent CI 1.10 to 1.81) compared to those exposed for less than ten years. Those exposed to estimated THM levels ≥50 ppb (μg/L) for 35 years had an odds ratio of 1.63 (95 percent CI 1.08 to 2.46). The results indicate that the risk of bladder cancer increases with both duration and concentration of THMs or associated disinfection byproducts in drinking water. The adjusted odds ratio for THM levels were: 0 to 25 ppb, 1.0 (reference); 25 to 74 ppb, 1.43 (95 percent CI 1.00 to 2.13); ≥ 75 ppb, 1.66 (95 percent CI 1.11 to 2.51); trend test ($p = 0.006$). The authors conclude that chlorination byproducts represent a potentially important risk factor for bladder cancer.

Poole (1997) prepared a report for the U.S. EPA based on Morris *et al*. (1992) and four additional studies including Ijsselmuiden *et al*. (1992), Vena *et al*. (1993), McGeehin *et al*. (1993), and King and Marrett (1996) to comment on the utility of the aggregated estimates for risk assessment purposes. U.S. EPA (1998h) evaluated this report and agreed with Poole (1997) that the studies were heterogeneous and thus were not appropriate to be combined into a single point estimate of risk (Craun, 1993; Farland and Gibb, 1993; Murphy, 1993).

Cantor *et al*. (1998) conducted a population-based case control study of bladder cancer in Iowa from 1986 to 1989 to evaluate the risk posed by consumption of tapwater containing chlorination byproducts. The study comprised 1,123 cases and 1,983 controls. After adjusting for potential confounders, the authors calculated an odds ratio for duration of exposure to chlorinated surface water of 1.0 (referent), 1.0, 1.1, 1.2, and 1.5 for 0, 1 to 19, 20 to 39, 40 to 59, and 60 and more years of surface water use, respectively (Lang *et al*., 1998; Kulldorff *et al*., 1999). For male subjects there were significant trends in odds ratio for bladder cancer for both total lifetime THM intake in grams ($p = 0.05$) and for lifetime average THM concentration in water in ppb ($p = 0.02$). For female subjects the trends for odds ratios were less significant ($p = 0.08$, $p = 0.04$, respectively). Among nonsmoking men and women there was no association of bladder cancer and chlorinated water consumption. Among smoking men, exposure to chlorinated water enhanced the risk of bladder cancer.

In a parallel study by Hildesheim *et al*. (1998), also conducted in Iowa, odds ratios were calculated for 560 colon cancer cases, 537 rectal cancer cases, and 1983 controls. For rectal cancer an association was observed with the duration of chlorinated water use, with adjusted odds ratios of 1.1, 1.6, 1.6, and 2.6 for 1 to 19, 20 to 39, 40 to 59, and ≥ 60 years of exposure, compared with no exposure. Rectal cancer was also associated with several measures of estimated lifetime THM exposure. The odds ratios for exposure to

lifetime average THM concentrations in the unit of μg/L and rectal cancer gave a statistically significant trend (p = 0.01), whereas the odds ratio for total lifetime THM intake in grams was not statistically significant (p = 0.08). No associations were seen for colon cancer. When other risk factors were analyzed jointly with THMs a larger relative risk was seen for rectal cancer among subjects with a low dietary fiber intake. The relative risk for ≥ 40 year exposure to chlorinated water was 2.4 and 95 percent CI 1.5 to 4.0 for low fiber intake versus 0.9 and 95 percent CI 0.4 to 1.8 for the high fiber intake with no exposure to chlorinated surface water.

Freedman *et al*. (1997) conducted a population-based case control study in Maryland to study the association of bladder cancer and exposure to chlorinated surface water sources. The Washington County Cancer Registry identified 294 bladder cancer cases in white residents between 1975 and 1992. White controls matched by age and gender were selected randomly from the census (N = 2,326). Households receiving chlorinated surface waters were designated as 'high exposure' and all others as 'low exposure.' Duration of exposure was based on length of residence in the census household prior to 1975. Odds ratios were calculated using logistic regression methods, adjusting for age, gender, tobacco use, and urbanicity. Bladder cancer risk was found to be weakly associated with duration of exposure to municipal water. The association was limited to male smokers. In eversmokers compared to neversmokers with low exposure, the odds ratios for increasing duration of exposure were: 1.3 for zero year; 1.4 for one to 10; 1.4 for 11 to 20; 1.7 for 21 to 30; 2.2 for 31 to 40; 2.8 for more than 40 years. While supporting some of the findings in previous studies, this study has no information on individual doses of treated water and disinfection byproducts.

In view of increasing glioma rates, Cantor *et al*. (1999) conducted a population-based case control study in Iowa of 375 brain cancer patients and 2,434 controls using a combination of postal questionnaire gathering information on lifetime residential history, sources of drinking water, beverage intake, and other potential risk factors, with historical information from water utilities and THM levels in recent samples to estimate the exposure to chlorination byproducts in drinking water. The analysis included 291 cases (77.6 percent) and 1,983 controls (81.5 percent), for whom water quality information was available for at least 70 percent of lifetime years. Proxies represented 74.4 percent of cases. The mean number and mean duration of places of residence were comparable between direct and proxy respondents, suggesting little contribution to bias. After multivariate adjustment, odds ratios for brain cancer were 1.0, 1.1, 1.6, and 1.3 for exposure to chlorinated surface water of zero, one to 19, 20 to 39, and ≥ 40 years (p for trend = 0.1). Among men, odds ratios were 1.0, 1.3, 1.7, and 2.5 (p trend = 0.04), and among women, 1.0, 1.0, 1.6, and 0.7 (p trend = 0.7). Similar findings were found with estimates of average lifetime level of THMs. The association was stronger among men with above-median tap water consumption.

King (1999) reviewed epidemiological studies that were methodologically most rigorous, assessing the magnitude of risk, causal nature, and implications of the relationship between disinfection byproducts and cancer. The review included those studies which permitted quantitation of this relationship, specifically case-control and cohort designs based on incident cases, interviews with study subjects, and measures of long-term exposure. Seven studies were identified which met the design criteria for review.

Relative risk estimates were derived from the studies and where appropriate, a pooled relative risk estimated was calculated. Bladder cancer was the site most frequently studied in relation to disinfection byproducts, and in these studies an association with increased risk was consistently reported. The summary measures from the bladder cancer studies were consistent in finding a moderate elevation in risk with 35 or more years of exposure to chlorination byproducts. Six studies showed increased risks in males and three studies in females. Results were pooled to obtain a relative risk of 1.5 with 95 percent CI 1.3 to 1.8 for ≥ 35 years of exposure to chlorinated drinking water. Inconsistencies were noted among study subgroups, e.g., smokers versus nonsmokers. The potential for occupational exposure was not explored.

King (1999) estimated the population attributable risk (PAR percent) for different levels of exposure prevalence. Assuming 25 percent of the population is exposed to disinfection byproducts, the population attributable risk would be 11 percent. For 100 percent exposure, the population attributable risk would be 33 percent. That is, up to 33 percent of current bladder cancer incidence could be due to disinfection byproduct exposure. Based on five bladder cancer studies (Cantor *et al*., 1985, 1998; McGeehin *et al*., 1993; King and Marrett, 1996; Freedman *et al*., 1997) with years of exposure to chlorinated surface water and THM levels, U.S. EPA (1998h) estimated a population attributable risk of two to 17 percent. The upper bound estimate of the number of potential bladder cancer cases/year potentially associated with exposure to disinfection byproducts in chlorinated surface water was about 1,100 to 9,300 based on 54,500 expected new bladder cancer cases in the U.S. projected for 1997 (U.S. EPA, 1998h). King (1999) concluded that the evidence pointed to a probable relationship between chlorination byproducts and bladder cancer, but identification of specific byproducts and mechanisms was lacking.

The results of a historical cohort study of 621,431 persons living in 56 towns in Finland support the magnitude of excess risks for rectal and bladder cancers found in earlier epidemiologic studies on chlorination byproducts (Koivusalo *et al*., 1997). The study assessed the relation between drinking water mutagenicity and cancer. Exposure to quantity of mutagenicity was calculated on the basis of historical information on raw water quality and water treatment practices using an empirical equation relating mutagenicity and raw water pH, $KMnO_4$ value and chlorine dose. Cancer cases were derived from the population-based Finnish Cancer Registry and follow-up time in the study started in 1970. Age, gender, time period, social class, and urban residence were taken into account in Poisson regression analysis of the observed numbers of cases using expected numbers of cases standardized for age and gender. Excess risks were calculated using a continuous variable for mutagenicity for 3,000 net revertants/L exposure representing an average exposure in a town using chlorinated surface water. After adjustment for confounding, a statistically significant excess risk was observed for women in cancers of the bladder with a relative risk of 1.48 and 95 percent CI 1.01 to 2.18, and rectum with a relative risk of 1.38 and 95 percent CI 1.03 to 1.85.

Koivusalo *et al*. (1998) studied the relationship between exposure to historical drinking water mutagenicity and cancer in Finland using a population-based case control study comprising 732 bladder cancer cases, 703 kidney cancer cases, and 914 controls obtained from the nationwide Finnish Cancer Registry for the years 1991 to 1992. Exposure to

drinking water mutagenicity was estimated by using information on past residence, past water source, and historical data on water quality and treatment. Odds ratios were calculated for an increase of 3,000 net revertants/L in average exposure from 1950 to 1987, adjusting for age, sex, socioeconomic status, and smoking in logistic regression models. A small, statistically significant, exposure-related excess risk was found for kidney cancer for men with an odds ratio of 1.49 and 95 percent CI 1.05 to 2.13 for 3,000 net revertants/L exposure level. For women, the association remained nonsignificant, with a lower odds ratio of 1.08 and 95 percent CI 0.69 to 1.68. For bladder cancer, the odds ratio for both men and women was 1.22 and 95 percent CI 0.92 to 1.62 for 3,000 net revertants/L exposure. A higher odds ratio of 2.59 and 95 percent CI 1.13 to 5.94 for 3,000 net revertants/L exposure was observed for male nonsmokers.

Colon and rectal cancer were examined in fewer studies meeting the design criteria, in four and two studies, respectively. For colon cancer, two studies gave relative risks of less than one and two a relative risk greater than 1.5 (Young *et al*., 1987). For rectal cancer, one study gave a relative risk less than one and the other a relative risk greater than two. Given the inconsistency in the results, no pooled relative risk estimates were made for these cancer sites. King *et al*. (2000) conducted a population-based case control study in southern Ontario, Canada from 1992 to 1994 to assess the relationship between chlorination byproducts in public water supplies and cancers of the colon and rectum including 767 colon cases, 661 rectal cases, and 1,545 controls with exposure information for at least 30 years. Interviews providing residence and water source histories were completed by 76 percent of eligible cancer cases and 72 percent of eligible controls. Supplemental data from municipal water supplies were used to estimate individual exposure to water source, chlorination status, and by-product levels as represented by THMs during the 40-year period before the interview. Among males, colon cancer risk was associated with cumulative exposure to THMs, duration of exposure to chlorinated surface water, and duration of exposure to a THM level ≥ 50 µg/L and 75 µg/L. Males exposed to chlorinated surface water for 35 to 40 years had an increased risk of colon cancer compared with those exposed for less than 10 years with an odds ratio of 1.53 and 95 percent CI 1.13 to 2.09. Males exposed to an estimated THM level of 75 µg/L for ≥ 35 years had double the risk of those exposed for less than 10 years with an odds ratio of 2.10 and 95 percent CI 1.21 to 3.66. In contrast, these relationships were not observed among females. No relationship was observed between rectal cancer risk and any of the measures of exposure to chlorination byproducts.

A review and epidemiological survey of adverse effects associated with chlorination byproducts in Spain conducted by Villanueva *et al.* (2001a,b) established a concern with an increase in various effects associated with drinking water chlorination, especially bladder cancer. Villanueva and colleagues have subsequently carried out an extensive series of studies on THM exposures and adverse health effects (Villanueva *et al.*, 2003a,b, 2004, 2006a, 2007a; Michaud *et al.*, 2007).

Villanueva *et al*. (2003a) conducted a meta-analysis of the best available data from Europe and North America population studies, with six case control studies including 6,084 incident bladder cancer cases and 10,816 controls, and two cohort studies including 124 incident bladder cancer cases. These authors reported that long-term consumption of chlorinated drinking water (more than 40 years) is associated with bladder cancer,

particularly in men, with an adjusted odds ratio of 1.4 and a 95 percent CI 1.1 to 1.9. In women there was an adjusted odds ratio of 1.2 and a 95 percent CI 0.7 to 1.8. The combined odds ratio for long term exposure of more than 40 years in both genders was 1.4 and a 95 percent CI 1.2 to 1.7. The combined estimate of slope for a linear increase in risk was 1.27 (95 percent CI 1.15 to 1.43) for 40 years of exposure in both sexes. These researchers also pointed out (Villanueva *et al.*, 2003b) that both THMs and haloacetic acids are present at elevated levels in chlorinated drinking water, which complicates attribution of risk to specific chemical contaminants.

Villanueva *et al*. (2004) pooled the primary data from six case control studies of bladder cancer that used THMs as a marker of disinfection byproducts with data on THM exposure and individual water consumption. There were two studies from the U.S. and one each from Canada, France, Italy, and Finland. The analysis included 2,806 cases and 5,254 controls, all of whom had known exposure measures for at least 70 percent of the exposure window of 40 years before the interview. Cumulative exposure to THMs was estimated by combining individual year-by-year average THM level and daily tap water consumption. There was an adjusted odds ratio of 1.24 in men exposed to an average of more than 1 µg/L THMs compared with those who had lower or no exposure, with a 95 percent CI 1.09 to 1.41. Estimated relative risks increased with increasing exposure, with an adjusted odds ratio of 1.44 and a 95 percent CI 1.20 to 1.73 for exposure higher than 50 µg/L. Similar results were found with other indices of THM exposure. Among women, THM exposure was not associated with bladder cancer risk, with an adjusted odds ratio of 0.95 and a 95 percent CI 0.76 to 1.20.

Villanueva *et al.* (2006a) compared exposure to tap water versus consumption of other fluids in a pooled evaluation of six case-control studies, related to bladder cancer risk. Total fluid intake was associated with a significantly increased risk of bladder cancer in men but not in women, albeit with very low odds ratios (males, OR = 1.08, CI 1.03-1.14, p for linear trend <0.001; females, OR = 1.04, CI 0.94-1.15, p = 0.7). The relationship was strengthened by considering only consumption of tap water (OR = 1.46, CI 1.20-1.78) for both genders combined, comparing consumption of >2 L/day versus ≤.0.5 L/day. These researchers also attempted to evaluate total lifetime exposures to THMs versus bladder cancer rates (Villanueva *et al*., 2007a), combining estimates of THM exposures through ingestion of water with estimates of inhalation and dermal absorption through showering, bathing, and swimming. They reported that long-term exposure to THMs was associated with an odds ratio of 2.10 (CI 1.09-4.02) for increased bladder cancer risk "for average household THM levels of >49 versus ≤8 µg/L."

However, a followup study by many of the same researchers (Michaud *et al*., 2007) provides contradictory results. The study reported that total fluid intake in a case-control study of bladder cancer cases in Spain was associated with a decrease in bladder cancer risk (OR = 0.62, CI = 0.40-0.95) for the highest consumption quintile versus the lowest. An inverse correlation was reported for exposure to THMs. The authors concluded that water intake was inversely associated with bladder cancer risk, regardless of THM exposure level.

Vinceti *et al*. (2004) analyzed the mortality of a cohort of 5,144 residents in Guastalla, northern Italy, who were supplied tap water with chloroform and THM at about 33.1 µg/L and 39.7 µg/L immediately after chlorination, respectively, and 51.7 µg/L and 70.7

µg/L 24 hours after chlorination, respectively, between 1965 and 1987. They suggested that consumption of drinking water with THM increases the risk of melanoma as shown in two previous cohort studies (Doyle *et al*., 1997; Koivusalo *et al*., 1997) and possibly prostate, breast, and ovary cancer as shown in three previous cohort studies (Wilkins and Comstock, 1981; Doyle *et al*., 1997; Koivusalo *et al*., 1997). However, the point estimates were statistically imprecise due to the limited number of deaths for some site-specific cancers as well as confounding smoking and other lifestyle factors. Using death rates of a nearby community as reference rates, the standardized mortality ratio from all cancers between 1987 and 1999 was slightly increased for males at 1.2 and 95 percent CI 1.1 to 1.4, and females at 1.1 and 95 percent CI 1.0 to 1.3. This was mainly due to a higher mortality from stomach, liver, lung, prostate and bladder cancer in males and from stomach, pancreas, breast and ovarian cancer and lymphocytic leukemia in females. Excess mortality from melanoma was observed in both males and females.

In a population-based case-control study of 486 incident cases of pancreatic cancer and 3,596 age- and sex-matched controls in Canada (Do *et al*., 2005), logistic regression analysis found no evidence of increased pancreatic cancer risk at higher chlorination byproduct concentrations, with all odds ratios smaller than 1.3. Exposure were estimated by linking lifetime residential histories to two different databases containing information on chlorination byproduct levels in municipal water supplies. Latency periods for pancreatic cancer induction of three, eight, or 13 years all gave null findings.

Bove *et al.* (2007) more recently examined the relation between concentrations of disinfection byproducts including THMs and the risk for urinary bladder cancer in a case-control study of 567 white men aged 35 to 90 years in western New York State. They used logistic regression to estimate odds ratios for effects of THM consumption on cancer risk. Higher THM consumption levels were associated with increased risk for cancer of the urinary bladder (OR = 2.34, 95 percent CI = 1.01 to 3.66). Results were most significant for bromoform (OR = 3.05, 95 percent CI = 1.51 to 5.69), and risk was highest (OR = 5.85, 95 percent CI = 1.93 to 17.46) for those who consumed the greatest amount of water with the oldest postdisinfected tap water (which might contain increased brominated byproducts).

Reviews of the earlier epidemiological studies (Wilkins *et al*., 1979; NRC, 1980; U.S. EPA, 1985a) concluded that none of them demonstrated an unequivocal association between presence of chloroform or total THMs in drinking water and an increased risk of cancer. Data analysis was limited by the indirect and often qualitative measures used (including estimation of exposures to chloroform or THMs), the inability of investigators to control for migration of exposed populations, and failure to account for smoking, dietary habits, and alcohol consumption. In addition, the scientific validity of the methodology used in epidemiological studies like these was seriously questioned (Feinstein, 1988). In subsequent reviews of the available epidemiological studies, the U.S. EPA (1994c, 1997b, 1998h, 2005) concluded that the most notable findings of the studies have been weak, but fairly consistent associations between exposure to chlorination byproducts in drinking water and cancers of the bladder, colon, and rectum. None of the studies have provided a definitive conclusion about the relationship between THM exposure and cancer, due to the presence of numerous potentially carcinogenic chemicals in the chlorinated drinking water. These data have been found to be

inconclusive with regard to the potential carcinogenicity of THMs in drinking water in humans by IARC (1999d), ATSDR (1997), and U.S. EPA (2001e), but the bladder cancer studies appear to provide the strongest evidence to date, according to U.S. EPA (2005).

Reproductive and Developmental Studies

Many epidemiological studies have investigated potential links between exposure to THM chlorination disinfection byproducts in drinking water and adverse reproductive or developmental outcomes in humans. In most cases, these studies have used either an ecologic measure based on chlorination practice of the communities or a composite measure of total THM concentration as the exposure metric. The studies have examined general categories of reproductive and developmental outcomes including spontaneous abortion, stillbirth, preterm delivery, low birth weight, growth retardation, and birth defects such as neural tube defects, oral cleft defects, respiratory and cardiac effects.

Aschengrau *et al*. (1989) conducted a case control study of the relationship between community drinking water quality and spontaneous abortion in 286 women through 27 weeks gestation compared with controls of 1,391 women having live births at or after 37 weeks of gestation in a hospital in Boston, Massachusetts from July 1976 to February 1978. The primary focus was on trace inorganic elements in public tap water from the communities where the women resided during pregnancy, based on maternal address. Treated surface water was compared to untreated ground or mixed water, and for surface water only, chlorination was compared to chloramination. However, no chemical analysis of disinfection byproducts was available. After adjustment for potential confounders, an increased frequency of spontaneous abortion was considered to be associated with surface water.

The same authors (Aschengrau *et al*., 1993) analyzed data from 2,348 pregnant women including 1,171 late adverse pregnancy outcome cases and 1,177 controls selected from 14,130 obsteric patients in the same Boston hospital between August 1977 and March 1980. Risks from exposure to water quality variables on the occurrence of congenital abnormalities (1,039 cases), stillbirth (77 cases), and neonatal death (55 cases) were analyzed. Again, available data on organic disinfection byproducts were too limited for analysis. Considering surface water only and comparing chlorinated to chloraminated water with higher total THMs in chlorinated water, increases were reported for stillbirth risk with water chlorination with an adjusted odds ratio of 2.6 and 95 percent CI 0.9 to 7.5 versus chloramination as the referent group. There was also an association of exposure to chlorination with increased risk for major malformations with an adjusted odds ratio of 1.5 and 95 percent CI 0.7 to 2.1, primarily of respiratory anomalies with an adjusted odds ratio of 3.2, CI 1.1 to 9.5, or urinary tract defects with an adjusted odds ratio of 4.1, CI 1.2 to 14.1.

Kramer *et al*. (1992) conducted population-based case control analyses of whether water supplies with high THM levels were associated with low birth weight, prematurity, or intrauterine growth retardation among residents of small towns in Iowa. Residents of small towns with a population between 1,000 to 5,000, where all of the public drinking water was derived from a single source, were studied. The chloroform or other THM concentration in tap water was estimated from a municipal tap water survey conducted in

1986 and 1987, a few years before the study period. Exposure was assigned based on the mother's town of residence at delivery, as reported on the birth certificate. Data on pregnancy outcomes for cases and controls were collected from birth certificates for nonHispanic white singleton births from January 1, 1989 to June 30, 1990. A separate analysis was conducted for each endpoint, with five randomly selected controls used for each affected newborn, low birth weight (159 cases, 795 controls), preterm delivery (342 cases, 1,710 controls), or intrauterine growth retardation (187 cases, 935 controls). The data were adjusted for maternal age, number of previous children, marital status, education, number of prenatal visits, and maternal smoking. A major shortcoming of the study was reliance on municipal surveys of THM concentrations in drinking water in assigning individual exposures, and inability to consider seasonal fluctuations or individual variability in water consumption, since only one annual measurement was taken. Residence in communities with chloroform levels exceeding 10 ppb was associated with increased risk for intrauterine growth retardation with an odds ratio of 1.8 with a 95 percent CI of 1.1 to 2.9.

Bove *et al*. (1995) evaluated the effects of public drinking water contamination on birth outcomes in 75 towns in northern New Jersey. This cross sectional study included 80,938 live births and 594 fetal deaths based on birth and fetal death certificates during the period 1985 to 1988. The same limitations noted for the Iowa study (Kramer *et al*., 1992) are applicable to this study except for a closer temporal relation between pregnancy and the measurement. Exposure to total THMs greater than 100 ppb, based on the average of quarterly tap samples concurrent with each birth's gestational period for the public water system serving the mother's town of residence at delivery, was associated with intrauterine growth retardation or small for gestational age with an odds ratio of 1.5 and 90 percent CI 1.2 to 1.9 (4,082 cases), and low birth weight with an odds ratio of 1.4 and 90 percent CI 1.0 to 2.0 (752 cases). Exposure to total THMs greater than 80 ppb was associated with central nervous system defects with an odds ratio of 2.6 and 90 percent CI 1.5 to 4.3 (118 cases), neural tube defects with an odds ratio of 3.0 and 90 percent CI 1.3 to 6.6 (56 cases), oral cleft defects with an odds ratio of 3.2 and 90 percent CI 1.2 to 7.3 (83 cases), and major cardiac defects with an odds ratio of 1.8 and 90 percent CI 1.0 to 3.3 (108 cases). Total THM concentrations greater than 100 ppb compared with less than 20 ppb were associated with a 70.4 g reduction in mean birth weight among term births with 90 percent CI -40.6 to -100.2.

Savitz *et al*. (1995) evaluated data from a population-based case control study of miscarriage, preterm delivery, and low birth weight in three communities in central North Carolina with respect to water source, amount consumed, and THM concentration, from September 1988 to April 1991. Telephone interviews were used for exposure to water and confounding factors without considering selection bias and recall bias. Water source was not related to any of the pregnancy outcomes. Increasing amount of water ingested was associated with decreased risks for all three outcomes. THM concentration and dose, as estimated by multiplying concentration monitored with amount recalled, were not related to pregnancy outcome except for an increased risk of miscarriage in the highest sixtile of THM concentration, with an adjusted odds ratio of 2.8 and 95 percent CI 1.1 to 2.7. However, the second to highest sextile was associated with an anomalously low risk of miscarriage, with an odds ratio of 0.2 and 95 percent CI 0.0 to 0.5, which did not correspond to an overall dose-response gradient.

Klotz and Pyrch (1998, 1999) studied neural tube defects and drinking water contaminants including disinfection byproducts. This case control study was based on births with neural tube defects reported to the New Jersey Birth Defects Registry in 1993 and 1994, matched against control births randomly sampled from all births statewide. The authors reported an elevated odds ratio for association of neural tube defects with either total THMs in drinking water (odds ratio = 2.1), or surface water source (odds ratio = 1.5). Statistically significant results were seen in the subjects exposed to the highest THM level of more than 40 ppb. A significant effect was also found when the analysis was limited to subjects with neural tube defects but no other malformation, with an odds ratio of 2.1 and 95 percent CI 1.1 to 4.0. The prevalence odds ratios for the highest tertile of total THMs versus the lowest was 1.6, with 95 percent CI 0.9 to 2.7. Sensitivity analyses in which analyses were restricted to isolated neural tube defect cases and mothers with known residence at conception yielded a stronger association with total THMs exposure, with a prevalence odds ratio of 2.1 and 95 percent CI 1.1 to 4.0.

Gallagher *et al*. (1998) reported a potential relation between exposure to THMs in the third trimester of pregnancy and retarded fetal growth based on a retrospective cohort study in Denver. A hydraulic model developed by the U.S. EPA was applied to the pipe networks of two surface water municipal systems to identify census blocks that were hydraulically similar to the total THM quarterly tap sample locations. Birth certificates from 1990 through 1993 were matched to historical water sample data with respect to time and location of maternal residence based on census block groups. After excluding births from all census block groups with no THM sample data and restricting to singleton white births greater than 400 g with 28 to 42 weeks of completed gestation, 1,893 live births within 28 census block groups were selected. A weak association of THM exposure during the third trimester with low birth weight was found with an odds ratio of 2.1 for the highest exposure level and 95 percent CI 1.0 to 4.8. In addition, a large increase in risk for term low birth weight at the highest exposure level was found with an odds ratio of 5.9 and 95 percent CI 2.0 to 17.0. No association was found between exposure and preterm delivery with an odds ratio of 1.0 for the highest exposure level and 95 percent CI 0.3 to 2.8 (Gallagher *et al*., 1998; Swan and Waller, 1998).

A series of studies in California examined risk factors for spontaneous abortion or early term miscarriage. Although inconsistencies in the reporting of tap water consumption suggest recall bias, bottled water drinkers appeared to have had unusually low risks, and tap water drinkers who received ground water may have had slightly higher risks based on external data from comparable studies (Hertz-Picciotto *et al*., 1989). The relation between reported prenatal water consumption and risk of spontaneous abortion was analyzed in an independent nested case control cohort among pregnant women who attended three prenatal clinics in three northern California counties between September 1981 and June 1982. In one evaluation, the life table-adjusted risk of spontaneous abortion for those drinking mainly bottled water was found to be 8.4 percent; the risk for those drinking mainly tap water was 12.5 percent. In another analysis, the Cox proportional hazards model was used to control for multiple confounders, yielding a hazard ratio for spontaneous abortion of 1.5 with 95 percent CI 1.1 to 2.0 for consumers of tap water compared with bottled water. Tap water drinkers whose home source of water included groundwater had the greatest risk of 13.8 and, after controlling for confounders, their hazard ratio was 1.7. A follow-up study reported that the association

was limited to women interviewed by telephone and was not observed among those who completed a mail questionnaire (Hertz-Picciotto *et al*., 1992).

Pregnancies during 1980 to 1985 in four areas of Santa Clara County, California, were studied by Wrensch *et al*. (1992). Two of the areas were exposed to solvent-contaminated drinking water during 1980 and 1981, and two were unexposed. Residents of a census tract that received drinking water from a well contaminated with solvents were shown to experience a spontaneous abortion rate over twice that occurring in an unexposed census tract. In addition, increased tap water consumption was associated with higher rates of spontaneous abortions in both the exposed and the unexposed tracts. There was an overall excess of spontaneous abortions among women who reported any tap water consumption during the first trimester of pregnancy compared with those who reported no tap water consumption, with an odds ratio of 4.0 and 95 percent CI 1.8 to 9.1, regardless of exposure to the contaminated water. The odds ratio for spontaneous abortion for women reporting any versus no tap water was 6.9 and 95 percent CI 2.7 to 17.7 after adjustment for numerous potential confounders using multiple logistic regression analyses. The elevated odds ratios of spontaneous abortion were seen among tap water drinkers who used no filters or softener-type filters but not among women who reported use of active filters. Spontaneous abortion rates were reduced in women who reported any versus no bottled water consumption with an odds ratio of 0.26 and 95 percent CI 0.16 to 0.43. Among women who reported no tap water consumption, no birth defects occurred among 263 live births; in comparison, among women who reported tap water consumption, four percent of 908 live births had defects ($p = 0.0001$). No relation between birth defects and bottled water use was observed (Wrensch *et al*., 1992).

Data from the study of Wrensch *et al*. (1992) were reanalyzed by Deane *et al*. (1992). The results confirmed the association between spontaneous abortions and cold tap water consumption that was seen in the original study. The observed effect was not related to maternal risk factors, nor was it a function of consumption of bottled water. After controlling for bottled water, the odds ratio for spontaneous abortions associated with consumption of tap water was 3.4 and 95 percent CI 0.6 to 19.4.

Windham *et al*. (1992) conducted a case control study to investigate whether drinking tap or bottled water during pregnancy affects the risk of spontaneous abortion. The study, involving 626 cases and 1,300 controls, ascertained cases from hospital pathology laboratory reports of pregnancies that began in 1986 and obtained controls from California birth certificates. The crude odds ratio for consumption of any versus no cold tap water at home during the first trimester was 1.2 and 95 percent CI 1.0 to 1.5, with no dose-response effect. The crude odds ratio for any bottled water consumption was 0.79 and 95 percent CI 0.65 to 0.96, with a downward trend by amount consumed.

Because preliminary data suggested a relation between risk of spontaneous abortion and tap water consumption during pregnancy (Hertz-Picciotto *et al*., 1989, 1992; Deane *et al*., 1992; Petitti, 1992; Wrensch *et al*., 1992; Zierler, 1992), the California Department of Health Services (DHS) included questions on prenatal water consumption in all reproductive studies conducted between 1982 and 1988 (Swan *et al*., 1992). Even though evidence for biased recall was presented (Neutra *et al*., 1992; Swan *et al*., 1992), results from four of the five retrospective studies conducted between 1982 and 1988 by the California DHS suggest that women abstaining from tap water or drinking bottled water

during the first trimester of pregnancy may be at reduced risk of spontaneous abortion (Swan *et al*., 1992). Fetal resorption frequencies seen in an accompanying toxicology study were consistent with these epidemiologic findings, although not conclusive. Tap and bottled water samples from these study areas were analyzed for agents that might account for these findings. Differences in trace element composition and biological activity were observed, but the reproductive significance of these differences is unknown (Swan *et al*., 1992; Zierler, 1992).

Swan *et al*. (1998) examined the potential association between amount and source of water consumed and spontaneous abortion in Santa Clara and Walnut Creek in the San Francisco Bay area, and Fontana in southern California. The study participants were recruited from three branches of a large managed health care organization that were located in areas that received either mixed, surface, or groundwater (Swan *et al*., 1998; Waller *et al*., 1998; Waller and Swan, 1999; Whorton, 1999). Participants were recruited for the study when they scheduled their first prenatal exam after confirmation of pregnancy. A group of 5,342 participants completed a telephone interview that collected information on demographics, previous pregnancy history, employment status, consumption of tap and bottled water, substance use (alcohol, tobacco, and caffeine), and other factors. Each participant was at least 18 years of age, at 13 or less weeks of gestation, spoke English or Spanish, and knew with certainty the date of her last menstrual period at enrollment. A total of 5,144 pregnancies remained for analysis following adjustment for elective termination of pregnancy, ectopic or molar pregnancies, and multiple gestations (Waller *et al*., 1998).

Swan *et al*. (1998) found a statistically significant association of consumption of cold tap water with spontaneous abortion in the Santa Clara area with an adjusted odds ratio of 2.17 and 95 percent CI 1.22 to 3.87. Neither tap nor bottled water consumption altered the risk of spontaneous abortion in the Walnut Creek or Fontana subjects. The Swan *et al*. (1998) study confirms an earlier retrospective study of pregnancy outcomes in the Santa Clara area (Neutra *et al*., 1992; Wrensch *et al*., 1992).

Waller *et al*. (1998), using the same study population, examined exposure to THMs and spontaneous abortion as pregnancy loss at 20 or less weeks of gestation in a prospective study of 5,144 pregnant women. Seventy-eight water utilities provided data on THMs, and total THM levels were calculated by averaging all measurements taken by the subject's utility during her first trimester. THM exposure was estimated from the participant's daily tap water intake at eight weeks gestation. Each woman was assigned an exposure classification for total THMs and individual THMs. A high exposure to total THMs was defined as drinking five or more glasses of cold tap water/day and having a total THMs level of 75 ppb or higher. Low exposure to total THMs was defined as either 1) drinking less than five glasses of cold tap water/day, 2) having a total THMs level of less than 75 ppb, or 3) receiving water from a utility that provided 95 percent or greater groundwater. Waller *et al*. (1998) found that women who drank at least five glasses/day of cold tap water containing at least 75 ppb total THMs had an increased spontaneous abortion risk with an adjusted odds ratio of 1.8 and 95 percent CI 1.1 to 3.0. The study authors found no additive or other effect from showering or swimming; therefore, no odds ratio adjustment was made for these variables. Increased risk of spontaneous

abortion was noted starting at a total THM concentration of approximately 75 ppb. An estimated 18.4 percent of the study participants were exposed at or above this level.

To reduce exposure misclassification due in part to limitations inherent in using utility sampling records, Waller *et al*. (2001) performed unweighted, weighted, and subset analyses using two exposure assessment methods, one based on utility-wide sampling averages, and one based on measurements from the utility sampling site closest to the subject's residence, to reestimate total THM exposure for 4,212 participants in a preexisting study of risk factors for spontaneous abortion. The weighted and subset analyses were based on within-utility variance in total THMs for the utility-wide average approach, and the distance between the subject's residence and sampling site for the closest-site approach. In general, the utility-wide average methods produced odds ratios ≥ the closest-site methods. Odds ratios obtained using the utility-wide average, but not the closest-site, approach also became progressively stronger in the weighted and subset analyses. A dose-response was seen between spontaneous abortion and an exposure metric incorporating both total THM concentration using the utility-wide average approach and personal ingestion, with spontaneous abortion rates ranging from 8.3 to 13.7 percent (unweighted), 7.9 to 16.6 percent (variance weighted), and 6.6 to 23.1 percent (low-variance subset). Utility-wide average total THM exposure estimates together with variance-based weights and subsets are relatively simple exposure assessment techniques, which may increase the epidemiologic usefulness of utility sampling records.

Wennborg *et al*. (2000) studied adverse pregnancy outcomes in biomedical laboratory workers exposed to solvents in Sweden from 1990 to 1994 in a questionnaire-based study (N = 1,052). There was a marginally significant increase in risk of spontaneous abortion of pregnancies in women who had worked with chloroform with an odds ratio adjusted for mother’s age and previous miscarriages of 2.3 and 95 percent CI of 0.9 to 5.9, based on 13 cases in 856 pregnancies with exposure to chloroform and 60 cases in 770 pregnancies without chloroform exposure. Exposure to chloroform presumably occurred primarily by inhalation in the laboratory workers, but was not quantified. In addition to chloroform, laboratory workers were exposed to other solvents, bacteria and viruses, and radioactive isotopes, none of which, however, showed any relationship to spontaneous abortion in this cohort. The odds ratio for large for gestational age infants in association with the mother's laboratory work was 1.9 with a 95 percent CI of 0.7 to 5.2. This study found no evidence for a relationship between maternal chloroform exposure and offspring birth weight or malformations.

Yang *et al*. (2000) found a significant relationship with an odds ratio of 1.34 and 95 percent CI of 1.15 to 1.56 between the use of chlorinated water, indicated by total THMs, and preterm delivery in Taiwan. The study compared 14 chlorinating municipalities (more than 90 percent of the population served chlorinated water), and 14 matched nonchlorinating municipalities (less than five percent of the population served chlorinated water). The chlorinating and nonchlorinating municipalities were similar in urbanization and sociodemographic characteristics. The study population comprised 18,025 women residing in the 28 municipalities who had a first parity singleton birth between January 1, 1994 and December 31, 1996, including 719 preterm delivery and 456 term delivery with low birth weight babies, and for which complete information on maternal age, education,

gestational age, birth weight, and sex of the baby was available. Yang *et al*. (2000) considered that there was no association between consumption of chlorinated drinking water and the risk of low birth weight.

A population-based cohort study (King *et al*., 2000) of 49,756 singleton deliveries in Nova Scotia, Canada, between 1988 and 1995 found significant increases in relative risk adjusted for maternal age and smoking for total stillbirths with a relative risk of 1.56 and 95 percent CI 1.04 to 2.34; and asphyxia-related stillbirths, primarily due to premature detachment of the placenta, with a relative risk of 3.15 and 95 percent CI 1.64 to 6.03, associated with exposure to high drinking water levels of chloroform (>100 ppb). Exposure to total THM or chloroform was assigned by relating the mother's residence at the time of delivery to the total THM or chloroform in the public water supply serving the residence. Exposure concentrations were estimated using samples collected from within the water distribution system during routine monitoring. Maternal age, parity, smoking during pregnancy, infant's sex, and neighborhood family income were examined as potential confounders. Relative risks were adjusted for smoking and maternal age. The reference group was exposed to low chloroform levels in their drinking water (<50 ppb). A potential limitation of this study was misclassification of exposure as a result of mobility within the study population (estimated by the authors to affect less than 10 percent of study subjects). This study did not examine early fetal death such as spontaneous abortion because the perinatal database employed in this investigation contained information only on fetuses that weighed 500 g or more.

In the same cohort, Dodds and King (2001) found no evidence for any connection between birth defects, including neural tube defects, cardiovascular anomalies, and cleft defects, and chloroform levels in drinking water, except for an increase in chromosomal abnormalities in the mid-dose group exposed to 75 to 99 ppb of chloroform with a relative risk of 1.9 and 95 percent CI 1.1 to 3.3, but not in the high dose group exposed to more than 100 ppb with a relative risk of 1.4 and 95 percent CI 0.8 to 2.8. The study used the Nova Scotia Atlee perinatal database which included infant diagnoses among stillborn and liveborn infants up to the time of discharge from the hospital after birth. Information from the database was supplemented with data on prenatally diagnosed congenital anomalies obtained from pregnancy terminations. Data from pregnancy terminations were included because, in Nova Scotia, approximately 80 percent of neural tube defects are detected before birth and the pregnancy is electively terminated. Exposure periods were selected on a defect-specific basis to target the interval before or during gestation when exposure to a potential developmental toxicant or mutagen might have the greatest effect on developmental or genotoxic endpoints. The intervals used for analysis of neural tube defects were one month before and one month after conception; for analysis of cardiac defects and cleft defects the first two months of pregnancy was used; and for analysis of chromosomal abnormalities three months before pregnancy was used. Estimates of relative risks and 95 percent CI were determined using Poisson regression models. Maternal age, parity, maternal smoking, and neighborhood family income were assessed as potential confounders. The concentration categories for chloroform were less than 5 ppb, 5 to 9 ppb, 10 to 19 ppb, and ≥ 20 ppb. Even in these studies, where chloroform was specifically measured, no conclusions can be reached due to the confounding presence of unmeasured contaminants in the drinking water and other limitations of the study such as small sample size in the high exposure group.

Using Swedish health registers linked to information on municipal drinking water composition, Cedergren *et al*. (2002) reported that drinking water disinfection byproducts in ground water have been associated with a small increased risk for congenital cardiac defects based on individual data on drinking water characteristics obtained from 58,669 women that gave birth. Among the infants born, 753 had a cardiac defect. The risk for cardiac defects increased with increasing THM concentrations ($p = 0.0005$).

Hwang *et al*. (2002) conducted a nationwide cross-sectional study of 285,631 Norwegian births in 1993-1998. Risks of birth defects versus four chlorination by-product exposure categories were compared on the basis of chlorination (yes/no) and water color (mg Pt/liter), representing the amount of natural organic matter: high (chlorination, ≥20), medium (chlorination, 10-19.9), and low (chlorination, <10) exposure, with no chlorination and low color (<10) as the reference category. The risks of any birth defect at medium and high categories combined was increased by logistic regression analysis (adjusted OR 1.13, 95 percent CI 1.01-1.25), and cardiac (OR 1.37, 95 percent CI 1.00-1.89) respiratory system (OR 1.89, 95 percent CI 1.00-3.58), and urinary tract (OR 1.46, 95 percent CI 1.00-2.13) defects were significantly associated with exposure. For specific birth defects, only ventricular septal defects were significantly elevated, with an exposure-response pattern, yielding adjusted ORs of 1.63 (95 percent CI 1.02-2.58) for the medium and 1.81 (95 percent CI 1.05-3.09) for the high exposure categories. Risk of neural tube defects was related to high color (adjusted OR 2.60, 95 percent CI 1.30-5.26).

Two case control maternal interview studies conducted for California births from 1987 through 1991 were used to investigate whether infants whose mothers were periconceptionally exposed to drinking water containing THMs were at greater risk of congenital malformations (Shaw *et al*., 1991, 2003). One study comprised 538 infants and fetuses with neural tube defects and 539 nonmalformed control infants, and the second study included an additional 265 infants with neural tube defects, 207 infants with conotruncal heart defects, 409 infants with orofacial clefts, and 481 control infants. Municipal water companies provided estimated THM levels for a particular residence and specific periconceptional time period using quarterly monitoring measurements. Risk of neural tube defects in the first study was inversely associated with total THM exposure, but the second study did not show the same inverse relationship for neural tube defects. There were no positive associations of neural tube defects or the other malformations with total THMs as estimated from continuous models. Elevated risks were observed for the lowest exposure category at 1 to 24 ppb, but risks were either not substantially elevated or were imprecise for higher exposure levels. Thus no evidence was observed for an exposure-response relation.

Wright *et al*. (2003) conducted a cross sectional study of 56,513 singleton infants born to Massachusetts residents during 1990, estimating the mother's average THM exposures during each trimester of pregnancy. They reported that increased average total THM exposures for the entire pregnancy and for the second trimester were associated with the factor "small for gestational age" and reductions in birth weight, after adjusting for potential confounding variables. Pregnancies with average total THM exposure over 80 ppb, compared to those with equal to and less than 60 ppb, were associated with a 32 g reduction in birth weight. There was a 23 g reduction in birth weight in infants born to mothers exposed to greater than 80 ppb total THM during the second trimester. For each

20 ppb increase in total THM, the estimated reduction in birth weight was 2.8 g for pregnancy average exposure and 2.6 g for second trimester exposure. An increased risk of small for gestational age births for pregnancy average was associated with total THM levels greater than 80 ppb with an odds ratio of 1.14 and 95 percent CI 1.02 to 1.26. An increased risk for second trimester exposure was associated with total THM levels greater than 80 ppb with an odds ratio of 1.13 and 95 percent CI 1.03 to 1.24.

Whitaker *et al*. (2003a,b) developed a modelling approach which provided robust quarterly estimates of ecological exposure to THMs in a situation where the raw data were too sparse to base exposure assessment on empirical summaries alone. Statistical models were constructed using sparse routinely collected THM measurements to obtain quarterly estimates of mean THM concentrations for each water zone. THM concentration estimates were typically highest in the third quarter from July to September, and varied considerably between water sources. The modelled THM estimates led to slightly stronger and more precise estimates of association with risk of still birth and low birth weight than did the raw annual means.

A population-based case control study in Nova Scotia and Eastern Ontario, Canada (Dodds *et al*., 2004), on 112 women with a stillborn infant versus 398 random women with live births as controls found evidence for an increased risk of stillbirth associated with exposure to chlorination byproducts through ingestion and showering and bathing, although there was not a clear dose-response relationship. Subjects were interviewed, and women with a public water source provided a residential water sample. Risks were related to residential THM level in tap water and to a total exposure metric incorporating tap water ingestion, showering, and bathing. Women with a residential total THM level of 80 ppb or more had twice the risk of a stillbirth compared with women with no exposure to THMs with an adjusted odds ratio of 2.2 and 95 percent CI 1.1 to 4.4. The highest quintile of total THM exposure using the total exposure metric was associated with an adjusted odds ratio of 2.4 and 95 percent CI 1.2 to 4.6 compared with women not exposed to THMs. However, a monotonic dose-response relationship was not seen.

Infante-Rivard (2004) reported findings suggesting that exposure to THMs above 29.4 µg/L can affect fetal growth in genetically susceptible newborns. This Montreal hospital-based case control study included 493 cases of intrauterine growth restriction defined as birth weight below the 10th percentile for gestational age and gender by Canadian standards, with 472 control babies delivered at the same hospital with birth weights ≥ the 10th percentile, matched for gestational age, race, and gender. Exposure to total and specific THMs was estimated using regular monitoring data. Residential history, water drinking, shower habits during pregnancy, and known risk factors for intrauterine growth restriction were determined with a face-to-face interview with all mothers. Mothers and newborns were characterized for polymorphisms in the CYP2E1 gene G1259C and the 5,10-methylenetetrahydrofolate reductase (MTHFR) gene C677T. Exposure to specific and total THMs from drinking water, determined for 458 cases and 426 controls, did not result in an increased risk of intrauterine growth restriction. Significant effect modification was observed between newborns with and without the CYP2E1 variant. Among newborns with the variant, the adjusted odds ratio for intrauterine growth restriction associated with exposure to average total THMs above the 90th percentile (29.4 µg/L) was 13.2 with a 95 percent CI 1.2 to 146.7.

A case control study with incident cases was performed by Aggazzotti *et al*. (2004) in nine towns near Modena, Italy, between October, 1999, and September, 2000, with 1,194 subjects including 343 preterm births between the 26th and 37th week, 239 small for gestational age at term of 37th completed week and weight less than the lowest 10th percentile, and 612 controls. Exposure was assessed both by a questionnaire on mothers' habits during pregnancy and by water sampling at mothers' homes. The median THM level was 1.10 ppb. Preterm birth showed no association with chlorination byproducts. A weak association was found between small for gestational age at term with THM levels ≥ 30 ppb, with adjusted odds ratios 1.38 and 95 percent CI 0.92 to 2.07.

Wright *et al*. (2004) used birth certificate data on 196,000 infants to examine the effect of third-trimester exposures to town-average concentrations of total THM, chloroform, and BDCM from 1995 to 1998 for 109 Massachusetts towns. Mean birth weight, mean gestational age, intrauterine growth retardation or small for gestational age infancy, and preterm delivery were evaluated. Reductions in mean birth weight of 12 to 18 g for maternal THM exposures greater than the 90th percentile compared with those less than the 50th percentile were observed. Birth weight reductions were detected for chloroform exposures greater than 20 ppb and total THM exposures greater than 40 ppb. Elevated THMs were associated with increases in gestational duration and a reduced risk of preterm delivery. Evidence of an exposure-response effect of THMs on risk of small for gestational age was found, with odds ratios ranging from 1.09 to 1.23 for BDCM exposures greater than 5 ppb.

Yang (2004) reported an association between the consumption of chlorinated drinking water and the risk of preterm delivery in a bigger study in Taiwan. The study areas included 113 ‘chlorinating municipalities’ (defined as more than 95 percent of the population served chlorinated water) and 15 ‘nonchlorinating municipalities’ (less than 5 percent of the population served chlorinated water). The study population comprised 182,796 women residing in the 128 municipalities who had a first parity singleton birth between January 1994 and December 1996 for which complete information was available on maternal age, education, gestational age, birth weight, and sex of the baby.

Savitz *et al.* (2005) evaluated the hypothesis that exposure to disinfection byproducts causes pregnancy loss. Pregnancy outcome was evaluated in 3132 women in three southeastern U.S. locations with varying levels of disinfection byproducts in drinking water. Tap water in the three communities was sampled frequently and analyzed for THMs, haloacetic acids (HAA9), and total organic halides (TOX). Chemical levels in the drinking water were linked to specific pregnancy periods for each participant, and their reports of drinking water consumption and other activities that may result in exposure, such as showering and bathing. Methodological improvements over previous studies are cited by the authors as consisting of better assessment of disinfection byproduct exposures, a wider range of exposures evaluated, and an improved quality of assessment of pregnancy outcome. In this study, women who drank 5 or more glasses per day of tap water with >75 μg/L of THMs had the same miscarriage risk (odds ratio of 1.0) as all other women. However, odds ratios of 1.6 and 1.7 for risk of pregnancy loss were found for the upper quartile of exposures to BDCM and DBCM, respectively, compared to the lower three exposure quartiles. This can be compared to odds ratios of 2.0 and 1.3 in the study of Waller *et al.* (1998) for the same comparison. Savitz *et al.* (2005) concluded

that results for THMs were generally not supportive of an association with pregnancy loss "with the possible exception of an increased risk for losses at >12 weeks' gestation." The results for BDCM, HAA9, and the other groups of DBPs provided some support for elevated risk that varied across pregnancy time window, exposure index, and agent. They concluded that BDCM results were marginally stronger than those for THMs, and the results for TOX had the most consistent association with pregnancy loss, both for tap water concentration and ingested amount.

Toledano *et al*. (2005) investigated the association between THMs and risk of stillbirth and low birth weight in three regions in England, using birth cohort data for 1992 to 1998. Estimates of quarterly THM concentrations in different water zones, categorized as low (< 30 μg/L), medium (30-59 μg/L), or high (≥ 60 μg/L), were linked to approximately 1 million routine birth and stillbirth records using maternal residence at time of birth. Estimates across the three regions using a random-effects model to allow for between-region heterogeneity in exposure effects showed small excess risks in areas with high THM concentrations for stillbirths (odds ratio = 1.11, 95 percent CI 1.00 to 1.23), low birth weight (OR = 1.09; 95 percent CI 0.93 to 1.27), and very low birth weight (OR = 1.05, 95 percent CI 0.82-1.34). Among the individual THMs, chloroform showed a pattern of risk similar to the total THMs, but no association was found with concentrations of BDCM or total brominated THMs.

A population-based case control study including 112 stillbirth cases and 398 live birth controls conducted in Nova Scotia and Eastern Ontario, Canada, found no association between haloacetic acid exposures and stillbirth risk after controlling for THM exposures (King *et al*., 2005). Estimates of daily exposure to total and specific THMs or haloacetic acids were based on household water samples and questionnaire information on water consumption at home and work.

Nieuwenhuijsen *et al.* (2008) reported an increased risk of various congenital anomalies associated with trihalomethane (THM) exposure in drinking water supplies in England and Wales. They obtained congenital anomaly data from the National Congenital Anomalies System, regional registries, and the national terminations registry, and THM data from the water companies. Total THMs (< 30, 30 to < 60, ≥ 60 μg/L), total brominated THMs (< 10, 10 to < 20, ≥ 20 μg/L), and bromoform (< 2, 2 to < 4, ≥ 4 μg/L) were assigned to the place of residence for the first trimester of pregnancy. Data were included on 22,828 cases of congenital anomalies out of 2,605,226 live births, stillbirths, and terminations. Fixed- and random-effects models were evaluated for groups of anomalies as well as for isolated and multiple anomalies, adjusted for sex, maternal age, and socioeconomic status. No statistically significant trends were observed across exposure categories for either the grouped or more restricted sets of anomalies. However, for isolated defects, there were small but significant ($p < 0.05$) excess risks for high-exposure total THMs for ventricular septal defects (OR 1.43, 95 percent CI 1.00-2.04) and for bromoform for major cardiovascular defects (OR 1.18, 95 percent CI 1.00-1.39) and gastroschisis (OR 1.38, 95 percent CI 1.00-1.92). The authors concluded that they had found little evidence of a relationship between THM concentrations in drinking water and risk of congenital anomalies.

Hwang *et al.* (2008) assessed the effect of water disinfection byproducts on the risk of a number of common birth defects in a population-based cross-sectional study of 396,049

births in 2001-2003, using information from the Taiwanese Birth Registry and Waterworks Registry. The risk of eleven of the most common defects was evaluated for four total trihalomethane (TTHMs) exposure categories, high (TTHMs 20+ µg/L), medium (10-19 µg/L), low (5-9 µg/L), and a reference category of 0-4 µg/L. Hwang *et al.* also conducted a meta-analysis of combined results from this and previous studies, focusing on the same birth defects. Risks at the high exposure category were evaluated compared to the reference by multivariate logistic regression analysis. Ventricular septal defects (OR 1.81, 95 percent CI 0.98-3.35), cleft palate (OR 1.56. 95 percent CI 1.00-2.41), and anencephalus (OR 1.96, 95 percent CI 0.94-4.07) were elevated in the high exposure compared to the reference category. In the meta-analysis, the summary odds ratio for ventricular septal defects (1.59, 95 percent CI 1.21-2.07) was consistently elevated. The study suggests that prenatal exposure to disinfection by-products increases the risk of ventricular septal defects, cleft palate, and anencephalus, with the evidence on ventricular septal defects most consistent in the three studies evaluated.

Chisolm *et al.* (2008) conducted a study of birth defects in western Australia based on observations that water supplies in that region contain relatively high THM levels, particularly the brominated forms. Rates of birth defects were compared across cities with various THM levels. Water samples from 47 areas were classified as low total TTHM (< 60 µg/L), medium (60 to < 130 µg/L), and high (≥ 130 µg/L). On average, 92 percent of the THMs were brominated. Birth registry-based data on total births and birth defects for 2000-2004 from postal codes corresponding to water sample collection sites were compared by binomial logistic regression. The frequency of defects was compared aggregately and separately for the TTHM exposure groups, adjusting for maternal age and socioeconomic status. Women living in high-TTHM areas showed an increased risk of any birth defect (OR 1.22, 95 percent CI 1.01-1.48) and for the category of any cardiovascular birth defect (OR 1.62, 95 percent CI 1.04-2.51), compared with women living in low-TTHM areas.

In related work, Villanueva *et al.* (2006b) studied THM exposure sources for pregnant women in Spain as part of a study on the association of different reproductive outcomes with exposure to total THMs. They found that pregnant women in this area primarily consumed bottled water (~90 percent), using tap water mostly for cooking. Thus, the main THM exposure routes appeared to be dermal absorption and inhalation resulting from other water uses in the home such as showering, plus use of outdooe swimming pools. A similar study of pregnant women in France in 2004 and 2005 (Villanueva *et al.*, 2007b) found that 90 percent of water ingestion was bottled. The multiroute exposure estimates found 64 percent of THM exposure from showering, 23 percent from swimming in pools, 12 percent from bathing, and only one percent from drinking water. The showering, swimming, and bathing estimates are the sum of both dermal and inhalation exposures.

One available study addresses the effects of THM on semen quality (Fenster *et al*., 2003). Total THM levels, assigned based on water utility measurements taken during the 90 days preceding semen collection, were not associated with decrements in semen quality based on continuous semen parameters, adjusting for potential confounders by using repeated measures analyses, analyzed from 157 healthy men of couples without known risk factors for infertility. Percent normal morphology decreased and percent head

defects increased at higher levels of an ingestion metric of multiplying total THM levels by cold home tap water consumption. At the highest ingestion level, at more than 160 ppb, equivalent to more than two glasses/day of water containing 80 ppb, a difference of -7.1 with 95 percent CI -12.7 to -1.6 was observed for percent morphologically normal sperm compared with the lowest level at less than and equal to 40 ppb.

One study addresses menstrual cycle changes and suggests that THM exposure may affect ovarian function (Windham *et al*., 2003). In a prospective study, 403 premenopausal women collected urine samples daily during an average of 5.6 cycles for measurement of steroid metabolites to define menstrual parameters such as cycle and phase length. Subjects were asked about consumption of various types of water as well as other habits and demographics. A THM level was estimated for each cycle based on residence and quarterly measurements made by water utilities during a 90-day period beginning 60 days before the cycle start date. A monotonic decrease in mean menstrual cycle length with increasing total THMs in drinking water was found. The adjusted decrement was 1.1 days (95 percent CI -1.8 to -0.40) at more than 60 ppb, compared to ≤ 40 ppb. This shortening occurred during the first half of the cycle before ovulation as reflected by a reduced follicular phase length with a difference of -0.94 day (95 percent CI -1.6 to -0.24). A decrement in cycle and follicular phase length of 0.18 days (95 percent CI -0.29 to -0.07) per 10 ppb increase in total THMs was found. No changes were noted in bleed length or in cycle regularity. Incorporating tap water consumption showed a similar pattern of reduced cycle length with increasing THM exposure.

Overview of studies

Hertz-Picciotto *et al*. (1992) and Neutra *et al*. (1992) discussed potential sources of bias and confounding factors in the environmental epidemiological studies. One of the limitations of these studies is that the small number of adverse outcomes reduces the precision of risk estimates. Although each of the studies has limitations and the results are not consistent enough to establish a causal relationship between THMs and adverse outcomes, the evidence appears adequate to establish some level of concern and need for further studies (Nuckols *et al*., 1995; Reif *et al*., 1996; Mills *et al*., 1998; Nieuwenhuijsen *et al*., 2000a; Graves *et al*., 2001; Reif and Bachand, 2001; Bove *et al*., 2002; Health Canada, 2004).

A major criticism of the studies is inadequate characterization of exposure that distorts exposure-response relationships and misestimates the risk (Nuckols *et al*., 1995; Reif *et al*., 1996; Mills *et al*., 1998; Swan and Waller, 1998; IPCS, 2000; Nieuwenhuijsen *et al*., 2000b; Graves *et al*., 2001; Lynberg *et al*., 2001; Reif and Bachand, 2001; Arbuckle *et al*., 2002; Bove *et al*., 2002; Hwang and Jaakkola, 2003; Health Canada, 2004; Hinckley *et al*., 2005; Wright and Bateson, 2005). Total or individual THMs may not be a good marker to represent disinfection byproducts and selection of the appropriate indicator(s) for exposure is problematic (Swan and Waller, 1998). In general, total THMs were monitored routinely, i.e., quarterly, as a marker for chlorination byproducts instead of specific compounds, but in some studies exposure was unquantified. The limited exposure characterization has been inadequate to definitively demonstrate an association of small magnitude (Graves *et al*., 2001). Although exposures may be estimated from maternal household location, errors are introduced in assigning residential exposure

because of temporal and spatial variability in concentrations of disinfection byproducts within the distribution system (Singer, 2001). There are also errors in estimating individual doses because of unmeasured variability in individual exposure parameters (Reif and Bachand, 2001). In addition, the exposure to THMs from sources other than ingestion was generally not characterized, the importance of which has been made glaringly obvious by the studies of Villanueva *et al.* (2006b, 2007b) showing that, in the two European populations which they studied, drinking water was not the major source of THM exposure.

Disinfected tap water contains more than five hundred disinfection byproducts depending on the geographic distribution and sources of the water, and seasonal and temperature variations have been observed (Singer *et al*., 1995). A significant amount of the chemicals that makes up the total organic halide and total organic carbon portions of the disinfection byproducts has not been identified. It is possible that one or more co-occurring compounds may be responsible for any observed trends. While some of these studies have identified an association of chloroform, bromoform, BDCM, or DBCM with adverse reproductive outcomes, establishing a causal relationship is more problematic, for these reasons. However, observing positive associations must raise concern for possible human developmental and reproductive effects of disinfection byproducts as a whole, or for any combination of the four THMs, that should be addressed in research designs (Simmons *et al*., 2002).

Misclassification of exposure was identified as the primary limitation of these studies by Nieuwenhuijsen *et al*. (2000a,b). Exposure concentrations for most subjects were based on test results for a single day, and thus do not reflect potential variation in THM levels over time. The complex chemical interrelationships between THMs and other parameters within a municipal water distribution system have not been well studied. Difficulties in obtaining accurate and reliable information on personal activity and water consumption patterns exist in every study. Imprecise exposure measures coupled with a lack of information about other possible sources of THM exposure, including showering, may have caused associations to be underestimated. However, since these exposures would only increase the overall levels in the exposed group, not including them would only result in a bias towards the null, i.e., an underestimate of the risk from exposure. In addition, most of the studies cannot resolve the issue of whether some or all of the relations between drinking water contaminants and adverse birth outcomes are causal or due to chance or bias, since no data on smoking, alcohol consumption, occupation, etc., are available. Limited data are available on the identity, occurrence, toxicity, pharmacokinetics and interactions of THMs and the many other byproducts of chemical disinfection. Differences in the susceptibility of populations, and different approaches in exposure assessment, may contribute to the heterogeneity of the results.

Keegan *et al*. (2001) reported that routinely collected data can be used to obtain exposure estimates for epidemiological studies at a small area level, based on five years of total THM readings routinely collected 11.1 times a year on average for compliance with statutory limits by one water company in northwest England from 1992 to 1996, providing 15,984 observations for statistical analysis in 288 water zones. Between 1992 and 1996 the total THM annual zone means were less than half the statutory

concentration, at approximately 46 µg/L. However, most of the exposure estimates have been far less rigorous than in this study.

Wright and Bateson (2005) observed a trade-off between bias and precision in the weighted exposure analyses, with the least biased effects estimates having the widest CIs in a sensitivity analysis of relative risk estimates using local area mean disinfection byproduct exposures with Monte Carlo simulations to generate data representing 100 towns, each with 100 births (N = 10,000). Effect attenuation due to intrasystem variability was most evident in absolute and relative terms for larger odds ratios. Each town was assigned a mean total THM exposure value with a mean ± standard deviation 45 ± 28 ppb based on a variable number of sampling locations ranging from two to 10. True maternal total THM exposure was randomly assigned from a lognormal distribution using that town's true mean value. The effect of a 20 ppb increase in total THM exposure on the risk of small-for-gestational age infancy using the true maternal exposure compared to various weighting measures of the town mean exposures was compared. The exposure metrics included: (1) unweighted town mean, (2) town mean weighted by the inverse variance of the town mean, (3) town mean weighted by the inverse standard deviation of the town mean, (4) town mean weighted by 1-(standard deviation of sites per town/mean across all towns), and (5) a randomly selected value from one of the sites within the town of residence. To estimate the magnitude of misclassification bias from using the town mean concentrations, the true exposure odds ratios (1.00, 1.20, 1.50, and 2.00) were compared to the mean exposure odds ratios from the five exposure scenarios. Misclassification bias from the use of unweighted town mean exposures ranged from 19 to 39 percent, increasing in proportion to the size of the true effect estimates. Weighted town mean total THM exposures were less biased than the unweighted estimates of maternal exposure, with bias from 0 to 23 percent. The weighted town mean analyses showed that attenuation of the true effect of disinfection byproduct exposure was diminished when town mean concentrations with large variability were downweighted.

Kramer *et al*. (1992), Bove *et al*. (1995), and Wright *et al*. (2003) found evidence of increased risk of intrauterine growth retardation or small for gestational age with THM exposures. However, Kramer *et al*. (1992) and Savitz *et al*. (1995) did not find an association with low birth weight. A series of California studies (Waller *et al*., 1998, 2001; Windham *et al*., 1992, 2003) found an early-term miscarriage rate of 15.7 percent for women who drank five or more glassess of cold water containing more than 75 µg/L total THMs, compared to a miscarriage rate of 9.5 percent for women with a lower exposure to total THMs. U.S. EPA (1994c, 1997b, 1998h) concluded that the epidemiological results were inconclusive with regard to the association between exposure to chlorinated water and adverse reproductive and developmental effects

Reif and Bachand (2001) reviewed eight studies that used a quantitative approach to exposure assessment (Shaw *et al*., 1991, 1992; Kramer *et al*., 1992; Bove *et al*., 1992, 1995; Klotz and Pyrch, 1999; Savitz *et al*., 1995; Gallagher *et al*., 1998; Waller *et al*., 1998; Dodds *et al*., 1999) and supplemented with studies that used a qualitative approach to exposure assessment (Aschengrau *et al*., 1989, 1993; Kanitz *et al*., 1996; Magnus *et al*., 1999) to conclude that the epidemiological evidence was insufficient to infer a causal relationship between exposure to disinfection byproducts and adverse reproductive

outcome. However, the authors could not rule out a modest association due to the limitations in exposure assessment.

Bove *et al*. (2002) conducted a qualitative review of 14 studies on THMs for their adverse birth effects in humans and some of their follow-ups (Aschengrau *et al*., 1989, 1993; Shaw *et al*., 1991; Kramer *et al*., 1992; Bove *et al*., 1995; Savitz *et al*., 1995; Kanitz *et al*., 1996; Gallagher *et al*., 1998; Waller *et al*., 1998; Dodds *et al*., 1999; Klotz and Pyrch, 1999; Magnus *et al*., 1999; Kallen and Robert, 2000; King *et al*., 2000; Yang *et al*., 2000; Dodds and King, 2001; Jaakkola *et al*., 2001). They concluded that the THM studies provide some evidence for associations with neural tube defects, "small for gestational age," and spontaneous abortions. However, the inability to accurately assess exposures to these contaminants that would distort exposure-response trends and tend to bias results toward the null hypothesis were major shortcomings.

Hwang and Jaakkola (2003) evaluated the epidemiological evidence from 1966 to 2001 for adverse effects of prenatal exposure to chlorination byproducts on birth defects. For five studies that provided estimates of the effect of exposure on one or several birth defects by Aschengrau *et al*. (1993), Bove *et al*. (1995), Dodds *et al*. (1999), Magnus *et al*. (1999), and Klotz and Pyrch (1998, 1999), the authors calculated summary odds ratios and 95 percent CIs using the Mantel-Haenszel method for a fixed effects model, and the DerSimonian-Laird method for a random effects model. The results of this meta-analysis provide evidence for an effect of exposure to chlorination byproducts on the risk of any birth defect, and of neural tube and urinary system defects. The results for respiratory system, major cardiac, and oral cleft defects were heterogeneous and inconclusive. Taken together, these studies do not establish a causal relation between THM exposure and adverse pregnancy outcomes.

The weight of evidence reviewed by Craun (1993), Reif *et al*. (1996), Mills *et al*. (1998), Nieuwenhuijsen *et al*. (2000a,b), Graves *et al*. (2001), Reif and Bachand (2001), Bove *et al*. (2002), and Hwang and Jaakkola (2003) suggested a positive association with exposure to disinfection byproducts for intrauterine growth retardation or small for gestational age, and for urinary tract anomalies. The analyses found inconsistent or very weak results for all congenital anomalies or birth defects, all central nervous system anomalies, neural tube defects, spontaneous abortion, and stillbirth. The weight of evidence is that no association with exposure to disinfection byproducts exists for preterm delivery, some specific congenital anomalies, and neonatal death.

POTENTIALLY SENSITIVE SUBPOPULATIONS

Children and the Fetus

A growing body of scientific evidence indicates that infants, children and the fetus may suffer disproportionately from some environmental health risks. Processes for uptake, distribution, and elimination can vary considerably with age and development. Greater risk of health effects might occur if the enzymes responsible for bioactivation of chloroform, bromoform, BDCM, or DBCM were more active in fetuses, neonates, and/or children than in adults, or if enzymes for inactivation were less active. This section evaluates available data for developmental expression and catalytic activity of the key

metabolizing enzymes CYP2E1 and CYP2B1/2 that are known or anticipated to bioactivate chloroform, bromoform, BDCM and DBCM (in rodents).

CYP2E1

Toxicity of the THMs has been shown to be at least partly related to bioactivation by the cytochrome P450 isoform CYP2E1 (U.S. EPA, 1994a; Van Vleet and Schnellmann, 2003). Studies of human fetal liver have produced contradictory results, but suggest that CYP2E1 protein is either not expressed or is expressed at levels lower than in adults (Hakkola *et al*., 1998). Carpenter *et al*. (1996) detected immunoreactive CYP2E1 protein in liver samples from fetuses 16 to 24 weeks in gestational age. The immunoreactive protein exhibited a slightly lower molecular weight than CYP2E1 in adult liver. CYP2E1 mRNA was not detectable by reverse transcriptase polymerase chain reaction (RT-PCR) in a fetal liver sample of 10 weeks gestational age, but expression was confirmed in a fetal liver sample of 19 weeks gestational age. The catalytic capability of CYP2E1 in human fetal microsomes varied from 12 to 27 percent of that in adult microsomes. Treatment of fetal hepatocytes in primary culture with ethanol or clofibrate increased catalytic activity approximately two-fold compared to untreated cells.

Vieira *et al*. (1996) detected small amounts of CYP2E1 mRNA in fetal liver samples (approximately five to 10 percent of the levels in adult liver) collected from fetuses aged 14 to 40 weeks, but did not detect immunoreactive CYP2E1 protein. Other studies have failed to detect either CYP2E1 protein or mRNA in fetal liver samples (Cresteil *et al*., 1985; Komori *et al*., 1989; Jones *et al*., 1992; Juchau and Yang, 1996). The factors contributing to these inconsistent results are unknown, but may include sensitivity of the tests used, interindividual variability, or the existence of factors other than developmental stage that control expression.

Information on CYP2E1 mRNA, protein, or catalytic activity in human fetal tissues other than the liver is limited. Vieira *et al*. (1998) detected CYP2E1 mRNA at very low levels in human fetal lung and kidney. Juchau *et al*. (1998) summarized studies which indicate that CYP2E1 is expressed in human embryonic brain tissue. Brzezinski *et al*. (1999) demonstrated that relatively low levels of CYP2E1 mRNA, immunoreactive protein, and catalytically active protein are present during early fetal development.

Viera *et al*. (1996) investigated age-related variations in human CYP2E1 protein levels and catalytic activity from birth through adulthood. These authors observed a rapid increase in the immunoreactive CYP2E1 microsomal content within 24 hours after birth that was independent of the gestational age of the newborn. The CYP2E1 protein level gradually increased during the first year and reached the adult level in children aged one to 10 years. CYP2E1 catalytic activity increased within 24 hours after birth and steadily increased during the first year. Adult levels of catalytic activity were reached at ages ranging from one to 10 years.

A preliminary Canadian study (Infante-Rivard *et al*., 2002) in 170 children between 0 and nine years old diagnosed between 1980 and 1983 in Quebec suggested that the risk of childhood acute lymphoblastic leukemia associated with THMs in drinking water may be modified by polymorphisms in the GSTT1 and CYP2E1 genes involved in THM metabolism. The risk was elevated among children homozygous for GSTT1 deletion null genotype. The interaction odds ratio (IOR) for a postnatal average of total THM above

the 95th percentile with GSTT1 null genotype was 9.1 (95 percent CI 1.4 to 57.8). Risks for those carrying the CYP2E1 variant G-1259C, known as the allele CYP2E1*5, were elevated for exposure during pregnancy and the postnatal period for an average exposure to total THM at or above the 75th percentile; the interaction odds ratio was 9.7 (95 percent CI 1.1 to 86.0). These results contrast with those from an earlier case-control analysis (Infante-Rivard *et al*., 2001), in which no increased risk of acute lymphoblastic leukemia was observed with THM exposure. Infante-Rivard *et al*. (2002) found no similar results in the literature, underscoring the need for other studies as well as showing the potential usefulness of combining exposure and genetic information in such studies.

Animal studies of CYP2E1 expression during development have also reported variable results. Carpenter *et al*. (1996) reported that CYP2E1 is expressed in fetal rat liver and placenta and that catalytic activity is increased by 1.5 to 2.4 fold in rat pups exposed to ethanol in utero or via lactation. Other authors have reported that hepatic CYP2E1 gene transcription in rats is activated at birth and that the amount of CYP2E1 reaches a peak prior to weaning (see Ronis *et al*., 1996). The protein level then falls to approximately 25 percent of the peak level and remains stable into adulthood (Ronis *et al*., 1996).

CYP2B1/2 (Rodents)

The closely related CYP isoforms 2B1 and 2B2 are also believed to participate in the metabolism of chloroform in rats, though generally only at higher doses (ILSI, 1997; U.S. EPA, 1997b,d, 1998c). The relevance of metabolism by CYP2B1/2 to human health is presently uncertain, since these isoforms have not been reported in human adult or fetal tissues (Nelson *et al*., 1996; Juchau *et al*., 1998).

Omiecinski *et al*. (1990) detected low levels of CYP2B isoform mRNA in fetal rat liver on gestation day 15. Although the mRNA expression levels were "substantially lower" at day 15 than later in development, expression was clearly inducible by pretreatment of pregnant rats with phenobarbital. Both constitutive and phenobarbital-induced levels of mRNA increased with developmental age, reaching maximal levels at approximately three weeks postpartum. CYP2B activity was not measured in this study, so it is not known whether changes in mRNA levels were paralleled by changes in catalytic activity.

Juchau *et al*. (1998) reviewed a series of experiments that tested for CYP2B1/2 catalytic activity in fetal rat tissues. The combined data indicated that if CYP2B isoforms are expressed in fetal rats, they likely occur at biologically insignificant levels. This conclusion is supported by the results of Asoh *et al*. (1999), who also found very low levels of CYP2B catalytic activity in fetal rat liver.

Gebremichael *et al*. (1995) investigated the postnatal developmental profile of CYP2B1 in SD rats. CYP2B1 activity was detectable as early as seven days postnatally and exhibited a variable pattern of expression (no clear trend evident) at Days 14, 21, 50, and 100. Asoh *et al*. (1999) examined induction of CYP2B isoforms in neonatal rats. CYP2B catalytic activity was markedly higher at five days after birth relative to levels observed in fetal hepatic tissue. Oral or intraperitoneal administration of phenobarbital to pregnant rats increased the level of CYP2B expression and activity in neonates. Overall, these findings suggest that CYP2B isoform activity is likely to be lower in fetuses than in

neonates or adults, but that increased levels of activity may be observed in fetuses and neonates exposed to inducing drugs or chemicals.

Other Sensitive Subpopulations

Factors in addition to age and developmental stage may also be associated with altered levels of CYP2E1 activity in humans. These factors potentially include genetic polymorphisms, health status, and prior or concurrent exposure to other inducers. Identification of these factors may define additional subpopulations of concern for exposure to the THMs.

Genetic Polymorphisms

Significant interethnic differences exist in CYP2E1 polymorphism (Ronis *et al*., 1996; Lieber, 1997) which might influence susceptibility to toxic effects of the THMs. The presently known CYP2E1 polymorphisms are located in the 5'-flanking (noncoding) regions of the gene, while the coding regions of the gene which specify the protein amino acid sequence appear to be well conserved among ethnic groups (Ronis *et al*., 1996). The c2 polymorphism is reported to be associated with higher transcriptional activity, protein levels, and catalytic activity of CYP2E1 than the more common wild-type allele (Lieber, 1997); the highest c2 allele frequency was found in Taiwanese (0.28) and Japanese (0.19 to 0.27) populations. The frequency of this polymorphism in African Americans, European Americans, and Scandinavians is lower, ranging from 0.01 to 0.05. Efforts to link the occurrence of the c2 allele to higher rates of CYP2E1-mediated liver disease have yielded inconsistent results. Thus, the functional significance of CYP2E1 polymorphism is presently uncertain, and it is not possible to estimate relative risks for different ethnic populations exposed to THMs.

Altered Physiological or Health States

The known physiological functions of CYP2E1 include lipid metabolism and ketone utilization (Lieber, 1997). Induction of this isoform is observed in many conditions that elevate circulating levels of lipids, including consumption of a high fat or low carbohydrate diet, starvation, obesity, and insulin-dependent diabetes. Among the groups likely to be affected by such conditions, diabetics constitute the most clearly defined susceptible population. Induction of CYP2E1 in uncontrolled insulin-dependent diabetes is well studied. In animals, this induction results in elevated levels of CYP2E1 in the liver, kidney, and lung (Ioannides *et al*., 1996). As a result of induction, diabetic animals are more susceptible to the toxicity of some chemicals metabolized by CYP2E1. This phenomenon has been documented for chloroform and other halogenated compounds (Ioannides *et al*., 1996). Because the animal and human orthologues of CYP2E1 show similar substrate specificity and bioactivation potential, it is conceivable that some diabetic humans may also be more susceptible to CYP2E1-mediated toxicity. Increased toxicity would be anticipated only in poorly controlled or uncontrolled diabetics (Ioannides *et al*., 1996), as CYP2E1 levels are reduced by insulin therapy.

Alcohol Consumption

The CYP2E1 isoform participates in the metabolism of ethanol in humans and animals. Chronic alcohol consumption is reported to result in up to 10-fold induction of this enzyme (Lieber, 1997). This observation suggests that concurrent exposure to ethanol or

THMs may increase susceptibility to adverse health effects. This potential interaction is of concern because concurrent exposure to THMs and ethanol is likely to occur in a significant number of people. Wang *et al.* (1994) reported that a single 100 mg/kg oral dose of ethanol administered to rats significantly increased the toxicity of chloroform. Hepatotoxicity of halogenated industrial solvents (e.g. carbon tetrachloride, bromobenzene, and vinylidene chloride) and anesthetics (enflurane and halothane) is increased in heavy drinkers (Lieber, 1997), with a pattern of damage that is consistent with selective expression and induction of CYP2E1 in certain regions of the liver.

Concurrent Exposures to Other Inducers

CYP2E1 is highly inducible by a wide range of drugs and chemicals. Prior or concurrent exposure to such inducers may contribute to increased toxicity from THMs. Known inducers include certain therapeutic agents (acetaminophen, isoniazid), volatile anesthetics (halothane, isoflurane), and solvents (acetone, benzene, carbon tetrachloride, trichloroethylene) (Raucy, 1995).

Synergy and Antagonism

Since the human population exposures to THMs are mostly to the mixture rather than to the individual chemicals, it is important to evaluate the potential synergy and antagonism effects among the individual THMs. Da Silva *et al*. (1999a) studied the blood kinetics of THMs and observed that the kinetics differed whether the oral administration was singly or combined. A single dose of 0.25 or 0.5 mmol/kg of each THM alone or a quaternary mixture of 0.25 mmol/kg of each THM was administered by gavage to adult male SD rats. The combined administration of THMs led to a significant increase in blood concentration attributed to pharmacokinetic interactions between two or more THMs present simultaneously. In a follow-up study with six binary THM mixtures (Da Silva *et al*., 1999b, 2000), a single dose of each THM at 0.5 mmol/kg alone or of a binary mixture containing 0.5 mmol/kg of each THM was administered by gavage to male SD rats. The authors observed that each binary mixture gave increased blood concentrations of the individual components compared to single administration and that the effect was time-dependent. The interaction varied among the mixtures, especially during the first hour following administration. Among the four THMs, bromoform and DBCM kinetics appeared to be more sensitive to the "mixture effect" and to exert the greatest impact on the second THM in the mixture. Da Silva *et al*. (2000) suggested that this is probably the consequence of a mutual metabolic inhibition between the THMs, based on simulation exercises conducted with physiologically based toxicokinetic models.

Pereira and Kramer (1999) studied interactions between chloroform and haloacetic acids. It was known that administration of chloroform by gavage induces liver cancer in mice, but when administered in drinking water it was not carcinogenic and prevented tumors initiated by diethylnitrosamine or ethylnitrosourea. In this study, male and female $B6C3F_1$ mice were initiated with 30 mg/kg of N-methylnitrosourea (MNU) on day 15 of age. From four weeks of age, the mice received 3.2 g/L dichloroacetic acid or 4.0 g/L trichloroacetic acid together with 0, 800, or 1,600 mg/L chloroform in the drinking water. All animals were sacrificed after a 36 week exposure and their livers and kidneys examined for tumors. The yield of dichloroacetic acid-promoted tumors was reduced by chloroform in both male and female mice. Liver tumors promoted by dichloroacetic acid

were reduced by 1,600 mg/L chloroform from 2.72 ± 0.41 to 0.76 ± 0.28 and from 0.60 ± 0.14 to 0.00 tumors per animal in males and females, respectively. The yield of trichloroacetic acid-promoted liver tumors was not affected in either sex. In contrast, chloroform greatly increased the yield of kidney tumors promoted by dichloroacetic acid in male mice, i.e., from 0.16 ± 0.07 to 1.38 ± 0.41 tumors per animal. Chloroform did not affect the yield of kidney tumors in trichloroacetic acid-promoted mice, which remained at about 0.7 tumors per animal. Kidney tumors were not observed in female mice. Thus, chloroform in water can on the one hand interfere with the formation of liver tumors promoted by dichloroacetic acid, and on the other hand stimulate dichloroacetic acid-promoted kidney tumors. Chloroform apparently has no effect on the carcinogenicity of trichloroacetic acid.

Skrzypinska-Gawrysiak *et al*. (1995) reported a diurnal rhythm in the hepatotoxicity of chloroform in mice. The animals were administered 300 or 600 mg/kg chloroform by oil gavage at 10 AM, 2 PM, and 6 PM, and hepatotoxicity was evaluated 18 or 24 hours later. Serum alanine aminotransferase and liver malonaldehyde (MDA) were higher after the evening administration compared to the morning administration. The decrements of glutathione levels in the liver followed a similar pattern.

Wang *et al*. (1994) observed that rats pretreated with a single dose of ethanol at 2 g/kg at 4 PM and challenged at 10 AM the following day with chloroform by oral, inhalation or i.p. routes, responded differently depending on the route of administration. The pretreatment increased the metabolism of chloroform about 1.5 fold, with no effect on liver microsomal protein, cytochrome P450, or glutathione contents. Compared at the same dose level, the area under the blood chloroform concentration × time curve (AUC) was higher following i.p. than oral administration, but ethanol had no significant effect on the blood concentration of chloroform at any dose by any route of administration. Chloroform produced route-dependent hepatotoxicity, and ethanol consumption potentiated this chloroform-induced hepatotoxicity, particularly by the oral route.

MECHANISM OF ACTION, CYTOTOXICITY, TISSUE REGENERATION

Animal studies have demonstrated that each of the THMs can produce tumors in liver or kidney. The main area of dispute involves mechanism of these tumors, and whether human risk should be estimated using linear or non-linear extrapolation. A summary of the mechanistic considerations is provided here for chloroform, the best-studied – and most contentious – of these chemicals.

Chloroform-related liver tumors included hepatocellular carcinomas in male and female $B6C3F_1$ mice treated by gavage in corn oil for 78 weeks (NCI, 1976); neoplastic nodules and hepatic adenofibrosis in female Wistar rats, and hepatic adenofibrosis in male Wistar rats, exposed to chloroform in drinking water (Tumasonis *et al*., 1985, 1987). Kidney tumors, tubular cell adenomas or carcinomas were found in male BDF1 mice exposed to chloroform in air (Nagano *et al*., 1998); male ICI mice treated with chloroform by gavage in toothpaste or arachis oil (Roe *et al*., 1979); male Osborne-Mendel rats treated by gavage in corn oil (NCI, 1976); and male Osborne-Mendel rats exposed to chloroform in drinking water (Jorgenson *et al*., 1982, 1985). These data provide sufficient evidence of

the carcinogenicity of chloroform in experimental animals (IARC, 1991b, 1999d; U.S. EPA, 2001e, 2005c).

It has been suggested that the tumor formation is secondary to cytotoxicity and tissue regeneration that have been observed at the high doses associated with the liver and kidney tumors (ILSI, 1997; U.S. EPA, 1998c). However, it is also possible that formation of tumors is not dependent on the cytotoxicity and tissue regeneration. It is not unusual to observe a multiplicity of effects when laboratory animals are exposed to high doses of toxicants. High doses of agents are typically employed in cancer bioassays because of the limited ability to detect statistically significant increases in tumors in small populations of animals. The highest dose in the bioassay aims at achieving the maximum tolerated dose, optimizing the ability of the study to detect weak carcinogens without producing toxicity that compromises the relevance of the bioassay. The rationale behind the use of high doses in cancer bioassays is discussed in more detail elsewhere (U. S. EPA, 1999a; McConnell, 1989).

Chloroform Cytotoxicity and Cellular Regeneration

A number of studies have demonstrated cytotoxicity in the liver and kidney as indicated by increased organ weights, histopathology, and increases in serum enzymes that reflect hepatotoxicity, following chloroform administered in air, in drinking water, or corn oil by gavage to rats or mice. In general, cytotoxicity in the liver and kidney appears to increase with dose. The effect of vehicle, administration route, and exposure duration on cytotoxicity is ambiguous. In many studies, the severity of toxicity appears to be transitory and diminishing with time. Histopathology and other effects observed initially are absent or much less evident with continual exposure, suggesting development of tolerance to chloroform with time (Nagano *et al.*, 1998; Yamamoto *et al.*, 1999).

While chloroform's toxicity and carcinogenicity have been attributed by some to use of a corn oil vehicle (and not observed when drinking water is employed), other factors may be involved (Bull *et al.*, 1986; Condie *et al.*, 1986; Simmons *et al.*, 1996; Raymond and Plaa, 1997; Science Advisory Board, 2000). In several studies, animals given chloroform in drinking water received a substantially lower dose than animals given the toxicant in a corn oil vehicle. Also, when a drinking water vehicle was employed, the animals tend to initially drink substantially less water (presumably due to taste aversion) and therefore initially receive a lower dose. With time, water consumption increases and the dose of chloroform they consume increases. The reduced toxicity, histopathology or cellular regeneration observed after several weeks in these animals may simply reflect tolerance to the toxic effects.

The relationship between cytotoxicity and tissue regeneration, indicated by an increase in DNA synthesis as measured by labeling index (the proportion of cells in S phase labeled) has been extensively studied following chloroform administration. While increases in labeling index tended to occur at high doses of chloroform, the increases often did not reflect the pattern of toxicity observed in the animals. Toxicity has been observed without an apparent increase in labeling index. Toxicity may increase with continual exposure while the labeling index diminishes. Increased labeling index has been observed without toxicity being evident. Finally, cytotoxicity and increases in the

labeling index have been observed without an observed tumorigenic response. Discussion of noteworthy studies on the possible links between cytotoxicity and labeling index and the occurrence of tumors in the liver and kidney of rats or mice administered chloroform follows.

Larson *et al.* (1993)

Hepatotoxicity, nephrotoxicity and effects on labeling index were investigated in male F344 rats (0, 34, 180, 477 mg/kg) and female $B6C3F_1$ mice (0, 34, 238, 477 mg/kg) following the administration of one dose of chloroform in corn oil by gavage. Changes in plasma enzymes were measured in three rats per dose group, while labeling index was measured in five rats or mice per dose group. In the male rat, hepatotoxicity (as indicated by histopathology and increased plasma enzyme activity) was most prominent at 477 mg/kg but appeared to occur at lower doses. An increase in the labeling index of the rat liver was only observed at the highest dose (477 mg/kg but not 34 or 180 mg/kg). Nephrotoxicity was observed at doses of 34 mg/kg or greater. Increased labeling index in the rat kidney was observed at 180 mg/kg and 477 mg/kg (no information was provided on changes at 34 mg/kg). While toxicity in the kidney became more severe at 477 mg/kg, the increase in labeling index was diminished (when compared to the increase in labeling index in animals administered 180 mg/kg-day).

These findings (increase in toxicity and labeling index) are not inconsistent with the occurrence of tumors in the rat kidney and not in the rat liver in the NCI (1976) study, because the high dose of 477 mg/kg-day is well above the doses tested by NCI in the rat. However, this study was limited to effects following a single dose. Hepatotoxicity, nephrotoxicity, and perhaps increases in labeling index in the rat, could very well have occurred if animals were chronically exposed to doses of chloroform like those in the NCI (1976) study.

Hepatotoxicity was reported in female mice given a single dose of 238 mg/kg and was clearly evident in mice that received 350 mg/kg-day or 477 mg/kg-day. The authors reported that the increase in labeling index was most marked in animals receiving 350 mg/kg, but did not make clear whether presumed increases in other dose groups were significant. No renal lesions were detected in the mice, but significant increases in labeling index were observed, albeit much smaller increases than observed in the liver. No information was provided concerning the doses at which labeling index increased in the kidney. The effects in the liver were at doses comparable to those that resulted in tumors in female mice in the NCI (1976) study. However, similar to the rat study, chloroform was administered only once to the animal. Hepatotoxicity (and perhaps nephrotoxicity) and increases in labeling index in the mouse may occur at lower doses in animals chronically exposed to chloroform, such as those in the NCI (1976) study.

Larson *et al.* (1993) suggested that their findings support the hypothesis that tumors occurred in the kidney of male rats and the liver of female mice in the NCI (1976) study because of toxicity and regeneration of the injured tissues that resulted from the high doses. They proposed that the tumors are linked to chloroform's cytotoxic effects and the subsequent tissue regeneration. Therefore, tumors should be observed only at dose levels at which these effects begin to occur. However, the study findings are only partly consistent with the hypothesis. Hepatotoxicity in the rat appeared to have occurred at a

dose(s) below the high dose, although increased labeling index was only observed at the high dose. The high dose is much higher than the doses used in the NCI study, so the lack of rat liver tumors in the NCI (1976) study is consistent with the increase in labeling index only at the high dose level. However, the increase in labeling index only in the high dose group is inconsistent with appearance of toxicity in this tissue at lower doses.

The animals were treated only once, whereas multiple daily doses of chloroform have yielded toxicity and regeneration at much lower dose levels. If chronic exposure yielded hepatotoxicity and increased labeling index at doses at which tumors were not observed in the NCI (1976) study, this finding could suggest that tumors are not directly related to hepatotoxicity and labeling index.

Effects observed in the mouse are also difficult to interpret. It is unclear when increases in labeling index occurred in the mouse liver. Also no lesions of the kidney were observed while increased labeling index was reported. This suggests that labeling index is not related to tumors, given that significant increases in kidney tumors were not observed in female mice in the NCI (1976) or Matsushima (1994) studies. Neither nephrotoxicity nor increased labeling index should be observed according to the hypothesis, because tumors did not occur in mouse kidney at the dose levels used in the NCI (1976) study.

Larson *et al.* (1994b)

Chloroform was given to male $B6C3F_1$ mice at 0, 34, 90, 138 or 277 mg/kg-day, three to five mice/dose group, in corn oil by gavage for four or five days/week for three weeks. Histopathology and labeling index were determined in the liver and kidney. Substantial pathology was observed in the liver of mice in all dose groups after four days of exposure but by three weeks the liver appeared normal in the 34 mg/kg-day and three of five mice in the 90 mg/kg-day groups. Histopathology was evident in the liver in the two highest dose groups at three weeks. The labeling index in the liver was significantly increased in all dose groups after four days. Labeling index at three weeks was increased only in the two highest dose groups, with labeling index being comparable to the level observed at four days in the highest dose group. In this study the changes in labeling index in the liver appear to be consistent with histopathology in the liver.

Microscopic and macroscopic renal lesions were evident in all dose groups after four days of exposure. Lesions in the kidney were less evident after three weeks. Labeling index was elevated in all dose groups after four days but increases in labeling index were only observed in the renal cortex of animals administered 138 and 277 mg/kg-day after three weeks. Labeling index at three weeks was below that observed at four days in the two highest dose groups.

Larson *et al.* (1994c)

Chloroform was administered in corn oil (0, 3, 10, 34, 90, 238 or 477 mg/kg-day) or drinking water (0, 60, 200, 400, 900, 1,800 ppm) to separate groups of female mice for four days or three weeks (five mice/group). Hepatotoxicity and nephrotoxicity as well as changes in labeling index in these tissues were investigated. The study has been cited as providing important evidence for the hypothesis that links chloroform-induced hepatic and renal tumors to cytotoxicity and tissue regeneration (Bruckner and Warren, 2001).

The findings of this study are offered as an explanation for why tumors in the liver occurred when chloroform was administered in corn oil (NCI, 1976) and not when chloroform was administered in drinking water (Jorgenson *et al*., 1985).

Liver – Toxicity

Chloroform administered in corn oil to female mice yielded evidence of hepatotoxicity at four days and three weeks post-administration. Liver weights and serum alanine aminotransferase and sorbitol dehydrogenase activity were significantly increased at the two highest doses four days post-administration. Three weeks post-administration, liver weights were increased at the three highest doses and serum enzyme activities were markedly increased at the four highest doses. Histopathology appeared to increase with doses of ≥ 34 mg/kg-day and became more severe after three weeks.

Administration of chloroform in drinking water initially resulted in a substantial decrease in water consumption at the higher concentrations, which was most likely due to taste aversion. Drinking water consumption increased with time. For water concentrations of 400, 900 and 1,800 ppm, the authors reported average daily doses of 53.5, 80.9 and 105 mg/kg-day (four days of exposure) and 82.5, 184 and 329 mg/kg-day (three weeks of exposure), respectively. After three weeks, significant increases in liver weight were observed at the three highest exposure levels. This finding is consistent with the increase in dose once water intake increased. No increases in serum enzymes were observed at four days or three weeks. Mild histopathology effects were observed in the three highest dose groups at four days but not at three weeks. Vehicle-dependent differences in histopathology at three weeks of exposure may reflect the initial reduced dose the mice received when chloroform was administered in their drinking water. The administration of a smaller dose of chloroform has been observed to foster tolerance to toxic effects of subsequent larger doses (Nagano *et al.*, 1998; Yamamoto *et al.*, 1999).

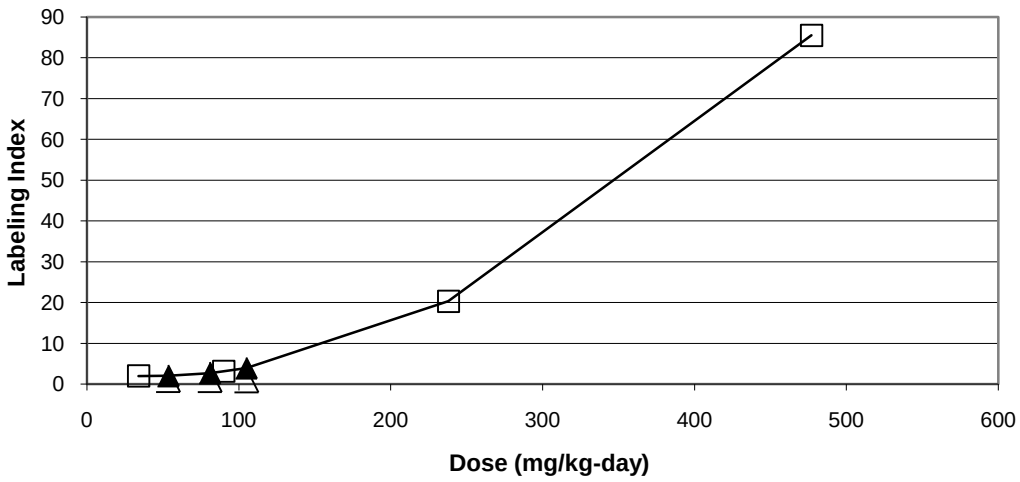
Liver – Labeling Index

Chloroform administered at doses of 238 and 477 mg/kg-day to female mice in corn oil for four days or three weeks resulted in markedly increased labeling index in the liver. Interestingly, labeling index was not increased at lower doses (34 and 90 mg/kg-day) in animals that displayed hepatotoxicity at three weeks. Unlike toxicity, the increase in labeling index was considerably greater at four days.

Increases in labeling index were not observed in mice receiving chloroform in drinking water. Doses at four days were relatively low because drinking water intake was reduced (probably due to taste aversion). Contrary to the conclusion reported by Bruckner and Warren (2001), the doses at which increased labeling index was observed in corn oil were considerably greater, and not “comparable,” to the high doses of chloroform administered in drinking water, particularly during the first four days (see Figure 3). Thus, at the lower doses received by the female mice in drinking water at four days, the lack of response (increase in labeling index) is consistent with the lack of response in animals receiving comparable doses in corn oil. At four days, there is no evidence that the vehicle influenced labeling index.

The dose response relationship for labeling index was determined for mice receiving chloroform in corn oil for four days. Various relationships were fitted to the four highest doses and the resulting labeling index using SPSS software. A cubic relationship [labeling index = 2.8375 - 0.042475(dose) + 0.000521(dose)^2 - 0.0000001439(dose)^3] yielded the best fit (Figure 3). Using the dose response relationship observed in mice receiving chloroform in corn oil for four days, the labeling indexes associated with the doses of chloroform administered in drinking water (provided by the study authors) were predicted. Various doses of chloroform received by the mice in drinking water and the predicted labeling indexes (based on if the dose had been administered in a corn oil vehicle), and observed labeling index responses are shown in Figure 3. The labeling indexes observed in the liver of mice receiving chloroform in drinking water were consistent with predicted labeling index in mice receiving an equivalent dose in corn oil.

Figure 3. Labeling Index in the Liver after Four Days of Exposure

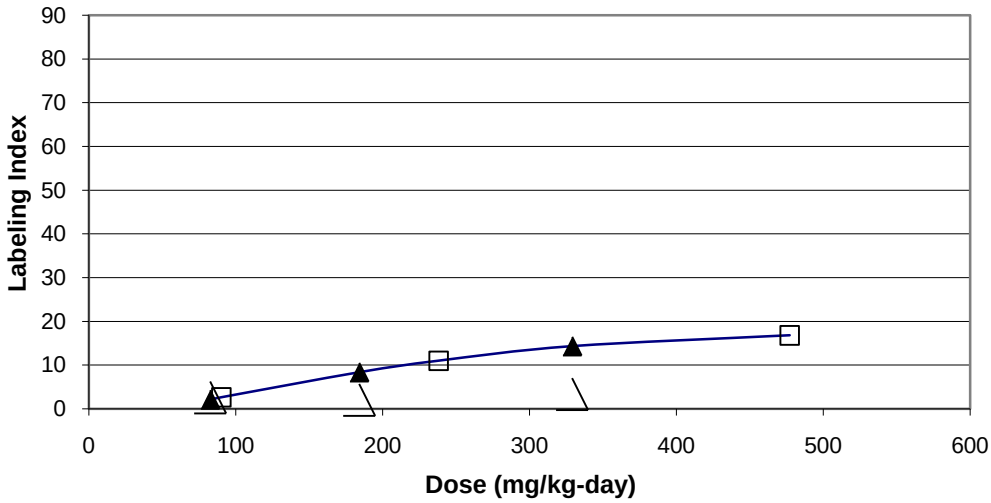


Legend:
Open square: observed labeling index in mice administered chloroform in corn oil
Black line: dose response curve based on doses administered in corn oil
Black triangle: predicted labeling index if doses were administered in corn oil
Open triangle: observed labeling index when doses were administered in drinking water

After three weeks, chloroform administered to female mice in corn oil by gavage yielded a considerably lower labeling index response compared to that observed at day four (Figure 4). Because daily water consumption had increased substantially in the mice over the three weeks, the average daily dose they received in their drinking water was much larger than that during the first four days of exposure. For drinking water concentrations of 400, 900 and 1,800 ppm, the authors calculated average daily doses of 53.5, 80.9 and 105 mg/kg-day (four days of exposure) and 82.5, 184 and 329 mg/kg-day (three weeks of exposure), respectively.

Various relationships were fitted to the three highest doses and the resulting labeling index in mice exposed to chloroform in corn oil for three weeks using SPSS software. A quadratic relationship [labeling index = $-4.20 + 0.083668(\text{dose}) - 0.000083(\text{dose})^2$] yielded the best fit (Figure 4). Using the dose response relationship observed in mice receiving chloroform in corn oil for three weeks, the labeling indexes associated with the doses of chloroform administered in the drinking water were predicted (assuming the dose had been administered in corn oil). Various doses of chloroform received by the mice in their drinking water and the predicted and observed labeling index responses are shown in Figure 4.

Figure 4. Labeling Index in the Liver after Three Weeks of Exposure



Legend:
Open square: observed labeling index in mice administered chloroform in corn oil
Black line: dose response curve based on doses administered in corn oil
Black triangle: predicted labeling index if drinking water doses were administered in corn oil
Open triangle: observed labeling index when doses were administered in drinking water

A small increase in the labeling index was predicted to occur but was not observed in the liver of mice receiving the highest dose of chloroform in drinking water (and perhaps the medium dose). The predicted labeling index assumes equivalent bioavailability at comparable doses of chloroform administered in corn oil or drinking water. The lack of observed response could perhaps have been due to the loss of a portion of the dose as the mice drank the water. Additional studies that employ the same method of exposure (e.g., water and corn oil vehicles both administered by gavage) or that measure blood chloroform levels would be useful in resolving this question.

At three weeks, the labeling indexes in mice receiving chloroform in corn oil were substantially reduced from levels observed at four days. Therefore, the difference between the predicted and observed labeling index at three weeks was not marked. It is unclear if there is a real difference in response due to the vehicle at three weeks.

The administration of chloroform in corn oil resulted in cytotoxicity in the liver as indicated by increased liver weights, histopathology, and increased serum enzyme levels. Hepatotoxicity appeared to be dose-dependent and was more severe at three weeks post-administration. The increase in labeling index did not correlate with hepatotoxicity. Labeling index was only increased at the two highest doses, although toxicity occurred at lower doses. The increase in labeling index was most notable at four days post-administration and had markedly decreased by three weeks. The reduced labeling index at three weeks could have been due to severe damage to the liver, preventing the proliferation of hepatocytes. However, mice receiving lower doses of chloroform in corn oil should have displayed increased labeling index at three weeks, given the evidence of cytotoxicity in these animals. Increased labeling index was not observed at lower doses as hepatotoxicity developed, so this explanation appears to be unsatisfactory.

Kidney

No increase in kidney weights, macroscopic, or microscopic lesions were observed in mice treated with chloroform in corn oil. Increased labeling index was observed in the kidney (cortex and medulla) at the high dose of chloroform in corn oil four days post-administration but no increase was evident by three weeks. In mice given chloroform in their drinking water, no toxic effects on the kidney were observed. However, chloroform given in drinking water did marginally increase labeling index in the renal medulla after four days (900 and 1,800 ppm) and three weeks (200, 400, 900 and 1,800 ppm). Increases in labeling index in the kidney were observed in mice receiving the toxicant in corn oil or drinking water without evidence of toxicity. While toxicity and increased labeling index were observed following chloroform administration, this study did not provide much evidence of a direct link between these two effects. Increases in labeling index were observed in the liver at high doses when the compound was given in corn oil. However, increases in labeling index were absent at lower doses when toxicity was observed after three weeks of exposure. Labeling index in the liver was substantially reduced at three weeks when the toxicity was most severe. In the kidney, increased labeling index was observed without evidence of toxicity.

The administration of chloroform resulted in increased tumors in female mouse liver but not in the kidney. In this study, increases in labeling index were observed in the female mouse kidney. Thus there does not appear to be a direct link between changes in labeling index and tumors in the female mouse kidney.

Larson *et al.* (1995a)

Liver

Chloroform was administered to male F344 rats in their drinking water (0, 60, 200, 400 900, 1,800 ppm) or in corn oil (0, 3, 10, 34, 90 or 180 mg/kg-day) by gavage (five rats/dose group). Changes in labeling index and evidence of toxicity were evaluated in the liver and kidney four days and three weeks post-administration. After four days of administration of chloroform in corn oil, increases in liver weight (except at the lowest dose), increases in serum alanine aminotransferase and sorbitol dehydrogenase activity, and mild histopathology were observed at the two highest doses. No microscopic alterations were observed at 10 mg/kg-day or less. After three weeks minimal hepatotoxicity was evident, only in the high dose group. When chloroform was

administered in drinking water, mild histopathology was observed after four days at 900 and 1,800 ppm (average daily doses of 68.1 and 57.5 mg/kg-day, respectively). Changes were only observed at 1,800 ppm after three weeks. No changes in serum enzyme activities were evident at four days or three weeks.

At four days, the labeling index in the liver was significantly increased when chloroform was administered in corn oil at doses of 90 and 180 mg/kg-day. Mild hepatotoxicity at lower doses was not accompanied by increased labeling index in the liver. No increase in labeling index was observed when chloroform was given in drinking water, but a reduced water consumption at four days resulted in an average daily dose in drinking water of 68.1 or 57.5 mg/kg-day (for 900 and 1,800 ppm, respectively).

Analogous to the results in the mouse study (Larson *et al.*, 1994c), the highest doses of chloroform administered in drinking water would not have resulted in a significant increase in labeling index in the liver if administered in corn oil (data not shown). After three weeks, based on drinking water consumption, the doses of chloroform associated with 900 and 1,800 ppm were 62.3 and 106 mg/kg-day, respectively. At three weeks, an increased labeling index was observed at the high dose with chloroform in corn oil but no increase was observed with chloroform in drinking water. No increase would be expected at three weeks for chloroform in water (all other things being equal), given that the highest dose of chloroform administered in water was only slightly larger than a dose administered in corn oil that did not yield a significant increase in labeling index in the liver. Therefore, as in the mouse, there is no evidence in the rat that administration of chloroform in corn oil results in a greater increase in labeling index in the liver compared to a drinking water vehicle.

Kidney

Dose-dependent degenerative changes were observed in the kidneys of male rats receiving chloroform doses of 34 mg/kg-day or greater in corn oil. The changes were greatest at four days. Administration of chloroform in corn oil resulted in an increase in labeling index in the kidney only at the highest dose at four days, but not at three weeks. Thus cytotoxicity did not necessarily trigger a regenerative response in the kidney.

Little histopathology was observed when the compound was given in drinking water but "more foci of regenerating tubules in the cortex" were reported in rats treated with 200 ppm or greater after three weeks. No increase in labeling index was detected at four days or three weeks when the compound was given in drinking water.

Larson *et al.* (1995b)

Female F344 rats were gavaged with 0, 34, 100, 200 or 400 mg/kg-day of chloroform in corn oil, at five rats/dose group). Four days or three weeks post-administration, the rats were sacrificed and the liver and kidney were examined for histopathology and changes in labeling index. Four days post-administration, slight liver histopathological changes were observed in rats exposed to 100 or 200 mg/kg-day with mild effects in rats receiving 400 mg/kg-day. At three weeks the high-dose group exhibited slight to mild changes and slight effects were observed in livers of rats receiving 200 mg/kg-day. The labeling index in the liver was significantly increased in rats exposed to 100 mg/kg-day or greater at four days and three weeks. Substantial histopathology was observed in the kidneys

after 200 or 400 mg/kg-day for four days. No cytotoxic effects were observed in the kidney at 100 mg/kg-day or less. After three weeks, the severity of the kidney lesions was less pronounced in rats given 200 or 400 mg/kg-day. Labeling index in the kidney was significantly increased at four days or three weeks at 100 mg/kg-day or more. Unlike the toxicity, labeling index appeared to be greater after three weeks in the 100 and 200 but not the 400 mg/kg-day dose groups.

Larson *et al.* (1996)

Male and female B6C3F$_1$ mice were exposed to chloroform at 0, 0.3, 2, 10, 30, or 90 ppm in air for six hours/day. Three to 15 female mice/dose group were exposed for four days, three, six or 13 weeks, seven days/week. Groups of male mice were exposed for three or seven weeks, seven days/week. Separate groups were exposed for only five days/week for 13 weeks. Based on standard body weights of 0.0316 kg and 0.0246 kg for male and female B6C3F$_1$ mice respectively, and inhalation rates of 0.053 and 0.04 m^3/day for male and female mice, respectively (U.S. EPA, 1988), we estimated the doses based on an assumed pulmonary absorption of 50 percent of the inhaled chloroform (Table 48).

Table 48. Absorbed Dose Associated with Exposure of Mice to Chloroform in Air

Concentration (ppm)	Males (mg/kg-day)	Females (mg/kg-day)
0	-	-
0.3	0.3	0.3
2	2	2
10	10.5	10
30	31	30
90	94	90

In female mice, liver weights were significantly increased only at 90 ppm chloroform (absorbed dose of 90 mg/kg-day) for three to 13 weeks. Liver histopathology was observed at lower doses and appeared to be dose-dependent. Interestingly, histopathology appeared to be most severe in female mice exposed for four days. Hepatic lesions were observed at absorbed doses as low as 10 mg/kg-day at four days but at the low doses little histopathology was apparent after three weeks of exposure. Only at the highest dose were moderate lesions evident at 13 weeks. Increased liver labeling index was observed at absorbed doses of 30 and 90 mg/kg-day but not at lower doses. At an absorbed dose of 30 mg/kg-day, the increase was greatest at three weeks and then declined and was not elevated after 13 weeks of exposure. At 90 mg/kg-day, the labeling index increased after four days of exposure and remained elevated through 13 weeks. The pattern of labeling index was not consistent with the cytotoxicity in the liver. While hepatotoxicity was observed in mice at 10 mg/kg-day, an increased labeling index was not observed until the 30 mg/kg-day dose. At 30 mg/kg-day, hepatotoxicity was more severe at four days and then declined, while labeling index was much higher at three weeks and then declined. At 90 mg/kg-day, both hepatotoxicity and labeling index were

elevated from four days to 13 weeks of exposure. No pathology was detected in the kidney nor was there an increase in labeling index in the kidney at any of the doses.

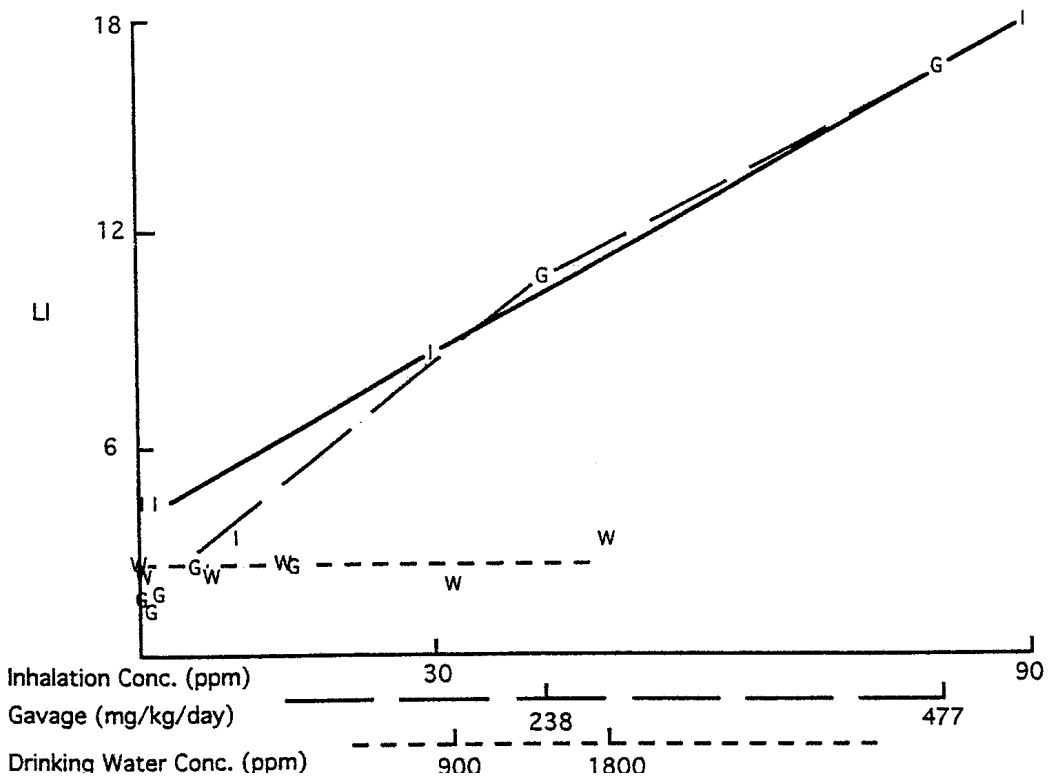
In male mice, liver weights were significantly increased only in mice receiving an absorbed dose of 31 and 94 mg/kg-day of chloroform for 13 weeks, but not three weeks. Liver histopathology was also evident in males at a dose of 31 and 94 mg/kg-day and was about the same or greater after 13 weeks compared to three weeks of exposure. Increased labeling index in the liver was only evident at 94 mg/kg-day; increases in labeling index peaked at three weeks (no data for four days) and were lower at six and 13 weeks.

As with females, the change in labeling index did not appear to reflect hepatotoxicity. While hepatotoxicity was observed in males receiving doses of 31 and 94 mg/kg-day of chloroform for three or 13 weeks, increases in labeling index were only associated with the highest dose. Also while labeling index was much higher after three weeks at 94 mg/kg-day, hepatotoxicity was equal or more severe after 13 weeks of exposure.

Exposure of male mice to chloroform by inhalation resulted in minimal to mild kidney pathology at an absorbed dose of 31 and 94 mg/kg-day after three and 13 weeks of exposure. Labeling index in the kidney was also elevated in both dose groups, and unlike the histopathology, the labeling index was much higher after three weeks of exposure. Interestingly, in animals only exposed for five days/week, the labeling index was elevated at the dose of 10.5 mg/kg-day (no data for the 31 mg/kg-day dose group).

Larson *et al*. (1996) evaluated the influence of route of administration on hepatic labeling index after three weeks of exposure to chloroform (Figure 5). Labeling indicies are shown on the Y-axis and doses, expressed as concentration in water, air, or mg/kg-day in corn oil) are displayed on the X-axis. It is very difficult to make a meaningful comparison of the different metrics plotted on the X-axis.

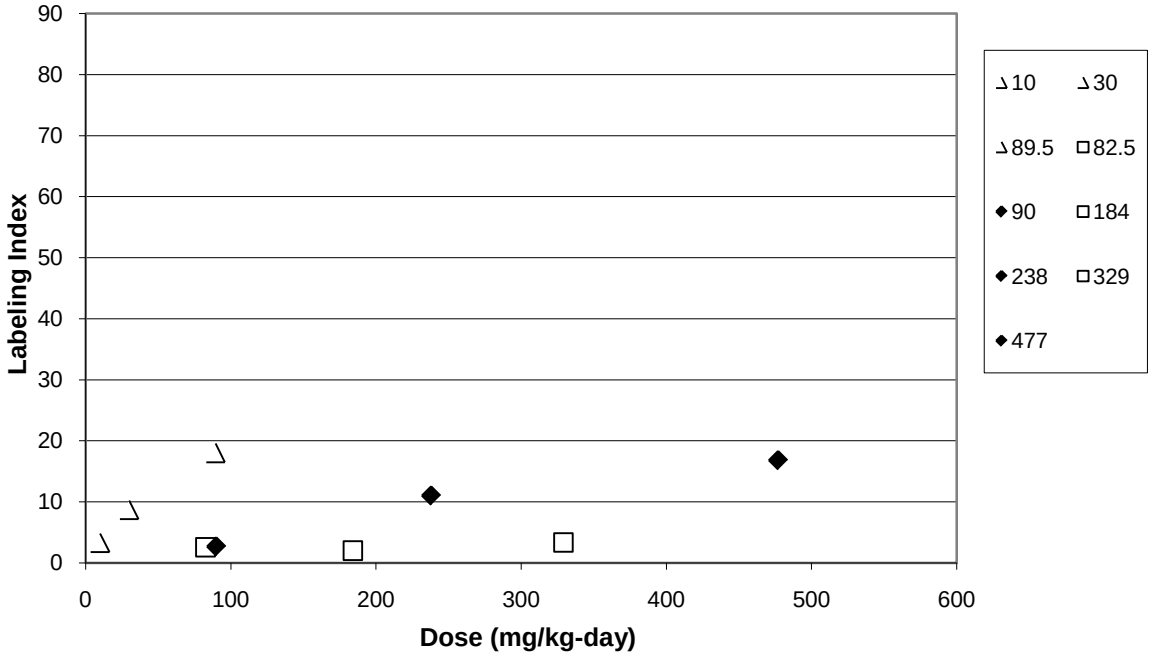
Figure 5. Labeling Index in Female Mice Exposed to Chloroform for Three Weeks



From Larson *et al*., 1996.
I- Inhalation route; W-Drinking water and G-Gavage in corn oil.

Changes in labeling index based on the same metric such as administered dose would appear to yield a much more meaningful comparison. We plotted labeling index in female mice exposed to chloroform by inhalation (Larson *et al*., 1996), in drinking water, and by gavage in corn oil (Larson *et al*., 1994b,c) for three weeks versus dose, in Figure 6. The labeling indexes in female mice after three weeks (and not four days) were selected because male mice were not included in Larson *et al* (1994b,c), while labeling index measurements in female mice were not performed after four days of exposure in Larson *et al*. (1996). While these data are very limited, the dose response relationship based on findings in the three studies suggests that the labeling index is highest in animals exposed to chloroform in air for six hours. As discussed earlier, it is less clear if there is a significant difference in the labeling index when the compound is administered in corn oil or in drinking water. Perhaps the prolonged and more constant delivery of chloroform by inhalation over six hours accounts for the more pronounced increase in labeling index in these animals.

Figure 6. Labeling Index in Female Mice Exposed to Chloroform for Three Weeks



Legend:
Black diamond: observed labeling index in mice administered chloroform in corn oil
Open triangle: observed labeling index in mice exposed to chloroform in the air
Open square: observed labeling index in mice administered chloroform in drinking water

Templin *et al.* (1996b)

Eleven week old male or female BDF_1 mice were exposed to chloroform at 0, 0.3, 5, 30, or 90 ppm in air for six hours/day for four consecutive days. This strain was selected because it was employed in the two year cancer bioassay in which chloroform was administered by inhalation. Eighteen hours after the exposure, the animals were sacrificed and the liver and kidney were examined for histopathology. Labeling index was determined in the kidney and liver. Separate groups of male mice were exposed to chloroform for five days/week for two weeks.

Based on standard mouse body weights, 0.0223 kg for male and 0.0204 kg for female, inhalation rates of 0.037 and 0.033 m^3/day for male and female BAF_1 mice, respectively (U.S. EPA, 1988), and an assumed 50 percent absorption, we estimated the doses associated with inhalation exposures (Table 49).

Table 49. Absorbed Dose Associated with Exposure of BAF_1 Mice to Chloroform by Inhalation (Templin *et al*., 1996b)

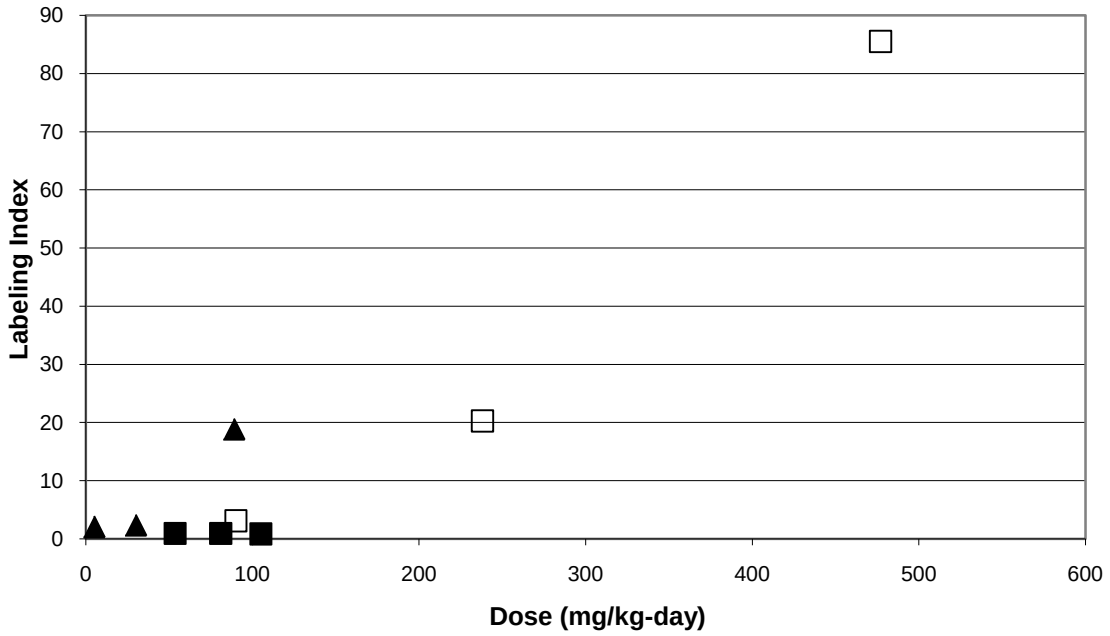
Concentration (ppm)	Males (mg/kg-day)	Females (mg/kg-day)
0	-	-
0.3	0.3	0.3
5	5	5
30	31	30
90	93	89

No significant liver lesions were observed in either male or female animals exposed to chloroform at or below 31 or 30 mg/kg-day. Substantial histopathology was observed in both male and female mice exposed to 93 or 89 mg/kg-day for four days. The severity of the lesions in the female mice was reported to be less than that observed in male mice. Labeling index after four days was markedly increased in both male and females exposed to 93 or 89 mg/kg-day and in males exposed to 30 mg/kg-day of chloroform. The increase in labeling index at 31 mg/kg-day in males was not consistent with the lack of toxicity in the liver at this dose.

No toxicity or increase in labeling index was observed in the kidney of female mice. Cytotoxicity as well as increased labeling index was observed in the kidney of male mice exposed to 31 or 93 mg/kg-day for four days.

Although a different mouse strain was employed in this study (compared to Larson *et al*., 1994b,c), labeling indexes at four days in livers of female mice were compared (using an absorbed dose metric) to chloroform administered in corn oil or drinking water (Figure 7). The two lower doses in the inhalation study, which did not result in increases in the labeling index, are not inconsistent with what was observed when the compound was administered by the oral route. The labeling index associated with high dose in the inhalation study is equivalent to (or may be greater than) the labeling index associated with corn oil administration.

Figure 7. Labeling Index in Liver of Female Mouse Exposed for Four Days



Legend:
Open square: observed labeling index in mice administered chloroform in corn oil
Black triangle: observed labeling index in mice exposed to chloroform in the air
Black square: observed labeling index in mice administered chloroform in drinking water

Templin *et al.* (1996a)

Nine-week old male or female F344 rats were exposed to chloroform at 0, 2, 10, 30, 90 or 300 ppm in air for six hours/day. Five to eight male rats/dose group) were exposed for four days or three, six or 13 weeks and female rats for three or 13 weeks. The doses associated with these air levels were determined using standard estimates of body weight (0.18 and 0.12 kg), ventilation rates (0.19 and 0.14 m^3/day) for male and female F344 rats, respectively, and an assumption of 50 percent absorption (Table 50). Lesions in the livers of male and female rats were evident in the two high dose groups and became more severe with duration of exposure in males exposed to 90 or 300 ppm. Lesions in the female rats occurred in both the 90 and 300 ppm groups and were minimally severe at 90 ppm and moderately severe at 300 ppm. Labeling index in the liver was significantly increased only in male and female rats exposed to 300 ppm. The labeling index in the liver peaked at 13 weeks in the females and at six weeks in the males. In the liver, the increase in labeling index was consistent with the increase in toxicity in rats exposed to 300 ppm, although toxicity observed in male and female rats exposed to 90 ppm was not associated with an increase in labeling index. In addition, in the male rat, labeling index peaked at six weeks while the toxicity was more severe at 13 weeks. The increased toxicity and labeling index at 300 ppm is at an absorbed dose comparable to that used in two-year cancer bioassays, although the NCI (1976) did not detect an increase in liver tumors in the Osborne Mendel rats in the bioassay. Tumasonis *et al*. (1985, 1987)

observed hepatic adenofibrosis in Wistar rats at a comparable lifetime dose administered in drinking water.

Table 50. Doses Associated with Inhalation Exposure in Fischer 344 Rats (Templin *et al*., 1996a)

	Absorbed dose (mg/kg-day)	
Concentration (ppm)	**Males**	**Females**
10	6.5	7
30	20	22
90	59	65
300	200	220

Kidney lesions appeared at concentrations above 10 ppm in both male and female rats. Lesions in the female rat were reported to be less severe than lesions in the male rat. Labeling index in the kidney was significantly increased in both sexes exposed to 30 ppm or greater, whereas lesions in the kidney occurred at a lower concentration. Labeling index was greatest in animals exposed to 300 ppm of chloroform (males and females), with 90 ppm greater than 30 ppm. The increases in labeling index in the kidney in this study were not consistent with the occurrence of tumors in the rat. Yamamoto *et al*. (1994) did not observe tumors in the kidney, while NCI (1976) observed tumors in the kidney of male but not female Osborne-Mendel rats. Jorgenson *et al*. (1982, 1985) also observed kidney tumors in male Osborne-Mendel rats.

Templin *et al.* (1996c)

Male Osborne Mendel or F344 rats were administered a single dose of 0, 10, 34, 90, 180 or 477 mg/kg of chloroform in corn oil by gavage (five or six rats/dose group). Two days post-administration, the animals were sacrificed and the liver and kidney were examined for histopathology and changes in labeling index. While an identical protocol was used for the two strains of rat, the studies were conducted in different laboratories and at different times due to disease concerns.

No significant lesions were observed in kidneys of Osborne Mendel rats, while minimum to mild histopathology was observed in kidneys of the high-dose F344 rats. Unlike the histopathology, an increase in labeling index was observed in kidneys of both strains. Statistically significant increases were observed in Osborne Mendel rats receiving 34 mg/kg or more, while statistically significant increases were observed in the F344 rats that received doses of 90, 180 or 477 mg/kg. No pathology or increased labeling index was observed in the liver at any doses in the Osborne Mendel rats, while increased labeling index and hepatotoxicity was observed only in the liver of F344 rats administered 477 mg/kg-day.

Constan *et al.* (2002)

Female $B6C3F_1$ mice were exposed to chloroform in air at 10, 30 or 90 ppm from two to 18 hours for seven days (five mice/dose group). We estimated doses associated with

inhalation exposures based on a standard body weight of 0.0246 kg, an inhalation rate of 0.04 m^3/day (U.S. EPA, 1988), and an assumed pulmonary absorption of 50 percent of the inhaled chloroform. Changes in body and relative liver weight, liver histopathology and labeling index in the liver were investigated. While an increased labeling index was observed in animals exposed to 30 ppm for six or 12 hours/day (average daily doses of 30 and 60 mg/kg-day, respectively) and animals exposed to 90 ppm for two or six hours/day (average daily doses of 30 and 90 mg/kg-day, respectively), treatment-related histopathology was reported in only three of the four groups. Severe histopathology was observed in animals exposed to 90 ppm for six hours, minimal changes in animals exposed to 30 ppm for 12 hours, and occasional changes in animals exposed to 30 ppm for six hours. The investigators concluded: "…measurement of regenerative cell proliferation is a more sensitive indicator of chloroform-induced cellular damage than histopathological examination."

Pereira (1994)

Female $B6C3F_1$ mice, seven weeks of age (from 8 to 190 animals/dose group), received chloroform in drinking water (1,800 ppm), in corn oil (263 mg/kg-day) by gavage, or in drinking water (1,800 ppm) plus corn oil containing no chloroform by gavage for up to 159 days. Other groups of control animals received corn oil containing no chloroform or no treatment. Body weight, drinking water consumption, and labeling index in the mouse liver as well as organ weight and histopathology were determined. In a separate study, labeled chloroform was administered in water or corn oil by gavage to fasted mice, and the uptake and binding of chloroform to macromolecules were determined in the liver and kidney at various times (up to four hours) post-administration.

Chloroform in the drinking water markedly reduced drinking water consumption between day zero and seven. Water consumption then increased, but remained significantly below control through days 134 to 140, the last days consumption was monitored. The average daily dose was 0.49 mg/kg-day on day 0-7, 12.66 mg/kg-day from day 8-14, and after day 14, ranged from 203 to 272 mg/kg-day. Corn oil administered to animals receiving chloroform in their drinking water had no effect on the animals' water consumption. Animals receiving chloroform in their drinking water had reduced body weight, which returned to control levels by week 33. The mean body weights of animals that received chloroform in corn oil by gavage were not significantly different from control.

Significant hepatotoxicity was observed in animals that received chloroform in corn oil but by day 12 the hepatotoxicity was less severe. At day 33 and day 159 very little toxicity was observed in the animals. Changes in the hepatocytes were observed in animals receiving chloroform in drinking water (with or without corn oil). The livers of both groups appeared no different than control on day 33 or 159.

Labeling index was significantly elevated only in animals receiving chloroform in corn oil by gavage. The labeling index peaked at day five through day 12 post-administration and then declined to half-peak values on day 33. At day 159, labeling index was one-tenth peak levels. It is unclear if labeling index was significantly elevated above control values at this time.

Vehicle-dependent differences in labeling index after 12 days of exposure may reflect the initial reduced dose that the animals received when chloroform was administered in their

drinking water vehicle. Administration of a smaller chloroform dose fostered tolerance to toxicity of subsequent larger doses (Nagano *et al.*, 1998; Yamamoto *et al.*, 1999).

Peak chloroform levels in liver were markedly higher, and occurred earlier, in animals administered the compound by gavage in a water vehicle, compared to corn oil. Chloroform levels in the liver associated with the different vehicles were comparable by 40 minutes post-administration. The binding of chloroform administered in water to liver macromolecules was higher for the first 40 minutes post-administration and then was comparable for the remaining portion of the study. After gavage administration in water, chloroform levels were substantially higher in the kidney at all times (up to 240 minutes, the last time point of the study) than when it was administered in the corn oil vehicle. Binding to macromolecules was also markedly higher throughout the 240 minutes of the study when the compound was administered in water.

The much higher peak chloroform levels in liver and more binding to macromolecules after gavage in water, compared to administration in corn oil, is not consistent with lack of hepatotoxicity or increase in labeling index in the liver.

Melnick *et al.* (1998)

Groups of eight to nine-week-old female B6C3F$_1$ mice (ten mice/dose group) were exposed to various doses of one of the four THMs administered in corn oil by gavage five days/week for three weeks. Equivalent doses of chloroform, BDCM, DBCM, and bromoform (0.46, 0.92 and 2 mmol/kg (as well as 4.0 mmol/kg of chloroform) were administered. For chloroform the equivalent doses were 55, 110, 238 and 477 mg/kg-day. Effects on hepatic labeling index and hepatotoxicity as indicated by changes in liver weight, histopathology and changes in serum enzymes were investigated.

Changes in hepatotoxicity as indicated by increased liver weight, serum alanine aminotransferase and sorbitol dehydrogenase activity, and perhaps histopathology appeared to be dose-dependent and equivalent on a mmol/kg-day basis for chloroform, BDCM and DBCM but not bromoform. Changes in labeling index were both dose- and compound-related with increased labeling index highest for chloroform followed by BDCM, DBCM, and bromoform.

The dose-related change in labeling index did not correspond with the changes in indicators of liver toxicity (serum enzyme activity and increased liver weight) with dose. While the four THMs yielded similar dose-response relationships for hepatotoxicity indicators, labeling index was not equivalent at the same dose for the various THMs. These finding suggest that the increase in labeling index is not directly related to the increase in hepatotoxicity.

Chloroform Toxic Effects

Statistically significant increases in tumors have been observed in the Osborne-Mendel rat kidney at a dose of 180 mg/kg-day (males), and in the B6C3F$_1$ mouse liver at doses of 138 or 277 mg/kg-day (males) and 238 or 477 mg/kg-day (females), respectively (NCI, 1976). Statistically significant increases in tumors in Osborne-Mendel rat kidney were also observed by Jorgenson *et al*. (1985) at a dose of 160 mg/kg-day. Statistically significant increases in renal tumors were observed in male ICI mouse administered 60

mg/kg-day of chloroform in a toothpaste vehicle (Roe *et al*., 1979). Statistically significant increases in tumors were not reported at lower doses (in these and other studies), but given the insensitivity of the animal bioassay in detecting tumors in small populations of animals, this finding is not surprising.

A number of effects have been observed in various tissues in addition to hepatic and renal toxicity, in the range of chloroform doses administered in the cancer bioassays. This multiplicity of effects is typical of many toxicants. Chloroform administration has been associated with effects such as changes in body and organ weights, water consumption, hematological changes, suppressed immunity, and sedation (see Table 51 below). These effects may not themselves be considered to be toxic effects, but may indicate that toxic effects are occurring. In addition, it should be noted that occurrence of a toxic effect does not establish that the effect is a precursor to, causative of, caused by the same mechanism, or related to the mechanism that produced tumors in the liver or kidney.

Table 51. Toxic Effects After Oral Administration of Chloroform to Rats and Mice*

Species/sex	Exposure	System	LOAEL (mg/kg-day)		Reference
			Less serious**	More serious	
Rat	1 dose	hematological	546		Chu *et al*., 1982a
Rat/Male	4 doses	decreased body weight	180		Larson *et al*., 1995a
Rat/Female	10 doses	hematological	100		Ruddick *et al*., 1983
Rat/Female	10 doses	decreased body weight		100	Ruddick *et al*., 1983
Rat/Female	10 doses	gastric erosions		516	Thompson *et al*., 1974
Rat/Female	10 doses	alopecia	126		Thompson *et al*., 1974
Mouse	14 dose	suppressed immunity	50		Munson *et al*., 1982
Rat/Female	1 doses	reduced lymphocytes	1071		Chu *et al*., 1982b
Mouse	1 dose	ataxia, anesthesia, brain hemorrhage		500	Bowman *et al*., 1978
Mouse/Male	10 doses	taste aversion	30		Landauer *et al*., 1982
Rat/Female	10 doses	increased resorptions		316	Thompson *et al*., 1974
Rat	10 doses	decreased fetal weight	400		Ruddick *et al*., 1983
Rat/Male	28 days	hematological	193		Chu *et al*., 1982b
Rat/Male	90 days	decreased body weight	160		Jorgenson and Rushbrook, 1980

Species/sex	Exposure	System	LOAEL (mg/kg-day)		Reference
Rat/Male	21 days	decreased body weight	106		Larson *et al*., 1995a
Rat/Female	3 weeks	respiratory	34		Larson *et al*., 1995b
Rat	91 days	hematological	410		Palmer *et al*., 1979
Mouse	90 days	depressed immunity	50		Munson *et al*., 1982
Mouse	60 days	neurological	100		Balster and Borzelleca, 1982
Rat	91 days	gonadal atrophy		410	Palmer *et al*., 1979
Mouse/Male	105 days	epididymal degradation	41		Gulati *et al*., 1988
Rat/Male	1,260 days	50 percent reduced weight gain		200	Tumasonis *et al*., 1985, 1987
Mouse/Female	78 weeks	pulmonary inflammation	238		NCI, 1976
Mouse/Female	78 week	cardiac thrombosis	238		NCI, 1976

*Adapted from Table 2-2 in "Toxicological Profile for Chloroform" (ATSDR, 1997).
**As defined by ATSDR, "less serious" effects are those that are not expected to cause significant, dysfunction or death, or those whose significance to the organism is not entirely clear. "Serious" effects are those that evoke failure in a biological system and can lead to morbidity or mortality (e.g., acute respiratory distress or death).

Relationship Among Cytotoxicity, Cellular Regeneration, and Tumors

It has been inferred that the occurrence of tumors in the kidney and liver in animals exposed to chloroform is due to (or is secondary to) cytotoxicity and tissue regeneration (U.S. EPA, 1998g; ILSI, 1997). If true, no tumors would occur at exposure levels at which no cytotoxicity and tissue regeneration occurs, and a dose response assessment would focus on determining the dose at which cytotoxicity, tissue regeneration and therefore tumor formation does not occur. In the case of chloroform, tumors, cytotoxicity, and evidence of tissue regeneration (and other effects) have been observed in the liver and kidney of animals administered high doses. Furthermore, evidence of cytotoxicity and tissue regeneration has been observed at doses associated with tumors in rats and mice. However, statistically significant increases in tumors were only observed in a few studies that employed similar dose levels. Many other effects (Table 51) also occur at the dose levels associated with tumors. Thus, observed cytotoxicity and tissue regeneration at the dose(s) of chloroform associated with tumors is not in itself compelling evidence that the effects are causally related. More persuasive evidence of a relationship between the effects would include a consistency of the appearance of cytotoxicity, tissue regeneration, and then tumors; consistency in the dose response that characterizes the appearance of these effects: and a consistent temporal relationship for the appearance of these effects (Tables 52 and 53).

Table 52. Consistency or Inconsistencies of the Changes in Cytotoxicity, Labeling Index, and the Occurrence of Tumors in the Liver of Rats and Mice

Study	Species	Vehicle	Sex	Relationship Between Cytotoxicity, Labeling Index and Occurrence of Tumors	Comment
Larson *et al.*, 1993	Rat	Corn oil	M	Differences in dose-response	Cytotoxiciy at lower doses without comparable increase in labeling index
	Mouse	Corn oil	F	Inadequate data	
Templin *et al.*, 1996c	Rat F344	Corn oil	M	Consistent changes in cytotoxicity and labeling index	
	Rat Osborne Mendel	Corn oil	M	Consistent changes in cytotoxicity and labeling index	
Larson *et al.*, 1995b	Rat F344	Corn oil	F	Differences in dose-response	Little cytotoxicity but increased labeling index at mid doses
				Divergent responses	Increased labeling index but no tumors at site
Larson *et al.*, 1994b	Mouse	Corn oil	M	Consistent changes in cytotoxicity and labeling index	
Larson *et al.*, 1994c	Mouse	Corn oil	F	Divergent responses	Cytotoxicity with no increase in labeling index at middle doses
				Temporal differences when response occurred	Labeling index peaked at 3 days, cytotoxicity more severe at 3 weeks
	Mouse	Drinking water	F	Divergent responses	Mild cytotoxicity with no increase in labeling index
Larson *et al.*, 1995a	Rat	Corn oil	M	Differences in dose response	Cytotoxicity with no increase in labeling index at middle dose
				Divergent responses	Increased labeling index but no tumors at site
	Rat	Drinking	M	Divergent responses	Cytotoxicity with no increase in labeling index

Study	Species	Vehicle	Sex	Relationship Between Cytotoxicity, Labeling Index and Occurrence of Tumors	Comment
		water		Divergent responses	Cytotoxicity with no tumors at this site
Larson *et al.*, 1996	Mouse	Air	M	Differences in dose response	Cytotoxicity with no increase in labeling index at mid dose (30 ppm)
				Temporal differences when responses occurred	Labeling index peaked at 3 weeks, cytotoxicity remained elevated at 13 weeks in high dose group
	Mouse	Air	F	Differences in dose response	Cytotoxicity but no increase in labeling index at mid dose (10 ppm)
				Temporal differences when responses occurred	Cytotoxicity more severe at four days while labeling index higher at mid dose (30 ppm) after three weeks
Templin *et al.*, 1996a	Rat	Air	M	Differences in dose response	Cytotoxicity but no increase in labeling index at mid dose
				Temporal differences when responses occurred	Labeling index peaked at 6 weeks, cytotoxicity more severe at 13 weeks
				Divergent responses	Increased labeling index but no tumors at site
	Rat	Air	F	Differences in dose response	Cytotoxicity but no increase in labeling index at mid doses
				Divergent responses	Increased labeling index but no tumors at site
Templin *et al.*, 1996b	Mouse	Air	M	Differences in dose response	Little cytotoxicity but increased labeling index at the mid dose
	Mouse	Air	F	Consistent changes in cytotoxicity and labeling index	
Constan *et al.*, 2002	Mouse	Air	F	Differences in dose response	No cytotoxicity but increased labeling index in one dose group

Table 53. Consistency or Inconsistencies of the Changes in Cytotoxicity, Labeling Index, and the Occurrence of Tumors in the Kidney of Rats and Mice

Study	Species	Vehicle	Sex	Relationship Between Cytotoxicity, Labeling Index and Occurrence of Tumors	Comments
Larson *et al.*, 1993	Rat	Corn oil	M	Differences in dose response	Cytotoxicity increased with dose, labeling index decreased at higher dose
	Mouse	Corn oil	F	Divergent responses	No cytotoxicity but increased labeling index
				Divergent responses	Increased labeling index but no tumors at site
Templin *et al.*, 1996c	Rat F344	Corn oil	M	Differences in dose response	Dose dependent increase in labeling index at all doses, Cytotoxicity only at high dose
	Rat Osborne Mendel	Corn oil	M	Divergent responses	No cytotoxicity but dose dependent increase in labeling index
Larson *et al.*, 1995b	Rat F344	Corn oil	F	Differences in dose response	No cytotoxicity but increased labeling index at middle dose
				Divergent responses	Cytotoxicity, increased labeling index but no tumors at this site
				Temporal differences when responses occurred	Cytotoxicity but not labeling index diminished at three weeks
Larson *et al.*, 1994b	Mouse	Corn oil	M	Consistent changes in cytotoxicity and labeling index	At four days
				Differences in dose response	Cytotoxicity but no increased labeling index in low dose groups at three weeks
Larson, *et al.*, 1994c	Mouse	Corn oil	F	Divergent responses	No cytotoxicity but increased labeling index at high dose
				Divergent responses	Increased labeling index but no tumors at site

Study	Species	Vehicle	Sex	Relationship Between Cytotoxicity, Labeling Index and Occurrence of Tumors	Comments
	Mouse	Drinking water	F	Divergent responses	No cytotoxicity but increased labeling index at higher doses
				Divergent responses	Increased labeling index but no tumors at site
Larson *et al.*, 1995a	Rat	Corn oil	M	Differences in dose response	Dose dependent cytotoxicity, increase labeling index only at high dose
	Rat	Drinking water	M	Divergent responses	Mild cytotoxicity, no increase in labeling index
Larson *et al.*, 1996	Mouse	Air	M	Divergent response (perhaps)	Marked increased labeling index at 3 weeks but minimal cytotoxicity at three or 13 weeks in mid (30 ppm) dose group
	Mouse	Air	F	No cytotoxicity or changes in labeling index	
Templin *et al.*, 1996a	Rat	Air	M	Differences in dose response	Cytotoxicity but no increase in labeling index at low dose
	Rat	Air	F	Differences in dose response	Cytotoxicity but no increase in labeling index at low dose
				Divergent responses	Cytotoxicity, increased labeling index but no tumors at site
Templin *et al.*, 1996b	Mouse	Air	M	Consistent changes in cytotoxicity and labeling index	
	Mouse	Air	F	Consistent changes in cytotoxicity and labeling index	

Consistency

If tumors are secondary to cytotoxicity and tissue regeneration, then neither tumors nor regeneration should occur without the occurrence of cytotoxicity. Furthermore, cytotoxicity should be followed by tissue regeneration and then tumors. However, increased labeling index (a measure of tissue regeneration) without cytotoxicity was observed in the kidney of animals administered chloroform (Larson *et al.*, 1993; Templin *et al.*, 1996c). Conversely, following chloroform administration, cytotoxicity was observed in the kidney (Larson *et al.*, 1995a) or liver (Larson *et al.*, 1994c, 1995a) without an increase in labeling index.

Following chloroform administration, increased cytotoxicity or labeling index have been observed in the rat liver (Larson *et al.*, 1993; 1995a,b; Templin *et al*., 1996a), female mouse kidney (Larson *et al.*, 1993; 1994c), and female rat kidney (Templin *et al.*, 1996a; Larson *et al.*, 1995b), tissues where no statistically significant increases in tumors were observed in cancer bioassays.

Dose Response

Comparable (parallel) dose-response relationships for cytotoxicity, tissue regeneration and tumor formation would provide more compelling evidence that the effects are related. Conversely, evidence that the effects occur at or peak at substantially different doses suggests that the effects may be unrelated. Because tumors were observed at most at two doses, dose-response information that characterizes this effect is very limited. However, as discussed above, there is considerable information regarding the appearance of cytotoxicity and tissue regeneration in the liver and kidney.

Following chloroform administration, increased labeling index was observed at dose levels where cytotoxicity was not observed in the liver (Templin *et al.*, 1996b; Constan *et al.*, 2002; Larson *et al.*, 1995b) or kidney (Templin *et al.*, 1996c; Larson *et al.*, 1995b). Conversely, cytotoxicity was observed at dose levels where increased labeling index or comparable increased labeling index was not observed in the liver (Larson *et al.*, 1993; 1995a, 1996; Templin *et al.*, 1996a) or kidney (Larson *et al.*, 1993; 1994b, 1995a; Templin *et al.*, 1996a). The dose-response relationship for labeling index for various THMs did not appear to parallel the hepatotoxicity dose-response relationship for these related compounds (Melnick *et al.*, 1998). Suggestions that labeling index is a more sensitive measure of cytotoxicity is not supported by studies where toxicity was observed at doses without an increase in labeling index in the liver (Templin *et al.*, 1996; Larson *et al*., 1995a) or kidney (Larson *et al.*, 1993, 1994c, 1995a, 1996; Templin *et al.*, 1996a).

Temporal Pattern

Given the hypotheses that cytotoxicity and tissue regeneration are antecedent to tumor formation, the predicted temporal pattern of toxicity, tissue regeneration, and then tumors should be observed. While toxicity did precede or accompany the increase in labeling index in certain studies (Larson *et al.*, 1995b, 1993), increases in labeling index preceded toxicity or peaked and then were reduced or returned to near background levels, while toxicity continued or became more severe in the liver (Larson *et al.*, 1994c, 1996; Templin *et al.*, 1996a). Serum alanine aminotransferase and sorbitol dehydrogenase level peaked at three weeks at 238 and 477 mg/kg-day while hepatic labeling index peaked at

34 days and was markedly reduced at these dose levels at three weeks. Suggestions that the labeling index decreased because increased toxicity prevents regeneration were not supported by the findings that labeling index was not increased at lower doses where toxicity was not as severe (Larson *et al.*, 1994a).

Vehicle Effects

Because of volatility, palatability, or water solubility concerns, many bioassays have employed corn oil as a vehicle to administer test articles. Potential confounding effects from using corn oil as a vehicle are unclear. While some investigators have observed increased or enhanced toxicity when a toxicant was administered in corn oil (Bull *et al.*, 1986; Condie *et al.*, 1986), others have reported equivocal results (Raymond and Plaa, 1997; Simmons *et al.*, 1996). Corn oil administered alone did not result in significant increases in tumors in the rat kidney or liver (NTP, 1994).

Cytotoxicity and tissue regeneration in liver and kidney of mice and rats following chloroform administration have been attributed to the use of a corn oil vehicle. It has been concluded that these effects are not observed when chloroform was administered in drinking water (Science Advisory Board, 2000). However, our reevaluation of the findings of Larson and coworkers (1994a,b, 1996a,b, 1996) does not fully support this conclusion. As we showed above, the doses administered in drinking water were below doses of chloroform administered in corn oil. Thus the initial increases in cytotoxicity and labeling index in corn oil and their absence when the compound was administered in water can be accounted for by a difference in the doses. Interestingly, equivalent doses administered by the inhalation route appear to yield a greater increase in labeling index response. Further study of this effect is warranted.

Any vehicle-dependent differences (drinking water vs. corn oil) in toxicity and labeling index observed after a couple of weeks of exposure may also reflect a difference in the dose of chloroform the animals initially received due to the marked reduction in their drinking water intake. The administration of a smaller dose of chloroform has been observed to foster tolerance to subsequent larger doses (Nagano *et al.*, 1998; Yamamoto *et al.*, 1999). In most studies, the labeling index and often toxicity was substantially diminished after several weeks of exposure, indicating that the animals were better able to tolerate chloroform.

The difference in cytotoxicity and regenerative response after chloroform was administered in corn oil has been attributed to higher tissue levels of chloroform in the kidney and liver (Bruckner and Warren, 2001). However, more rapid chloroform uptake into the liver and kidney and more binding to macromolecules have been observed when chloroform was administered in water (compared to corn oil) in a different study (Pereira, 1994) and are not consistent with this finding. The findings of Pereira (1994) are not unexpected, given that lipids are known to delay gastric emptying.

Carcinogenic Mechanism of Action: Conclusion

The time course and pattern of toxicity and tissue regeneration following chloroform administration raises doubts that these effects are responsible for tumors in the rat and mouse. In most instances toxicity and labeling index peaked after only a few days or at

most a few weeks. A noteworthy increase in toxicity and labeling index occurred after the administration of a single dose of chloroform (Larson *et al.*, 1993, 1994a). Given that toxicity and increases in labeling index usually occurred immediately after the animals were exposed to chloroform, and then rapidly diminished, the importance of continued exposure to chloroform appears to be unclear. Tumors might be expected after a brief or repeated short-term exposures, which is a pattern more reminiscent of a strong initiator and not a chemical that has been surmised to act by a nongenotoxic mechanism. However, the data are insufficient to distinguish among the various possibilities.

In rodents, chloroform is both a hepatic and renal toxin and a carcinogen. As is apparent from the discussion of short-term test results, there is little evidence that chloroform directly interacts with or damages DNA *in vivo*. As a consequence, there has been speculation that the carcinogenic activity of chloroform is due to an epigenetic (nongenotoxic) process (Reitz *et al*., 1980, 1982; Pereira *et al*., 1982, 1984a,b, 1985). This hypothesis is based on the theory that chloroform may act as a promoter of previously initiated cells by virtue of regenerative hyperplasia which occurs in response to renal and hepatic toxicity (i.e., chloroform-induced cell death).

Reitz *et al*. (1980, 1982) examined the ability of chloroform to induce cell regeneration using ^{3}H-thymidine uptake as a measurement technique. Their data indicate that orally administered chloroform (15, 60, or 240 mg/kg) induced a dose-dependent increase in replicative DNA synthesis 48 h postexposure in liver and kidney of $B6C3F_1$ mice. Similarly, a single oral dose of chloroform (180 mg/kg) administered to Osborne-Mendel rats induced a 160 percent increase in cell regeneration in liver and a 40 percent increase in kidney. When Reitz *et al*. (1980, 1982) examined the livers of mice (N = 2) 48 hours after a single oral dose of 240 mg/kg chloroform, there was evidence of induced tissue damage and increased mitosis. The liver was not measurably affected by doses of 15 or 60 mg/kg. The kidneys of these animals exhibited necrotic lesions after a dose of 240 mg/kg and epithelial regeneration after 60 or 240 mg/kg of chloroform. Moore *et al*. (1982) used gross tissue uptake of ^{3}H-thymidine at 96 hour postexposure as a measurement of cell regenerative activity. They observed that a single dose of chloroform (approximately 18, 60, or 200 mg/kg) in corn oil or toothpaste induced a dose-related increase in cell regeneration in liver and kidney of male Swiss albino mice.

To determine if chloroform induces regenerative hyperplasia, Pereira *et al*. (1984b) administered a single ip dose of chloroform to rats and mice (50, 75, 225, 375, 575, or 750 mg/kg). Measurements of hepatic ornithine decarboxylase activity and hepatic and renal DNA synthesis, measured as incorporation of ^{3}H-thymidine, were made in both species as indices of hyperplasia. Chloroform induced a dose-related increase in hepatic ornithine decarboxylase activity in the liver of rats and mice (measured 18 hours after treatment). However, the magnitude of this effect was species-dependent. The maximum response in mice (a 10-fold increase) occurred after a dose of 375 mg/kg whereas in rats the maximum response (a 52-fold increase) was measured after 750 mg/kg. In rats, hepatic DNA synthesis was *inversely* related to the quantity of chloroform administered. In the rat kidneys, DNA synthesis also decreased with increasing dose of chloroform, but to a much smaller extent than was observed in the liver. DNA synthesis in the liver and kidney of mice was stimulated by a dose of 50 mg/kg chloroform; Pereira *et al*. (1984b) measured a 3.1-fold increase in hepatic DNA

synthesis, and a 6.7-fold increase in renal DNA synthesis. With chloroform doses greater than 50 mg/kg, DNA synthesis decreased. Because there is not a consistent relationship between induction of hepatic ornithine decarboxylase activity and DNA synthesis, nor any clear dose response relationship for induction of DNA synthesis alone, these data do not provide a clear association between administration of chloroform and stimulation of cell proliferation in the liver and kidney. However, hepatectomy or toxicity-induced hepatocellular replication in rodents does not reach a maximum until 36 to 72 hours after initial damage to liver (Leevy *et al*., 1959; Gerhard, 1973; Rabes *et al*., 1973; Schultze *et al*., 1973). Because Pereira *et al*. (1984b) measured DNA synthesis 24 hours after chloroform dosing, it is not directly apparent whether these measurements adequately evaluate the ability of chloroform to induce replicative DNA synthesis in rats and mice.

We conclude that the strength of the evidence is insufficient to support a non-genotoxic mechanism of action, secondary to cytoxicity and tissue regeneration for chloroform. For bromoform, BDCM, and DBCM, the mechanistic studies are less detailed, and similarly are inadequate to demonstrate threshold mechanisms for tumor induction.

DOSE RESPONSE ASSESSMENT

Chloroform

Noncarcinogenic Effects

Human data identified the central nervous system, liver, and kidney as the primary targets of concern after long-term exposure to chloroform at sub-anesthetic levels (e.g., Bomski *et al*., 1967; Phoon *et al*., 1983; Li *et al*., 1993). Most of the studies involved occupational exposure to chloroform, which is presumed to have occurred primarily by inhalation exposure, although some oral and dermal exposure may have occurred as well. Given that exposure to chloroform in drinking water will occur primarily though the oral route and that the human data are associated with high levels of occupational exposure, these data are insufficient to derive a proposed PHG for chloroform.

Animal studies support the findings from the human studies that the liver and kidney are important targets of chloroform toxicity following repeated exposure to relatively low levels (e.g., Torkelson *et al*., 1976; Condie *et al*., 1983; Heywood *et al*., 1979; Hard *et al*., 2000). Central nervous system effects have not been well studied in repeated exposure animal studies, and as a result, there is limited evidence regarding the occurrence in animals of subtle neurological effects, such as those reported in chronically exposed workers (e.g., Balster and Borzelleca, 1982). Obvious central nervous system depressive effects are associated with acute high-level exposure (e.g., Whitaker and Jones, 1965; Bowman *et al*., 1978).

Animal studies also found the nasal epithelium to be a sensitive target of chloroform (e.g., Templin *et al*., 1996a; Larson *et al*., 1995b). Nasal lesions have been observed after inhalation and oral (gavage) exposure. The appearance of these lesions following gavage exposure in rodents is likely to be related to direct exposure of the nasal epithelium to chloroform vapor volatilizing from the test material in the gut, rather than a

systemic effect of ingested chloroform. Therefore, the nasal lesions were not considered a potential critical effect for PHG derivation.

Liver and kidney effects from chloroform were observed in rats, mice, guinea pigs, rabbits, and dogs (e.g., Torkelson *et al*., 1976; Heywood *et al*., 1979; Templin *et al*., 1996a; Larson *et al*., 1996). Characteristic pathological changes in the liver include congestion, enlargement, fatty infiltration, and centrilobular necrosis. Related observations were increased levels of serum biomarkers for hepatotoxicity (e.g., alanine aminotransferase, aspartate aminotransferase, sorbitol dehydrogenase), cell proliferation in the liver (increased bromodeoxyuridine labeling index), and jaundice. In the kidney, chloroform produced a variety of degenerative, necrotic, and regenerative lesions in the proximal tubules. Other indicators of nephrotoxicity were increased blood urea nitrogen and cell proliferation in the kidney. Sensitivity, particularly for renal lesions, varied with species, strain, and sex of the animals tested.

The lowest reliable adverse effect level for chronic oral exposure identified in animals was a LOAEL of 15 mg/kg-day for hepatic effects in dogs treated with chloroform in toothpaste for over seven years (Heywood *et al*., 1979). This study found increased serum alanine aminotransferase throughout the study at 30 mg/kg-day and after week 130 at 15 mg/kg-day. Histopathological examination revealed a significant dose-related increase in the incidence of fatty cysts of moderate or marked severity in both male and female dogs at 15 and 30 mg/kg-day. A NOAEL was not identified. This endpoint is supported by a subchronic (12 to 18 week) study in dogs reported in the same paper, which found a dose-related spectrum of hepatic effects: occasional increases in serum alanine aminotransferase at the low dose of 30 mg/kg-day; increased liver weight and slight fatty change at 45 mg/kg-day; hepatocellular hypertrophy, fatty degeneration, and increases in several serum enzyme indicators of hepatotoxicity at 60 mg/kg-day; jaundice and loss of general condition at 120 mg/kg-day.

Further support comes from the vast array of animal studies that found similar effects on the liver in other species. Hepatic effects in other species occurred at doses of about 35 mg/kg-day and higher (Larson *et al*., 1994c; Condie *et al*., 1983; DeAngelo, 1995; Melnick *et al*., 1998; Bull *et al*., 1986). Renal lesions in other species were also seen at doses of about 35 mg/kg-day and up (Larson *et al*., 1994b; Hard *et al*., 2000).

There is evidence for development effects of chloroform. In oral studies, the effects were limited to mild fetotoxicity (decreased fetal body weight, delayed ossification, increased skeletal variations) at high doses that also produced overt toxic effects in the dams (Thompson *et al*., 1974; Ruddick *et al*., 1983). Therefore, the developing organism does not appear to be an especially sensitive target for chloroform, and a PHG based on liver effects would be expected to be protective against developmental effects as well.

Effects on reproductive function were reported in one multigeneration rodent study at very high doses (Borzelleca and Carchman, 1982). No such effects were observed in a second study that employed considerably lower doses, but still produced hepatotoxicity at the high dose (NTP, 1988). These data show that if chloroform produces reproductive effects, it is only at high doses, so that a PHG based on liver effects would be expected to be protective against reproductive effects as well.

The LOAEL of 12.9 mg/kg-day (15 mg/kg-day × 6/7 days/week) from the chronic dog study by Heywood *et al*. (1979) was used as the basis for the U.S. EPA's reference dose (RfD) of 0.01 mg/kg-day for chloroform (U.S. EPA, 2001e, 2008c), with application of an uncertainty factor of 1,000. U.S. EPA (2001e, 2008c) also performed benchmark dose modeling of the Heywood *et al*. (1979) data, despite limitations of this data set for that procedure. The dose corresponding to a 10 percent incidence of liver effects, known as the $BMDL_{10}$, was estimated as 1.0 mg/kg-day. When used with the applicable uncertainty factor of 100, this also produced an RfD of 0.01 mg/kg-day.

Carcinogenic Effects

For dose response determination, four long-term studies were identified in which the administration of chloroform resulted in a statistically significant increase in liver tumors of male or female mice (NCI, 1976), kidney tumors of male rats (NCI, 1976; Jorgenson *et al*., 1985), or kidney tumors in male mice (Roe *et al*., 1979). PBPK modeling was utilized to determine if internal dose metrics such as liver or kidney metabolized chloroform dose yielded better fits to observed tumor data than the applied dose. A basic assumption of PBPK modeling for dose-response analysis is that if a given dose metric gives a better fit to the observed data than the applied dose it probably represents a process more closely related to the tumorigenic response. The final analysis employed the PBPK model of Corley *et al*. (1990) modified to accommodate both drinking water input and the larger animal size of the Jorgenson *et al*. (1985) study. This model is considered adequately validated and was suitable for animal dose modeling. A more detailed description of the PBPK model is found in the appendix.

Dose response relationships were determined for chloroform dose metrics using U.S. EPA (1995b, 2000a) BMDS software. This software has been available in successive versions for many years and has essentially replaced commercial software used previously by OEHHA (e.g., ToxRisk, Global86, etc.). The use of BMDS is essentially the same for the initial assessment of quantal dose-response data for noncancer and cancer analysis. In the former case the 95 percent lower confidence limit on the 5 percent response level ($BMDL_{05}$) is treated as a no observable effect level (NOAEL) and appropriate uncertainty factors are applied to produce a health protective dose level. For cancer risk assessment a $BMDL_{10}$ is used as a point of departure for low dose extrapolation and to calculate a cancer potency, e.g., potency or slope factor = $0.1/BMDL_{10}$.

Available quantal models in the U.S. EPA BMDS program (Version 1.3) were fit to combined incidence of adenomas and carcinomas in the liver of male or female $B6C3F_1$ mice (NCI, 1976) or kidneys of male Osborne-Mendel rats (NCI, 1976; Jorgenson *et al*., 1985) or male ICI mice (Roe *et al*., 1979). BMDS modeling results were evaluated according to the criteria outlined in U.S. EPA (2000a). Acceptable fits ($p > 0.1$) are shown in Tables 54 to 58.

Table 54. Dose Modeling of Chloroform Based on Applied Dose and Combined Incidence of Liver Adenomas and Carcinomas in Male B6C3F$_1$ Mice (NCI, 1976)

Model	Chi-square	P value	BMD$_{10}$[a] (mg/kg-day)	BMDL$_{10}$[b] (mg/kg-day)
Gamma	2.25	0.1335	78.0	56.1

[a]BMD$_{10}$ = maximum likelihood estimate of the dose producing a 10 percent extra risk of hepatic adenomas and adenocarcinomas
[b]BMDL$_{10}$ = lower 95 percent CI on the BMD$_{10}$

Table 55. Dose Modeling of Chloroform Based on Applied Dose and Combined Incidence of Liver Adenomas and Carcinomas in Female B6C3F$_1$ Mice (NCI, 1976)

Model	Chi-square	P value	BMD$_{10}$ (mg/kg-day)	BMDL$_{10}$ (mg/kg-day)
Gamma	0.05	0.9749	16.0	12.6
Log logistic	0.0	1	49.4	3.5
Multistage	0.05	0.9749	16.0	12.6
Log probit	0	1	38.9	0.052
Quantal linear	0.05	0.9749	16.0	12.6
Weibull	0.05	0.9749	16.0	12.6

Table 56. Dose Modeling of Chloroform Based on Applied Dose and Combined Incidence of Kidney Adenomas and Carcinomas in Male Osborne-Mendel Rats (NCI, 1976)

Model	Chi-square	P value	BMD$_{10}$ (mg/kg-day)	BMDL$_{10}$ (mg/kg-day)
Gamma	0	1	44.1	24.2
Logistic	0.32	0.5726	49.3	37.4
Log logistic	0	1	44.1	25.2
Multistage	0.23	0.8901	39.7	21.9
Probit	0.17	0.6832	47.1	35.2
Log probit	0	1	44.2	28.5
Quantal linear	3.0	0.2226	22.8	15.5
Quantal quadratic	0.23	0.8901	39.6	32.6
Weibull	0	1	44.0	23.9

Only one model yielded an acceptable fit for liver tumors in the male mouse (Table 54). Several of the models yielded acceptable fits for the other tumor data sets, and similar

BMDL estimates. The estimates of $BMDL_{10}$ based on tumors in the female mouse liver were lower than the estimates of $BMDL_{10}$ based on tumors in the male rat or male mouse kidney. The $BMDL_{10}$ estimates based on tumors in the male mouse kidney were lower than estimates based on kidney tumors in the Osborne-Mendel rat. Several of the models consistently yielded equivalent estimates of $BMDL_{10}$ within each of the studies. One of these consistent models, the Multistage model, was selected to evaluate if a given dose metric yielded better fits to the tumor data or yielded substantially different estimates of $BMDL_{10}$ in any of the four studies. Use of a metabolized dose, tissue metabolized dose or tissue-bound dose did not yield better fits to the quantal tumor response in the liver or kidney nor yield substantially different estimates of the $BMDL_{10}$ (data not shown).

Table 57. Dose Modeling of Chloroform Based on Applied Dose and Combined Incidence of Kidney Adenomas and Carcinomas in Male Osborne-Mendel Rats (Jorgenson *et al*., 1985)

Model	Chi-square	P value	BMD_{10} (mg/kg-day)	$BMDL_{10}$ (mg/kg-day)
Gamma	0.25	0.8843	128.0	93.8
Logistic	0.74	0.8630	135.6	111.1
Log logistic	0.26	0.8776	128.1	93.7
Multistage	0.38	0.8249	129.4	98.1
Probit	0.59	0.8982	133.2	105.6
Log probit	0.13	0.9370	125.9	89.3
Quantal linear	2.41	0.4918	166.0	104.6
Quantal quadratic	0.47	0.9262	128.9	99.1
Weibull	0.28	0.8688	128.8	95.1

Table 58. Dose Modeling of Chloroform Based on Applied Dose and Combined Incidence of Kidney Adenomas and Carcinomas in Male ICI Mice (Roe *et al*., 1979)

Model	Chi-square	P value	BMD_{10} [a] (mg/kg-day)	$BMDL_{10}$ [b] (mg/kg-day)
Gamma	0	1	53.0	33.0
Logistic	0	0.9996	57.8	43.9
Log logistic	0	0.9996	56.4	33.0
Multistage	0.06	0.9722	49.1	34.0
Probit	0	0.9997	55.8	40.9
Log probit	0	0.9996	53.1	31.5
Quantal linear	2.51	0.2849	35.1	20.6
Quantal quadratic	0.71	0.7021	41.8	32.0
Weibull	0	0.9997	56.7	33.6

Given that metabolized dose metrics did not yield better fits or substantially different estimates of $BMDL_{10}$ than the lifetime time-weighted average (LTWA) dose, the derivation of the cancer slope factor employed $BMDL_{10}$ estimates based on LTWA dose. The $BMDL_{10}$s in animals were converted to a human equivalent dose based on a ratio of body weights to the ¾ power (Table 59). A geometric mean of the four human equivalent $BMDL_{10}$s (4.9 mg/kg-day) was derived $(1 \times 7 \times 28 \times 3)^{1/4}$. The cancer slope factor or cancer potency is equal to 0.1 ÷ mean human equivalent $BMDL_{10}$, which is 0.1/4.9 mg/kg-day = 0.02 $(\text{mg/kg-day})^{-1}$. The potency or slope factor is used to calculate theoretical lifetime excess cancer risk for any lifetime low dose, e.g., Risk (unitless) = Dose (mg/kg-d) x Potency $(\text{mg/kg-d})^{-1}$.

Table 59. Summary Data for Chloroform Cancer Dose Response Assessment

Study	Species	Sex	Tumor site	$BMDL_{10\ (animal)}$ (mg/kg-day)	$BMDL_{10\ (human\ equiv)}$[a] (mg/kg-day)
NCI (1976)	Mouse	Female	Liver	7.6	1
NCI (1976)	Rat	Male	Kidney	25	7
Jorgenson *et al*. (1985)	Rat	Male	Kidney	98	28
Roe *et al.* (1979)	Mouse	Male	Kidney	20	3

[a] $BMDL_{10\ (human\ equiv)} = BMDL_{10\ (animal)} \times (\text{body weight}_{animal} \div \text{body weight}_{human})^{¼}$
Body weight of Osborne-Mendel rat = 460 grams, male ICI mouse = 29 grams, and female $B6C3F_1$ mouse = 35 grams.

Human Cancer Studies

Epidemiological studies have produced weak but consistent associations between exposure to chlorination byproducts in drinking water and cancers of the bladder, colon, rectum (e.g., Cantor *et al*., 1978; Gottlieb *et al*., 1981; Young *et al*., 1981, 1987; Zierler *et al*., 1988; McGeehin *et al*., 1993; Doyle *et al*., 1997; Hildesheim *et al*., 1998; Infante-Rivard *et al*., 2001, 2002). However, none of these studies has specifically investigated the relationship between chloroform and cancer, nor could any conclusions about chloroform be reached due to the confounding presence of numerous other potentially carcinogenic chemicals in the chlorinated drinking water. These data have been found to be inconclusive with regard to the potential carcinogenicity of chloroform by IARC (1999d), ATSDR (1997), and U.S. EPA (2001e, 2008c).

Another complicating factor is the inconsistency between the animal THM data and human epidemiological data on disinfection byproducts, the former indicating liver, kidney, and large intestine as the prime target sites, and the latter indicating bladder, colon, and rectum as target sites. However, site concordance is not assumed *a priori* in human risk assessment based on animal studies unless specific mechanistic data suggest site specificity.

Bromoform

Noncarcinogenic Effects

The experimental database for noncarcinogenic effects of bromoform consists of three subchronic studies and three chronic studies. In addition, data from two studies of developmental or reproductive toxicity were reviewed for possible use in dose response assessment. These candidate studies are summarized in Table 60.

Table 60. Candidate Studies for Dose Response Assessment of the Noncarcinogenic Effects of Bromoform

Reference	Species, sex	N	Dose	Route	Exposure duration	NOAEL (mg/kg-day)	LOAEL (mg/kg-day)
Subchronic Studies							
Chu *et al*. (1982b)	Rat M, F	20	Male 0, 0.65, 6.1, 57, 218 Female 0, 0.64, 6.9, 55, 283	Drinking water	90 days	57	218 hepatic lesions in males
NTP (1989a)	Rat M, F	10	0, 12, 25, 50, 100, 200	Gavage corn oil	13 weeks 5 days/week	25	50 hepatic vacuolization in males
NTP (1989a)	Mouse M, F	10	0, 25, 50,100, 200, 400	Gavage corn oil	13 weeks 5 days/week	100	200 hepatic vacuolization in males
Chronic Studies							
Tobe *et al*. (1982)	Rat M, F	40	Male 0, 22, 90, 364 Female 0, 38, 152, 619	Diet	24 months	22	90 serum chemistry changes, gross liver lesions
NTP (1989a)	Rat M, F	50	0, 100, 200	Gavage corn oil	103 weeks 5 days/week	--	100 decreased body weight.; liver histopathology
NTP (1989a)	Mouse M, F	50	Male 0, 50, 10 Female 0, 100, 200	Gavage corn oil	103 weeks 5 days/week	-	100 decreased body weight, mild liver histopathology in females
Reproductive Studies							
Ruddick *et al*. (1983)	Rat F	9-14	0, 50, 100, 200	Gavage corn oil	Gestation days 6 - 15	100	200 sternebral variations
NTP (1989b)	Mouse	50	0, 50, 100, 200	Gavage	105 days	100	200

Reference	Species, sex	N	Dose	Route	Exposure duration	NOAEL (mg/kg-day)	LOAEL (mg/kg-day)
	M, F			corn oil			decreased F_1 postnatal survival; organ weight changes, and liver histopathology

The results from a subchronic study conducted in F344/N rats (NTP, 1989a) were selected for quantification of noncarcinogenic effects. This study was selected because the LOAEL was the lowest among the candidate studies, it included an adequate number and range of dose groups and it examined more sensitive toxic endpoints than the study of Tobe *et al*. (1982). The chronic NTP (1989a) study was not used because it employed higher doses that yielded a near 100 percent response (hepatic vacuolization) at the doses tested. However, the consistency in the findings of hepatic vacuolization in the chronic and subchronic studies is notable.

A NOAEL and a LOAEL of 25 mg/kg-day and 50 mg/kg-day, respectively, were identified, based on hepatic vacuolization in the liver of male rats (NTP, 1989a). The NTP results were also assessed using a benchmark dose approach. Available models in the U.S. EPA (1995b, 2000a) BMDS program (Version 1.3.1) were fit to the hepatic vacuolization incidence data in male rats. BMDS modeling results were evaluated according to the criteria outlined in U.S. EPA (2000a). Almost all of the models yielded acceptable fits (Table 61). All but one of the models yielded nearly identical estimates of BMD_{10}, ranging from 6 to 11 mg/kg-day, and $BMDL_{10}$, ranging from 3 to 7 mg/kg-day.

Table 61. Dose Response for Bromoform-induced Hepatic Vacuolization in Male Fischer 344/N Rats

Model	Chi-square	P value	AIC	BMD_{10}[a] (mg/kg-day)	$BMDL_{10}$[b] (mg/kg-day)
Gamma	2.18	0.7033	65.87	6.2	3.6
Logistic	2.48	0.6478	66.14	10.2	6.6
Log logistic	2.40	0.4937	68.44	6.2	1.3
Multistage	2.19	0.5334	67.85	6.8	3.7
Probit	2.45	0.6536	66.09	10.9	7.4
Log probit	2.71	0.6076	66.48	11.1	5.9
Quantal linear	2.18	0.7033	65.87	6.2	3.6
Quantal quadratic	3.45	0.4857	67.15	27.8	19.0
Weibull	2.18	0.7033	65.87	6.2	3.6

[a]BMD_{10} = maximum likelihood estimate of the dose producing a 10 percent extra risk of hepatic vacuolization
[b]$BMDL_{10}$ = lower 95 percent CI on the BMD_{10}

Carcinogenic Effects

The experimental database for bromoform carcinogenicity consists of three bioassays. The results of a well-conducted two-year gavage study of bromoform in F344/N rats (NTP, 1989a) were selected for quantification of carcinogenic effects. The two-year study in $B6C3F_1$ mice (NTP, 1989a) was not used because it did not identify treatment-related tumors. The data of Theiss *et al*. (1977) were considered unacceptable for risk assessment based on the use of an injection route of administration and lack of a clear dose response relationship for tumorigenicity.

To determine which exposure measure is most useful to determine a dose response relationship for bromoform, the same methodology that was used for chloroform was applied, for the combined tumors in large intestine. The PBPK rat model of da Silva *et al.* (1999a) was used to yield the time-weighted metabolized dose. Because total metabolized dose metric did not yield a better fit to the tumor data than the applied dose, an LTWA dose metric was utilized in all subsequent bromoform dose response analyses.

Cancer slope factors were determined for bromoform using U.S. EPA (1995b, 2000a) BMDS. Models were fitted to combined incidence of adenomatous polyps and adenocarcinomas in the large intestine of female F344/N rats exposed to bromoform (Table 24). BMDS modeling results were evaluated according to the criteria outlined in U.S. EPA (2000a). Acceptable fits were obtained with several models with BMD_{10}s from 90 to 111 mg/kg-day and $BMDL_{10}$s from 54 to 98 mg/kg-day (Table 62). Four of the models yielded near identical estimates of $BMDL_{10}$ (≅ 68 mg/kg-day).

Table 62. Dose Response for Bromoform Combined Incidence of Adenomatous Polyps and Adenocarcinomas in the Large Intestine of Female Fischer 344/N Rats

Model	Chi-square	P value	BMD_{10}[a] (mg/kg-day)	$BMDL_{10}$[b] (mg/kg-day)
Gamma	0	1	109.2	69.1
Quantal linear	1.72	0.4239	89.7	54.2
Logistic	0.16	1	119.1	98.0
Log logistic	0	1	110.1	69.1
Probit	0.07	0.7846	115.3	93.0
Log probit	0	1	107.5	76.8
Quantal quadratic	0.33	0.8483	103.9	80.8
Multistage	0.33	0.8483	103.9	65.0
Weibull	0	1	110.9	69.1

[a]BMD_{10} = maximum likelihood estimate of the dose producing a 10 percent extra risk of adenomatous polyps and adenocarcinomas
[b]$BMDL_{10}$ = lower 95 percent CI on the BMD_{10}

A linear approach to extrapolating estimates of human cancer risk at low doses is

recommended, because there is evidence that bromoform has genotoxicity and there is insufficient evidence of a mode of action that supports a nonlinear dose response relationship. The $BMDL_{10}$ (68 mg/kg-day) is converted to a human equivalent dose of 16 mg/kg-day using a body weight to the ¾ power scaling factor[4] Linear extrapolation from the human equivalent $BMDL_{10}$ of 16 mg/kg-day to the origin results in a cancer slope factor of 0.006 per mg/kg-day (calculated as 0.1 ÷ $BMDL_{10}$ human).

Bromodichloromethane

Noncarcinogenic Effects

The findings of three chronic and seven developmental or reproductive toxicity studies were considered for dose response assessment of noncarcinogenic effects of BDCM, as summarized in Table 63. Results from the subchronic study by Chu *et al*. (1982b) were not considered because of inadequate verification of the concentrations of BDCM in drinking water and reporting of histopathological data.

Table 63. Candidate Studies for Dose Response Assessment of the Noncarcinogenic Effects of BDCM

Reference	Species, strain, sex	N	Dose (mg/kg-day)	Route	Exposure duration	NOAEL (mg/kg-day)	LOAEL (mg/kg-day)
Chronic Studies							
NTP (1987)	Rat F344/N M, F	50	0, 50, 100	Gavage Oil	102 weeks 5 days/week	-	50 lesions of kidney and liver
NTP (1987)	Mouse $B6C3F_1$ M, F	50	0, 25, 50	Gavage Oil	102 weeks 5 days/week	-	25 lesions of liver, kidney, and thyroid
Aida *et al*. (1992b)	Rat Wistar M, F	40	Male 0, 6, 26, 138 Female 0, 8, 32, 168	Diet	24 months	-	6 liver fatty degeneration and granuloma
Reproductive and Developmental Studies							
Narotsky *et al*. (1997)	Rat F344 F	13-14	0, 75, 100	Gavage oil water	Gestation days 6 - 15	25	50 full litter resorption
Klinefelter *et al*. (1995)	Rat F344 M	?	0, 22, 39	Drinking water	52 weeks	22	39 decreased sperm velocities
Bielmeier *et al*. (2001)	Rat F344 F		8-11	Gavage aqueous	Gestation day 9	-	75 full litter resorption
Christian *et al*. (2001a)	Rabbit F	25	0, 1.4, 13, 36, 55	Drinking water	Gestation days 6 - 29	55 development	-

[4]Scaling factor = [rat body weight ÷ human body weight]^{¼} = (0.25 kg ÷ 70 kg)^{¼} = 0.24.

Reference	Species, strain, sex	N	Dose (mg/kg-day)	Route	Exposure duration	NOAEL (mg/kg-day)	LOAEL (mg/kg-day)
Christian *et al.* (2001b)	Rat M, F	10	Variable by physiological stage	Drinking water	Males 64 days Females 74 days	50 ppm 16.3 - 41.4[a]	150 ppm 23.5 - 40.3[a] decreased pup body weight
Christian *et al.* (2001a)	Rat Sprague-Dawley F	25	0.0, 2.2, 18.4, 45.0, 82.0	Drinking water	Gestation days 6 - 21	45	82 reduced ossification occurring with maternal toxicity
Christian *et al.* (2002)	Rat Sprague-Dawley M, F	30	Variable by physiological stage	Drinking water	Two generations	50 ppm 4.1 - 12.6[a]	150 ppm 11.6 - 40.2[a] delayed sexual maturation with reduced body weight

[a]The estimated average daily dose is presented as a range because it varied widely by life stage, so a biologically meaningful average dose could not be calculated for the duration of treatment.

The chronic study in Wistar rats (Aida *et al*., 1992b) was selected for quantification of noncarcinogenic effects. The LOAEL of 6 mg/kg-day, based on liver fatty degeneration and granuloma in male rats, was the lowest among the candidate studies. Results were also assessed using the Benchmark Dose approach (U.S. EPA, 1995b, 2000a). Available BMDS models were fitted to the incidence of fatty liver at the various doses, with the highest dose censored because it yielded a one hundred percent response. Several models yielded acceptable fits for estimates of the dose that produced a 10 percent extra risk of hepatic lesions (fatty degeneration) (BMD_{10}, as shown in Table 64). Models with acceptable fits yielded essentially equivalent estimates of the BMD_{10} (1 to 3 mg/kg-day) and $BMDL_{10}$, the lower 95 percent CI on the BMD_{10} (0.6 to 1.5 mg/kg-day).

Table 64. Dose Response for Hepatic Lesions in Wistar Rats Treated with BDCM

Model	P value	Chi-square	BMD_{10}[a] (mg/kg-day)	$BMDL_{10}$[b] (mg/kg-day)
Log logistic	1	0	2.78	0.58
Multistage	1	0	1.55	0.79
Weibull	1	0	1.82	0.79
Gamma	1	0	1.97	0.79
Log probit	1	0	2.84	1.40
Quantal linear	0.8844	0.25	1.21	0.78

[a]BMD_{10} = maximum likelihood estimate of dose producing a 10% extra risk of hepatic lesions
[b]$BMDL_{10}$ = Lower 95 percent CI on the BMD_{10}

Carcinogenic Effects

The experimental database for BDCM carcinogenicity consists of eight oral bioassays. Data obtained in the two-year gavage studies of BDCM in F344/N rats and $B6C3F_1$ mice (NTP, 1987) were selected for quantification of carcinogenic effects.

To determine which measure of exposure is most appropriate for establishing a dose response relationship for tumors in the rat, the PBPK rat model of Lilly *et al*. (1998) was applied to the male and female rat kidney tumors (NTP, 1987). The model incorporated a four compartment gastrointestinal tract submodel for pulsed delivery of BDCM to the liver. All of the dose metrics (lifetime time weighted average applied dose, total metabolized dose, and kidney metabolized dose) gave adequate fits ($p < 0.05$) to the quantal tumor response in the rat kidney by quantal probit regression. The total metabolized dose metric gave inadequate fits ($p < 0.05$) when applied to the large intestine tumors in male and female rats. Because the metabolized dose metric did not yield better dose response relationships than use of the applied dose, a LTWA dose metric was utilized in all subsequent dose response analyses for BDCM.

Data obtained in the two-year gavage studies of BDCM in F344/N rats and $B6C3F_1$ mice (NTP, 1987) were selected for calculation of carcinogenic effects. Cancer slope factors were determined using U.S. EPA (1995b, 2000a) BMDS software to estimate the BMD_{10}. BMDS models were fitted to combined incidence of adenoma and adenocarcinomas in the large intestine of male F344/N rats (Table 65) or combined hepatocellular adenomas and carcinomas in female $B6C3F_1$ mice (Table 66) because these two sites had the greatest tumor incidences.

Table 65. Dose Response for Combined Adenomas and Adenocarcinomas in the Large Intestine of Male Fischer 344/N Rats Treated with BDCM

Model	Chi-square	P value	BMD_{10}[a] (mg/kg-day)	$BMDL_{10}$[b] (mg/kg-day)
Gamma	0	0.9999	27.0	20.7
Logistic	0.70	0.4043	25.6	20.0
Log logistic	0	1	27.7	22.3
Multistage	3.55	0.1696	17.1	12.5
Probit	0.27	0.6032	25.1	19.0
Log probit	0	1	28.1	22.9
Quantal quadratic	3.55	0.1696	17.1	15.2
Weibull	0	1	24.7	18.1

[a]BMD_{10} = maximum likelihood estimate of the dose producing a 10 percent extra risk of adenomas and adenocarcinomas
[b]$BMDL_{10}$ = lower 95 percent CI on the BMD_{10}

Table 66. Dose Response for Combined Hepatocellular Adenomas and Carcinomas in Female B6C3F$_1$ Mice Treated with BDCM

Model	Chi-square	P value	BMD_{10}[a] (mg/kg-day)	$BMDL_{10}$[b] (mg/kg-day)
Gamma	0	0.9698	13.7	10.5
Multistage	0	0.9698	13.7	10.5
Probit	2.09	0.1482	27.5	22.7
Log probit	0.23	0.6320	24.1	19.0
Quantal linear	0	0.9698	13.7	10.5
Weibull	0	0.9698	13.7	10.5

[a]BMD_{10} = maximum likelihood estimate of the dose producing a 10 percent extra risk of hepatic adenomas and carcinomas
[b]$BMDL_{10}$ = lower 95 percent CI on the BMD_{10}

BMDS models with acceptable fits for the rat intestinal tumors yielded similar estimates of BMD_{10} (ranging from 17 to 28 mg/kg-day) and $BMDL_{10}$ (ranging from 12 to 23 mg/kg-day). The models with acceptable fits to the mouse combined hepatocellular adenomas and adenocarcinomas yielded similar estimates of BMD_{10} (ranging from 14 to 28 mg/kg-day) and $BMDL_{10}$ (ranging from 10 to 23 mg/kg-day). Cancer slope factors were derived from $BMDL_{10}$s of 20 and 10 (mg/kg-day) for male rat intestinal tumors and tumors of the liver in female mice, respectively, because six of the models yielded similar results for intestinal tumors in the male rat and four of the models yielded similar results for female mouse liver tumors. The rat and mouse $BMDL_{10}$ were converted to a human equivalent dose (Table 67), using the scaling relationship of (human body weight ÷ rodent body weight)^{¾}.

Table 67. Summary Data for BDCM Cancer Dose Response Assessment

Species	Sex	Tumor site	$BMDL_{10}$ (mg/kg-day)[1]	Adjusted $BMDL_{10}$ human equivalent (mg/kg-day)[1]	Cancer slope factor $(mg/kg/day)^{-1}$
Rat	Male	Large intestine	20	5.4 [a]	0.02
Mouse	Female	Liver	10	1.6 [b]	0.06

[a]converted to a human equivalent dose using the relationship [reference rodent body weight (0.38 kg) ÷ reference human body weight (70 kg)]$^{1/4}$
[b]converted to a human equivalent dose using the relationship [reference rodent body weight (0.035 kg) ÷ reference human body weight (70 kg)]$^{1/4}$

Dibromochloromethane

Noncarcinogenic Effects

The experimental database for noncarcinogenic effects of DBCM consists of four subchronic studies and three chronic studies, summarized in Table 68. In addition, data from three studies of developmental or reproductive toxicity were reviewed for possible use in dose response assessment.

Table 68. Candidate Studies for Dose Response Assessment of the Noncarcinogenic Effects of DBCM

Reference	Species	Route	Sex	N	Duration	Dose (mg/kg-day)	NOAEL (mg/kg-day)	LOAEL (mg/kg-day)
Subchronic Studies								
Chu *et al*. (1982b)	Rat Sprague-Dawley	Drinking water	M	20	90 days	0, 0.57, 6.1, 49, 224	49	224 hepatic lesions
			F	20	90 days	0, 0.69, 7.5, 59, 237	59	237 hepatic lesions
NTP (1985)	Rat F344/N	Gavage corn oil	M, F	10	13 weeks	0, 15, 30, 60, 125, 250	30	60 hepatic lesions in males
	Mouse $B6C3F_1$	Gavage corn oil	M, F	10	13 weeks	0, 15, 30, 60, 125, 250	125	250 hepatic and renal lesions in males
Daniel *et al*. (1990)	Rat Sprague-Dawley	Gavage corn oil	M, F	10	90 days	0, 50, 100, 200	-	50 hepatic lesions in males and renal lesions in females
Chronic Studies								
Tobe *et al*. (1982)	Rat Wistar SPF	Diet	M	40	2 years	0, 12, 49, 196	12	49 decreased body weight gain
			F	40	2 years	0, 17, 70, 278	17 Fischer	70 decreased body weight gain
NTP (1985)	Rat F344	Gavage corn oil	M, F	50	2 years	0, 40, 80	--	40 hepatic lesions
	Mouse B6C3F1	Gavage corn oil	M, F	50	105 weeks	0, 50, 100	--	50 hepatic and thyroid lesions

Data from a subchronic study in male and female F344/N rats (NTP, 1985) were selected for quantification of noncarcinogenic effects. Rats were gavaged with 0, 15, 30, 60, 125, or 250 mg/kg-day in corn oil five days/week for 13 weeks. The NOAEL and LOAEL of 30 and 60 mg/kg-day were based on vacuolar change (fatty metamorphosis) in the liver of male rats (Table 69). Adjusted to a continuous dose basis, the NOAEL and LOAEL values are 21.4 and 42.8 mg/kg-day. Selection of this study is supported by the number and range of tested doses compared to the chronic studies (Tobe *et al*., 1982; NTP, 1985), more sensitive endpoints compared to Tobe *et al*. (1982), and more thorough documentation of administered doses than Chu *et al*. (1982b).

Table 69. Hepatic Vacuolar Change in Male Fischer 344 Rats Administered DBCM in a 13 Week Corn Oil Gavage Study (NTP, 1985)

Dose (mg/kg-day)	Frequency
0	4/10
15	7/10
30	8/10
60	10/10
125	10/10
250	10/10

For comparison, the dose response relationship for histopathological changes in the livers of male rats was also assessed using a BMD approach (U.S. EPA, 1995b, 2000a). Nine BMDS models fit to the incidence data in Table 69 gave estimates of the BMD_{10} for hepatic lesions (Table 70) of 2.1 to 8.9 mg/g-day, all with acceptable fits to the data ($p > 0.1$). The $BMDL_{10}$ values generated by the models were similar, ranging from 1.3 to 2.1 mg/kg-day; seven of the $BMDL_{10}$ values were essentially identical.

Table 70. BMD Modeling of Hepatic Lesion Incidence in Wistar Rats Given DBCM

Model	P value	BMD_{10}[a] (mg/kg-day)	$BMDL_{10}$[b] (mg/kg-day)
Gamma	0.900	5.5	1.3
Logistic	0.974	3.7	2.1
Log logistic	0.804	7.7	0.60
Multistage	0.936	4.0	1.3
Probit	0.983	3.6	2.3
Log probit	0.851	7.6	2.2
Quantal linear	0.958	2.1	1.2
Quantal quadratic	0.949	8.6	5.9
Weibull	0.916	5.4	1.3

[a]BMD_{10} = maximum likelihood estimate of dose for 10 percent extra risk of hepatic lesions
[b]$BMDL_{10}$ = lower 95 percent CI on the BMD_{10}

Carcinogenic Effects

The experimental database for DBCM carcinogenicity consists of two oral bioassays conducted in rats and mice (NTP, 1985) using corn oil gavage. The study in male and female F344/N rats did not identify compound-related tumors in either sex. The study conducted in $B6C3F_1$ mice observed a statistically significant increase in the incidence of hepatocellular carcinomas in high dose males, hepatocellular adenomas in high-dose females, and in combined adenomas and carcinomas in both sexes. Hepatocellular adenomas occurred in 14/50 control and 10/50 high dose male mice; hepatocellular carcinomas occurred in 10/50 control and 19/50 high dose males; survival was too poor for tumor quantitation in low-dose males due to a dosing error. Hepatocellular adenomas occurred in 2/50 control, 4/49 low dose and 11/50 high dose female mice; hepatocellular carcinomas occurred in 4/50 control, 6/49 low dose and 8/50 high dose female mice. The incidence of liver neoplasms in the high dose was significantly greater than controls ($p = 0.004$, pairwise comparisons; $p = 0.003$ trend test).

Voronin *et al*. (1987b) evaluated carcinogenicity of DBCM in drinking water in CBA × C57B1 mice, with treatments up to 400 ppm for 104 weeks. No tumor occurred at increased incidence in the treated mice. However, this study was poorly reported (IARC, 1991c).

Table 71. Carcinogenicity Studies of DBCM Administered Orally to Mice or Rats

Study	Applied dose	Tumor site and type	Incidence	Significance
NTP (1985) Male B6C3F1 mice	0, 50, 100 mg/kg-day	hepatocellular adenoma; carcinoma; neoplasms; neoplasms (adjusted) Lymphoma	14/50, 5/50, 10/50; 10/50, 9/50, 19/50; 23/50, 14/50, 27/50; 26/50, 38/50, 37/50 9/50, 4/50, 0/50	accidental overdose in mid dose (50 mg/kg) group $p = 0.03$ decrease $p = 0.06$
NTP (1985) Female B6C3F1 mice	0, 50, 100 mg/kg-day	Liver, adenoma; carcinoma; neoplasms	2/50, 4/49, 11/50; 4/50, 6/49, 8/50; 6/50, 10/49, 19/50	$p = 0.003$ $p = 0.003$
Voronin *et al*. (1987b) M, F CBA×C57B1 mice	0, 0.04, 4.0, 400 mg/L in drinking water	No increase in tumor rates		
NTP (1985) M, F F344 rats	0, 40, 80 mg/kg-day	No increase in tumor rates		

Dose response relationships were determined for DBCM (based on LTWA dose) using the U.S. EPA (1995b, 2000a) BMDS software. Available models were fitted to combined incidence of liver adenomas and carcinomas in female $B6C3F_1$ mice (NTP, 1985). Four of the models yielded statistically acceptable fits of the LTWA doses to the liver tumors of female mice (Table 72). However, upon visual inspection, the quantal linear model was observed to yield an inferior dose-response relationship compared to the other three models. The logistic and probit models yielded similar $BMDL_{10}$ estimates that were slightly more conservative than the $BMDL_{10}$ estimate yielded by the quantal quadratic model. The $BMDL_{10}$ estimate generated by the logistic and probit models, 24 mg/kg-day, was then employed to calculate an equivalent human dose. The human equivalent $BMDL_{10}$ was derived based on a ratio of body weight to the ¾ power, $(0.035kg \div 70\ kg)^{1/4} = 0.1495$. Human equivalent $BMDL_{10} = BMDL_{10}$ (mouse) × $(0.035kg \div 70\ kg)^{1/4} = 3.6$ (mg/kg-day). The cancer slope factor = 0.1 ÷ 3.6 (mg/kg-day) = 0.03 $(mg/kg/day)^{-1}$.

Table 72. Modeling of Applied Dose and Combined Incidence of Liver Adenomas and Carcinomas in Female $B6C3F_1$ Mouse Treated with DBCM (NTP, 1985).

Model	Chi-square	P value	BMD_{10}[a] (mg/kg-day)	$BMDL_{10}$[b] (mg/kg-day)
Logistic	0.07	0.7853	31.7	24.6
Probit	0.12	0.7289	30.3	23.3
Quantal linear	0.58	0.4477	23.7	15.1
Quantal quadratic	0.02	0.8810	39.1	30.7

[a] BMD_{10} = maximum likelihood estimate of the dose producing a 10 percent extra risk of liver adenomas and carcinomas
[b] $BMDL_{10}$ = lower 95 percent CI on the BMD_{10}

CALCULATION OF PROPOSED PHGS

Noncarcinogenic Effects

Calculation of a public health protective concentration (C, in mg/L) for a chemical in drinking water for noncarcinogenic endpoints follows the general equation:

$$C = \frac{NOAEL/LOAEL \times BW \times RSC}{UF \times DWC} = mg/L$$

where,

NOAEL/LOAEL = no observed adverse effect level or lowest observed adverse effect level;

BW	=	body weight (a default of 70 kg for adult male, 60 kg for adult female, or 10 kg for a child);
RSC	=	relative source contribution (default of 20 percent, with most values in the range of 20 to 80 percent; U.S. EPA, 1989a);
UF	=	uncertainty factors (default values of 10 each for interspecies extrapolation, human variability, use of subchronic data, use of a LOAEL, and an incomplete database have been commonly used);
DWC	=	daily water consumption rate (default values of 2.0 L/day for 60 to 70 kg adult and 1.0 L/day for 10 kg child, although higher values of L equivalents [L_{eq}/day] can be used to account for inhalation and dermal exposure through other household uses of tap water.

In selecting the uncertainty factors for chronic effects, it is customary to apply an uncertainty factor in cases where adequate data are not available from full lifetime exposures and data from short-term or subchronic exposures of animals must be used. An uncertainty factor of up to 10 is recommended for interspecies extrapolation of effects seen in experimental animals to humans to account for potential differences in the response of humans and animals to a chemical exposure.

Exposed individuals are known to vary in response to toxic chemicals and drugs as a result of age, disease state, and genetic constitution (e.g., polymorphisms in metabolizing enzymes). An uncertainty factor of up to 10 for human variability is considered prudent based on the exposure of the general population to chemicals in drinking water.

An additional uncertainty factor of 10 could be applicable to account for carcinogenicity. OEHHA has previously applied an additional factor of 10 for other PHGs in situations where either a nonlinear dose extrapolation was applied to a carcinogen or where both linear and nonlinear approaches were used. Current OEHHA (and U.S. EPA) policy is to apply a maximum of 3,000 for combined uncertainty factors, except in extraordinary circumstances.

A relative source contribution value of 80 percent was selected for calculation of the proposed health protective concentration for each of the four THMs. This value represents the percentage of total exposure that could be attributable to drinking water. The use of the 80 percent value reflects the conclusion that most exposure to these compounds will occur as a result of THM formation during disinfection of drinking water. Although the available data suggest that exposure from other environmental media is relatively low, use of 80 percent is considered prudent given the uncertainty regarding exposure from media other than disinfected tap water.

Carcinogenic Effects

For carcinogens, the following general equation can be used to calculate the public health protective concentration (C) for a chemical in drinking water (in mg/L):

$$C = \frac{R \times BW}{CSF \times DWC} = mg/L$$

where,

R	=	*de minimis* level for lifetime excess individual cancer risk, a default of 10^{-6} (not more than one excess cancer case in a million people exposed for a lifetime);
BW	=	adult body weight, a default of 70 kg;
CSF	=	cancer slope factor derived from the lower 95 percent confidence interval on the 10 percent tumor dose (LED_{10}), CSF = 0.1 ÷ $LED_{10,}$ converted to human equivalent (in $[mg/kg\text{-}day]^{-1}$) using $BW^{3/4}$ scaling;
DWC	=	drinking water consumption rate, a default of 2 L/day for ingestion; higher values may be used to account for inhalation or dermal exposure, expressed as L_{eq}/day.

Chloroform

Noncarcinogenic Effects

The most sensitive noncancer endpoint for chloroform was a LOAEL of 12.9 mg/kg-day (15 mg/kg-day × 6/7 days/week for hepatic effects (fatty lesions) in dogs exposed for over seven years (Heywood *et al*., 1979). An uncertainty factor of 1,000 was applied to the LOAEL for this effect, including a factor of 10 to extrapolate from dogs to humans, 10 to protect sensitive human subpopulations, and 10 for use of a LOAEL. No factors were needed for study duration or database because the critical study was chronic duration and the database for chloroform is extensive.

A relative source contribution of 80 percent was selected for calculation of a health-protective concentration. This value reflects the conclusion that most exposure to this compound will occur as a result of its formation during disinfection of drinking water.

Based on data for water consumption by the general population in the Western Region of the U.S. (OEHHA, 2000), a water ingestion amount of 2.0 L/day was assumed. The contributions from inhalation and dermal exposure to chloroform in tap water in the home were 0.5 L_{eq}/day and 0.5 L_{eq}/day, respectively (see Exposure Section). Adding up exposure by the different routes leads to a total drinking water consumption of 3.0 L_{eq}/day for chloroform.

Using these values, a public health protective concentration for noncarcinogenic effects of chloroform can be calculated as:

$$C = \frac{12.9\ mg/kg\text{-}day \times 70\ kg \times 0.80}{1{,}000 \times 3.0\ L_{eq}/day} = 0.24\ mg/L = 200\ \mu g/L\ (ppb)\ (rounded)$$

Alternatively, a BMD_{10} of 1.0 mg/kg-day (see Dose Response Assessment section) and an uncertainty factor of 100 could be used, based on a factor of 10 to extrapolate from dogs to humans and 10 to protect sensitive human subpopulations. This calculation yields the equivalent health protective concentration:

$$C = \frac{1.0 \text{ mg/kg-day} \times 70 \text{ kg} \times 0.80}{100 \times 3.0 \text{ L}_{eq}\text{/day}} = 0.19 \text{ mg/L} = 200 \text{ µg/L (ppb) (rounded)}$$

Thus, it is estimated that a health protective level of 200 ppb of chloroform in drinking water would be protective against all noncancer effects, including potential effects in sensitive subpopulations. Because the concentration to protect against carcinogenicity of chloroform is lower than this value, the proposed PHG is based on the cancer calculation.

Carcinogenic Effects

As noted in the Dose Response Assessment section, the calculation of a health protective concentration of chloroform in drinking water for carcinogenic effects is based on animal studies rather than on the human epidemiological data. The cancer slope factor was based on an increase in incidence of tumors in female mouse liver (NCI, 1976) and in kidneys of male rats (NCI, 1976; Jorgenson *et al*., 1985) and male mice (Roe *et al.*, 1979). A geometric mean of the human equivalent BMD_{10}s from the four studies was employed to derive a slope factor of 0.02 $(\text{mg/kg-day})^{-1}$ for chloroform. A drinking water ingestion rate of 2.0 L/day is used plus the contribution from inhalation and dermal exposures via showering, bathing and other household uses of tap water, which is equivalent to another 1.0 L_{eq}/day. The health-protective calculation is thus:

$$C = \frac{70 \text{ kg} \times 10^{-6}}{0.02 \ (\text{mg/kg-day})^{-1} \times 3.0 \text{ L}_{eq}\text{/day}} = 1.2 \times 10^{-3} \text{ mg/L} = 1.0 \text{ µg/L (rounded)}$$

This value of 0.001 mg/L or 1.0 µg/L or 1.0 ppb is lower and more health-protective than the non-cancer value, and therefore is proposed as the PHG for chloroform. The corresponding values at 10^{-5} and 10^{-4} risk would be 10 and 100 µg/L or ppb, respectively. The proposed PHG based on cancer would also be protective against all noncancer effects and is judged to be adequately protective of sensitive populations..

Bromoform

Noncarcinogenic Effects

The most robust data available for calculation of the bromoform non-cancer level were from a 13 week subchronic study in F344 rats. Results from a chronic bioassay in F344/N rats were available but a NOAEL was not determined in this study. A NOAEL

of 25 mg/kg-day was identified based on the occurrence of hepatic vacuolization in the 13 week rat study (NTP, 1989a).

An uncertainty factor of 1,000 is applied to this critical effect, including a factor of 10 to extrapolate from a subchronic study, 10 for extrapolation from rats to humans, and 10 to protect sensitive human subpopulations. Use of an uncertainty factor of 10 for interspecies differences is considered appropriate because there are no human toxicokinetic data for bromoform and only limited data in animals. Because little is known about individual variation in bromoform metabolism and toxicity, the use of an uncertainty factor of 10 for human variability is considered prudent based on the widespread exposure of the general population to disinfected tap water.

A relative source contribution value of 80 percent was selected to reflect the conclusion that most exposure to this compound will occur as a result of bromoform formation during disinfection of drinking water. Based on data for water consumption by the general population in the Western Region of the U.S. (OEHHA, 2000), a drinking water ingestion amount of 2.0 L/day was assumed. Contributions from inhalation and dermal exposure to bromoform in tap water in the home are estimated as 0.4 L_{eq}/day and 0.7 L_{eq}/day, respectively (see Exposure section), which leads to a total drinking water consumption of 3.1 L_{eq}/day for bromoform.

Using these values, the health-protective level for noncarcinogenic effects is calculated as follows:

$$C = \frac{25 \text{ mg/kg-day} \times 70 \text{ kg} \times 0.80}{1{,}000 \times 3.1 \text{ L}_{eq}\text{/day}} = 0.45 \text{ mg/L} = 450 \text{ ppb}$$

Therefore, a health-protective level (C) for non-carcinogenic effects of bromoform based on the rat NOAEL is estimated to be 0.45 mg/L, or 450 ppb.

For comparison, a health protective concentration was also calculated using the BMD_{10} of 4 mg/kg-day obtained using the U.S. EPA BMDS program (see Dose-Response Assessment section). A composite uncertainty factor of 1,000 was used, which includes the same factors discussed above. The values used for body weight, the relative source contribution, and water intake were identical to those used in the NOAEL/LOAEL approach. The health protective level for noncarcinogenic effects can then be calculated as follows:

$$C = \frac{4 \text{ mg/kg-day} \times 70 \text{ kg} \times 0.80}{1{,}000 \times 3.1 \text{ L}_{eq}\text{/day}} = 0.07 \text{ mg/L} = 70 \text{ ppb}$$

The health protective level of 0.07 mg/L, or 70 ppb, obtained using the BMD_{10} approach is approximately six-fold lower than that obtained using the subchronic NOAEL. Because the concentration to protect against carcinogenicity of bromoform is lower than this value, the proposed PHG is based on the cancer calculation.

Carcinogenic Effects

As noted in the Dose Response Assessment section, the calculation of a health protective concentration of bromoform in drinking water for carcinogenic effects is based on tumors observed in animal studies rather than on the human epidemiological studies. The most sensitive cancer endpoint for bromoform was an increase in the combined incidence of adenomatous polyps and adenocarcinomas in the large intestine of female F344/N rats (NTP, 1998a). The water concentration equivalent to a negligible lifetime risk of 10^{-6} was calculated using the cancer slope factor obtained from the U.S. EPA BMDS software. The health protective concentration was calculated using oral data only, as no data on the carcinogenicity of bromoform via the inhalation route are available. The health-protective value can thus be calculated as follows:

$$C = \frac{70 \text{ kg} \times 10^{-6}}{0.005 \text{ (mg/kg-day)}^{-1} \times 3.1 \text{ L}_{eq}\text{/day}} = 0.005 \text{ mg/L} = 5 \text{ ppb}$$

This value of 0.005 mg/L or 5 μg/L or 5 ppb derived from the linear cancer extrapolation model and a *de minimis* 10^{-6} lifetime theoretical cancer risk is proposed as the PHG for bromoform. The corresponding values at 10^{-5} and 10^{-4} risk would be 50 and 500 μg/L or ppb, respectively. The proposed PHG based on cancer would also be protective against all noncancer effects and is judged to be adequately protective of sensitive populations.

Bromodichloromethane

Noncarcinogenic Effects

The most robust data available for calculation of the public health protective concentration of BDCM in drinking water are from a 24-month chronic study in Wistar rats. A LOAEL of 6 mg/kg-day was identified based on hepatic lesions in the male rats (Aida *et al*., 1992b). None of the chronic studies available for BDCM identified a NOAEL (i.e., in each case potentially adverse effects were observed at the lowest dose tested). Inclusion of a total uncertainty factor of 1,000 based on factors of 10 for the use of a LOAEL, for interspecies differences, and for human variability was considered prudent for protection of human health.

A relative source contribution value of 80 percent was selected for calculation of the proposed health protective concentration to address potential nontap water sources of exposure to BDCM, including outdoor air and food. The 80 percent value reflects the conclusion that most exposure to this compound will occur as a result of BDCM formation during disinfection of drinking water.

Based on data for water consumption by the general population in the Western Region of the U.S. (OEHHA, 2000), a drinking water ingestion amount of 2.0 L/day was assumed. The contributions from inhalation and dermal exposure to BDCM in tap water in the home were 0.5 L_{eq}/day and 0.6 L_{eq}/day, respectively (see Exposure section), which yield a combined drinking water consumption of 3.1 L_{eq}/day for BDCM.

Using these values, the health protective concentration for noncarcinogenic effects is calculated as:

$$C = \frac{6\ \text{mg/kg-day} \times 70\ \text{kg} \times 0.80}{1{,}000 \times 3.1\ L_{eq}/\text{day}} = 0.11\ \text{mg/L} = 100\ \text{ppb (rounded)}$$

Therefore, a health-protective level (C) based on a LOAEL for non-carcinogenic effects of BDCM is estimated to be 0.1 mg/L, or 100 ppb. This level is not considered to protect the public against the carcinogenic effects of BDCM.

For comparison, a health protective concentration was also calculated using the BMD_{10} of 0.8 mg/kg-day obtained using the U.S. EPA BMDS program, as shown in the Dose Response Assessment section. A composite uncertainty factor of 100 was used, which includes a factor of 10 for extrapolation of data from animals to humans and a factor of 10 for individual human variability. The other values were identical to those used in the traditional NOAEL/LOAEL approach. The health protective concentration for noncarcinogenic effects can then be calculated as:

$$C = \frac{0.8\ \text{mg/kg-day} \times 70\ \text{kg} \times 0.80}{100 \times 3.1\ L_{eq}/\text{day}} = 0.14\ \text{mg/L} = 100\ \text{ppb (rounded)}$$

The health protective value of 0.14 mg/L, or 100 ppb (rounded), obtained using the LED_{10} is essentially the same as the value of 100 ppb (rounded) obtained using the subchronic LOAEL. This 100 ppb level is not considered to protect the public against the potential carcinogenic effects of BDCM. Because the concentration to protect against carcinogenicity of BDCM is lower than this value, the proposed PHG is based on the cancer calculation.

Carcinogenic Effects

As noted in the Dose Response Assessment section, the calculation of a health protective concentration of BDCM in drinking water for carcinogenic effects is based on tumors observed in animal studies rather than on the human epidemiological studies. The health protective concentration equivalent to a negligible lifetime risk of 10^{-6} was calculated using the cancer slope factor obtained using the U.S. EPA BMDS (1995b, 2000a) software. As summarized in Table 67, cancer slope factors were calculated both for combined incidence of adenoma and adenocarcinomas in the large intestine of male F344/N rats (Table 65) and for combined hepatocellular adenomas and carcinomas in female $B6C3F_1$ mice (Table 66) obtained from the NTP (1987) two-year gavage studies of BDCM. The mouse data yielded the higher cancer slope factor and thus the lower health protective value. Using the human equivalent cancer slope factor derived from the BMD_{10} from liver tumors in female $B6C3F_1$ mice, the health protective value for protection against carcinogenic effects of BDCM is calculated as:

$$C = \frac{70\ \text{kg} \times 10^{-6}}{0.06\ (\text{mg/kg-day})^{-1} \times 3.1\ L_{eq}/\text{day}} = 0.0004\ \text{mg/L} = 0.4\ \text{ppb}$$

This value of 0.0004 mg/L or 0.4 μg/L or 0.4 ppb derived from the linear cancer extrapolation model and a *de minimis* 10^{-6} lifetime theoretical cancer risk is proposed as the PHG for BDCM. The corresponding values at 10^{-5} and 10^{-4} risk would be 4 and 40 μg/L or ppb, respectively. The proposed PHG based on cancer effects would also be protective against all noncancer effects and is judged to be adequately protective of sensitive populations.

Dibromochloromethane

Noncarcinogenic Effects

For DBCM, the most robust data available for calculation of the proposed public health protective concentration in drinking water based on noncarcinogenic effects were from a 13-week subchronic study in F344 rats (NTP, 1985). Chronic data were available for F344/N rats and $B6C3F_1$ mice; the LOAEL value in these studies was the lowest dose tested, which was 40 and 50 mg/kg-day, respectively. A NOAEL of 30 mg/kg-day was derived based on fatty metamorphosis in liver in male F344/N rats administered DBCM five days per week for 13 weeks (NTP, 1985), which corresponds to 21.4 mg/kg-day adjusted for daily exposures. A combined uncertainty factor of 1,000 was considered appropriate to account for the use of subchronic toxicity data, for interspecies differences, and for human variability.

Based on data for water consumption by the general population in the Western Region of the U.S. (OEHHA, 2000), water ingestion of 2.0 L/day was assumed. The contributions from inhalation and dermal exposure to DBCM in tap water in the home were 0.5 L_{eq}/day and 0.7 L_{eq}/day, respectively. Adding up exposure by the different routes leads to a total drinking water exposure equivalent of 3.2 L_{eq}/day.

The public health protective concentration for noncarcinogenic effects was then calculated as follows:

$$C = \frac{21.4\ \text{mg/kg-day} \times 70\ \text{kg} \times 0.80}{1{,}000 \times 3.2\ L_{eq}/\text{day}} = 0.37\ \text{mg/L} = 400\ \text{ppb (rounded)}$$

The health-protective concentration for DBCM based on a noncancer endpoint is estimated as 0.4 mg/L or 400 ppb. This 400 ppb level is not considered to protect the public against the potential carcinogenic effects of DBCM.

For comparison, a health protective concentration was also calculated using the $BMDL_{10}$ value of 2.3 mg/kg-day for hepatic vacuolization obtained using the U.S. EPA BMDS program, as shown in the Dose Response Assessment section. This value corresponds to

1.6 mg/kg-day when adjusted to daily exposures. The other values were identical to those used in the traditional NOAEL/LOAEL approach. This calculation is as follows:

$$C = \frac{1.6 \text{ mg/kg-day} \times 70 \text{ kg} \times 0.80}{1{,}000 \times 3.2 \text{ L}_{eq}\text{/day}} = 0.03 \text{ mg/L} = 30 \text{ ppb}$$

The health-protective value of 0.03 mg/L, or 30 ppb, obtained using the $BMDL_{10}$ is approximately 10-fold lower (after rounding) than that obtained using the subchronic NOAEL above. This 30 ppb level is not considered to protect the public against the potential carcinogenic effects of DBCM. Because the concentration to protect against carcinogenicity of DBCM is lower than this value, the proposed PHG is based on the cancer calculation.

Carcinogenic Effects

As noted in the Dose Response Assessment section, calculation of health protective concentrations of DBCM in drinking water for carcinogenic effects is based on tumors observed in animal studies rather than on the human epidemiological studies. Only one DBCM data set was suitable for dose response analysis. The female mouse liver tumors from the NTP (1985) study (Table 71) were analyzed using the U.S. EPA BMDS software (Table 72). Water ingestion rate is assumed to be 2.0 L/day and the contribution from inhalation and dermal exposures via showering, bathing and other household uses of tap water is assumed to be 1.2 L_{eq}/day (see Table 9 in Exposure Assessment section). An estimated health-protective concentration of DBCM was calculated as follows:

$$C = \frac{70 \text{ kg} \times 10^{-6}}{0.03 \text{ (mg/kg-day)}^{-1} \times 3.2 \text{ L}_{eq}\text{/day}} = 0.0007 \text{ mg/L} = 0.7 \text{ ppb}$$

This value of 0.0007 mg/L or 0.7 μg/L or 0.7 ppb derived from the linear cancer extrapolation model and a *de minimis* 10^{-6} lifetime theoretical cancer risk is proposed as the PHG for DBCM. The corresponding values at 10^{-5} and 10^{-4} risk would be 7 and 70 μg/L or ppb, respectively. The proposed PHG based on cancer effects would also be protective against all noncancer effects and is judged to be adequately protective of sensitive populations.

RISK CHARACTERIZATION

Carcinogenic Effects of Trihalomethanes

Mechanistic evidence and considerations applicable to cancer risk assessments for the THMs is summarized below:

- While chloroform has generally tested negative in standard genotoxicity assays, positive results have been reported with appropriate metabolic activation, e.g., in a

mouse host-mediated assay with *Salmonella*, or in fungal systems such as *Saccharomyces cerevisiae* or *Aspergillus nidulans*. The brominated THMs are more active in several genotoxicity assays than chloroform and the findings are more compelling that a genotoxic mode of action is operative for these compounds.

- Several authors have interpreted the findings of numerous studies as supporting the hypothesis that the mode of carcinogenic action of chloroform is largely or completely due to oxidative metabolism leading to cytotoxicity and cell proliferation in the liver and probably also in the kidney. Others have also discussed the uncertainty about contributions of other non-cytotoxic mode of actions based on reductive or conjugative metabolism of chloroform. Kidney rather than liver appears to be the more relevant target organ for carcinogenicity. Andersen *et al*. (1998) and Melnick and Kohn (1998) discussed the pros and cons of the cytotoxicity mode of action. OEHHA's re-evaluation of the studies found that the evidence is not consistent with tumors in the kidney or in the liver being secondary to cytotoxicity and tissue regeneration.
- There is evidence that some chemical carcinogens may induce cancers in animals through nongenotoxic mechanisms (Klaunig *et al*., 2000). For example, dichloroacetic acid appears to be carcinogenic acting through nongenotoxic mechanisms at the lower doses of 0.5 and 1 g/L, at which genotoxicity was not observed (ILSI, 1997; Carter *et al*., 2003). Dichloroacetic acid is carcinogenic to male and female $B6C3F_1$ mouse and male F344 rat in the liver (DeAngelo *et al*., 1999). Carter *et al*. (2003) analyzed the dose response of dichloroacetic acid-induced histopathological changes in livers of male mice. Because all classes of premalignant lesions and carcinomas were found at various doses, the data are consistent with the conclusion that the carcinogenesis at lower doses must be occurring through nongenotoxic mechanisms.
- **Animal and Human Tumor Site Concordance**. Cancer bioassays of individual THMs in rodents using different dosing vehicles and treatment modalities show cancers of the liver, kidney, and large intestine. There is a lack of concordance in the site of tumors in mice and rats. Epidemiology studies of chlorinated drinking water that contains THMs and other disinfection byproducts indicate a modest but consistent increase in urinary bladder cancer risks (relative risk 1.5) for individuals consuming chlorinated water for over 35 years. The evidence for colon or rectal cancer is less consistent. There is little overall concordance between THM-induced tumor sites in rodents and human cancer epidemiology of chlorination disinfection byproducts.
- **Relative Source Contribution (RSC) for Trihalomethanes.** The maximum default RSC of 0.8 used in the calculation of the health protective water concentration for non-carcinogenic endpoints is supported by estimates of airborne exposure of California residents to THMs. DHS (1990) estimated a median chloroform concentration in ambient air of 0.06 ppb and a population-weighted concentration of 0.03 ppb for 20.3 million people. Assuming 20 m^3/day inhalation intake and a 50 percent pulmonary absorption, this represents an airborne exposure of about 1.5 to

3 μg/day. Waterborne exposure estimates from U.S. EPA (1998k) would be 15 μg/L × 3 L_{eq}/day = 45 μg/day.

- **Interspecies Extrapolation**. Due to lack of tumor site concordance, the use of PBPK models to estimate human risk based on animal tumor response data and internal dose metrics is problematic. To estimate risk of any human cancer based on the animal data, OEHHA used the interspecies scaling of body weight to the ¾ power to account for both potential pharmacokinetic and pharmacodynamic differences, i.e., differences in tumorigenic response to THM exposure. The difference between scaled and unscaled values is about four-fold for rat-based and seven-fold for mouse-based values.
- **Interactions of Trihalomethanes**. Laboratory studies indicate that some THMs can interfere with metabolism of other THMs and that their metabolism can be potentiated by other dietary or environmental agents, e.g., ethanol. It is uncertain what, if any, of these effects may occur at environmentally relevant concentrations. However, it needs to be emphasized that this risk assessment is based on individual chemicals studied under relatively controlled conditions, while human exposures to THMs occur in complex mixtures containing hundreds of identified and unidentified compounds at low environmental concentrations.
- **Dose Metric**. Since there are uncertainties about PBPK model structure (gastrointestinal uptake), metabolic parameters and kinetic relationships (Michaelis-Menten or more complex), and mode(s) of action, this assessment has taken an empirical approach of using metrics that fit the individual tumor data sets the best. The choice of other metrics did not result in substantially different risk estimates.

Chloroform

The proposed PHG of 1.0 μg/L or 1.0 ppb for chloroform was based on several studies of carcinogenicity in experimental animals, calculated from the liver and kidney tumors (Jorgenson *et al*., 1985; NCI, 1976; Roe *et al*., 1979). Epidemiological studies suggest an association of exposure to disinfection byproducts with tumors in humans.

Sources of uncertainty in the development of the proposed PHG for chloroform in drinking water are also the general issues of uncertainty in any risk assessment. These sources include knowledge of the mode of action, human variability, intraspecies extrapolation, dose-response extrapolation, and relative source contribution. Additional sources of uncertainty specific to chloroform include the influence of other THMs present in drinking water on chloroform metabolism and toxicity, and the suitability of the carcinogenicity database.

Chloroform is listed as a chemical known to the State of California to cause cancer under Proposition 65, with a no-significant risk level of 20 μg/day (OEHHA, 2001). U.S. EPA (2001e, 2008c) reviewed the available data for carcinogenicity and assigned chloroform to Group B2, probable human carcinogen. However, the final conclusion of a lengthy evaluative process was that human carcinogenic hazard would apply only to high doses – i.e., that this chemical should be treated as a threshold carcinogen. This is summarized in IRIS (U.S. EPA, 2008c) as:

> "[C]hloroform is *likely to be carcinogenic to humans by all routes of exposure* under high-exposure conditions that lead to cytotoxicity and regenerative hyperplasia in susceptible tissues…. Chloroform is *not likely to be carcinogenic to humans by any route of exposure* under exposure conditions that do not cause cytotoxicity and cell regeneration. This weight-of-evidence conclusion is based on: 1) observations in animals exposed by both oral and inhalation pathways which indicate that sustained or repeated cytotoxicity with secondary regenerative hyperplasia precedes, and is probably required for, hepatic and renal neoplasia; 2) there are no epidemiological data specific to chloroform and, at most, equivocal epidemiological data related to drinking water exposures that cannot necessarily be attributed to chloroform amongst multiple other disinfection byproducts; and 3) genotoxicity data on chloroform are essentially negative, although there are some scattered positive results that generally have limitations such as excessively high dose or with confounding factors. Thus, the weight-of-evidence of the genotoxicity data on chloroform supports a conclusion that chloroform is not strongly mutagenic, and that genotoxicity is not likely to be the predominant mode of action underlying the carcinogenic potential of chloroform."

This conclusion was reached after highly contentious discussions culminating in a lawsuit against U.S. EPA, which forced them to vacate their prior judgement that uncertainty regarding carcinogenic mechanisms for chloroform would require them to treat it as a non-threshold agent. The litigation is discussed in more detail under "Other Regulatory Standards" below.

IARC (1999d) concluded that there is sufficient evidence for carcinogenicity of chloroform in experimental animals and inadequate evidence in humans and assigned chloroform to Group 2b, possibly carcinogenic in humans. Although the U.S. EPA (2001e, 2008c) and IARC (1999d) conclusions indicate considerable uncertainty regarding the carcinogenicity of chloroform, OEHHA considers use of cancer data for development of the PHG to be appropriate based on our updated literature review, the IARC classification, and OEHHA's prior assessment of the available data under Proposition 65.

For calculation of the proposed PHG for chloroform it was assumed that the drinking water consumption rate of an adult is 2.0 L/day, based on data for consumption by the general population in the Western Region of the U.S. (OEHHA, 2000). An additional exposure equivalent of 1.0 L_{eq}/day was included to account for inhalation and dermal uptake resulting from other household activities. A total consumption of 3.0 L_{eq}/day could either overestimate or underestimate actual human exposure. Too few measurement data are available for exposure from media other than tap water to permit a quantitative assessment of the relative source contribution. Use of a relative source contribution of 80 percent accounts for possible minor exposures via media other than tap water.

Chloroform was reported as detected in 4.5 percent of samples (536/11, 848) in California's Water Quality Monitoring Database for public drinking water systems that use either ground or surface water sources (Storm, 1994). The mean concentration in samples with reported chloroform was 6.20 µg/L, which is well above the proposed PHG

of 1.0 µg/L. This suggests that levels of chloroform in the California water supply may be of public health significance.

Bromoform

The proposed PHG of 5 ppb for bromoform was based on carcinogenic effects in F344/N rats following two years of exposure to bromoform by corn oil gavage (NTP, 1989a). Adenatomous polyps and adenocarcinomas in the large intestine of female rats were the most sensitive effects observed. These tumors are uncommon in rats administered a corn oil vehicle (NTP, 1989a). Sources of uncertainty in development of the proposed PHG for bromoform in drinking water are the general issues of uncertainty in any risk assessment, including interspecies and dose-response extrapolations. Additional sources of uncertainty specific to bromoform include the influence of other THMs in drinking water on bromoform metabolism and toxicity and the suitability of the carcinogenicity database for risk estimation.

Chlorinated tap water may contain the four THMs in varying proportions. These compounds are thought to be metabolized by at least two common P450-mediated pathways (the oxidative and reductive pathways). There is some evidence to suggest that brominated THMs are also metabolized by a glutathione *S*-transferase theta mediated pathway, which has a low affinity for chloroform. At present, there is little or no experimental data on interactions among THMs with respect to metabolism or manifestations of toxicity. Thus the effect of concurrent exposure is unknown.

The database for bromoform carcinogenicity includes two oral studies in rats and mice. NTP (1989a) found clear evidence of carcinogenicity in female rats and some evidence of carcinogenicity in male rats, based on increased incidence of uncommon tumors of the large intestine. The same investigators found no evidence for carcinogenicity in male or female mice, although a maximum tolerated dose may not have been achieved in males in that bioassay. The bioassay data in rats are supported by positive results for bromoform genotoxicity in some *in vitro* and *in vivo* assays and by formation of lung tumors in Strain A mice exposed to bromoform by injection (Theiss *et al*., 1977).

Bromoform is listed as a chemical known to the State of California to cause cancer under Proposition 65 (OEHHA, 2002). U.S. EPA (2005, 2008a) reviewed the available data for carcinogenicity and assigned bromoform to Group B2, probable human carcinogen, based on the clear evidence in female rats and structural similarity to other THMs verified as probable or possible carcinogens. IARC (1991b, 1999b) concluded that there is limited evidence for carcinogenicity in experimental animals and inadequate evidence in humans and assigned bromoform to Group 3, not classifiable as to carcinogenicity in humans. Although the IARC (1999b) classification indicates some uncertainty regarding the carcinogenicity of bromoform, OEHHA considers use of cancer data for development of the PHG to be appropriate based on the U.S. EPA classification and OEHHA's prior assessment of the available data under Proposition 65.

In calculating the proposed PHG for bromoform, it was assumed that the drinking water consumption rate of an adult is 2.0 L/day (OEHHA, 2000). An additional exposure equivalent of 1.1 L_{eq}/day was included to account for inhalation and dermal uptake

during other household activities. A total consumption of 3.1 L_{eq}/day could either overestimate or underestimate actual human exposure.

Bromodichloromethane

The proposed PHG of 0.4 ppb for BDCM was based on carcinogenic effects in female $B6C3F_1$ mice exposed to BDCM for two years by corn oil gavage (NTP, 1987). Induction of hepatic tumors (combined adenomas and carcinoma) was selected because they were the most sensitive effects observed. Sources of uncertainty in development of the proposed PHG for BDCM in drinking water are the general issues of uncertainty in any risk assessment, including interspecies and dose-response extrapolations. Additional specific sources of uncertainty for BDCM include the influence of other THMs in drinking water on BDCM metabolism and toxicity, the suitability of the carcinogenicity database, and human health concerns raised by epidemiological studies.

The database for BDCM carcinogenicity includes two well-conducted oral studies in rats and mice. NTP (1987) found clear evidence of carcinogenicity in male and female F344/N rats exposed to BDCM by corn oil gavage for two years, based on increased incidence of tumors in the large intestine and kidney. Clear evidence of carcinogenicity was also obtained in male and female $B6C3F_1$ mice, based on increased incidence of kidney and liver tumors, respectively. The NTP bioassay findings are supported by positive results for BDCM genotoxicity obtained in some *in vitro* and *in vivo* assays. Therefore, use of cancer data for development of the PHG is considered appropriate.

The database on human health effects of BDCM is limited. Some recent epidemiologic studies found weak associations between consumption of disinfected tap water containing BDCM and increased risk of adverse pregnancy outcomes. Such studies are confounded by coexposures to a great variety of identified and unidentified disinfection byproducts.

For calculation of the proposed PHG for BDCM, it was assumed that the drinking water consumption rate of an adult is 2.0 L/day (OEHHA, 2000). An additional exposure equivalent of 1.1 L_{eq}/day was included to account for inhalation and dermal uptake during showering, bathing, and other household activities. A total consumption of 3.1 L_{eq}/day could either overestimate or underestimate actual human exposure.

Dibromochloromethane

The proposed PHG of 0.7 ppb for DBCM was based on carcinogenic effects observed in female $B6C3F_1$ mice following two years of exposure to DBCM by corn oil gavage (NTP, 1985). Induction of hepatic tumors (combined adenomas and carcinoma) was selected because they were the most sensitive effects. Sources of uncertainty in development of the proposed PHG for DBCM in drinking water are the general issues of uncertainty in any risk assessment, including interspecies and dose-response extrapolations. Additional sources of uncertainty specific to DBCM include the influence of other THMs in drinking water on DBCM metabolism and toxicity, the suitability of the carcinogenicity database, and the limited availability of data on human health effects.

The database for DBCM carcinogenicity consists of two oral exposure studies conducted in rats and mice (NTP, 1985). NTP (1985) found no evidence for carcinogenicity in male

and female rats, equivocal evidence in male mice, and some evidence in female mice based on increased incidence of hepatic tumors. Other data relevant to evaluation of the carcinogenic potential of DBCM include positive results in some mutagenicity tests and structural similarity to bromoform and BDCM. Clear evidence for the carcinogenic potential of these closely-related brominated THMs has been obtained in one or more well-conducted oral exposure studies.

The database on human health effects of DBCM is limited. Recent epidemiologic studies have found no association or weak associations between consumption of disinfected tap water containing DBCM and increased risk of adverse pregnancy outcomes. Two additional studies were unable to examine potential associations with DBCM because its low concentration in the tap water. Such studies are confounded by coexposures to a great variety of identified and unidentified disinfection byproducts. No occupational exposure studies were identified in the materials reviewed for this document.

In calculating the proposed PHG for DBCM, it was assumed that the tap water intake rate of an adult is 2.0 L/day (OEHHA, 2000). An additional exposure equivalent of 1.2 L_{eq}/day was included to account for inhalation and dermal uptake during showering, bathing, and other household activities. A total consumption of 3.2 L_{eq}/day could either overestimate or underestimate actual human exposure to DBCM.

Other Cancer Slope Factors

Earlier values of oral cancer slope factors from U.S. EPA and OEHHA for the individual THMs that were employed to develop health based criteria or in the development of standards are summarized in Table 73. In addition to the values in this table, oral and inhalation cancer slope factors of 0.019 and 0.031 $(mg/kg\text{-}day)^{-1}$, respectively, have been used to derive no significant risk levels (NSRLs) for chloroform under California's Proposition 65 in Title 22, Section 12705c, based on a 1990 risk assessment. For BDCM, an oral or inhalation slope factor of 0.13 $(mg/kg\text{-}day)^{-1}$ has been used as the basis of a NSRL under Proposition 65 in California based on an U.S. EPA risk assessment.

Table 73. Comparison of Oral Cancer Slope Factors for Individual Trihalomethanes

Chemical, organization; key Studies	Animal sex, species, tumor site	Dose response dose metric, low dose extrapolation method	Interspecies extrapolation method	Calculation	Final cancer slope factor $(mg/kg\text{-}day)^{-1}$
Chloroform					
DHS (1990): Jorgenson *et al*. (1985), NCI (1976), Roe *et al*. (1979), Tumasonis *et al*. (1985)	Male rat Kidney Male mouse Kidney Male and Female rat Liver	TWA* applied dose, Metabolized dose; LMS	$BW^{2/3}$	a complicated weighted average of all 15 relevant values including 2 dose metrics	0.031
U.S. EPA (2008c): Jorgenson *et al*. (1985)	Male rat Kidney	TWA applied dose; LMS**	$BW^{2/3}$	a single study value which discounts all other data	0.006
OEHHA (2000) proposed: Jorgenson *et al*. (1985), NCI (1976), Roe *et al.* (1979)	Male rat Kidney Male mouse Kidney	TWA metabolized dose, TWA applied dose, TWA target tissue dose; LED_{10}	$BW^{3/4}$	a single site, 3 studies, 3 dose metrics with a geometric mean	0.065
Bromoform					
U.S. EPA (2008a): NTP (1989a)	Female rat Large intestine	TWA applied dose; LMS	$BW^{2/3}$	single site	0.0079
OEHHA (1993), Brown *et al*. (1993): NTP (1989a)	Female rat Large intestine	TWA applied dose; LMS	$BW^{2/3}$	single site	0.007
OEHHA (2000) proposed: NTP (1989a)	Female rat Large intestine	TWA applied dose; LED_{10}	$BW^{3/4}$	single site	0.0025

BDCM					
U.S. EPA (2008b): NTP (1987)	Male mouse Kidney	TWA applied dose; LMS	$BW^{2/3}$	single site	0.062
OEHHA (1993), Brown *et al*. (1993): NTP (1987)	Male rat Large intestine	TWA applied dose; LMS	$BW^{2/3}$	single most relevant site	0.03
OEHHA (2000) proposed: NTP (1987)	Male rat Large intestine	TWA applied dose; LED_{10}	$BW^{3/4}$	single most relevant site, more sensitive sex	0.029
DBCM					
NTP (1985)	Female mouse Liver	TWA applied dose; LMS	$BW^{2/3}$	single site	0.084
OEHHA (1993), Brown *et al*. (1993): NTP (1985)	Female mouse Liver	TWA applied dose; LMS	$BW^{2/3}$	single site	0.04
OEHHA (2000) proposed: NTP (1985)	Female mouse Liver	TWA applied dose; LED_{10}	$BW^{3/4}$	single Site	0.041

*TWA: Time-weighted average
**LMS: 95 percent upper bound on the low dose slope by the linearized multistage model (q_1*), extra risk
LED_{10}: 95 percent lower bound on the dose yielding a 10 percent tumor incidence for model free low dose extrapolation ($0.1 \div LED_{10} = CSF$)
BW^y: body weight power scaling to adjust animal values to human equivalence

OTHER REGULATORY STANDARDS

U.S. EPA Drinking Water Standards

After the U.S. Congress passed the Safe Drinking Water Act in 1974 to help fulfill the need for clean and safe water, chloroform was shown to be carcinogenic in rodents in 1976. This prompted the U.S. EPA (1979a,b) to develop a drinking water rule that provided guidance on the levels of total THMs allowed in drinking water. The U.S. EPA set an interim MCL of 0.1 mg/L or 100 μg/L or 100 ppb for total THMs as the annual average sum of the concentrations of chloroform, bromoform, BDCM, and DBCM. In 1992 the U.S. EPA initiated a negotiated rulemaking to develop an approach that would reduce exposure to disinfectants and disinfection byproducts without undermining the control of microbial pathogens. This resulted in a proposed Stage 1 disinfection byproduct rule (U.S. EPA, 1994c), which was finalized four years later (U.S. EPA, 1998i,k).

As part of the Stage 1 Rule, the U.S. EPA lowered the MCL to 0.08 mg/L for total THMs, effective for large surface water systems serving more than 10,000 people on January 1, 2002, and effective for all other ground water systems and small surface water systems on January 1, 2004. The U.S. EPA (1998d,e,f,g,i,k) also established MCLGs of zero for chloroform, bromoform, and BDCM, and 0.06 mg/L (60 μg/L or 60 ppb) for DBCM under the Stage 1 rules. On January 14, 2002, U.S. EPA (2002) finalized the Long Term 1 Enhanced Surface Water Treatment Rule to improve control of microbial pathogens and address risk trade-offs with disinfection byproducts. In July 2003, U.S. EPA proposed the Stage 2 Disinfectants and Disinfection Byproducts (DBP) Rule focusing on public health protection to reduce the variability of exposure to disinfection byproducts, specifically total THMs and five haloacetic acids. The final Stage 2 DBP rule, published in the Federal Register on January 4, 2006, is one part of the Microbial and Disinfection Byproducts Rules that address risks from microbial pathogens and disinfectants/disinfection byproducts (U.S. EPA, 2006). The Stage 2 DBP rule limits exposure to DBPs, specifically total trihalomethanes (TTHM) and five haloacetic acids which can form in water through disinfectants used to control microbial pathogens. This rule applies to all community water systems and nontransient noncommunity water systems that add a primary or residual disinfectant or deliver water that has been disinfected by a primary or residual disinfectant other than UV light.

The Stage 1 Rules initially established an MCLG of zero for chloroform in 1998 (U.S. EPA, 1998i,k); however, the U.S. EPA removed the zero MCLG for chloroform effective May 30, 2000, in accordance with a court order (U.S. EPA, 1998c,g,i; 2000d). The MCLG had been challenged by the Chlorine Chemistry Council and Chemical Manufacturers Association, and the U.S. Court of Appeals for the District of Columbia Circuit found that the U.S. EPA had not used the best available, peer-reviewed science to set the MCLG as required by the Safe Drinking Water Act (U.S. EPA, 2000e). The Court found the scientific evidence to indicate that chloroform produces carcinogenic effects only when human exposures exceed certain minimum or threshold levels and issued an order vacating the zero MCLG (U.S. EPA, 2000e). Under the Stage 2 Rule, the MCLG for chloroform has been set at 0.07 mg/L (U.S. EPA, 2006).

Under the Stage 1 Rules, the U.S. EPA (1998i,k) also established MCLGs of zero for bromoform and BDCM, and a MCLG of 0.06 mg/L for DBCM; these levels were maintained in the Stage 2 Rule (U.S. EPA, 2006). The zero MCLG for bromoform is based on a weight of evidence classification that bromoform is a Group B2 chemical (probable human carcinogen) based on a consideration of all relevant health data including cancer and noncancer effects (U.S. EPA, 1998d, 2008a), including a NTP (1989a) chronic animal carcinogenicity study (U.S. EPA, 1998d). The MCLG of zero for BDCM is based on the classification of this chemical as a Group B2 chemical, probable human carcinogen (U.S. EPA, 1998c,e, 2008b), derived from inadequate human data and sufficient evidence of carcinogenicity in animal studies. The MCLG of 0.06 mg/L for DBCM is based on the classification of this chemical as a Group C chemical, a possible human carcinogen (U.S. EPA, 1998c), derived from inadequate human data and limited evidence of carcinogenicity in animal studies (U.S. EPA, 2008d).

Other Drinking Water Standards and Guidelines

In 2000, WHO established Tolerable Daily Intake (TDI) values for disinfectant byproducts in drinking water as described below for chloroform, bromoform, and DBCM (WHO, 2000).

In Canada, the interim maximum acceptable concentration (IMAC) for total THMs is 0.1 mg/L in public and private drinking water supplies based on the potential cancer risk associated with chloroform (Environment Canada, 1995). This standard was based on using optimum methods for conventional water treatment processes to reduce total THM concentrations to below 0.1 mg/L, and cancer potency estimates for chloroform. The 0.1 mg/L standard is based on combined incidence of renal tubular cell adenomas or adenocarcinomas in rats administered chloroform in drinking water. The standard is within a range of potential health risk that is considered to be essentially negligible. The maximum acceptable concentration is designated as interim until such time as the risk from other disinfection byproducts are ascertained.

The European Union (1998a) established a standard of 100 µg/L for total THMs in drinking water in Directive 98/83/EC dated November 3, 1998, on the quality of water intended for human consumption, to be achieved within 10 years of December 25, 1998. The interim standard for five to 10 years after publication of the directive was 150 µg/L. This supercedes the previous upper limit in water of 30 µg/L for total organohalogen compounds, which include THMs and other halogenated compounds. For residual total THMs in drinking water, Austria and Italy set a guideline goal at 30 µg/L, Germany at 10 to 25 µg/L (Hydes, 1999), and WHO (2004) at 1 µg/L.

U.S. FDA (2001) has established a regulatory limit of 0.08 mg/L in bottled water for total THMs (21 CFR 165.110).

The California Department of Public Health has had an MCL of 0.1 mg/L (100 µg/L) for total THMs under Title 22, CCR, Section 64439 since 1983 (DHS, 2002, DPH, 2008). DPH published a Proposed Disinfectants/Disinfectants Byproduct Rule on May 1, 2002, to start the process for adopting the new and anticipated federal MCLs for disinfection byproducts. This was finalized to provide a California MCL of 0.080 mg/L on June 17, 2006 (DPH, 2007).

Most of the other U.S. states have completed adoption of the federal MCL for total THMs of 0.1 mg/L. Florida, New Hampshire, and Wisconsin established a drinking water guideline of 6.0 µg/L for chloroform (HSDB, 2002b). Florida and New Hampshire established a drinking water guideline of 4.0 µg/L for bromoform (HSDB, 2002d). The drinking water guideline for bromoform in Arizona, Minnesota, and Wisconsin is 0.19, 40.0, and 4.4 µg/L, respectively (HSDB, 2002d). Arizona established a drinking water guideline of 0.19 µg/L for BDCM and Minnesota established a guideline value of 6 µg/L (HSDB, 2002a). Florida, New Hampshire, and Wisconsin have established a drinking water guideline of 0.6 µg/L for BDCM (HSDB, 2002a). Arizona, Florida, Minnesota, and Wisconsin have established drinking water guidelines of 0.19, 1, 10, and 60 µg/L, respectively, for DBCM (HSDB, 2002c).

Chloroform

Chloroform is listed under Proposition 65 in California as a chemical known to the State to cause cancer (OEHHA, 2005). OEHHA has promulgated a no significant risk level for chloroform associated with 1×10^{-5} lifetime excess cancer risk for the purposes of Proposition 65, as an intake level of 20 µg/day by ingestion and 40 µg/day by inhalation as regulated under 22 CCR 12705 (DHS, 1991a; OEHHA, 2002).

WHO (2000, 2004) has established a drinking water guideline of 200 µg/L for chloroformand a Tolerable Daily Intake (TDI) for chloroform in drinking water of 10 µg/kg body weight. The drinking water standard for chloroform in China is 60 µg/L (Wei and Wang, 2004; Zhao *et al*., 2004).

The U.S. EPA (2000b) drinking water health advisory for chloroform recommends that a 10 kg child not exceed 4 mg/L for one day and 10 day exposures. A drinking water equivalent level (DWEL) of 0.4 mg/L for chloroform is based on an oral reference dose (RfD) value of 0.01 mg/kg-day and a 70 kg human who consumes two L/day of drinking water (U.S. EPA, 2000b). A DWEL is a lifetime exposure concentration protective of adverse, noncancer health effects that assumes all of the exposure to a contaminant is from drinking water (U.S. EPA, 2000b). U.S. EPA (2000b) estimated that a concentration of 0.6 mg/L of chloroform in drinking water corresponds to a lifetime cancer risk of one in 10,000 (superseded by the evaluation of U.S. EPA, 2001e).

The U.S. EPA (1999b) recommended an ambient water quality criterion for chloroform for protection of human health based on "the consumption of water and organisms of less than 5.7 µg/L, and for consumption of organisms only to be less than 470 µg/L, at a cancer risk of 10^{-6}."

The California Air Resources Board (ARB) has listed chloroform as a toxic air contaminant(TAC) and as a chemical to be reported under the Air Toxics Hot Spots Program and the California Toxic Release Inventory (ARB, 1999). A unit risk of 5.3×10^{-6} $(\mu g/m^3)^{-1}$ has been developed for chloroform (DHS, 1990). An oral cancer potency of 0.031 $(mg/kg\text{-}day)^{-1}$ was also developed using the linearized multistage model, based on the Jorgenson *et al*. (1985), NCI (1976), Roe *et al*. (1979), and Tumasonis *et al*. (1985) studies. A weighted average value was derived from each of the Jorgenson *et al*. (1985) (one third) and NCI (1976) studies (one third) and the remainder from the Roe *et al*. and Tumasonis *et al*. studies (DHS, 1990, 1991b).

The American Conference of Governmental Industrial Hygienists (ACGIH) recommends a threshold limit value (TLV) for chloroform of 10 ppm for an eight hour time-weighted average (TWA) for a 40 hour week (ACGIH, 2001). ACGIH (2001) has classified chloroform as an A3 carcinogen, a confirmed animal carcinogen with unknown relevance to humans. The National Institute of Occupational Safety and Health (NIOSH, 2002) has a short-term exposure limit (STEL) of 2 ppm chloroform for a 15 minute time-weighted average (NIOSH, 2002). NIOSH has recommended an immediately dangerous to life and health (IDLH) value of 500 ppm chloroform (NIOSH, 2002). Additionally, NIOSH has designated chloroform as a potential occupational carcinogen.

The U.S. Occupational Safety and Health Administration (OSHA) has mandated a permissible exposure level (PEL) of 50 ppm chloroform as a ceiling limit (OSHA, 1997) for general, construction, and maritime industries

ATSDR (1997) has derived a minimum risk level (MRL) of 0.1 ppm for acute inhalation exposure to chloroform, a MRL of 0.05 ppm for intermediate duration inhalation exposure, and a minimum risk level of 0.02 ppm for chronic inhalation exposure. ATSDR (1997) has also derived a MRL of 0.01 mg/kg-day for chronic oral exposure to chloroform.

U.S. EPA (2008c, last updated 10/19/01) concludes that chloroform is a Group B2 probable human carcinogen, and that chloroform is likely to be carcinogenic to humans by all routes of exposure under high-exposure conditions that lead to cytotoxicity and regenerative hyperplasia in susceptible tissues. The U.S. EPA (2008c) oral reference dose (RfD) of 0.01 mg/kg-day for chloroform is judged to be protective against cytolethality and regenerative hyperplasia, and thus to provide adequate protection against both cancer and non-cancer effects. No reference concentration (RfC) has been derived.

The U.S. EPA regulates chloroform under the Clean Air Act (CAA) and lists it as a hazardous air pollutant (HAP) (U.S. EPA, 1990), based on serious health effects, including cancer, from ambient air exposure to chloroform (U.S. EPA, 1994b). For chloroform, the U.S. EPA establishes a threshold quantity of 20,000 pounds for accidental release prevention (40 CFR 68.130), and a toxicity endpoint of 0.49 mg/L (40 CFR 68, Appendix A) (U.S. EPA, 1997b,c,e, 2000c,d).

Chloroform has been designated as a toxic waste for commercial chemical products, manufacturing chemical intermediates, or off-specification commercial chemical products (U.S. EPA, 1980c) and as a toxic pollutant under the Clean Water Act (U.S. EPA, 1981). Additionally, U.S. EPA designated a maximum concentration of chloroform for hazardous waste characterization of 6.0 mg/L.

IARC (1991b, 1999d) has classified chloroform as a Group 2B chemical, possibly carcinogenic to humans, based on inadequate evidence in humans and sufficient evidence in experimental animals for carcinogenicity. Additionally, IARC (1991b) has classified chlorinated drinking water as a Group 3 chemical, not classifiable as to carcinogenicity to humans, based on inadequate evidence of carcinogenicity in humans and experimental animals. NTP (2001) has classified chloroform as reasonably anticipated to be a human carcinogen, based on sufficient evidence of carcinogenicity in experimental animals and inadequate evidence for carcinogenicity in humans. Chloroform has been listed as a

Priority Substance under the Canadian Environmental Protection Act (Meek *et al*., 2002). The German Commission for the Investigation of Health Hazards of Chemical Compounds in the Work Area (also called MAK Commission) classified chloroform into Category 4 in 1998. Category 4 chemicals are suspected carcinogens known to act by nongenotoxic mechanisms, which are considered to not contribute significantly to the risk of cancer to man, if an appropriate exposure limit such as the MAK value is observed. The MAK value of 0.5 mL/m^3 or 2.5 mg/m^3 for chloroform was established in 1999 (Greim and Reuter, 2001).

Bromoform

OEHHA has listed bromoform as a chemical known to the State to cause cancer under Proposition 65 (OEHHA, 2002). OEHHA has proposed a draft no significant risk level of 64 µg/day for bromoform associated with 1×10^{-5} lifetime excess cancer risk (OEHHA, 2005).

WHO (2000, 2004) has established a drinking water guideline value of 100 µg/L for bromoform and a TDI of 25 µg/kg body weight. The drinking water standard for bromoform in China is 100 µg/L (Wei and Wang, 2004).

The U.S. EPA (2000b) drinking water health advisory for bromoform recommends that a 10 kg child not exceed 5 mg/L for a one day exposure and 2 mg/L for a 10 day exposure. Additionally, based on an oral reference dose (RfD) value of 0.02 mg/kg-day for bromoform, and a 70 kg human who consumes two liters of drinking water, a drinking water equivalent level (DWEL) of 0.7 mg/L was derived (U.S. EPA, 2000b). U.S. EPA (1999b) has recommended a surface water quality criterion for bromoform for the protection of human health based on "for consumption of water and organisms to be less than 4.3 µg/L, and for consumption of organisms only to be less than 360 µg/L, based on a 10^{-6} carcinogenicity risk."

California ARB (1999) has listed bromoform as a toxic air contaminant since 1993 (, and as a chemical to be reported under the Air Toxics "Hot Spots" Program and the California Toxic Release Inventory.

ACGIH recommends a threshold limit value of 0.5 ppm for an eight hour time weighted average for a 40 hour week (ACGIH, 2001) for bromoform. ACGIH (2001) has designated bromoform with a skin notation based on potential hazards from contact with vapors or direct skin contact and has classified bromoform as an A3 carcinogen, a confirmed animal carcinogen with unknown relevance to humans.

NIOSH issued a recommended exposure limit (REL) of 0.5 ppm for a 10 hour time-weighted average for a 40 hour week and an immediately dangerous to life and health value of 850 ppm (NIOSH, 2002). NIOSH also has designated bromoform with a skin notation and recommends personal protective clothing to avoid exposure. OSHA has mandated a permissible exposure level (PEL) of 0.5 ppm for an eight hour time-weighted average for a 40 hour week (OSHA, 1997), with a skin designation.

The U.S. EPA regulates bromoform under the Clean Air Act (CAA) and it is listed as a hazardous air pollutant (HAP) (U.S. EPA, 1990). CERCLA has designated bromoform as a hazardous substance (U.S. EPA, 2001c).

IARC (1991b, 1999b) has concluded that there is limited evidence of carcinogenicity in animals and inadequate evidence in humans and has categorized bromoform as a Group 3 chemical, not classifiable as to carcinogenicity in humans. NTP (2001) has classified bromoform as reasonably anticipated to be a human carcinogen based on sufficient evidence of carcinogenicity in experimental animals and inadequate evidence for carcinogenicity in humans. Bromoform is classified by the U.S. EPA (1994a, 2008a) as a Group B2 chemical, probable human carcinogen.

Bromodichloromethane

OEHHA has listed BDCM under Proposition 65 in California as a chemical known to the State to cause cancer (OEHHA, 2005). OEHHA has promulgated a no significant risk level for BDCM associated with a 1×10^{-5} lifetime excess cancer risk at an intake level of 5 μg/day as regulated for Proposition 65 (OEHHA, 2002).

WHO (2000, 2004) established a guideline for drinking water quality of 60 μg/L for BDCM associated with an excess lifetime cancer risk of 10^{-5} or 6 μg/L for a 10^{-6} excess lifetime cancer risk. The drinking water standard for BDCM in China is 60 μg/L (Wei and Wang, 2004).

The U.S. EPA (2000b) drinking water health advisory for BDCM recommends that a 10 kg child not exceed 6 mg/L for one day and 10 day exposures. Additionally, based on an oral reference dose (RfD) value of 0.02 mg/kg-day for BDCM, and a 70 kg human who consumes two liters of drinking water per day, a drinking water equivalent level (DWEL) of 0.7 mg/L is derived (U.S. EPA, 2000b).

The U.S. EPA (1999b) has recommended an ambient water quality criterion for BDCM for the protection of human health based on "the consumption of water and organisms to be less than 0.56 μg/L, and for the consumption of an organism only to be less than 46 μg/L, based on carcinogenicity of 10^{-6} risk."

ACGIH (2001), NIOSH (2002), and OSHA (1997) have not recommended or mandated occupational exposure limits for BDCM. The U.S. EPA has designated BDCM as a toxic pollutant (U.S. EPA, 1981).

IARC (1991a, 1999a) has classified BDCM as a Group 2B chemical, possibly carcinogenic to humans, based on sufficient evidence in experimental animals for carcinogenicity. NTP (1998b) has classified BDCM as reasonably anticipated to be a human carcinogen based on sufficient evidence of carcinogenicity in experimental animals. BDCM is classified by the U.S. EPA (1994a, 2008b) as a Group B2 chemical, probable human carcinogen.

Dibromochloromethane

DBCM was listed on January 1, 1990 under Proposition 65 in California as a chemical known to the State to cause cancer based on the U.S. EPA classification of the chemical as a Group B2 probable human carcinogen (OEHHA, 2005). As a result of the U.S. EPA's subsequent reclassification of DBCM as a Group C carcinogen and a review of the evidence of carcinogenicity by the State's Proposition 65 review panel, known as the Carcinogen Identification Committee, the chemical was removed from the Proposition 65

list as a chemical known to cause cancer on October 29, 1999. A no significant risk level for DBCM of 7 μg/day had been adopted into regulation when it was on the Proposition 65 list; when DBCM was delisted, the no significant risk level was rescinded.

WHO (2000, 2004) has established a guideline for drinking water quality of 100 μg/L for DBCM and a TDI of 30 μg/kg. The drinking water standard for DBCM in China is 100 μg/L (Wei and Wang, 2004).

The U.S. EPA (2000b) drinking water health advisory for DBCM recommends that a 10 kg child not exceed 6 mg/L for one day and 10 day exposures. A drinking water equivalent level (DWEL) of 0.7 mg/L for DBCM is derived based on an oral reference dose (RfD) value of 0.02 mg/kg-day and a 70 kg human who consumes two L of drinking water per day (U.S. EPA, 2000b). Additionally, a concentration of 0.06 mg/L of DBCM in drinking water is not expected to cause any adverse noncarcinogenic effects for a lifetime of exposure, and a concentration of 0.04 mg/L corresponds to an estimated lifetime cancer risk of one in 10,000 (U.S. EPA, 2000b).

The U.S. EPA (1999b) has recommended an ambient water quality criterion for DBCM for protection of human health based on consumption of water and organisms to be less than 0.41 μg/L, and for consumption of aquatic organisms only to be less than 34 μg/L, based on 10^{-6} carcinogenicity risk.

ACGIH (2001), NIOSH (2002), and OSHA (1997) have not recommended or mandated occupational exposure limits for DBCM. CERCLA has designated DBCM as a hazardous substance (U.S. EPA, 2001a,c).

IARC (1991c, 1999c) has classified DBCM as a Group 3 chemical, not classifiable as to its carcinogenicity to humans, based on limited evidence in experimental animals for carcinogenicity. NTP (2001) has not classified DBCM as a human carcinogen. DBCM is classified by the U.S. EPA (1994a, 2008d) as a Group C chemical, possible human carcinogen.

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APPENDIX

The PBPK model for chloroform was constructed using Powersim 2.01 software for Windows (MicroWorlds, Cambridge, MA). Model parameters are given in Table A1, and 24 hour simulation results for the Jorgenson *et al*. (1985) study are shown in Table A2 (nominal doses in mg/kg-day, model doses in moles for a 0.46 kg rat, and moles of metabolites and macromolecular binding (MMB) in both liver and kidney model compartments at 24 hour).

TableA1. PBPK Model Parameters for Rat Drinking Water Exposure to Chloroform

Parameter	**Rat 0.46 kg**
Tissue Volumes: percent body weight, L	
Liver: 2.53 (V_L)	0.011638a
Kidney: 0.71 (V_K)	3.266×10^{-3}
Fat: 6.30	0.02898
Vessel Rich Group 4.39	0.020194
Muscle: 77.07	0.3545
Tissue Flows: percent cardiac output, L/hour	
Liver: 25.0	2.127b
Kidney: 25.0	2.127
Fat: 2.0	0.1702
Vessel Rich Group: 29.0	2.4673
Muscle: 19.0	1.61652
Cardiac output, L/hour	8.508
Alveolar ventilation, L/hour	8.508
Partition Coefficients, dimensionless	
Blood/Air	20.8
Liver/Blood	1.0144
Kidney/Blood	0.5288
Fat/Blood	9.7596
Vessel Rich Group/Blood	0.66826
Metabolism	
Vmax Liver, mol/hour	2.20×10^{-5}b
Km, *M*	4.55×10^{-6}
A (kidney/liver)	0.052
Vmax Kidney, mol/hour = A × (V_K/V_L) × Vmax Liver	3.22×10^{-7}
f_{MMB} Liver/hour	0.00104
f_{MMB} Kidney/hour	0.0086
Input Rate Constants	
KSI: Stomach to Intestine/hour	0.009
KAI: Intestine to Liver	1.4×10^{-4}
KAS: Stomach to Liver	0.3

Table A2. PBPK Model Simulation of Chloroform Metabolism in the Liver and Kidney of the Rat following Drinking Water Exposure in the Jorgenson *et al*. (1985) Study

Applied dose, mg/kg-day	Model dose, mol/day	Liver metabolites, mol/day	Liver metabolites bound to macro-molecules, mol/day	Kidney metabolites, mol/day	Kidney metabolites bound to macro-molecules, mol/day	Mixed venous concentration at 12 hour after dosing, *M*
18	5.94×10^{-5}	5.8×10^{-5}	1.26×10^{-6}	4.14×10^{-7}	8.09×10^{-8}	1.00×10^{-7}
38	1.46×10^{-4}	1.07×10^{-4}	2.27×10^{-6}	9.64×10^{-7}	1.87×10^{-7}	2.27×10^{-7}
79	3.04×10^{-4}	1.64×10^{-4}	3.32×10^{-6}	1.72×10^{-6}	3.18×10^{-7}	5.75×10^{-7}
155	5.98×10^{-4}	2.17×10^{-4}	4.17×10^{-6}	2.44×10^{-6}	4.27×10^{-7}	1.61×10^{-6}

The values in Table A2 were converted into three dose metrics for dose response analysis (Table A3). The first metric is the total metabolized dose in liver and kidney, the sum of the four central rows in Table A2. The use of this metric would involve the assumption that all or part of the metabolites generated in the liver might also be active in the kidney. The second metric is the kidney metabolized dose, the sum of the metabolites generated in the kidney and bound to macromolecules (MMB). This metric involves the assumption that only metabolites generated in the kidney are likely to be involved in carcinogenesis there. Both of these metrics are calculated on a body weight basis (mg/kg-day). The final dose metric is based only on the bound metabolites in the kidney and is expressed as mg MMB/L_{kidney}-day. This metric involves the assumption that only metabolites bound in the kidney are related to the carcinogenic effects seen. All the metrics are in chloroform equivalents (i.e., 119.39 g/mol).

Table A3. Dose Metrics Derived from PBPK Simulations of Chloroform Drinking Water Exposure in Rats (Jorgenson *et al*., 1985)

Applied dose, mg/kg-day	Total metabolized dose, mg/kg-day	Kidney metabolized dose, mg/kg-day	Kidney metabolites bound to macromolecules dose, mg MMB/L_{kidney}-day
18	15.5	0.13	2.96
38	28.7	0.29	6.84
79	43.9	0.53	11.6
155	58.1	0.74	15.6

The simulation results and dose metrics for the NCI (1976) male rat cancer study are shown in Table A4. For the studies involving oil gavage administration of chloroform the KAS input rate constant was 0.6/hour. The dose metrics derived from the mouse studies are given in Table A5. In this case a PBPK model similar to that of Corley *et al.* (1990) was used. Since limited information was available on the uptake kinetics of chloroform from toothpaste base used in the studies of Roe *et al*. (1979), the 24-hour simulations were conducted with KAS values for oil (0.6/hour), water (5.0/hour), and an intermediate value of 1.0/hour. The latter value was chosen for calculation of the final toothpaste dose metrics. The data set with arachis oil used the KAS value of 0.6/hour.

Table A4. PBPK Model 24-Hour Simulations and Chloroform Dose Metrics for NCI (1976) Male Rat Cancer Bioassays

Model dose, mol/day	Liver metabolites, mol/day	Liver metabolites bound to macro-molecules, mol/day	Kidney metabolites, mol/day	Kidney metabolites bound to macro-molecules, mol/day
0	0	0	0	0
3.46×10^{-4}	1.28×10^{-4}	2.78×10^{-6}	1.51×10^{-6}	2.93×10^{-7}
6.92×10^{-4}	1.65×10^{-4}	3.43×10^{-6}	2.01×10^{-6}	3.73×10^{-7}
Applied dose mg/kg-day	**Lifetime time-weighted average dose, mg/kg-day**	**Total metabolized dose, mg/kg-day**	**Kidney metabolized dose, mg/kg-day**	**Kidney metabolites bound to macro-molecules dose, mg/L_{kidney}-day**
0	0	0	0	0
90	48.2	18.2	0.25	5.7
180	96.4	23.7	0.33	7.3

Table A5. PBPK Derived Dose Metrics for Chloroform Dosed Mice

Study	Applied dose, mg/kg-day	Lifetime time-weighted average dose, mg/kg-day	Total metabolized dose, mg/kg-day	Liver or kidney metabolized dose, mg/kg-day	Liver or kidney metabolites bound to macro-molecules dose, mg/L-day
NCI (1976) Male liver	0, 138, 277	0, 83, 167	0, 43.5, 57.9	0, 43.5, 57.8	0, 46.7, 60.9
NCI (1976) Female liver	0, 238, 477	0, 143, 287	0, 54.8, 69.1	0, 54.8, 69.1	0, 58.3, 74.2
Roe *et al*. (1979) Male kidney, toothpaste vehicle	0, 17, 60	0, 11.2, 39.5	0, 10.3, 28.7	0, 0.24, 0.98	0, 2.94, 11.87